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Rockefeller University

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146.	Specificity of synaptic connections	Poster	Tue AM
139.	Sprouting and sprouting mechanisms	Poster	Tue AM
54.	Synaptogenesis I	Slide	Mon PM
147.	Synaptogenesis II	Poster	Tue AM
106.	Transmitter phenotypic plasticity I	Slide	Tue AM
278.	Transmitter phenotypic plasticity II	Poster	Wed PM
155.	Transplantation I	Slide	Tue PM
269.	Transplantation II	Slide	Wed PM
349.	Transplantation III	Poster	Thu AM
396.	Transplantation IV	Poster	Thu PM
397.	Transplantation V	Poster	Thu PM
219.	Trophic agents I	Slide	Wed AM
300.	Trophic agents II	Poster	Wed PM
299.	Trophic agents: nerve growth factor	Poster	Wed PM
111.	Trophic agents: nerve growth factor receptors I	Slide	Tue AM
298.	Trophic agents: nerve growth factor receptors II	Poster	Wed PM
163.	Trophic interactions I	Slide	Tue PM
301.	Trophic interactions II	Poster	Wed PM
302.	Trophic interactions III	Poster	Wed PM
218.	Visual system: cortical development and plasticity I	Slide	Wed AM
373.	Visual system: cortical development and plasticity II	Poster	Thu PM
34.	Visual system: development and plasticity of retina and optic nerve	Poster	Mon AM
122.	Visual system: subcortical development and plasticity I	Poster	Tue AM
164.	Visual system: subcortical development and plasticity II	Slide	Tue PM

Theme B: Cell Biology

Session Number	Session Title	Type	Day and Time
293.	Blood brain barrier I	Poster	Wed PM
342.	Blood brain barrier II	Poster	Thu AM
257.	**Brain spectrin: structure, location and function**	**Symp.**	**Wed PM**
408.	Cell biology: axonal transport I	Slide	Fri AM
424.	Cell biology: axonal transport II	Poster	Fri AM
423.	Cell biology: staining and tracing	Poster	Fri AM
393.	Cell-surface macromolecules	Poster	Thu PM
73.	Cellular aspects of disease I	Poster	Mon PM
74.	Cellular aspects of disease II	Poster	Mon PM
15.	Cellular aspects of disease: general	Slide	Mon AM
43.	Functions of glia I	Poster	Mon AM
45.	Functions of glia II	Poster	Mon AM
112.	Functions of glia III	Slide	Tue AM
44.	Glial and neuronal membranes	Poster	Mon AM
189.	Metabolic studies	Poster	Tue PM
4.	Molecular biology of gene expression and nucleic acids I	Slide	Mon AM
62.	Molecular biology of gene expression and nucleic acids II	Poster	Mon PM
102.	Molecular biology of gene expression and nucleic acids III	Slide	Tue AM
188.	Molecular biology of gene expression and nucleic acids IV	Poster	Tue PM
271.	Molecular biology of gene expression and nucleic acids V	Slide	Wed PM
379.	Molecular biology of gene expression and nucleic acids VI	Poster	Thu PM
392.	Molecular biology of gene expression and nucleic acids VII	Poster	Thu PM
311.	Structure and function of identified cells	Poster	Wed PM

Theme C: Excitable Membranes, Transduction and Synaptic Transmission

Session Number	Session Title	Type	Day and Time
17.	Action potentials and ion channels I	Poster	Mon AM
18.	Action potentials and ion channels II	Poster	Mon AM
49.	Action potentials and ion channels III	Slide	Mon PM
154.	Action potentials and ion channels IV	Slide	Tue PM
211.	Action potentials and ion channels V	Slide	Wed AM
261.	Action potentials and ion channels VI	Slide	Wed PM
327.	Action potentials and ion channels VII	Poster	Thu AM
328.	Action potentials and ion channels VIII	Poster	Thu AM
367.	Action potentials and ion channels IX	Poster	Thu PM
368.	Action potentials and ion channels X	Poster	Thu PM
410.	Action potentials and ion channels XI	Slide	Fri AM
99.	**Biosynthesis and localization of ion channels and pumps**	**Symp.**	**Tue AM**
235.	CNS neurons: vertebrates and invertebrates	Poster	Wed AM
294.	Drug effects on receptors I	Poster	Wed PM
295.	Drug effects on receptors II	Poster	Wed PM
185.	Pharmacology of synaptic transmission I	Poster	Tue PM
197.	Pharmacology of synaptic transmission II	Poster	Tue PM
312.	**Phosphoinositide hydrolysis, protein kinases and synaptic transmission**	**Symp.**	**Thu AM**
174.	Photoreceptors	Poster	Tue PM
8.	Postsynaptic mechanisms I	Slide	Mon AM
196.	Postsynaptic mechanisms II	Poster	Tue PM
198.	Postsynaptic mechanisms III	Poster	Tue PM
199.	Postsynaptic mechanisms IV	Poster	Tue PM
12.	Presynaptic mechanisms I	Slide	Mon AM
227.	Presynaptic mechanisms II	Poster	Wed AM
228.	Presynaptic mechanisms III	Poster	Wed AM
195.	Synaptic structure and function I	Poster	Tue PM
406.	Synaptic structure and function II	Slide	Fri AM
153.	**The neurobiology of epilepsy**	**Symp.**	**Tue PM**

Theme D: Neurotransmitters, Modulators, and Receptors

Session Number	Session Title	Type	Day and Time
158.	Acetylcholine	Slide	Tue PM
245.	Acetylcholine: behavior	Poster	Wed AM
244.	Acetylcholine: presynaptic mechanisms	Poster	Wed AM
246.	Acetylcholine: receptor actions	Poster	Wed AM
394.	Adrenergic receptors	Poster	Thu PM
79.	Anatomical localization of peptides I	Poster	Mon PM
80.	Anatomical localization of peptides II	Poster	Mon PM
81.	Anatomical localization of peptides III	Poster	Mon PM
157.	Anatomical localization of peptides IV	Slide	Tue PM
47.	**Ascorbate: a vitamin as neuromodulator**	**Wksh.**	**Mon PM**
248.	Behavioral pharmacology I	Poster	Wed AM
249.	Behavioral pharmacology II	Poster	Wed AM
250.	Behavioral pharmacology III	Poster	Wed AM
251.	Behavioral pharmacology IV	Poster	Wed AM

326.	Behavioral pharmacology V	Slide	Thu AM
108.	Catecholamine receptors	Slide	Tue AM
38.	Catecholamines I	Poster	Mon AM
314.	Catecholamines II	Slide	Thu AM
37.	Catecholamines: dopamine release and metabolism	Poster	Mon AM
378.	Catecholamines: effects of toxins	Poster	Thu PM
377.	Catecholamines: electrophysiological studies	Poster	Thu PM
209.	Catecholamines: metabolism of dopamine and effects of MPTP	Slide	Wed AM
167.	Catecholamines: synthesis and metabolic enzymes	Poster	Tue PM
134.	Characterization of cholinergic receptors I	Poster	Tue AM
265.	Characterization of cholinergic receptors II	Slide	Wed PM
40.	Characterization of nicotinic cholinergic receptors I	Poster	Mon AM
67.	Characterization of nicotinic cholinergic receptors II	Poster	Mon PM
247.	Cholinergic anatomy	Poster	Wed AM
173.	Coexistence of transmitters	Poster	Tue PM
276.	Cyclic nucleotides	Poster	Wed PM
411.	Dopamine neurons: electrophysiological studies	Poster	Fri AM
55.	Dopamine receptors I	Slide	Mon PM
133.	Dopamine receptors II	Poster	Tue AM
273.	Dopamine receptors III	Poster	Wed PM
107.	Excitatory amino acid mechanisms	Slide	Tue AM
264.	Excitatory amino acid receptors: pharmacology	Slide	Wed PM
92.	Excitotoxins	Poster	Mon PM
9.	GABA and benzodiazepine receptor mechanisms	Slide	Mon AM
53.	GABA and benzodiazepine receptors: autocoid interactions	Slide	Mon PM
181.	GABA and benzodiazepine receptors: behavioral studies	Poster	Tue PM
182.	GABA and benzodiazepine receptors: molecular characterization	Poster	Tue PM
183.	GABA and benzodiazepine: physiology	Poster	Tue PM
180.	GABA and benzodiazepine: receptor binding	Poster	Tue PM
414.	GABA pathways	Poster	Fri AM
118.	Glutamate pathways	Poster	Tue AM
20.	Glutamate receptor physiology	Poster	Mon AM
93.	Glutamate receptors	Poster	Mon PM
14.	Interactions between neurotransmitters I	Slide	Mon AM
66.	Interactions between neurotransmitters II	Poster	Mon PM
277.	Interactions between neurotransmitters III	Poster	Wed PM
225.	Localization: acetylcholine, amino acid and neuropeptide receptors	Poster	Wed AM
39.	Localization: catecholamine and serotonin receptors	Poster	Mon AM
376.	Localization: opiate, benzodiazepine, steroid and other receptors	Poster	Thu PM
350.	Metabolism of biogenic amines	Poster	Thu AM
243.	Metabolism of neurotransmitters and modulators	Poster	Wed AM
223.	Modulators I	Poster	Wed AM
274.	Modulators II	Poster	Wed PM
214.	Motor systems and sensorimotor integration: vestibular system II	Slide	Wed AM
226.	Muscarinic receptor characterization and purification	Poster	Wed AM
21.	NMDA: receptors	Poster	Mon AM
357.	**Neuronal acetylcholine receptors**	**Symp.**	**Thu PM**
48.	Opiates, endorphins and enkephalins: anatomy and chemistry I	Slide	Mon PM
115.	Opiates, endorphins and enkephalins: anatomy and chemistry II	Poster	Tue AM
116.	Opiates, endorphins and enkephalins: physiological effects I	Poster	Tue AM
215.	Opiates, endorphins and enkephalins: physiological effects II	Slide	Wed AM
279.	Opiates, endorphins and enkephalins: physiological effects III	Poster	Wed PM
320.	Opiates, endorphins and enkephalins: physiological effects IV	Slide	Thu AM
375.	Opiates, endorphins and enkephalins: physiological effects V	Poster	Thu PM

100.	**Opioids in hippocampus**	**Wksh.**	**Tue AM**
210.	Peptide biosynthesis and metabolism I	Slide	Wed AM
286.	Peptide biosynthesis and metabolism II	Poster	Wed PM
287.	Peptide biosynthesis and metabolism III	Poster	Wed PM
104.	Peptide receptors I	Slide	Tue AM
229.	Peptide receptors II	Poster	Wed AM
230.	Peptide receptors III	Poster	Wed AM
41.	Physiological effects of peptides I	Poster	Mon AM
63.	Physiological effects of peptides II	Poster	Mon PM
359.	Physiological effects of peptides III	Slide	Thu PM
404.	Physiological effects of peptides IV	Slide	Fri AM
11.	Receptor modulation: up and down regulation I	Slide	Mon AM
83.	Receptor modulation: up and down regulation II	Poster	Mon PM
117.	Receptor modulation: up and down regulation III	Poster	Tue AM
170.	Receptor modulation: up and down regulation IV	Poster	Tue PM
364.	Regional localization of receptors and transmitters	Slide	Thu PM
101.	Serotonin I	Slide	Tue AM
159.	Serotonin II	Slide	Tue PM
336.	Serotonin III	Poster	Thu AM
120.	Serotonin and histamine	Poster	Tue AM
119.	Serotonin receptors	Poster	Tue AM
337.	Serotonin: electrophysiological studies	Poster	Thu AM
123.	Transmitter cytochemistry I	Poster	Tue AM
281.	Transmitter cytochemistry II	Poster	Wed PM
213.	Transmitter cytochemistry and immunohistochemistry	Slide	Wed AM
156.	Transmitters and receptors in disease I	Slide	Tue PM
338.	Transmitters and receptors in disease II	Poster	Thu AM
339.	Transmitters and receptors in disease III	Poster	Thu AM
340.	Transmitters and receptors in disease IV	Poster	Thu AM
68.	Transmitters in invertebrates I	Poster	Mon PM
69.	Transmitters in invertebrates II	Poster	Mon PM
260.	Transmitters in invertebrates III	Slide	Wed PM
224.	Uptake, storage and secretion I	Poster	Wed AM
275.	Uptake, storage and secretion II	Poster	Wed PM

Theme E: Endocrine and Autonomic Regulation

Session Number	Session Title	Type	Day and Time
144.	Cardiovascular regulation: CNS pathways I	Poster	Tue AM
315.	Cardiovascular regulation: CNS pathways II	Slide	Thu AM
266.	Cardiovascular regulation: agonists, receptors and post-ganglionic	Slide	Wed PM
142.	Cardiovascular regulation: blood pressure I	Poster	Tue AM
161.	Cardiovascular regulation: blood pressure II	Slide	Tue PM
143.	Cardiovascular regulation: heart, cerebral flow and post-ganglionic	Poster	Tue AM
145.	Cardiovascular regulation: hypothalamus	Poster	Tue AM
82.	Endocrine and autonomic regulation: respiratory regulation I	Poster	Mon PM
135.	Endocrine and autonomic regulation: respiratory regulation II	Poster	Tue AM
91.	Neural control of immune system I	Poster	Mon PM
291.	Neural control of immune system II	Poster	Wed PM
168.	Neuroendocrine control: other I	Poster	Tue PM
399.	Neuroendocrine control: other II	Poster	Thu PM
5.	Neuroendocrine control: pituitary I	Slide	Mon AM
124.	Neuroendocrine control: pituitary II	Poster	Tue AM
217.	Neuroendocrine control: pituitary III	Slide	Wed AM

282.	Neuroendocrine control: pituitary IV	Poster	Wed PM
322.	Neuroendocrine control: pituitary V	Slide	Thu AM
382.	Neuroendocrine control: pituitary VI	Poster	Thu PM
412.	Neuroendocrine control: pituitary VII	Poster	Fri AM
208.	**Regulation by brain of its own blood flow and metabolism: intrinsic neural control of the cerebral circulation**	**Symp.**	**Wed AM**
178.	Regulation of autonomic function I	Poster	Tue PM
289.	Regulation of autonomic function II	Poster	Wed PM
321.	Regulation of autonomic function III	Slide	Thu AM
360.	Regulation of autonomic function IV	Slide	Thu PM

Theme F: Sensory Systems

Session Number	Session Title	Type	Day and Time
216.	Auditory system I	Slide	Wed AM
361.	Auditory system II	Slide	Thu PM
344.	Auditory system: cochlear nucleus	Poster	Thu AM
346.	Auditory system: cortex and systems	Poster	Thu AM
347.	Auditory system: evoked potentials	Poster	Thu AM
345.	Auditory system: higher centers	Poster	Thu AM
343.	Auditory system: periphery	Poster	Thu AM
370.	Chemical sensory systems: CNS	Poster	Thu PM
323.	Chemical sensory systems: olfaction	Slide	Thu AM
369.	Chemical sensory systems: sense organs	Poster	Thu PM
236.	Invertebrate sensory processing	Poster	Wed AM
64.	Pain modulation I	Poster	Mon PM
105.	Pain modulation II	Slide	Tue AM
171.	Pain modulation III	Poster	Tue PM
280.	Pain modulation IV	Poster	Wed PM
13.	Pain: central pathways I	Slide	Mon AM
65.	Pain: central pathways II	Poster	Mon PM
57.	Retina I	Slide	Mon PM
114.	Retina II	Slide	Tue AM
175.	Retina III	Poster	Tue PM
176.	Retina IV	Poster	Tue PM
177.	Retina V	Poster	Tue PM
89.	Somatic afferents I	Poster	Mon PM
90.	Somatic afferents II	Poster	Mon PM
88.	Somatic afferents: nociceptors	Poster	Mon PM
222.	Somatosensory cortex and thalamocortical relationships I	Slide	Wed AM
386.	Somatosensory cortex and thalamocortical relationships II	Poster	Thu PM
387.	Somatosensory cortex and thalamocortical relationships III	Poster	Thu PM
172.	Spinal cord	Poster	Tue PM
403.	**Spinal cord nociceptive pathways**	**Symp.**	**Fri AM**
87.	Subcortical somatosensory pathways I	Poster	Mon PM
263.	Subcortical somatosensory pathways II	Slide	Wed PM
6.	Subcortical visual pathways I	Slide	Mon AM
283.	Subcortical visual pathways II	Poster	Wed PM
284.	Subcortical visual pathways III	Poster	Wed PM
285.	Subcortical visual pathways IV	Poster	Wed PM
36.	Visual cortex I	Poster	Mon AM
121.	Visual cortex II	Poster	Tue AM
162.	Visual cortex III	Slide	Tue PM
324.	Visual cortex IV	Slide	Thu AM
372.	Visual cortex V	Poster	Thu PM
405.	Visual cortex VI	Slide	Fri AM

Theme G: Motor Systems and Sensorimotor Integration

Session Number	Session Title	Type	Day and Time
129.	Control of arm and hand movements I	Poster	Tue AM
353.	Control of arm and hand movements II	Poster	Thu AM
187.	Control of posture and movement I	Poster	Tue PM
268.	Control of posture and movement II	Slide	Wed PM
385.	Control of posture and movement III	Poster	Thu PM
72.	Cortex	Poster	Mon PM
354.	Disorders of motor systems and neural prostheses	Poster	Thu AM
330.	Invertebrate motor systems	Poster	Thu AM
241.	Locomotion	Poster	Wed AM
60.	Motor systems and sensorimotor integration: basal ganglia I	Slide	Mon PM
179.	Motor systems and sensorimotor integration: basal ganglia II	Poster	Tue PM
240.	Motor systems and sensorimotor integration: basal ganglia III	Poster	Wed AM
334.	Motor systems and sensorimotor integration: basal ganglia IV	Poster	Thu AM
362.	Motor systems and sensorimotor integration: basal ganglia V	Slide	Thu PM
417.	Motor systems and sensorimotor integration: basal ganglia VI	Poster	Fri AM
127.	Motor systems and sensorimotor integration: cerebellum I	Poster	Tue AM
160.	Motor systems and sensorimotor integration: cerebellum II	Slide	Tue PM
383.	Motor systems and sensorimotor integration: cerebellum III	Poster	Thu PM
126.	Motor systems and sensorimotor integration: oculomotor system I	Poster	Tue AM
297.	Motor systems and sensorimotor integration: oculomotor system II	Poster	Wed PM
325.	Motor systems and sensorimotor integration: oculomotor system III	Slide	Thu AM
416.	Motor systems and sensorimotor integration: sensory integration	Poster	Fri AM
71.	Motor systems and sensorimotor integration: vestibular system I	Poster	Mon PM
3.	**Multidimensional analyses of sensorimotor systems**	**Wksh.**	**Mon AM**
128.	Muscle I	Poster	Tue AM
296.	Muscle II	Poster	Wed PM
352.	Postural control	Poster	Thu AM
186.	Reflex function	Poster	Tue PM
70.	Spinal cord and brainstem I	Poster	Mon PM
109.	Spinal cord and brainstem II	Slide	Tue AM
242.	Spinal cord and brainstem III	Poster	Wed AM
384.	Spinal cord and brainstem IV	Poster	Thu PM
418.	Spinal cord and brainstem V	Poster	Fri AM

Theme H: Structure and Function of the CNS

Session Number	Session Title	Type	Day and Time
50.	Brain metabolism I	Slide	Mon PM
125.	Brain metabolism II	Poster	Tue AM
380.	Brain metabolism III	Poster	Thu PM
381.	Brain metabolism IV	Poster	Thu PM
306.	Clinical CNS neurophysiology: epilepsy	Poster	Wed PM
307.	Clinical CNS neurophysiology: evoked potentials	Poster	Wed PM
31.	Comparative neuroanatomy I	Poster	Mon AM
32.	Comparative neuroanatomy II	Poster	Mon AM
94.	Cortical and subcortical organization I	Poster	Mon PM
95.	Cortical and subcortical organization II	Poster	Mon PM

388.	Cortical and subcortical organization III	Poster	Thu PM
259.	Diseases of the nervous system: Alzheimer	Slide	Wed PM
30.	Diseases of the nervous system: dementia	Poster	Mon AM
24.	Diseases of the nervous system: epilepsy I	Poster	Mon AM
25.	Diseases of the nervous system: epilepsy II	Poster	Mon AM
23.	Diseases of the nervous system: epilepsy, kindling	Poster	Mon AM
26.	Diseases of the nervous system: myelin, neuron, synapse	Poster	Mon AM
267.	Diseases of the nervous system: trauma, tumor, basal ganglia	Slide	Wed PM
27.	Diseases of the nervous system: trauma, tumor, infection	Poster	Mon AM
22.	Diseases of the nervous system: vascular, ischemia	Poster	Mon AM
341.	Limbic system and hypothalamus I	Poster	Thu AM
363.	Limbic system and hypothalamus II	Slide	Thu PM
413.	Limbic system and hypothalamus III	Poster	Fri AM
358.	**Neuroglia: function, development and diversity**	**Wksh.**	**Thu PM**

Theme I: Neural Basis of Behavior

Session Number	Session Title	Type	Day and Time
19.	Alcohol: behavioral studies	Poster	Mon AM
78.	Alcohol: metabolic aspects	Poster	Mon PM
77.	Alcohol: molecular and genetic studies	Poster	Mon PM
130.	Antidepressants	Poster	Tue AM
132.	Anxiolytics	Poster	Tue AM
61.	Biological rhythms I	Slide	Mon PM
148.	Biological rhythms II	Poster	Tue AM
166.	Biological rhythms III	Slide	Tue PM
233.	Biological rhythms IV	Poster	Wed AM
292.	Biological rhythms V	Poster	Wed PM
152.	**Cellular substrates of learning: vertebrate and invertebrate mechanisms compared.**	**Symp.**	**Tue PM**
96.	Circuitry and pattern generation I	Poster	Mon PM
220.	Circuitry and pattern generation II	Slide	Wed AM
169.	Effects of chronic drug administration and neurotoxicology I	Poster	Tue PM
329.	Effects of chronic drug administration and neurotoxicology II	Poster	Thu AM
422.	Effects of chronic drug administration and neurotoxicology III	Poster	Fri AM
165.	Feeding and drinking I	Slide	Tue PM
221.	Feeding and drinking II	Slide	Wed AM
351.	Feeding and drinking III	Poster	Thu AM
391.	Feeding and drinking IV	Poster	Thu PM
419.	Feeding and drinking V	Poster	Fri AM
420.	Feeding and drinking VI	Poster	Fri AM
231.	Hormonal control of behavior I	Poster	Wed AM
232.	Hormonal control of behavior II	Poster	Wed AM
316.	Hormonal control of behavior III	Slide	Thu AM
317.	Human behavioral neurobiology	Slide	Thu AM
389.	Human behavioral neurobiology: disorders	Poster	Thu PM
390.	Human behavioral neurobiology: methods	Poster	Thu PM
194.	Interhemispheric relations I	Poster	Tue PM
415.	Interhemispheric relations II	Poster	Fri AM
16.	Invertebrate learning and behavior I	Slide	Mon AM
113.	Invertebrate learning and behavior II	Slide	Tue AM
237.	Invertebrate learning and behavior III	Poster	Wed AM
366.	Invertebrate learning and behavior IV	Slide	Thu PM
10.	Learning and memory: anatomy I	Slide	Mon AM

200.	Learning and memory: anatomy II	Poster	Tue PM
201.	Learning and memory: anatomy III	Poster	Tue PM
202.	Learning and memory: anatomy IV	Poster	Tue PM
270.	Learning and memory: anatomy V	Slide	Wed PM
191.	Learning and memory: pharmacology I	Poster	Tue PM
192.	Learning and memory: pharmacology II	Poster	Tue PM
193.	Learning and memory: pharmacology III	Poster	Tue PM
51.	Learning and memory: physiology I	Slide	Mon PM
140.	Learning and memory: physiology II	Poster	Tue AM
141.	Learning and memory: physiology III	Poster	Tue AM
149.	Learning and memory: physiology IV	Poster	Tue AM
313.	**Mechanisms of neuropeptide actions on behavior**	**Symp.**	**Thu AM**
308.	Monoamines and behavior I	Poster	Wed PM
309.	Monoamines and behavior II	Poster	Wed PM
310.	Monoamines and behavior III	Poster	Wed PM
253.	Motivation and emotion I	Poster	Wed AM
254.	Motivation and emotion II	Poster	Wed AM
255.	Motivation and emotion III	Poster	Wed AM
151.	**Multidisciplinary approach to neural function in the hypothalamic paraventricular nucleus**	**Symp.**	**Tue PM**
355.	Neural basis of behavior: aging I	Poster	Thu AM
356.	Neural basis of behavior: aging II	Poster	Thu AM
58.	Neuroethology I	Slide	Mon PM
84.	Neuroethology II	Poster	Mon PM
136.	Neuroethology III	Poster	Tue AM
131.	Neuroleptics	Poster	Tue AM
184.	Neuropeptides and behavior I	Poster	Tue PM
400.	Neuropeptides and behavior II	Poster	Thu PM
258.	**Physiological controls of mammalian vocalization**	**Symp.**	**Wed PM**
42.	Sleep	Poster	Mon AM
290.	Stress, hormones and the autonomic nervous system I	Poster	Wed PM
398.	Stress, hormones and the autonomic nervous system II	Poster	Thu PM
402.	**The neurobiology of sleep: implications for sleep disorders**	**Symp.**	**Fri AM**
207.	**The new pharmacology of anxiety: looking beyond the benzodiazepines**	**Symp.**	**Wed AM**

201.11 PARVALBUMIN IN DEVELOPING AVIAN VISUAL, AUDITORY AND VOCAL MOTOR NUCLEI. K. Braun*[1]; H. Scheich[1]; C.W. Heizmann[2]. (SPON: European Neuroscience Association). [1] Dept.Zoology,Techn.Univ.Darmstadt,FRG. [2] Inst.Pharmacol.Biochem, Univ. Zürich,CH.

The Ca-and Mg-binding protein parvalbumin (PV) has been immunocytochemically localized in a neuronal population and neuropil of the visual, auditory and vocal motor system of adult zebra finches (Braun K. et al, Cell Tissue Res 240:101+117,1985). The postnatal development of these systems was investigated using an antiserum against chicken PV as a neuronal marker.

At hatching day (day 0), in the telencephalon the visual ectostriatum and the auditory field L show a high density of PV-positive somata surrounded by dense neuropil. At about day 2, the auditory nucleus mesencephalicus lateralis, pars caudalis starts to exhibit some immunoreactive somata and neuropil that increase in number and density until adulthood. At about day 5, in the thalamic visual nucleus rotundus (Rt) and the auditory nucleus ovoidalis (Ov) show PV-positive punctate and fibrous varicose neuropil; in the telencephalic vocal motor nucleus RA immunoreactive somata and neuropil appear and increase in density until adulthood. In Rt the first PV-positive somata appear around day 12, their number increases until about day 28, when Rt has reached its adult appearance. From day 10 on Ov can be subdivided into a medial part, containing a higher density of PV-positive neuropil than the lateral part. At all stages the somata in Ov remain unlabeled. Starting around day 8 telencephalic vocal motor HVc shows PV-positive somata and some neuropil consisting mainly of dendritic processes (Zuschratter W. et al, Cell Tissue Res 241,77,1985). The marked increase of immunoreactive neuropil observed between day 14 and 28 parallels the large volume increase of HVc during that time. At that stage PV-positive fibrous structures, intermingled with some immunoreactive somata can be observed in the neostriatum mediating between the dorsal tip of field L and the developing HVc.

In Rt the appearance of PV-positive somata occurs shortly after eye-opening and correlates with the time of maximal sensitivity for visual imprinting between day 13-15 (Immelmann K.,Proc.XV.Int.Ornith.Congr.1972,317). The auditory and vocal motor nuclei reach a high density of PV-positive somata and neuropil during the time of song learning which remains until adulthood. Therefore it may be speculated that PV-containing neurons possess Ca-and Mg-dependent mechanisms involved in imprinting and learning.

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201.12 LESIONS IN THE DENTATE-INTERPOSITUS REGION OF THE RAT CEREBELLUM FAIL TO DISRUPT EITHER LEARNING OR RETENTION OF ONE-WAY ACTIVE AVOIDANCE. R. W. Skelton, Department of Psychology, University of Victoria, Victoria, B.C., Canada, V8W 2Y2.

The dentate-interpositus (DI) region of the cerebellum is essential for both acquisition and retention of classically conditioned eyeblink and leg flexion responses in the rabbit but is not essential for heartrate conditioning. Although this region may be the locus of the memory trace for many different discrete adaptive responses in many different animals, neither of these propositions have been tested. The present study examined the effects DI lesions on acquisition and performance of an active avoidance response by rats.

Under Nembutal anesthesia, 16 male hooded rats were given bilateral electrolytic lesions of the DI region of the deep cerebellar nuclei. Control animals (n=8) were sham operated (no current was passed through acutely implanted electrodes). After recovery from surgery, all animals were tested in a standard one-way active avoidance paradigm (10 sec tone precedes a 0.6 mA footshock) in which animals could avoid or escape the shock by jumping up 2 cm to the "safe" half of the box. Criterion performance was 9 avoidance responses in 10 consecutive trials.

The first 12 animals were operated after learning the response, and the lesions had no effect on retention. Performance in the lesioned animals was identical to both pre-lesion and control levels. Histological analysis showed that 6 of the 8 lesioned animals had complete, bilateral destruction of the DI region. (The data from the other two animals were included with the control data.)

The second 12 animals were operated prior to any exposure to the avoidance paradigm and subsequent learning proceeded at exactly the same rate for lesioned and unlesioned animals. Histological analysis showed that complete bilateral DI lesions were produced in 7 of the 8 experimental animals. (The data from the other animal was included with the control data.)

These results suggest that neither the lateral portion interpositus nucleus nor the medial portion of the dentate nucleus are essential for this form of learning in the rat. Perhaps a different region of the deep cerebellar nuclei encodes the learning of the avoidance response, or alternatively, this form of learning might not involve the cerebellum at all.

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201.13 EFFECTS OF LESIONS OF THE DORSAL AND MEDIAL CORTEX ON THE ACQUISITION EXTINCTION AND REACQUISITION OF A DISCRETE TRIAL OPERANT IN TURTLES. W. Grisham*[1] & A.S.Powers[2]. Bryn Mawr College, Bryn Mawr, PA 19010.

Previous research in this laboratory has shown that lesions of the dorsal cortex (DC) in turtles result in a deficit in the reversal of a simultaneous pattern discrimination. The reversal of a simultaneous discrimination involves several elements: the new S+ must acquire stimulus control properties, responding to the former S+ must be extinguished, and a partially extinguished behavior must be acquired. A deficit in any one of these elements could contribute to a reversal deficit. Accordingly, the effects of DC lesions were examined on the acquisition, extinction, and reacquisition of a discrete-trial operant. Medial cortex (MC) lesions were included as a control.

Turtles were given lesions aimed at the DC (n=7) or MC (n=3) or served as controls (n=7). Acquisition training was given using a response requirement of one press on a plastic key (FR1) for a reward of beef baby food. Training continued until the animals responded with a mean latency of 10 seconds or less for a daily session of 20 trials. The response requirement was then raised to FR2 until the same criterion was met or until 80 days of acquisition training had been completed. Extinction then began and continued until the animals responded on two or fewer trials in two consecutive 20-trial sessions. Reacquisition training was then begun and continued until the terminal level of original acquisition performance was attained.

Damage to the DC produced a deficit in original acquisition of the response. Spearman rank-order correlations showed that DC damage was significantly correlated with the number of days in acquisition whereas MC damage was not. This result is similar to the previous finding from this laboratory that DC lesions produce an acquisition deficit on a go-no-go discrimination due to a retardation in the acquisition of stimulus control properties by the S+. Neither DC nor MC damage was found to be significantly correlated with the number of days in extinction or reacquisition.

It was concluded that the deficit on the reversal of a simultaneous discrimination was due not to a deficit on extinction or reacquisition but rather to a retardation in the original acquisition of stimulus control properties by the new S+ coupled with a prepotent tendency to respond to the former S+. The results also suggest that the DC and MC are functionally different.

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201.14 THE RELATIVE ROLE OF ENTORHINAL AND PREFRONTAL CORTICAL SYSTEMS IN SPATIAL AND NONSPATIAL REFERENCE LEARNING. M. Rasmussen*, C.A. Barnes, and B.L. McNaughton (SPON: G. Rao). Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO 80309.

A major theory of hippocampal function is that it specifically involves the storage of spatial information (O'Keefe and Nadel, 1978). The prefrontal system has also been implicated as important for accurate performance on spatial tasks (Kolb, Sutherland, and Whishaw, 1983).

As a part of a comprehensive lesion by task investigation of the relationship between the hippocampal formation and the prefrontal cortex, a completely between subjects design is being used to assess acquisition of spatial and nonspatial versions of three tasks: reference memory, working memory and short-latency alternation. Five lesion groups (entorhinal, medial prefrontal cortex, mediodorsal thalamus, and two types of controls) are being tested, holding variables such as apparatus, reinforcer, distal sensory cues, strain and age of rats, and preliminary treatment constant. We report here the results for all lesion groups on the spatial and nonspatial reference memory problems.

At least 9 animals in each lesion group were tested for acquisition of two tasks requiring either spatial or nonspatial reference memory. A radial maze with one of eight arms baited was used. For the spatial version, half of the animals were required to locate the goal on the basis of distal cues alone. In the nonspatial version, half of the animals were required to locate the goal using a visual-tactile cue placed on one of the arms, chosen at random for each trial, making distal cues irrelevant.

For the control, prefrontal and thalamic groups, the average errors to acquisition were equivalent for the two tasks and did not differ among groups. This indicates that the tasks were equated for difficulty level. The entorhinal group was able to acquire the nonspatial version as readily as the other groups but made 3 times as many errors during acquisition of the spatial version. The data indicate that the deficit seen in entorhinal rats may derive from their inability to associate places with reinforcement contingencies or that they are unable to differentiate between the arms on the basis of distal cues.

This strongly supports conclusions drawn from previous studies that the role of the entorhinal cortex in reference learning is intrinsically spatial. Furthermore, the results strongly suggest that the medial frontal cortex and medial dorsal thalamus do not play an important role in spatial or nonspatial reference learning on this task.

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201.15 **SIMULTANEOUS TEMPORAL PROCESSING AND THE BASAL FOREBRAIN CHOLINERGIC SYSTEM.** W.H. Meck, R.M. Church, G.L. Wenk and D.S. Olton. Depts. of Psych., Columbia Univ., New York, NY 10027, Brown Univ., Providence, RI 02912 & The Johns Hopkins Univ., Baltimore, MD 21218.

The effects of bilateral ibotenic acid-induced lesions of the nucleus basalis magnocellularis (NBM) and medial septal area (MSA) on divided attention and temporal memory were studied in the rat. Subjects were trained both pre- and postoperatively on a discrete-trials timing procedure in which auditory and visual stimuli signalled two different fixed-interval schedules of food reinforcement (10 & 20 s). These two signals could be presented one at a time or simultaneously in a hierarchical fashion such that the 10-s signal was initiated at a random time during the 20-s signal. Light, sound, and light+sound trials occurred randomly with equal probability and food was made available on a random half of these trials. On peak trials, no food was given and the signal continued for 120 s. During peak trials the rat's response rate increases as a function of time since signal onset(s) until the time(s) that food is sometimes made available and then it declines in a fairly symmetrical fashion. The time of the response function mode during a peak trial is referred to as the peak time and it serves as an indicator of when the rat maximally expects food. When signals are presented one at a time rats normally place their peak times near the physical times of reinforcement. Similarly, when signals are presented together they time both intervals simultaneously and show bimodal response functions that are alligned with the correct times of reinforcement. If signals are interrupted by a gap, rats normally retain the duration of the signal before the gap and add it to the duration of the signal after the gap. In contrast, although rats with lesions of the NBM are able to time signals presented one at a time and are also able to bridge gaps in the signal presentations, they are unable to time two signals simultaneously. On the other hand, rats with lesions of the MSA are able to time signals presented one at a time and are also able to time two signals simultaneously, but are unable to bridge gaps in the signal presentations. These results suggest a behavioral differentiation of the basal forebrain cholinergic system such that the NBM is involved in divided attention and reference memory while the MSA is primarily involved in working memory.

201.16 LONG-LASTING INCREASES IN THE METABOLISM OF CORTICAL AREAS DURING MEMORY CONSOLIDATION IN MICE. C. Destrade, J. Sif * and M. Meunier * . Lab. Psychophysiologie, UA CNRS 339, Univ. Bordeaux I, 33405 Talence Cedex FRANCE.

In previous behavioral studies using operant conditioning tasks in mice, we have shown that memory consolidation processes may be defined as neural events occuring during the 3 hours which follow learning (Destrade et al., Neurosci. Lett., 7, 239, 1978). In the present experiment the ^{14}C-2-deoxy-D-glucose (2DG) technique was used to define in vivo, the neural structures which may mediate memory consolidation. Regional brain uptake of 2DG was assessed both immediately and 3 hr after the learning session.

Male mice of the BALB/c strain were implanted with a polyethylene catheter in the jugular vein. One week after surgery they were subjected to a food deprivation procedure and maintained at 83% of their ad libitum body weight. The learning task was an operant lever-press conditioning with food reward in a Skinner box. The animals were randomly assigned to control ("quiet" (n=2) and "sham conditioned" (n=3)) or experimental groups. The mice of the experimental groups were submitted to a first learning session (20 lever-presses on continuous reinforcement schedule) followed 24 hr later by a retention session (40 lever-presses). They were given a single intrajugular dose (5 µCi) of ^{14}C-2DG either immediately after (group A, n=3) or 3 hr after (group B, n=3) the end of the retention-learning session. Forty min later the animals were sacrificed and the brain processed for autoradiography. Local cerebral glucose utilization (LCGU) values for discrete brain regions from X-ray autoradiographs were obtained using a digital image processing system (Pericolor 2000, Numelec) and subjected to statistical analysis.

Compared to the values observed in control animals, LCGU was significantly increased in mice of both experimental groups in the medial septum, diagonal band, hippocampus (CA1, CA3, DG), thalamus (MD, CL, MV), mammillary bodies and cingulate cortex. However,comparing the 2 experimental groups, a further increase in LCGU in the subiculum, entorhinal, prefrontal and piriform cortex, was observed only in the animals of the group B, injected with 2DG 3 hr after the retention session.

These results indicate a functional activation of the Papez circuitry as a consequence of the acquisition of an operant learning task in mice, and produce direct evidence for a long-lasting activation of the subiculum and cortical areas during memory consolidation.

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201.17 [^{14}C]2-DEOXYGLUCOSE MAPPING OF REGIONAL BRAIN ACTIVITY DURING RETENTION OF RADIAL ARM MAZE TRAINING. R. F. Berman[1], R. P. Kesner[2], L. M. Partlow[3], L. H. Bush[3]*, R. W. Campbell[4]*, and L. J. Stensaas[5]. [1]Dept. Psychology, [4]Dept. Physiology, Wayne State Univ., Detroit, MI; [2]Dept. Psychology, [3]Dept. Pharmacology, [5]Dept. Physiology, University of Utah, Salt Lake City, Utah.

Regional mapping of [^{14}C]2-deoxyglucose (2-DG) uptake during retention of radial arm maze performance was studied in rats in order to examine selective activity in specific brain regions during an extended 1 hour retention interval. Specifically, we used a radial arm maze task in which rats were required to retain information about each specific daily maze trial for 1 hour. Briefly, rats were trained to enter 4 arms of an 8 arm radial arm maze at random in order to obtain food reinforcement. One hour later animals were placed back on the maze and required to enter the remaining 4 arms of the maze in order to obtain additional food. Animals were trained for approximately 16 weeks to a criterion of 3 consecutive days of errorless performance. They were then placed on the maze, again allowed to enter 4 arms at random, and were immediately injected with 25 uCi of [^{14}C]2-deoxyglucose via a chronically implanted jugular catheter. Animals were sacrificed after a 45 min isotope incorporation period and the brains removed, coded and frozen. Control rats were similarly allowed to enter 4 arms of the maze, but with food available from all arms when they were returned to the maze one hour later. Thus, control animals were not required to retain information about arms visited (or not visited) one hour earlier, while trained animals were. After reaching criterion on the maze control rats were injected with [^{14}C]2-DG and sacrificed using procedures identical to those described for trained animals. The frozen brains were sectioned, mounted on slides and exposed to X-ray film. The subsequent autoradiograms were analyzed by computerized densitometry for differential uptake of 2-DG. Fifteen brain regions were examined, including septum, caudate, medial prefrontal cortex, hippocampus, thalamus, hypothalamus, parietal cortex, amygdala, central gray, dorsal raphe, reticular formation, superior colliculus, occipital cortex, entorhinal cortex and locus coeruleus. The results demonstrated increased 2-DG uptake in the reticular formation of trained animals relative to controls ($p<.01$), with similar increases noted in neighboring structures, including central gray and dorsal raphe. No significant changes in 2-DG uptake were noted in other brain regions, including the hippocampus. These data point to the involvement of the reticular formation and other brainstem structures in extended retention of maze learning, and suggest that the reticular formation may play a role in controlled memory processing and selective attention.

201.18 IPSILATERAL DECREMENT IN LATERAL HYPOTHALAMIC SELF-STIMULATION AFTER UNILATERAL NEUROTOXIC LESION OF THE PREOPTIC AREA. J.P. Huston, C. Munoz* and S. Kiefer*. Psychology III, Univ. of Düsseldorf, 4000 Düsseldorf, Federal Republic of Germany.

We have previously reported (Muñoz et al. Brain Res., 358:85-95, 1985) a lateralized decrement of lateral hypothalamic self-stimulation (LH-SS) after unilateral radiofrequency lesions in the preoptic area (POA) of rats. The purpose of the present investigation was to examine whether the lesion effects were due to damage to the POA proper or instead, to damage to the fibers of passage, such as those of the medial forebrain bundle.

Fourteen male albino rats were bilaterally implanted with bipolar stimulation electrodes in the LH and with cannulae in the POA. They were tested for SS in the LH at two different current intensities (low and high). When the rate of responding was stabilized, they received a unilateral injection of ibotenic acid (5 µg/1 µl phosphate buffer, pH=7.4) through the cannula implanted in the POA. The animals were first tested for SS 24 hours post-injection, and then daily over the following 21 days at the two current intensities that were used before the lesion was made.

The ibotenic acid destroyed most of the cells of the POA in 9 rats (large lesion group, LPOA) while in the remaining 5, the area of neuronal depletion was smaller (small lesion group, SPOA). The rate of SS in the hemisphere ipsilateral to the side of the lesion was decreased in both groups, to levels of 27-30% of the preoperative lesion rate in the LPOA group, and 48-60% in the SPOA group. Self-stimulation did not recover to the pre-lesion values during the 21 days following the neurotoxin injection, unlike in our previous experiment with radiofrequency lesions in the POA. The rate of responding in the intact hemisphere increased above the pre-lesion levels.

These results strengthen the view of a critical role of the POA in LH-SS, and support the hypothesis that the path neurons of the POA region are part of a substrate for LH-SS.

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201.19 THALAMIC LESIONS AND REGIONAL BRAIN MONOAMINERGIC ALTERATIONS FOLLOWING AN ACUTE BOUT OF THIAMINE DEFICIENCY IN THE RAT.
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The neurobiological substrates implicated in the learning and memory deficits of Korsakoff's disease include mediodorsal nucleus of the thalamus, mammillary bodies, and central monoaminergic activity. In a rat model of Korsakoff's disease, induced by a two week bout of thiamine deficiency and daily pyrithiamine injections (0.5 mg/kg, i.p.), we observed both learning and performance deficits and a significant and long-lasting reduction in the norepinephrine (NE) content of cortex-hippocampus (Mair et al., Brain Res. 360: 273-284, 1985). Pathological examination of another group of treated rats demonstrated consistent lesions in the medial thalamus.

We report here examinations of the pathologic changes in left hemisphere and regional monoamine concentrations in right hemisphere of rats subjected to a similar bout of thiamine deficiency. These studies were conducted on an experimental (ExpA, N=17) and a control (CONT, N=12) group of rats tested on a number of behavioral tasks over a period of 7 months following treatment. The results of those behavioral studies are described in the accompanying abstract (Mair et al., Neuroscience Abstracts, 1986). A second group (ExpB, N=8) of more severely impaired and non-behaviorally tested experimental animals were also studied biochemically. These studies provided an opportunity to (1) determine the nature and distribution of lesions; (2) examine the monoamine content of eighteen brain areas including the hippocampus, amygdala, and individual cortical and subcortical structures; and (3) explore possible relationships between behavioral deficits, sites of pathologic change, and monoaminergic alterations in discrete brain areas.

Results obtained in the ExpA group show: (1) a high incidence (94%) of thalamic lesions, specifically within the intralaminar nuclei and ventral parts of the mediodorsal nucleus; (2) absence of detectable changes in the mammillary bodies, hippocampus, cortical areas or locus coeruleus; and (3) significant reduction in entorhinal NE concentration. These findings implicate damage to intralaminar and ventral mediodorsal regions of the thalamus in learning and memory deficits produced by thiamine deficiency. Reduced entorhinal NE may also contribute to the behavioral deficits and result from either thalamic lesions and/or changes in local physiological regulation. Signficiant NE reductions were observed in hippocampus, entorhinal cortex and olfactory bulb of ExpB animals. These changes may reflect more widespread monoaminergic changes in severely affected animals or may be the result of a lack of behavioral training.

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201.20 RELATIVE REGIONAL METABOLIC CHANGES IN THE BRAINS OF NEONATAL RATS DURING APPETITIVE OLFACTORY LEARNING.
D. Kucharski*, J.A. Browde, Jr.*, and W.G. Hall. Department of Psychology, Duke University, Durham, NC 27706.

The [14C] 2-deoxyglucose autoradiographic technique has been used in developmental studies to map neural effects of early experience (Coopersmith & Leon Sci., 225:849, 1984; Teicher, et al. Brain Res., 194:530, 1980) and to trace ingestive response systems in infant rats (Hall, submitted). In the present experiment, DG methods were used to identify brain systems showing altered metabolic activity during classical conditioning of an association between a novel odor and an ingestive milk reward. In this appetitive learning paradigm, rat pups express conditioned responses to the odor (e.g. mouthing, activation, and probing) similar to those that occur to milk. In addition, after just a few odor/milk pairings, pups come to prefer the originally aversive odor.

During a 1 hr DG incorporation period, trained pups (n = 8) received forward pairings of cedar odor with intraoral infusion of milk, while controls (n = 7) received backward pairings of the same stimuli. Because both groups received equivalent odor exposures and milk infusions, differences in the DG autoradiograms reflect only the 10-sec difference distinguishing forward conditioning (for which learning occurs) from backward conditioning. Pups were sacrificed and brain sections were prepared for autoradiography by standard methods. Forty-eight frontal sections (olfactory bulbs through medulla) from each brain were matched to a set of reference sections. In these sections, 140 neuroanatomical regions were defined (each on 3-10 sections) and their optical densities measured. Measurements were transformed to standard scores and averaged between brains to provide an index of relative optical density for each region. These averages could be used for between-group comparisons. Comparisons between additional groups provided information on the neural response to olfactory and ingestive stimulation.

Changes in neural activity during learning appeared in central amygdaloid nucleus, CA2 region of the hippocampus, the posterior region of the caudate-putamen, and olfactory tubercle. Activity in the anatomically-related tubercle and caudate-putamen may represent motor aspects of the transition from aversive to appetitive responding. Changes in limbic regions are consistent with findings from neural analyses of learning in adults and indicate a role for hippocampus and amygdala in infantile learning even though these regions may not be fully mature.

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LEARNING AND MEMORY: ANATOMY IV

202.1 THE INFRAPYRAMIDAL MOSSY FIBER PROJECTION IN THE HIPPOCAMPUS OF THE MOUSE: A POSITIVE CORRELATION WITH Y-MAZE AVOIDANCE LEARNING
H.-P. Lipp, H. Schwegler*, and Z. Hausheer-Zarmakupi*. Inst. of Anatomy, Univ. of Zürich, CH-8057 Zürich, Switzerland, and Inst. of Human Genetics, Univ. of Heidelberg, FRG.

The capacity of two-way avoidance (shuttlebox) learning in mice and rats correlates negatively with the extensiveness of the infrapyramidal mossy fiber projection (IIP-MF) on the basal dendrites of the pyramidal neurons in the hippocampal region CA3 (Schwegler H. et al., Science, 214: 817,1981; Lipp H.-P. et al., Science, 225: 80, 1984). This study tested whether the individual variability of the IIP-MF might be related to a genuine learning capacity. If it were so, one would also expect negative correlations of the IIP-MF with learning scores obtained in other devices. Twenty random-bred mice, taken from the F3-generation of a 5x5 diallel cross (progenitor strains BALB/c, C56BL/6, BA, DBA/2, C57BR), were tested for the performance of discrimination learning in a computer-controlled Y-maze. Wrong choices were punished by means of air puffs. The discriminanda, placed in the walls of the choice arms, had distinct visual and vibrissotactile cues. The mice were trained for 5 consecutive days, the main measure being the number of correct choices (total 144 trials). Complementary measures were side biases, choice strategies, decision time, number of comparisons, and one-way avoidance. Morphometry of hippocampal terminal fields, as visualized by Timm's silver sulfide stain, was performed on 10 sections taken from a mid-septotemporal level. The correlations between volumes of terminal fields and behavior scores were analysed by means of multiple regression. The number of correct choices was strongly correlated with the extensiveness of the IIP-MF projection: the more IIP-MF, the better discrimination ($r = 0.75$, $p < 0.001$). The IIP-MF correlated similarly, though weaker, with two joint measures, the number of comparisons between choice arms ($r = 0.53$, $p < 0.05$), and, more puzzling, with a side preference to the right ($r = 0.62$, $p < 0.01$). These results show that correlations of the IIP-MF and behavior are not restricted to tasks involving electrical shock, and that the behavioral processes correlated with the IIP-MF are unrelated to learning capacity per se. However, it might be that the IIP-MF are associated with behavior related to hesitation and doubt, mice with more IIP-MF being more hesitant in making decisions, and, perhaps, adhering more tenaciously to a particular behavioral strategy. This would be beneficial in situations of discrimination learning but disadvantageous in two-way avoidance. The data still fit several theories of hippocampal function. Current studies may rule out some of them. Supported by Swiss National Foundation for Scientific Research, SNF 3.041, 3.516 (Van der Loos), and by the Deutsche Forschungsgemeinschaft.

202.2 DISRUPTION OF ACQUISITION AND RETENTION OF THE CLASSICALLY CONDITIONED EYEBLINK RESPONSE IN RABBITS WITH ALUMINUM INDUCED NEUROFIBRILLARY TANGLES.
P. R. Solomon, T. Pingree*, A. Schwentker* and W. W. Pendlebury. Dept. of Psychology, Williams College, Williamstown, MA 01267, and Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405.

Aluminum exposure of the rabbit central nervous system produces widespread neurofibrillary tangle formation in the cortex, hippocampus, brain stem, and spinal cord. Although these tangles have been well characterized morphologically and biochemically, little is known about their effects on learning and memory.

In Experiment I, rabbits were classically conditioned to emit an eyeblink conditioned response (CR) in response to tone CS (500 msec, 85dB) paired with a corneal air puff (100 msec, 3 PSI) UCS until they attained a criterion of two consecutive sessions (100 CS-UCS pairings) of greater than 90% CRs. They then received via the intraventricular route, 1% $AlCl_3$ (100 µl), HCL (100 µl, to control for pH) or normal saline (100 µl). Ten days post-injection, they were tested for retention of the conditioned response. Relative to saline and HCL controls, $AlCl_3$ animals showed significant deficits in retention. Subsequent tests indicated that these deficits were not due to changes in sensory or motor processes, nor were they correlated with illness. These conditioning deficits were, however, correlated with the degree of pathology (i.e., number of tangled cells).

In Experiment II, rabbits were first exposed to intraventricular $AlCl_3$, HCL or saline. Five days post-injection, they were given 100 tone - air puff conditioning trials (as in Experiment I) per day for 5 days. Whereas saline and HCL rabbits readily acquired the eyeblink CR during this time, $AlCl_3$ animals were severely retarded. As in Experiment I, these conditioning deficits did not appear to be sensory or motor in nature, nor were they related to illness. Again, there was a correlation between conditioning deficit and extent of pathology.

Neuropathological examination of the tissue revealed widespread neurofibrillary tangle formation involving dorsal cortex, ventral hippocampus, multiple brainstem nuclei, and cervical spinal cord anterior horn cells. Involvement of the cortex showed a striking laminar distribution, primarily involving deep layers.

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202.3 LOCALIZATION OF NEURAL SUBSTRATES FOR VISUAL MEMORY WITH 2-DEOXYGLUCOSE: REGIONAL METABOLIC ACTIVATION OF VISUAL AND LIMBIC STRUCTURES AND LATERALIZATION OF LEARNING EFFECTS. F. Gonzalez-Lima, I. Steele Russell* and E.M. Gonzalez-Lima*. Dept. of Anat., Coll. of Med., Texas A&M Univ., College Station, TX 77843 and Dept. of Anat., Univ. Coll. London, London WC1E 6BT, U.K.

The ^{14}C-2-deoxyglucose (2DG) autoradiographic method was used to examine the functional activity in all structures of the rat brain following visual Pavlovian conditioning. The conditioned stimulus (CS) was a light flash (3 times/s) and the unconditioned stimulus (US) consisted of foot shock. Normal and split-chiasm hooded rats were conditioned monocularly with paired CS-US presentations. Control rats received either unpaired CS-US presentations or no stimulation. The rats were deprived of water overnight and tested for conditioned suppression of drinking. Then they were injected with 2DG and stimulated binocularly with the CS alone for 1 hr. Thus both eyes were equally stimulated during 2DG testing, but only one eye was previously stimulated during pre-training. Differences in 2DG uptake were examined quantitatively in each brain (interhemispheric comparison) and between brains (group comparison). The major findings consisted of specific patterns of metabolic activation observed in conditioned rats. The changes included the visual midbrain and limbic structures. In chiasm-cut rats, the side ipsilateral to the "trained" eye showed a striking enhancement of 2DG uptake in visual structures, including the pretectum (olivary and posterior nuclei) and superior colliculus (intermediate layers). The largest increase was observed in the olivary pretectal nucleus. Associative structures such as the hippocampal subiculum and the retrosplenial cortex showed dramatic enhancements restricted to the pretrained side of the brain. Similar changes were found in normal animals, but were restricted to the contralateral side. No significant changes were observed in the visual thalamocortical pathway (lateral geniculate, areas 17, 18, 18a) of split-chiasm rats. But in normal animals a sensitization effect was observed. The changes revealed by 2DG represent the first anatomical demonstration of brain substrates with localized metabolic alterations produced by visual conditioning. They may provide a useful approach to the study of visual memory localization and plasticity in two ways. First, by identification of structures normally involved in the learned response to conditioned light; and second, by identification of structures showing the lateralized effects of conditioned light in the lesioned brain.

202.4 PARASAGGITAL KNIFE CUTS THAT INTERRUPT SUBLENTICULAR AND POSTERIOR INTERNAL CAPSULE IMPAIR CARDIAC CONDITIONING BUT HAVE NO EFFECT ON CONCOMITANT EYEBLINK CONDITIONING. D.A. Powell, Dianne Levine* and Lisa James*. Neuroscience Lab., WJB Dorn VA Hospital and Univ. of South Carolina, Columbia, SC.

Previous experiments in several laboratories have demonstrated that forebrain (i.e., amygdala and prefrontal cortex) mechanisms are intimately involved in the bradycardia produced by classical conditioning contingencies. We have previously found that parasaggital knife cuts just medial to the amygdala, which interrupt the descending efferents from these structures, abolish learned bradycardia, and diminish the bradycardia associated with the orienting reflex (OR) in the rabbit. In the present experiment rabbits with similar knife cuts were exposed to classical conditioning parameters that are optimal for eyeblink conditioning (i.e. 450 msec ISI). A 500 msec, 1216 Hz, 75 db tone served as the conditional stimulus (CS), and a 250 msec, 3 mA paraorbital electric shock train was the unconditional stimulus (UCS). HR conditioning was greatly diminished in these animals but EB conditioning appeared normal compared to sham animals and a pseudoconditioning sham control group. However, significant bradycardia occurred after CS offset on test trials during which the UCS was not presented. The cardiac component of the OR was however not diminished in the lesioned animals. In a second experiment rabbits with coronal knife cuts, which interrupt these descending tracts as they pass through the lateral hypothalamus, and sham control animals, received a single session of differential cardiac conditioning with a 4 sec ISI. No discriminative cardiac responding was obtained in any experimental animal. These findings are compatible with our original study in which parasaggital knife cuts virtually eliminated differential cardiac responding when a 4 sec ISI was employed. Animals with knife cuts in both the present experiments showed normal cardiac responses to the unconditioned stimulus; however only the coronal cuts decreased responses to a novel unreinforced tone. These findings suggest that a system coursing through the lateral hypothalamus from upper forebrain areas is necessary for learned bradycardia but not for learned somatomotor changes. However, hypothalamic mechanisms are apparently not necessary for the control of learned bradycardia, since hypothalamic cells were intact in all of these experiments. The latter, however, may contribute to, but not be completely responsible for the bradycardia associated with orienting. The fact that significant bradycardia occured on test trials in lesioned animals when short ISIs were employed in experiment 1 may reflect a non-associative process similar to orienting in response to the omission of the UCS on these trials.

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202.5 HRP-WGA STUDIES OF PREMOTOR CEREBELLAR-BRAIN STEM PATHWAYS FOR THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE. M.E. Rosenfield and J.W. Moore. Dept. Psychology, University of Massachusetts, Amherst, MA 01003.

Lesions of cerebellar anterior nucleus interpositus (Yeo, C.H., et. al., Exp. Brain Res., 60: 87, 1985), brachium conjunctivum (McCormick, D.A., et. al., Brain Res., 244: 347, 1982), red nucleus (Rosenfield, M.E. and Moore, J.W., Behav. Brain Res., 10: 398, 1983), or the rubrobulbar tract (Rosenfield, M.E., et. al., Physiol. Behav., 34: 751, 1985) disrupt or prevent classical conditioning of the rabbit nictitating membrane (NM) response. Lesions of the supratrigeminal reticular formation also disrupt NM conditioned responses (Desmond, J.E. and Moore, J.W., Physiol. Behav. 28: 1029, 1982). Subsequent anatomical (Desmond, J.E., et. al., Brain Res. Bull., 10: 747, 1983) and physiological (Desmond, J.E. and Moore, J.W., Brain Res. Bull., 10: 765, 1983) also implicate the supratrigeminal reticular formation in NM conditioning: Neurons in this region (a) project bilaterally to the accessory abducens nuclei, loci of retractor bulbi motoneurons that mediate the conditioned response, and (b) fire in relation to conditioned responding.

In order to investigate whether the supratrigeminal reticular formation is innervated directly by the cerebellum via a descending limb of brachium conjunctivum (Woodson, W. and Angaut, P., Exp. Brain Res., 56: 167, 1984), WGA-HRP was implanted into cerebellar deep nuclei dentate and/or interpositus, red nucleus, or the supratrigeminal reticular formation of 10 rabbits. TMB reacted tissue reveals no evidence of a descending limb projection (terminals) to supratrigeminal reticular formation from cerebellum. Nor have we observed evidence of projections to supratrigeminal reticular formation from red nucleus. Generally, observed patterns of antero- and retrograde learning confirm previously published HRP data from our laboratory.

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202.6 AMYGDALA LESIONS BLOCK FEAR-ENHANCED STARTLE USING EITHER VISUAL OR AUDITORY CONDITIONED STIMULI. J.M. Hitchcock and M. Davis Depts. of Psychology and Psychiatry, Yale University, Conn. Mental Health Ctr., Ribicoff Res. Fac., 34 Park St., New Haven, CT 06508.

Considerable evidence indicates that the amygdala is involved in innate and conditioned fear. Lesions of the amygdala reduce innate and conditioned fear, stimulation of the amygdala elicits species-typical fear and defense reactions, and opiates or benzodiazepines injected directly into the amygdala have anxiolytic effects. Consistent with this, we have found that lesions of the amygdala block potentiated startle (Hitchcock & Davis, Beh. Neurosci., 100, 1986), a measure of fear conditioning in which a light that has previously been paired with shock enhances the amplitude of the acoustic startle reflex. Because a light was used as the conditioned stimulus (CS) in that study, it is possible that the effect of amygdala lesions on potentiated startle is limited to situations involving visual information. Therefore, the present study evaluated the effect of amygdala lesions on potentiated startle using an auditory CS.

In a preliminary experiment, we replicated and extended earlier findings (Galvani, Beh. Res. Meth. Instru., 2, 1970; Siegel, J. Comp. Phys. Psych., 64, 1967) that a tone is an effective CS for potentiated startle. In a second experiment, rats were given 10 tone-shock pairings on 2 successive days. At 24-48 hr following training, the rats received bilateral electrolytic lesions of the central nucleus of the amygdala or sham operations. Testing for potentiated startle occurred 4-5 days later. The amygdala lesions impaired potentiated startle using an auditory CS, consistent with previous findings using a visual CS. In a third experiment, rats were given 10 light-shock pairings and 10 tone-shock pairings in the same training session on 2 successive days. At 24-48 hr following training, the rats received amygdala lesions or sham operations. Five days later they were tested for potentiated startle using visual and auditory CSs in the same test session. The sham-operated rats showed potentiated startle with either the visual or auditory CS. Amygdala lesions blocked potentiated startle using both visual and auditory CSs.

These data indicate that the effect of amygdala lesions on potentiated startle is not specific to one sensory modality, consistent with the hypothesis that the amygdala is involved in processing multimodal information related to fear. Currently, we are attempting to delineate the visual and auditory pathways needed to sustain each type of potentiated startle by using the training and testing paradigm that employs both visual and auditory CSs in the same session. We are also investigating possible connections from these sensory pathways to the amygdala, and the pathway from the amygdala to the startle reflex pathway.

202.7 **MULTIPLE VISUAL PATHWAYS CAN MEDIATE NICTITATING MEMBRANE CONDITIONING TO A VISUAL CS.** **O. Koutalidis*, A. Foster* and D.J. Weisz. Department of Psychology, Yale University, New Haven, CT 06520.**

We investigated the CS pathway necessary for acquisition of the conditioned nictitating membrane response (NMR) in rabbit using a visual CS. Bilateral lesions using aspiration and ibotenic acid were made singly and in various combinations to dorsal and ventral lateral geniculate (LGd and LGv), superficial and deep layers of superior colliculus (SCs and SCd), the nuclei of the pretectal area (PTA), and visual cortex (VC). All of these receive direct retinal input except for VC and SCd. For technical reasons ibotenic acid lesions of LGd were combined with aspiration of VC to remove the LGd output. Animals were trained with a 600 ms light (281x) as the CS and a 100 ms air puff to the cornea as the UCS (ISI=500 msec). Following recovery from surgery, animals were trained for 100 trials per day either to acquisition criterion or a maximum of 14 days. Animals not acquiring by Day 14 were trained with an auditory CS to criterion, and then given 7 additional days of visual training.

Lesions restricted to single structures (LGv+LGd, PTA,SCs,VC) did not retard acquisition of conditioned NMRs. Combined lesions of SCs+PTA and LGv+LGd+PTA also did not slow the rate of conditioning. Lesions of the LGv+LGd+SCs (mean trials to criterion=980) retarded conditioning ($p<.05$) when compared to controls (mean=530), suggesting that the PTA was less effective in mediating NMR conditioning than were the lateral geniculate (LG) or SCs. Lesions of the SCs+SCd also slowed conditioning with no animal acquiring by Day 14 (or 1400 trials). The SCd receives input from the LGd via the VC and from the LGv, and it is possible that the combined SCs+SCd lesion disrupted the projections to the conditioning circuit of the SCs directly and of the LGd and LGv indirectly via SCd. As expected lesions of the PTA+SCs+LGv+LGd prevented conditioning over 21 days of training with the visual CS but not with the auditory CS. These data indicate that multiple pathways can carry information from the retina to the circuit mediating NMR conditioning, and they suggest that the PTA may be less important than LG or SCs.

202.8 STIMULATION OF RED NUCLEUS AS UNCONDITIONED STIMULUS DOES NOT LEAD TO CLASSICAL CONDITIONING OF EYEBLINK RESPONSE IN RABBIT. P. F. Chapman*, and R. F. Thompson (SPON: L. Ganz) Department of Psych. Stanford University, Stanford, CA 94305

The importance of the olivocerebellorubral system, as well as the pontocerebellar mossy fiber system to the conditioned eyeblink response in the rabbit has been well documented. Lesions of the cerebellar nuclei permanantly abolish the conditioned response (CR) (McCormick et al. Science, 1982) as do lesions of the red nucleus (Haley et al. Neuro. Abst., 1983). Furthermore, the use of direct stimulation of the climbing fiber system originating in the inferior olive as an unconditioned stimulus (US) and the mossy fiber system from the pontine or reticular nuclei as the conditioned stimulus (CS) will produce normal conditioning, indicating the sufficiency of transmitting this information to the cerebellum. Conversely, while stimulation of the anterior interpositus as the US will produce eyelid closure, the UR, it will not lead to the expression of conditined responses (CR), implying the necessity of processing the information in the cerebellum. In this study, we demonstrate that the red nucleus (RN) is involved in the expression of the conditioned response, but not necessary for the development or maintenance of learning related plasticity.

Subjects were male albino rabbits weighing between 2 and 3 kg. Electrodes were placed stereotaxically in the caudal RN, under asceptic conditions, and halothane anaesthesia. Following recovery (one week), electrode placement was tested by stimulating using 30 ms. trains of 0.2 msec pulses (200 ua) at 200 Hz. If stimulation produced closure of the contralateral eye, subjects exposed to paired presentations of a 1 kHz tone and stimulation of the RN, following the standard tone and air puff eyelid paradigm. Following five days of trainng, the animals exhibited no CRs. Subjects were then switched from stimulation to air puff training.

Animals that were trained with stimulation of the RN as the US failed to acquire CRs in the five training days. Animals learned normally, as compared to controls when air puff was substituted for stimulation. The normal acquisition of CR to air puff following RN stimulation is in contrast to significantly faster acquisition following stimulation of interpositus (Chapman, Neur. Abst., 1985).

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202.9 LESIONS IN THE PONTINE NUCLEAR REGION SELECTIVELY ABOLISH CLASSICALLY CONDITIONED EYELID RESPONSES IN RABBITS. J.E. Steinmetz, C.G. Logan, D.J. Rosen*, D.G. Lavond and R.F. Thompson. Department of Psychology, Stanford University, Stanford, CA, 94305.

Recent studies have demonstrated that mossy fibers project sufficient and necessary information to the cerebellum concerning occurrence of the conditioned stimulus (CS) during classical conditioning (Solomon et al., Bull. Psychon. Soc., 24, 1986; Steinmetz et al., Bull. Psychon. Soc., 23, 1985). In the present study, electrolytic lesions were placed in lateral regions of the pontine nuclei, an area that appears to receive auditory input, in hopes of selectively abolishing conditioned eyelid responses (CRs) established with an acoustic CS. Under halothane anesthesia, 28 albino rabbits were chronically implanted with bilateral lesioning electrodes into the region of the dorsolateral and lateral pontine nuclei. The animals were then classically conditioned with daily training sessions (108 trials/session) by pairing a tone CS with an airpuff unconditioned stimulus (US). After conditioning criterion was reached with the tone CS, the animals were trained to criterion using a light CS and the airpuff US. Bilateral electrolytic lesions were then placed into the lateral pontine nuclear region through the previously implanted lesioning electrodes. Twenty-four hours after lesioning, paired CS-US training was reinstated and continued for 8-12 days (4-6 days of tone training and 4-6 days of light training). Our results indicate that lesions placed in regions of the caudal dorsolateral and lateral pontine nuclei selectively abolish CRs established with the acoustic CS while leaving intact the light CR. Lesions placed in other regions of the pontine nuclei and in brainstem regions caudal to the pontine nuclei were not effective in abolishing the acoustic CR. These data support the possibility that information concerning the occurrence of the acoustic CS is relayed to the cerebellum via mossy fibers that originate in the region of the caudal pontine nuclei.

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202.10 RETENTION OF CLASSICAL CONDITIONING AFTER REMOVAL OF CEREBELLAR CORTEX. D.G. Lavond, J.E. Steinmetz, M.H. Yokaitis*, J. Lee*, & R.F. Thompson. Dept of Psych, Stanford Univ, Stanford, CA 94305.

Lesions of the cerebellar interpositus nucleus prevent learning and retention of a classically conditioned eyeblink without affecting the unlearned reflex (Clark, McCormick, Lavond & Thompson, Brain Res., 291:125-136, 1984) whereas large lesions of the cerebellar cortex alone do not abolish the learned behavior (McCormick & Thompson, Science, 223:296-299, 1984). However, lesions of Larsell's hemispheral lobule VI are said to abolish the learned response (Yeo, Hardiman & Glickstein, Exp. Brain Res., 60:99-113, 1985). In several subsequent studies we removed HVI without abolishing the learned response when using our training paradigms (Woodruff-Pak, Lavond & Thompson, Brain Res., 348: 249-260, 1985; Woodruff-Pak, Lavond, Logan, Steinmetz & Thompson, Neurosci. Abstr., 11:333, 1985).

The current study is our last attempt to substantiate the claim that cerebellar HVI is essential for classical conditioning. We replicated the paradigm used by Yeo et al. (91 dB SPL white noise CS; light CS; 2 mA, 60 Hz face-shock UCS; 550 ms CS; 50 ms UCS; 15 sec intertrial interval; 100 white-noise CS & 100 light CS trials per day; 5 days of training, cerebellar aspiration, 1 week recovery, 5 days of retraining). We retrained for 5 more days, then trained to criterion on our usual paradigm using first white noise then light CS (350 ms CS; 100 ms 2.1 N/cm^2 corneal airpuff; 108 trials per day; 20-40 sec intertrial interval). We present coronal sections of the cerebellum and inferior olive as used by Yeo et al., along with views from Larsell's monograph and Brodal's surface diagram, and figures of daily CR amplitude and percent of the behavior.

All rabbits relearned following the lesion. Four rabbits had complete HVI removal and 6 more rabbits had lesions comparable to a composite of the common area of lesion (cf. Lashley) derived from Yeo et al.'s published data. The pattern of inferior olive degeneration includes and exceeds that seen after injection of HVI with HRP (Yeo, Hardiman & Glickstein, Exp. Brain Res., 60: 114-126, 1985) but does not include the rostral IO seen in Yeo et al.'s lesion study. Stimulation of the rostral IO is an effective UCS (Steinmetz, Lavond & Thompson, Neurosci. Abstr., 11:982, 1985).

Our evidence indicates that complete removal of HVI, per se, does not permanently abolish the eyelid conditioned response.

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202.11 PRELIMINARY EVIDENCE FOR A PROJECTION FROM THE COCHLEAR NUCLEUS TO THE PONTINE NUCLEAR REGION. J.K. Thompson*, D.G. Lavond and R.F. Thompson. Department of Psychology, Stanford University, Stanford, CA 94305.

Current findings from lesions suggest that the pontine nuclei may serve as a relay for auditory CS information to the cerebellar memory trace circuit in eyelid conditioning in the rabbit (Solomon et al., Bull. Psychon. Soc., 24:75-78, 1986; Steinmetz et al., Neurosci. Abstr., in press, 1986).

In the present experiment we explore possible direct anatomical connections from auditory nuclei to the pontine nuclei, using retrograde transport of a fluorescent tracer. Injections were made in the lateral pontine nuclei and control injections in surrounding regions. The rabbits were anesthetized and stereotaxically injected (0.2 ul) with the fluorescent tracer Fast Blue. Click-evoked potential recordings from the injection needle tip were used to guide localization of the injection sites. Survival time was 8 days, at which time the rabbits were perfused with a paraformaldehyde-phosphate buffer solution. The brains were sectioned within 1-3 days.

Injections in the lateral pontine nuclear region resulted in marked labeling in the contralateral ventral cochlear nucleus, lesser labeling in the contralateral dorsal cochlear nucleus and some labeling (mostly ipsilateral) in the superior olivary nucleus. Control injections in the ventral nucleus of the lateral lemniscus resulted in labeling in contralateral dorsal cochlear nucleus and less in ventral cochlear nuclues, in agreement with earlier literature. These results suggest there may be a direct projection from the ventral cochlear nucleus to the contralateral lateral pontine nuclear region.

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202.12 ACOUSTIC RELATED RESPONSES RECORDED FROM THE REGION OF THE PONTINE NUCLEI. C.G. Logan*, J.E. Steinmetz and R.F. Thompson (SPON: N.H.Donegan). Department of Psychology, Stanford University, Stanford, CA 94305.

One requirement for a site of plasticity in Pavlovian conditioning is that it receive input about both the conditioned stimulus (CS) and the unconditioned stimulus (US). The cerebellum has been found to be essential for the rabbit conditioned eyeblink response, and there is strong evidence that the dorsal accessory olive (DAO) provides the cerebellum with US information via climbing fiber input. Recent studies indicate that mossy fiber input is both necessary and sufficient for relaying CS information to the cerebellum. Lesions of the middle cerebellar peduncle (MCP) abolish conditioned responses to tone, light, and tactile CSs, and stimulation of pontine nuclei can serve as an effective CS. The source of auditory CS input to the cerebellum, however, remains unknown. Since the pontine nuclei are the richest source of mossy fiber input projecting to the cerebellum via the MCP, we mapped the pontine nuclei and more caudal brainstem regions for auditory potentials evoked with a click stimulus.

New Zealand white rabbits were acutely maintained under halothane anesthesia (2-3%) throughout the mappings. A click was produced by passing a voltage pulse through a speaker which was located approximately 6 inches from the animal's ear. Evoked potentials were recorded at 1 mm increments in an area extending from 9.5-14.5 mm posterior to bregma, 18-24 mm ventral to bregma and 0-4 mm from midline. Auditory EPs with an onset latency of 3.5-5 msec were recorded in the ventrolateral region of the pontine nuclei and in a somewhat more rostral midline region of the pontine nuclei. Additionally, potentials were recorded from classic auditory areas including the superior olive, ventral nucleus of the lateral lemniscus and nucleus of the trapezoid body. Multiple and single unit recordings were also made in a group of awake, behaving rabbits through electrodes lowered through previously implanted manipulator bases. Units in the ventrolateral pontine region were found to respond with short latency to acoustic stimulus onset (to both a 1 kHz tone and white noise). Histological reconstruction for both groups was done on the basis of marking lesions placed in some of the auditory-responsive areas.

Together with lesion data involving ventrolateral regions of the pontine nuclei (Steinmetz et al., Neurosci. Abstr., 1986), these data suggest that this area may serve as an important relay to the cerebellum for acoustic CSs used during classical conditioning.

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202.13 EFFECTS OF ASPIRATION OF CEREBELLAR CORTEX ON RETENTION OF EYEBLINK CONDITIONING USING STIMULATION OF DIFFERENT MOSSY FIBER SOURCES AS CONDITIONED STIMULI. B. Knowlton, G. Beekman*, D. Lavond, J. Steinmetz & R. Thompson. Dept. of Psychology, Stanford University, Stanford, CA 94305.

Lesion, recording, stimulation and pharmacological studies have identified the essential involvement of the cerebellar interpositus nucleus in the acquistion and retention of the classically conditioned eyeblink in the rabbit. Lesion studies have less clearly demonstrated the essential involvement of the cerebellar cortex. This may be due to the nature of a peripheral CS. A tone or light CS probably reaches the cerebellar cortex and interpositus nucleus in parallel and terminates over a widespread area. The activation of a CS pathway that terminates primarily in a resticted area of either structure may provide a solution. Recently, rapid and robust associative conditioning has been demonstrated when electrical stimulation of 2 mossy fiber sources, the dorsolateral pontine nucleus (DLPN) or the lateral reticular nucleus (LRN) has been used as a CS. These brainstem nuclei differ in their cerebellar projections. The LRN projects predominately to the interpositus nucleus, while the DLPN project primarily to the cerebellar cortex. In the present study, stimulation of the DLPN or LRN was used as a CS to investigate the effect of unilateral aspirations of cerebellar cortex on retention of the CR. Adult male New Zealand rabbits were implanted with bipolar stimulating electrodes in the DLPN or LRN. Training consisited of 108 trials per day over 4 or 5 consecutive days with stimulation in the LRN or DLPN (both 200 Hz, 350 ms, 60-120 ua), paired with a coterminating airpuff (100 ms, 2.1 N/cm2). Unilateral aspiration of the ansoparamedian lobe resulted in an immediate and permanent abolition of the CR for all DLPN trained animals. All LRN trained animals relearned the task after surgery. These results suggest that the critical loci in the cerebellum for lesion abolition of the conditioned eyelid response depend on the patterns of projections of mossy fibers activated by the CS.

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202.14 **A COMPARATIVE BIOCHEMICAL STUDY OF THE SUPRA-AND SUBESOPHAGEAL DIVISIONS OF OCTOPUS BRAIN.** **J.D. Robertson and P. Lee*. Dept. of Anatomy, Duke Univ. Sch. of Med., Durham, N.C. 27710.** **J.Z. Young (The Anatomy of the Nervous System of Octopus vulgaris, Oxford Univ. Press, 1971) has shown that essentially all associative learning functions in the Octopus brain take place in the supraesophageal mass. Based upon extirpation experiments, the vertical lobe is considered essential for visual and the posterior buccal lobe for tactile learning. We have conducted an analysis of the proteins present in the two main divisions of the brain by SDS-polyacrylamide gel electrophoresis with the aim of identifying proteins that are peculiar to the supraesophageal division and hence possibly related to associative learning. Live specimens of Octopus vulgaris from the North Carolina coast were kept in running sea water tanks at the Duke Marine Laboratory in Beaufort until needed. All the tentacles of a live animal were rapidly severed with a scalpel. The supra- and subesophageal masses were then removed and homogenized separately on ice in 200 mM cacodylate buffer at pH 7.4 with or without protease inhibitors (0.1 mM PMSF, EDTA and a 1:10000 dilution of a stock solution of leupeptin, chymostatin, pepstatin A and antipain 25 mg/ml each). Protein concentration was determined by the method of Bradford. We found a group of four proteins with molecular weights in the range of 20000-30000 that are present in the supra- but weak or absent in the subesophageal homogenate. There is another group of three proteins in the 10000-20000 range that is very prominent in the supra- but weak in the subesophageal lobe. This latter group of proteins may include one considered to be histones by Giuditta et al. (J. Neurochem., 29:235, 1977) and the 17000 d O-1 Octopus brain specific protein described by Giuditta et al. [Scand. J. Immunol. 15(Supp. 9):241, 1982].** **Supported by a gift from R.J. Reynolds Industries. We acknowledge the technical assistance of Ms. Cheryl Bock.**

202.15 EFFECT OF ZONA INCERTA LESIONS ON TIMING: APPLICATION OF A NEW METHOD OF FUNCTIONAL MAPPING. J. L. Eberling and S. Roberts. Dept. of Psychology, Univ. of California, Berkeley, CA 94720

One method of assigning functions to brain areas has been to study a variety of tasks. A given area will be used in some tasks but not others; the pattern of use and non-use should suggest the function of the area. This method has worked reasonably well with areas close to the input and output, but not with more central areas; two major problems have been finding an appropriate set of tasks and deciding what processes two tasks have in common. Another method, used here on a small scale, avoids these problems. It begins with a purely behavioral result--that two measures of a single response can be changed independently. This result suggests that the response is generated by two distinct processes--distinct in the sense that one can be changed without changing the other--and that the two measures are sensitive to different processes. Determining which brain areas control each measure will suggest the location of the processes.

This experiment trained rats with the peak procedure, similar to a discrete-trials fixed-interval schedule. On some trials, the first response (level-press) after 40 s of signal (light or sound) produced food and the trial ended; on other trials, the signal lasted at least 120 s and ended without food. After training, response rate reached a maximum about 40 s after the start of the signal. Earlier work with this procedure has shown that peak time--the time of the maximum rate, measured from the start of the signal--and peak rate (the maximum rate) are independent: Each can be changed without changing the other (Roberts, J. Exp. Psychol.: Anim. Behav. Proc., 1981, 7, 242-268). This suggests that performance of this task depends on two distinct processes, one that determines peak time and another that determines peak rate. To begin to locate these processes, we made radio-frequency lesions: bilateral zona-incerta (ZI) lesions in some rats ($n = 5$) and bilateral lesions of similar size in the frontal cortex of other rats ($n = 8$). Another group of rats ($n = 18$) received sham operations. The ZI lesions increased peak time about 10% ($p < .05$); the frontal lesions and the sham operations did not reliably change peak time. This agrees with earlier work suggesting that the ZI is important for timing. Because ZI lesions had the same effect on timing light and timing sound, they probably did not change a sensory pathway. Neither ZI nor frontal lesions reliably changed peak rate; however, peak rate was measured much less precisely than peak time. The main conclusion is that the ZI is part of the process that determines peak time and that some of the frontal cortex (the region that we lesioned) is not.

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202.16 THE ROLE OF CORTICAL AND THALAMIC AUDITORY REGIONS IN DIFFERENTIAL BRADYCARDIAC CONDITIONING TO ACOUSTIC STIMULI IN RABBITS. T. W. Jarrell*, C. G. Gentile*, L. M. Romanski*, B. J. Rose*, P. M. McCabe, and N. Schneiderman. Dept. of Psychol., Univ. of Miami, Coral Gables, FL 33124.

The results of a previous study (Jarrell et al., Soc. Neurosci. Abstr., 11:1111, 1985) indicate that electrolytic lesions in the medial division of the medial geniculate nucleus (mMGN) prevent the acquisition of differential bradycardiac conditioned responses (CRs) to acoustic conditioned stimuli (CSs). The purpose of the present study was to examine further the role of mMGN in bradycardiac conditioning. In addition, the present experiment examined the possible contributions of the ventral division of the medial geniculate nucleus (vMGN) and auditory cortex to differential conditioning of bradycardia to tonal stimuli.

Electrodes were chronically implanted bilaterally in mMGN, vMGN, or auditory cortex. After 7 days of recovery from surgery, each animal received one differential Pavlovian conditioning session in which one tone (CS+) was paired with eyelid shock, and a second tone was presented alone (CS-). At the end of this session, electrolytic lesions were produced through the implanted electrodes. On the following day, the animals received another conditioning session.

Lesions had no effect on baseline heart rate or the bradycardiac orienting response. Each group of animals demonstrated differential bradycardiac conditioning during the prelesion session. During the postlesion session, animals with lesions in vMGN continued to demonstrate differential CRs. However, animals with mMGN or auditory cortex lesions failed to demonstrate differential conditioning during the postlesion session due to the fact that the postlesion response to the CS- was significantly larger.

The results of this study confirm our previous finding that mMGN lesions affect differential bradycardiac CRs to acoustic CSs. The fact that either auditory cortex or mMGN lesions enhanced responses to the CS- suggests that a corticothalamic pathway may be involved in the inhibition of responses to the CS-.

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202.17 IBOTENIC ACID LESIONS IN AMYGDALOID CENTRAL NUCLEUS PREVENT THE ACQUISITION OF DIFFERENTIALLY CONDITIONED BRADYCARDIAC RESPONSES IN RABBITS. C.G. Gentile*, L.M. Romanski*, T.W. Jarrell*, P.M. McCabe and N. Schneiderman. (SPON: W. H. Evoy) Dept. of Psych., Univ. of Miami, Coral Gables, FL 33124.

Previous work in our laboratory indicated that bilateral electrolytic lesions of the amygdaloid central nucleus (ACE) abolish differentially conditioned heart rate (HR) responses but do not abolish differential corneo-retinal potential (CRP) responses (Gentile, et. al., Behav. Brain Res., in press). It has also been demonstrated that radio frequency lesions of ACE produce an impairment in the acquisition of the conditioned bradycardiac response (Kapp, et. al., Physiol. Behav., 23:1109, 1979). The present study sought to determine whether previous results were due to destruction of cell bodies in ACE or fibers of passage.

Ibotenic acid, a substance that selectively destroys cell bodies but spares fibers of passage, was injected bilaterally into ACE or control sites. After approximately 7 days of recovery, animals were exposed to differential Pavlovian conditioning in which one tone (CS+) was paired with eyelid shock and another tone (CS-) was presented alone. Each animal received one conditioning session per day for 7 days.

ACE and control lesions had no effect on HR baseline, orienting response, unconditioned response, or CRP conditioning. Animals with control lesions showed differentially conditioned bradycardiac responses. However, animals in the ACE lesion group did not demonstrate differential bradycardiac responses to the acoustic stimuli. In addition, the bradycardiac response magnitude to both the CS+ and the CS- was attenuated in the ACE lesion group relative to controls.

These findings indicate that intrinsic neurons in ACE and not fibers of passage play an important role in differentially conditioned bradycardiac responses but not CRP conditioned responses.

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202.18 DESTRUCTION OF INTRINSIC NEURONS IN THE MEDIAL GENICULATE NUCLEUS PREVENTS THE ACQUISITION OF DIFFERENTIAL PAVLOVIAN CONDITIONING OF BRADYCARDIA IN RABBITS. L.M. Romanski*, T.W. Jarrell*, C.G. Gentile*, P.M. McCabe, and N. Schneiderman. Dept. of Psych., Univ. of Miami, Coral Gables, FL 33124.

Previous findings in our laboratory (Jarrell, et. al., Neurosci. Abstr., 11:1111,1985) indicate that electrolytic lesions of the medial component of the medial geniculate nucleus (mMGN) prevent the acquisition of differential bradycardiac conditioned responses (CRs) to acoustic conditioned stimuli (CSs), but do not prevent the acquisition of differentially conditioned corneo-retinal potential responses to the same CSs. The purpose of the present study was to determine whether the results of our previous experiment were due to destruction of intrinsic neurons in mMGN or fibers of passage.

Injections of 2-4 ug of ibotenic acid (IA), a substance that selectively destroys cell bodies without damaging fibers of passage, were made bilaterally in mMGN (n=6) or in control sites (n=7). In addition, control injections of 2% pontamine sky blue (400 nl) were made in mMGN (n=5). After 7 days of recovery, animals were subjected to differential Pavlovian conditioning in which one tone (CS+) was paired with eyelid shock and another tone (CS-) was presented alone. Each animal received one 60 trial conditioning session per day for 4 days.

IA lesions had no effect on baseline heart rate (HR), HR orienting responses (OR), or HR unconditioned responses (UR). In the control lesion and control injection group, the CS+ elicited a larger bradycardiac response than the CS-. However, animals with lesions in mMGN did not demonstrate differential bradycardiac responses to the CS+ and CS-. In this group, bradycardiac responses to the CS+ and CS- were similar in magnitude due to a reduction in the magnitude of the response to the CS+.

The major finding of the present study was that bilateral IA lesions in mMGN prevented the acquisition of differential bradycardiac CRs but did not affect the HR OR, UR or baseline. These data suggest that cell bodies in mMGN were responsible for the observed effect, not fibers of passage. Since bradycardiac responses were still present after the lesion, other auditory regions may be involved in the mediation of the bradycardiac CR.

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202.19 OPERANT CONDITIONING OF PRIMATE SPINAL REFLEXES: THE H-REFLEX. J.R. Wolpaw. Wadsworth Labs, NYS Dept Hlth, and Depts Neurol & Anat, Alb Med Coll, Albany, NY 12201.

Study of primate memory substrates, CNS alterations which preserve conditioned responses, requires an experimental model fulfilling two criteria. The alterations must be in an accessible location and must persist without input from other regions. The spinal cord is the most accessible and readily isolated part of the primate CNS. Our recent work (J Neurophysiol 50:1296-1319, 1983; Cell Molec Neurobiol 5:147-162, 1985) shows that the largely monosynaptic spinal stretch reflex (SSR) can be operantly conditioned, and suggests that conditioning causes intrinsic spinal alteration. This study tried to condition the H-reflex, the SSR's electrical analog. Goals were to show that spinal reflex conditioning does not need the muscle spindle and to show conditioning in the lumbosacral cord which is preferable to the cervical for studies of neuronal and synaptic mechanisms.

Nine Macaca nemestrina with triceps surae EMG electrodes learned to maintain a given level of background EMG. At random times, a nerve cuff stimulus elicited the H-reflex. In control mode, reward always followed. In HR↑ or HR↓ mode, it followed only if the H-reflex was above (HR↑) or below (HR↓) criterion. Monkeys worked 3,000-6,000 trials/day for 2-3 months. Background EMG and M response were stable throughout. The H-reflex was stable in the control mode.

In HR↑ or HR↓ mode, the H-reflex changed appropriately over at least 6 weeks. Change appeared to occur in two phases: an abrupt change on day 1, and much slower change which continued indefinitely. In HR↑ mode, the H-reflex rose to an average of 213% of control. In HR↓ mode, it fell to an average of 68%.

The results show that the H-reflex can be operantly conditioned. The reflex bypasses the muscle spindle, thus the spindle is not the site of change. The most probable site(s) are the Ia synapse on the alpha motoneuron, the motoneuron itself, and possibly other oligosynaptic paths from large afferents.

The salient features of H-reflex change, combined with other clinical and laboratory data on spinal cord plasticity, suggest that change is due to intrinsic spinal alteration. If so, this alteration is a memory substrate which should be accessible to detailed physiologic, anatomic, and biochemical study.

(Supported by NIH NS22189 and United Cerebral Palsy.)

202.20 AN AUTORADIOGRAPHIC ANALYSIS OF THE EFFERENT PROJECTIONS OF THE TEMPORAL POLE (AREA TG) OF THE MONKEY BRAIN. R. Insausti and D.G. Amaral. The Salk Institute, La Jolla, CA 92037 and the Dept. of Anatomy, Univ. of Navarra, Pamplona (Spain).

Area TG forms the ventromedial cap of the monkey temporal lobe and can be divided, on cytoarchitectonic grounds, into medial and lateral subdivisions. Because it is surrounded by the sphenoidal ridge of the temporal fossa, few studies have been carried out on its connectivity. However, by means of a transorbital approach, we have placed injections of ^{3}H-amino acids into different areas of the temporal pole of four macaque monkeys (Macaca fascicularis). The animals survived for two weeks and the brains were processed for the autoradiographic demonstration of the anterogradely transported tracer.

Projections to the frontal lobe were heaviest to the medial and orbital surfaces including areas 10, 14, 32, 25, 11, 13 and 12. There was only a minor projection to the dorsolateral frontal cortex which terminated primarily in area 46 at the fundus of the principal sulcus. Labeling was also observed at all levels of the cingulate cortex. In the temporal lobe, projections were heaviest to rostral levels of inferotemporal cortex (area TE) and to the superior temporal gyrus (area TA). At more caudal levels, the projections were confined primarily to the parahippocampal gyrus (areas TF and TH) and to the dorsal bank of the superior temporal gyrus. Areas 35 and 36 received prominent inputs as did the entorhinal cortex, presubiculum and subiculum of the hippocampal formation. The amygdaloid complex was also heavily innervated, especially the lateral, accessory basal and central nuclei. The rostral extent of the insular cortex received a projection which terminated in each of its major subdivisions. The injections of area TG also demonstrated several subcortical projections. There was particularly heavy labeling in the olfactory tubercle which continued into the dorsomedial aspect of the caudate nucleus. The region of heavy terminal labeling also continued caudally into the substantia innominata and covered the large cells of the basal nucleus of Meynert. In the hypothalamus, projections were most consistently observed to the lateral hypothalamic area. In the thalamus, the heaviest projections were directed to the medial nucleus of the pulvinar though the medial or magnocellular division of the mediodorsal nucleus also received a prominent projection. There were also lighter projections to several components of the midline thalamic nuclei. In the brain stem, projections were observed to the dorsolateral central gray and to the reticular formation. These data demonstrate that the temporal polar cortex gives rise to widespread cortical and subcortical structures many of which have been implicated in memory processing.

These studies were supported, in part, by NIH grants 16980, 20004 and by the Clayton Foundation for Research-California Division.

207 SYMPOSIUM. THE NEW PHARMACOLOGY OF ANXIETY: LOOKING BEYOND THE BENZODIAZEPINES. D.A. Bennett, CIBA-GEIGY Corporation and S. Cooper, Univ. of Birmingham (Chairpersons); D.P. Taylor, Bristol Myers Co.; F. Colpaert*, Janssen Pharmaceutica; D.E. Redmond, Jr., Yale Univ.; G.F. Koob, Scripps Clinic; S.E. File, Univ. of London.

For many years, benzodiazepines have been a mainstay in the treatment of anxiety, a disease state with increasing incidence. Recent concern has focused attention on their dependence liability and other side effects that detract from their therapeutic usefulness. At the same time, new research findings have clarified the various actions produced by partial benzodiazepine receptor agonism and have highlighted the role of other brain neurotransmitter/neuromodulator systems in anxiety. This symposium will give an overview of these research advances.

In the last two years with the development of specific serotonin modulating drugs, it has become possible to study the role of serotonin in anxiety. Colpaert (serotonin antagonists) and Taylor (serotonin agonists) will focus on this issue and on the drugs currently in clinical trials.

Bennett (partial agonists of benzodiazepine receptors) will focus on novel non-benzodiazepine drugs that interact with benzodiazepine receptors, yet do not produce the side effects associated with the benzodiazepine compounds.

Redmond (noradrenergic mechanisms in anxiety) and Koob (CRF, peptides and anxiety) will report on other neuronal systems involved in anxiety. Redmond will update the relationship of norepinephrine modulation to anxiety, while Koob will present evidence for a major involvement of CRF in anxiety. Both theoretical implications and practical aspects of this research will be discussed.

Finally, File will give an overview and will speculate on the therapeutic potential for non-benzodiazepine approaches to the treatment of anxiety.

208 REGULATION BY BRAIN OF ITS OWN BLOOD FLOW AND METABOLISM: INSTRINSIC NEURAL CONTROL OF THE CEREBRAL CIRCULATION. D.J. Reis, Cornell Univ. Med. Sch., New York, NY 10021.

The traditional view of the cerebral circulation is that local blood flow is tightly coupled to metabolism, in turn reflecting local neuronal activity. New evidence indicates that specific neuronal pathways, transmitters and neural networks intrinsic to brain participate in regulating flow and metabolism, often independently and either locally or globally. Recent developments are reviewed.

209.1 MASSIVE, RAPID, SPECIFIC DEGENERATION OF NIGRAL COMPACTA NEURONS INDUCED IN MICE BY INJECTION OF A 2'METHYL ANALOG OF MPTP. Arthur Hess, S.K. Youngster*and R.E. Heikkila. Departments of Anatomy and Neurology, Rutgers Medical School, University of Medicine and Dentistry, Piscataway, NJ 08854.

Administration of MPTP results in loss of dopamine in the neostriatum and reduction in neuronal cell number in the zona compacta of the substantia nigra of man, monkey and mouse. A methyl analog of MPTP (1-methyl-4-(2'methylphenyl)-1,2,3,6-tetrahydropyridine) is more potent and causes an even more severe loss of dopamine in the neostriatum than that induced by MPTP (Youngster et al, Eur. J. Pharmacol., 122, 283-287, 1986).

After 10 daily injections of 2'methyl-MPTP, the histofluorescence of the caudate was virtually depleted. The nucleus accumbens appeared unaffected. The zona compacta of the substantia nigra appeared severely depleted of dopaminergic cells, while the ventral tegmental area (A10) and the locus ceruleus appeared unaffected. Counts of neuronal cell number in the nigral compacta were performed:

	No. injections	No. cells-12 20μm sections
Mouse Male-C57 Black----	0	500
	4 (15mg/kg)	151
	9 (15mg/kg)	171
	5 (20mg/kg)	40
Mouse Female-C57 Black--	0	500
	1 (20mg/kg)	28
Rat Male-Sprague-Dawley-	0	1419- 15 sections
	14 (20mg/kg)	1377- 15 sections

Behavior was assessed subjectively by the investigator, as well as by allowing the animals to run on an activity wheel for one hr. Control mice made from 70-120 turns/15 min, reduced most frequently to 0-14 turns/15 min after one injection of 2'methyl-MPTP. An animal with 23 turns/15 min had 28 neurons in the compacta, one with 194 neurons performed at 84 turns/15 min. Inevitably, there were mice which did not react behaviorally to multiple doses of 2'-methyl-MPTP or which recovered from an initial deficit or which did not show decreases in neuronal number even after multiple injections. Animals showing behavioral deficits after 9 injections were still affected 50 days after the last injection and showed sharp decreases in neuronal number.

Insight into the mechanism of action of 2'methyl-MPTP and its apparent specific effect on nigral compacta neurons should prove of value in leading to an explanation of the mechanism whereby dopaminergic neurons degenerate spontaneously in human diseases.

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209.2 EFFECTS OF MPTP ON CENTRAL MONOAMINE SYSTEMS IN YOUNG AND AGED MICE. M. Gupta and D.L. Felten. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been shown to affect the nigrostriatal dopamine system in humans, non-human primates, and in rodents. Although Parkinson's disease in humans is characterized by degeneration of the nigrostriatal dopaminergic system, other monoamine systems also are known to be affected. Furthermore, the disease is a slowly progressive neurodegenerative disorder, and rarely occurs below the age of fifty. The present studies were undertaken to investigate if treatment of aged mice with MPTP affects monoamine systems in addition to the nigrostriatal dopaminergic system. Young adult (2 months old) and aged (21 months old) mice were given multiple injections of MPTP over a period of 2 days. Control animals received vehicle injections. All the control and treated animals were sacrificed either 2 or 3 days after the last injection. Brains were taken out and processed for fluorescence histochemistry of catecholamines or immunocytochemical staining for tyrosine hydroxylase (TH). Aged mice treated with MPTP showed severe physical symptoms characterized by marked akinesia, rigidity of hind-limbs, and an initial resting tremor of the whole body. These physical symptoms were less evident in the young adult mice treated with MPTP. Neuroanatomical studies using fluorescence histochemistry showed reduced numbers of fluorescent profiles and reduced intensity of fluorescence in the ventral tegmental area and in the locus coeruleus in the aged mice treated with MPTP, in addition to the nigrostriatal dopaminergic system that also was damaged in the young adult group treated with MPTP. These results show that aged mice are more sensitive to MPTP treatment than their younger counterparts, and more closely resemble the neuroanatomical and physical features seen in clinical Parkinson's disease.

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209.3 Effects of MPTP on Some Reducing systems in the Mammalian Brain. R.G. Fariello, G.T. Golden, T.N. Ferraro, M. DeMattei*. Neurology and Research, VAMC Coatesville PA 19320 and Thomas Jeff. Med. College, Phila. PA 19107.

One of the postulated mechanisms of MPTP toxicity implies the formation of highly reactive oxidative intermediates that cause damage to either membranes and/or cytoskeleton. Therefore we have studied the effects of MPTP on several reducing compounds in the substantia nigra (SN), neostriatum and cerebellum of mice, rats and cynomolgus monkeys. After daily systemic administration of various doses of MPTP, long enough to cause neostriatal dopamine (DA) depletion (according to a previously established protocol), rodents were decapitated and monkeys had their brain removed under heavy barbituate anesthesia. Brain regions were dissected on ice and immediately frozen at 70°C. Reduced and oxidized ubiquinones, tocopherols and reduced glutathione (GSH) were measured by HPLC with electrochemical detection and compared to values obtained from non MPTP treated animals. Neostriatal catecholamines were also measured by HPLC. Only GSH was found to be significantly decreased, specifically in the SN, whereas values of the other compounds did not differ from controls. Furthermore GSH depletion correlated with the degree of neostriatal DA depletion. We postulate that GSH nigral depletion is the most sensitve index of MPTP toxicity and can be a significant pathogenic factor in the expressivity of such neurotoxicity. Supported by VA funds.

209.4 Chronic MPTP in rats: Effects on food, water, body weight and spontaneous motor activity. G.T. Golden, R.G. Fariello, T.N. Ferraro, M. DeMattei* and P.F. Reyes*.Neurology & Research, VAMC, Coatesville PA 19320 and Thomas Jeff. Med. College, Phila. PA 19107

MPTP causes destruction of dopaminergic neurons in humans, monkeys, and mice and produces neurochemical, pathological and behavioral symptoms similar to idiopathic parkinsonism. Previous experiments have been unable to demonstrate MPTP induced impairment of locomotive function in the rat. We have chronically treated rats with high doses of MPTP, determined caudate levels of dopamine (DA), norepinephine (NE) and serotonin (5HT) and monitored daily food, water, body weight and spontaneous motor activity including stereopathy and rotational behavior.

Rats had daily food, water and body weights recorded. Animals were tested for spontaneous motor activity, stereopathy and rotational behavior under both light and dark cycles prior to beginning MPTP treatment and again at 15 and 30 days post MPTP treatment. Experimental group (N=11) was treated with MPTP by daily injection over a period of 42 days. Each rat was injected 29 times with 35 mg/kg daily with a 2 day break after every 5 injections. The control group (N=6) was kept under identical conditions but were injected with vehicle only. Levels of NE, DA, 5HT, DOPAC, HVA and 5HIAA were determined by reverse phase HPLC with electrochemical detection.

Chronic MPTP treatment resulted in a 45% mortality. MPTP treated animals progressively lost body weight, eventually stabilizing at 82% of control. Consistent with progressive loss of body weight, MPTP group also demonstrated corresponding reductions in daily food and water intakes; however, by the end of the experiment mean daily food and water intakes of MPTP and control animals were similar.

MPTP animals spent less time in ambulatory behavior when tested after 15 and 30 daily injections. After 15 injections MPTP treated animals showed a slight increase in time spent resting, however after 30 MPTP injections there was a decrease in time spent resting in both light and dark cycles which was correlated with an increase in time spent in stereotypic activity under both light and dark conditions. Rotational behavior, a measure of dopamine asymmetry in the nigrostriatal pathway, also showed abnormalities. Comparison of striatal biogenic amine neurotransmitters, revealed that chronic MPTP treatment significantly reduced the levels of DA,NE, 5HT, DOPAC and increased levels of Tyrosine & Tryptophan.

The present experiment reveals that chronic systemic administration of MPTP produces neurochemical changes in striatum, disturbances in food, water & body weight regulation & spontaneous locomotor disturbances in the rat. Supported with VA funds.

209.5 STRIATAL DOPAMINE DEPLETION BY AN AMINATED ANALOG OF MPTP. J.N. Johannessen, J.P. Bacon*, C.J. Markey*, C.C. Chiueh, and S.P. Markey. Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

The dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has generated considerable scientific curiosity because of its ability to mimic the natural lesion seen in Parkinson's disease. We are currently examining the toxicity of MPTP analogues containing amine groups at the 2', 3', and 4' position for two reasons. First, information on the structure activity relationships of MPTP analogues is valuable in delineating the mechanism of toxicity. Second, we would like to have an active MPTP analogue which can be bound to brain tissue using aldehyde fixation for use in anatomical studies.

Preliminary screening was performed in C57BL mice. While the 3'-NH_2-MPTP had a considerable degree of acute toxicity (most animals died at doses higher than 10 mg/kg) and the 2'-NH_2-MPTP produced akinesia, only the 4'-NH_2-MPTP caused the loss of striatal dopamine. Expressed as molar MPTP equivalents, 4 injections of 30 mg/kg at two hour intervals resulted in a 45% depletion 1 week later, while 4 x 50 mg/kg produced an 80% depletion. The dopamine loss was prevented by pargyline pretreatment.

To examine the effects of 4'-NH_2-MPTP in a more sensitive species, 3 doses (molar equivalents to 2.5, 5.0, and 10 mg/kg of MPTP) were injected into beagle dogs. The highest dose produced a transient motor deficit similar to that seen with MPTP, but slower in onset (3 days). Sacrificed 1 month after injection, the two highest dose groups exhibited striatal dopamine losses of 53% and 60%, respectively. One animal from each group showed > 90% dopamine depletion and loss of striatal tyrosine hydroxylase activity, indicating destruction of DA terminals in these animals. Neuropathological evaluation of these animals to determine if nigral cell loss has occurred is in progress.

The 4'-NH_2 derivative of MPTP is effective in producing dopamine depletion and the loss of tyrosine hydroxylase activity from the striatum of the C57BL mouse and the dog, and should be a useful tool for studying the mechanism of MPTP neurotoxicity.

C.J.M. was supported by the National Parkinson Foundation.

209.6 PRELIMINARY STRUCTURAL OBSERVATIONS MADE IN THE HUMAN CAUDATE NUCLEUS, FROM A PATIENT WITH A JUVENILE AND DRUG-REFRACTORY FORM OF PARKINSON'S DISEASE. Machado-Salas, J.P., Madrazo, I.*, Espinosa, J.*, Drucker, R.* and Colín, L.* Neuromorfología, Neurociencias, UIICSE, ENEP Iztacala, UNAM, Tlalnepantla 54030. Estado de México. Serv. Neurocirugía, La Raza, IMSS, and Instituto de Fisiología Celular, UNAM. MEXICO.

We have studied some fragments of human caudate nucleus, which were taken during the autologous implant of adrenal gland in the neostriatum of a patient with Parkinson's disease. Thsi patient, a 34-years-old white man, had developed a bilateral rigidity and a 5 cps resting tremor, without an apparent cause and a rather fast evolution. More recently he had shown a severe akinesia. Several pharmacological therapeutic schemes were used without success; furthermore, he developed severe signs of intolerance to the. One of us (J.P. M-S) discarded him as a candidate for stereotactic brain surgery, due to his advanced state of akinesia. Facing this difficult situation and based in preliminary observations made by the swedish group, it was decided , previous written consent, to implant his right adrenal medulla in a surgical bed made in the head of his right caudate nucleus.

Some pieces of tissue were exposed to formaldehyde vapors to induce histofluorescence, some other for rapid Golgi staining, and some samples more for luxol-fast blue and positive stain for electron-microscopy.

With the routine histological stains, we observed: gliosis, which was more pronounced in the myelinized tracts of the caudate, satellitosis and neuronophagia, dark neurons. These changes were moderate in degree. The rapid Golgi showed: Spiny neurons (12-25 microns) with somatodendritic distortion, abundant dendrites showing micronodulation, mainly in their distal part, and non-uniform loss of spines. No axonless neurons were observed; furthermore, aspiny large neurons were not also observed in this sample. We were struck by the fluorescence shown by the dopaminergic terminals and preterminals of this case, which appeared to be rather normal. These observations shall be complemented with our E/M studies.

Thus far, we have the impresion that, at least in this particular case, the Parkinsonic syndrome that he suffers seems to be correlated with a POSTSYNAPTIC impairment of the nigrostriatum system, rather than with a presynaptic one, which is the classical view.

209.7 TEMPORAL RESOLUTION OF DOPAMINE RELEASE IN THE RAT STRIATUM. R. Mark Wightman, Werner G. Kuhr* and Leslie J. May*. Department of Chemistry, Indiana University, Bloomington, IN 47405.

Release of dopamine in the rat striatum during medial forebrain stimulation has been measured with fast scan cyclic voltammetry. The working electrode is fabricated from a single carbon fiber and coated with Nafion. Individual voltammograms, which are recorded in 10 ms and repeated at 50 ms intervals, provide a characteristic "fingerprint" for the identification of dopamine. During stimulation of the medial forebrain bundle with 60 Hz, 2ms, biphasic pulse trains, dopamine is detected in the extracellular fluid. The rate of data acquisition is sufficiently fast that temporal profiles of the dopamine concentration are observed. The maximum concentration observed varies with working electrode position but is between 1 and 5 μM for a stimulation of 2 s duration. At a fixed anatomical location, the released amount varies in a linear manner with stimulus duration (0.25 to 5 s). When the stimulus train is terminated, the dopamine concentration rapidly falls to its initial concentration. The time course of the disappearance of dopamine follows the time course expected for Michaelis-Menton kinetics. Uptake inhibitors such as nomifensine (20 mg kg^{-1}) increase the amount of dopamine release and decrease the rate of disappearance of dopamine.. In addition, release following uptake inhibition can be observed following a single biphasic pulse. The transient duration of elevated dopamine concentration in the extracellular fluid and its sensitivity to uptake inhibition clearly indicate that cellular uptake of dopamine in the striatum is a major regulatory factor on the free diffusion of dopamine.

209.8 THE EFFECT OF DOPAMINE (DA) SYNTHESIS INHIBITION ON DOPAMINERGIC NEUROTRANSMISSION IN RABBIT STRIATAL SLICES. E.M. Parker and L.X. Cubeddu. Div. of Clinical Pharmacology, Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

Intraneuronal DA is thought to be compartmentalized into a functional, readily releasable pool of newly synthesized transmitter and a relatively inert storage pool of the amine. However, it is difficult to interpret unambiguously much of the previous data that have been used to support this notion. The goal of the present study was to test the importance of newly synthesized DA to the basal and electrically-evoked overflow of the transmitter. Superfused rabbit striatal slices were used in all experiments. Inhibition of DA synthesis by α-methyl-p-tyrosine (αMPT; 100 μM) or 3-iodotyrosine (3IT; 100 μM) reduced the basal efflux of endogenous DA and dihydroxyphenylacetic acid (DOPAC) by 60%. 3IT reduced the electrically-evoked overflow (3 Hz) of DA (60% decrease) and DOPAC (70% decrease). However, αMPT increased DA overflow 2-fold although DOPAC overflow was substantially reduced (80% reduction). Both synthesis inhibitors also reduced tissue DA levels by 35%. αMPT and 3IT (1-1000 μM) had no effect on monoamine oxidase (MAO) activity in rabbit brain homogenates (0.1 μM ^{14}C-DA as substrate); therefore, the ability of these agents to reduce DOPAC efflux and overflow was not due to inhibition of MAO. Although agents which interfere with striatal dopaminergic transmission generally increase electrically-evoked ^{3}H-ACh release, neither αMPT nor 3IT altered the electrically-evoked overflow of ^{3}H-ACh. However, the ability of the DA receptor antagonist sulpiride (1 uM) to enhance electrically-evoked ^{3}H-ACh release at 3 Hz or 10 Hz was decreased by 47% in the presence of either synthesis inhibitor. In order to determine the effects of the synthesis inhibitors on the release of preformed DA, the effects of αMPT and 3IT on transmitter release were examined in slices preloaded with ^{3}H-DA. Both 3IT and αMPT reduced the electrically-evoked overflow (3 or 10 Hz) of ^{3}H-DA by 20-40%. αMPT also increased basal ^{3}H-DA efflux by 30%. Our results indicate that DA synthesis inhibitors decrease stimulation-evoked DA release from striatal slices. Although this phenomenon may be due in part to depletion of a functional, newly synthesized transmitter pool, depletion of tissue DA stores as well as a direct effect of the synthesis inhibitors on the release process may also contribute. Furthermore, the postsynaptic consequences (i.e. dopaminergic modulation of ^{3}H-ACh release) of depletion of the putative functional pool are not marked.

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209.9 PHORBOL ESTERS ENHANCE CATECHOLAMINE SYNTHESIS IN RAT STRIATAL SYNAPTOSOMES. P. Onali and M.C. Olianas. Institute of Pharmacology University of Cagliari, Italy.

Phorbol esters, which are potent activators of Ca^{2+}-phospholipid-dependent protein kinase (protein kinase C), have been used as a tool to investigate the possible involvement of this phosphorylating enzyme system in the regulation of dopamine (DA) synthesis in rat striatum. DA synthesis was assayed by using total homogenates as synaptosomal source and measuring the release of $^{14}CO_2$ from L-[1-^{14}C] tyrosine. 4β-Phorbol 12-myristate 13-acetate (PMA) (1 μM) increased the conversion of tyrosine to dopamine by approximately 20%. No significant stimulatory effect was observed when L-[1-^{14}C] DOPA was used as substrate, suggesting that PMA increased tyrosine hydroxylase (TH) activity. Like PMA, 4β-phorbol 12,13-dibutyrate increased DA synthesis whereas 4β-phorbol and 4β-phorbol 13-acetate, two biologically inactive compounds, were ineffective. KCl (60 mM) stimulated DA synthesis by approximately 25%. Exposure of synaptosomes to PMA prior to KCl addition resulted in a more than additive increase (80%) of DA synthesis. This synergistic effect was dependent upon the concentration of PMA with an EC_{50} value of about 40nM and was also observed when PMA treatment was followed by either veratridine (48 μM) or d-amphetamine (10 μM). However, stimulation of DA synthesis by forskolin (10 μM), a potent activator of adenylate cyclase, was additive with that produced by PMA.To determine whether phorbol ester treatment affected the kinetic properties of striatal TH, synaptosomes were incubated with PMA, lysed by sonication and TH activity was assayed in the supernatant fraction with different concentrations of the pteridin cofactor 6-methyl-5,6,7,8-tetrahydropterin. PMA pretreatment produced an activation of TH with an approximately twofold increase of the enzyme affinity for the cofactor. These results indicate that activation of protein kinase C by phorbol esters enhances striatal DA synthesis by stimulating TH activity. The synergistic interaction between PMA and DA-releasing agents in increasing DA synthesis may result from the combination of protein kinase C-induced activation of TH and decreased end-product inhibition of the enzyme.

209.10 PERIPHERAL PLASMA HVA AND DOPAC CAN REFLECT DRUG-INDUCED CHANGES IN CENTRAL DOPAMINERGIC NEUROTRANSMITTER ACTIVITY. T.M. Abou-Nader*, R.W.von Borstel, R.J. Wurtman. (SPON: H. Lieberman,) Dept. of Brain and Cognitive Science, MIT, E25-604, Cambridge MA 02139.

We examined the plasma concentrations of the dopamine (DA) metabolites, homovanilic acid (HVA) and Dihydroxy-phenyl-acetic acid (DOPAC), as indicators of CNS DA turnover, since some experimental treatments which change brain HVA and DOPAC levels (e.g. Haloperidol), also have been found to cause parallel changes in plasma concentrations of these metabolites. The relative contribution of the CNS to the plasma changes could not be determined from these earlier studies since the test treatments also inhibit transport of HVA and DOPAC out of the brain. We measured plasma HVA and DOPAC using HPLC-EC detection, in 3 blood compartments: arterial, peripheral venous, and CNS venous. 400 gm male Sprague-Dawley rats were anesthetized and tracheostomized; the Carotid Artery (CA), Femoral Vein (FV), and Retroglenoid Vein (RV),(which collects only CNS blood), were cannulated.

Baseline HVA concentrations were (ng/ml): RV=2.49±.39, CA=1.93±.34, and FV=1.87±.27 with significant differences between the Retroglenoid and the carotid of the femoral. (RV-CA)=.53±.15 , (RV-FV)=.62±.23], and no difference between the carotid artery and the femoral vein, [(FV-CA)=.095±.15].

Subcutaneous injection of haloperidol [1mg/kg] increased HVA by 2-3 fold and DOPAC by 1.5-2.0 in the 3 blood compartments, with a significant 2-3 fold increase in the concentration difference between the retroglenoid and either the carotid or the femoral.

	HVA (ng/ml)			DOPAC (ng/ml)		
Time Post Injection (hrs)	0	1	2	0	1	2
RV - CA	.53±.15	2.50±1.00	1.77±.26	.51±.09	1.60±.37	1.60±.62
RV - FV	.62±.23	2.21±.50	1.30±.90	.27±.17	.99±.33	1.0±.11

The ratio between the different compartments however, did not show any significant change and remained for RV/CA and RV/FV at 1.2-1.5, for both HVA and DOPAC.This suggests that the relative contribution of the brain to these metabolites in the peripheral blood remains constant after haloperidol, reflecting increases in their production within both the CNS and the periphery.

With an estimated blood flow through the retroglenoid veins of 10 ml/mn, it is possible to calculate the CNS output of these metabolites in ug/hr:

Brain Output of HVA and DOPAC (mg/hr)			
Time Post Inj. (hrs)	0	1	2
HVA	.30	1.2	.90
DOPAC	.30	.90	.90

These observations indicate that measurement of peripheral plasma HVA and DOPAC may provide a useful index of central dopaminergic neurotransmission.

(Dr. Abou-Nader holds a Whitaker Health Science Fund Fellowship.)

209.11 **PROTEIN INGESTION RAISES TYROSINE LEVELS AND STIMULATES DOPA SYNTHESIS IN THE LIGHT-ADAPTED RAT RETINA.** M.H. Fernstrom, E.A. Volk*, and J.D. Fernstrom. Department of Psychiatry and Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15213.

Tyrosine hydroxylase activity in retina increases rapidly when rats are exposed to light. In such light-exposed, but not in dark-adapted rats, tyrosine (TYR) injection rapidly increases retinal TYR level and stimulates in vivo TYR hydroxylation rate (i.e., dopa accumulation rate). When fasting rats consume a high-protein meal, brain TYR levels are known to rise rapidly. We therefore wondered if ingestion of a protein meal by rats would similarly elevate retinal TYR levels, and if so, if such an increase would stimulate in vivo TYR hydroxylation rate in the light-adapted retina.

Groups of 6 male Sprague-Dawley rats, fasted overnight, were given free access at 9 am to either nothing, a carbohydrate meal (100% carbohydrate, 0% protein) or a 40% protein meal (60% carbohydrate, 40% protein). Ninety min later, the food was removed, and each animal received m-hydroxybenzylhydrazine (100 mg/kg ip) and was killed 30 min later. Retinas were removed for determination of TYR and dopa by HPLC. Serum TYR was assayed fluorimetrically. Ingestion of the protein meal produced large increases in serum and retinal TYR levels, and a significant rise in dopa accumulation rate. When rats consumed the carbohydrate meal, no increases in serum or retinal TYR level, or in dopa accumulation rate occurred. Moreover, when the study was repeated in the dark,. to cause TYR hydroxylase activity to be low, increments in serum and retinal TYR level occurred, but no increase in dopa accumulation rate was observed. These results suggest that a physiologic phenomenon, ingestion of a protein-containing meal, can produce a rise in retinal TYR level sufficient to stimulate TYR hydroxylation rate. The effect on hydroxylation rate occurs only under conditions in which the hydroxylase is activated. And, the effect cannot be ascribed simply to the act of consuming a meal, since no increases were observed when animals consumed the carbohydrate meal. Hence, TYR hydroxylase in this set of central nervous system dopamine neurons shows precursor dependence in a physiologic range of local TYR concentrations.

Treatment Group	Serum Tyrosine (nmol/ml)	Retinal Tyrosine (nmol/mg prot)	Retinal dopa Accumulation (nmol/mg prot)
Fasting	123 ± 6	1.60 ± 0.18	1.28 ± 0.04
Carbohydrate Meal	115 ± 5	1.23 ± 0.04	1.63 ± 0.18
Protein Meal	327 ± 27*	5.73 ± 0.75*	2.57 ± 0.24*

Data are means ± sem; * $P < 0.01$ compared to fasting values.

209.12 THE EFFECTS OF HIGH DOSES OF ALPRAZOLAM AND ADINAZOLAM ON DOPAMINE METABOLISM IN THE RAT. P.S.McQuade, T.K. Hilborn,*J.W.Richard* and M.Thakur*. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Quebec H4H 1R3 Canada.

Large doses of diazepam are reported to be beneficial in the treatment of schizophrenia (Beckmann, H., and Haas, S., Psychopharmacol. 71: 79, 1980). One of the problems with the use of diazepam, however, is the development of parkinsonian-like symptoms in patients on this regimen (Suranyi-Cadotte, B., Nestoros, J., Nair, N., Lal, S., and Gauthier, S., Biol. Psychiat. 20: 455, 1985). Therefore high doses of the triazolobenzodiazepines, alprazolam or adinazolam were administered to the rat in order to determine if they had dopaminergic effects similar to those of high doses of diazepam. Male Sprague Dawley rats (170-190 g) were injected intraperitoneally with 10 mg/kg of alprazolam and adinazolam either acutely or chronically once daily for twenty-one days. The animals were microwaved, the various tissues were dissected and the concentrations of dopamine (DA), homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT) were measured using capillary column negative chemical ionization gas chromatography-mass spectrometry. In the striatum after acute injection, alprazolam at doses of 1 and 10 mg/kg was without effect on the concentrations of DA and its metabolites. In contrast, diazepam at a dose of 10 mg/kg decreased HVA and 3-MT concentrations in the striatum. After the chronic administration of alprazolam and adinazolam, no changes were observed in 3-MT concentrations in the striatum but DA, HVA and DOPAC were all decreased (to approximately 60% of control) thus suggesting that these agents were inhibiting DA synthesis but not affecting DA release. In contrast the chronic administration of diazepam reduced both 3-MT and HVA concentrations. Challenging the chronically treated animals with an acute dose of either alprazolam or adinazolam (10 mg/kg; 60 minutes) resulted in a decrease in DOPAC, HVA and 3-MT concentrations. The acute challenge with diazepam of those animals that were chronically treated with diazepam produced a 50% decrease in 3-MT concentrations. Diazepam effects on the dopaminergic system appear to be mediated by its potentiation of GABAergic mechanisms while the triazolobenzodiazepines appear to be less effective in potentiating these effects, thus requiring very high doses before DA release is affected. These results suggest that alprazolam or adinazolam might be useful for either adjunctive or primary treatment of schizophrenia since these high doses of alprazolam or adinazolam appear to be more selective for synthesis modulation rather than for release modulation. (Supported by the Medical Research Council of Canada and the Upjohn Company of Canada).

210.1 PURIFICATION AND SEQUENCE DETERMINATION OF VARIOUS MOLECULAR FORMS OF DYNORPHIN A AND BOMBESIN-RELATED PEPTIDES IN BOVINE ADRENAL MEDULLA.

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The medullae of four hundred bovine adrenal glands were dissected immediately after removal from the animal and frozen in dry ice. The tissue was allowed to partially defreeze, and it was cut into small pieces (1-2 mm) before adding 10 vol of a solution of 0.1 M HCl - 0.01% thiodiethanol at 95°C. The temperature was maintained at 95°C for 15 min and the mixture was homogenized with a Waring blender (30 s) before neutralization and centrifugation. The clear supernatant was lyophilized, dissolved in a solution of 0.1% trifluoroacetic acid and passed through Sep Pak C18 cartridges (Waters). Peptides were then eluted with a solution of acetonitrile (35% in 0.1% trifluoroacetic acid), lyophilized and submitted to chromatography on a preparative high performance liquid chromatography (HPLC) column (SP - 5PW, cation exchanger, Waters). The elution and separation of peptides was achieved with a gradient of ammonium acetate (from 0.01 M to 1.4 M) with respect to pH (from 4.5 to 6.5). The eluted material was monitored for its content in dynorphin A and bombesin-related peptides by radioimmunoassay. Immunoreactive-(ir) bombesin and dynorphin were separated into two distinct fractions. Each one of them was resubmitted to successive analytical HPLC on SP-5PW and u-bondapak C18 columns (Waters). These later chromatograms yielded two peaks of ir-dynorphin and three peaks of ir-bombesin. The structural sequences of the dynorphin-immunoreactive materials corresponded quite closely to that of dynorphin A while that of one of the ir-bombesin peaks was identical to neuromedin C. These results demonstrate that bombesin and dynorphin-related peptides in bovine adrenal medulla exist under biologically active forms.
(Supported by the Canadian Heart Foundation and the Medical Research Council of Canada).

210.2 IDENTIFICATION AND SEQUENCE DETERMINATION OF A cDNA CLONE FOR AN OCTADECAPEPTIDE THAT INHIBITS BENZODIAZEPINE BINDING.

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Several laboratories, including our own, have reported the existence of endogenous proteins and peptides in brain that inhibit benzodiazepine ligand binding to membrane receptor. Partial amino acid sequence of one such protein, called diazepam binding inhibitor (DBI), is known (Ferrero, P. et al., Proc. Natl. Acad. Sci. USA 83: 827-831, 1986). An intriguing property of this protein is that it elicits "proconflict" responses in rodents.

In order to begin molecular characterization of this protein, we have used polyclonal antibody raised against a synthetic octadecapeptide (Gln-Ala-Thr-Val-Gly-Asp-Val-Asn-Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys) fragment of DBI, to screen a λgt11 expression library representing mRNA from the mouse cerebellum. A cDNA clone was isolated from an immunoreactive plaque, recloned into the plasmid pGEM-3, and sequenced. A nucleotide (nt) sequence encoding the octadecapeptide was identified within the 114 nt open reading frame of the cDNA. However, comparison of the cDNA sequence to the partial amino acid sequence of DBI (Alho, H. et al., Science 229: 179-182, 1985) shows no codon alignment in the amino acid sequences flanking on either side of the octadecapeptide. Since studies on fragments of DBI suggest that more than one copy of the octadecapeptide may be present, it is possible that our cDNA represents a region of DBI containing a second copy of the octadecapeptide flanked by different sets of amino acids. It is also possible that lack of correspondence between codons and amino acids flanking the octadecapeptide is attributable to species differences (amino acid sequence data for DBI is from the rat), but we think this is unlikely due to virtually complete lack of homology. As a third alternative our cDNA might correspond to a gene other than that representing DBI, which also shares the octadecapeptide sequence. This might very well be so if a family of DBI genes exists.

Northern blot analysis shows the cDNA is complementary to several brain mRNA species, ranging in size from 0.8 to 2 kilobases. In an attempt to determine if other biologically active peptides occupy the flanking regions of the octadecapeptide, we have undertaken a search for a clone containing a full length coding sequence.

210.3 SUBSTANCE P AND SUBSTANCE K BIOSYNTHESIS: EFFECTS OF METHAMPHETAMINE

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Substance P (SP) containing cell bodies in the striatum innervate the substantia nigra (SN). Microinfusions of SP into the SN activate both dopamine (DA) and non-dopaminergic neurons. The DA cell bodies in the SN, in turn, project forward and terminate in the striatum. Peripheral administration of dopaminergic drugs have been reported to chnage SP levels in the SN and the striatum, the direction and magnitude of the change depending upon the drug used and the exact dosing regimen.
The present study investigated the effects of methamphetamine on the levels of SP and the newly discovered tachykinin, substance K (SK), in the rat striatum and SN. SP and SK are cotransmitters encoded by the same gene. Changes in the levels of striatal preprotachykinin (PPT) mRNAs were also assessed.
Results indicate that subchronic administration (15mg/kg, ip. every 6h for 24h) of methamphetamine increases SP levels in both the SN and striatum by 50% and 30% respectively, replicating previously published data. SK levels were altered in an identical fashion in both tissues. SP and SK levels were found to be unchanged in the SN up to 6h after a single dose (15mg/kg) of methamphetamine. However, in the striatum the concentrations of both tachykinins were decreased by 30% within 3h. All of these alterations were preceded by an increase (80-100%) in the levels of PPT mRNAs in the striatum. In complementary studies these changes could be blocked by simultaneous administration of haloperidol (1mg/kg), suggesting that the methamphetamine-induced changes in peptide and PPT levels were DA dependent.
Methamphetamine elicits opposite effects to those obtained using the DA anatagonist haloperidol alone indicating further that the changes seen are DA receptor mediated. In conclusion, the changes in tachykinin levels in the SN and striatum occur as a consequence of an altered biosynthesis.

210.4 ASSAY AND CHARACTERIZATION OF TRH PRECURSOR PEPTIDES IN RAT BRAIN

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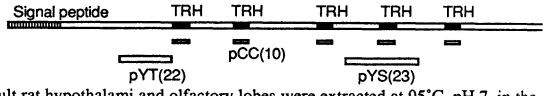
To determine whether the precursor for thyrotropin releasing hormone (preproTRH) is processed to peptides other than TRH we have raised antisera and developed radioimmunoassays for synthetic peptides corresponding to sequences within the cDNA deduced amino acid (aa) sequence of preproTRH. The predicted primary structure of preproTRH consists of a 255 aa sequence of which the amino terminal 24 aa form a probable signal peptide. The remaining proTRH sequence of 231 aa (MW=26,299) contains 5 putative TRH sequences (Gln-His-Pro-Gly) flanked by paired basic aa residues.

The 3 antisera used in this study were raised against 3 synthetic peptides, pCC(10), corresponding to a potential TRH sequence flanked by cysteine residues, pYT(22) and pYS(23), N-tyrosyl analogs of N-terminal and central sequences respectively. For RIA the pCC(10) and pYS(23) antisera were used with ^{125}I-pYS(23) tracer and pYS(23) standards. The pYT(22) RIA used ^{125}I-pYT(22) tracer and pYT(22) standards. These assays do not cross-react with TRH or other neural peptides.



Adult rat hypothalami and olfactory lobes were extracted at 95°C, pH 7, in the presence of protease inhibitors. Assay of these crude extracts showed immunoreactivity in all 3 RIA's. On Sephadex G-75, pH 7.4, rat hypothalamic extracts contained peaks of immunoreactive pCC(10) (ipCC) and immunoreactive pYS(23) (iPYS) coeluting at a position corresponding to a MW ~6,000 daltons as well as a peak of immunoreactive pYT(22) (ipYT) of MW ~4,500 daltons. After purification on Baker C4 wide pore cartridges, reverse phase HPLC of these peaks showed that the 6kD iPCC/ipYS and 4.5kD ipYT each chromatographed as single distinct, baseline resolved, peaks of immunoreactivity. In contrast, G-75 chromatography of olfactory lobe extracts revealed two peaks of ipCC. One eluted in the same position as a peak of ipYS and the hypothalamic 6kD ipCC/ipYS. The other peak fractions, at a position suggesting a MW of ~22-26kD, also contained a coeluting peak of ipYT.
We conclude: 1. ProTRH is processed to peptides other than TRH. 2. Rat olfactory lobe and hypothalamus may each process proTRH differently. 3. The larger ~22-26kD MW form observed in olfactory lobe may be intact or nearly intact proTRH. 4. The smaller 6kD MW ipCC/ipYS and 4.5kD ipYT forms may represent partially processed proTRH.

210.5 **Mesencepalic Dopamine Neurons Control The Expression Of Neuropeptide mRNAs In The Rat Forebrain.** M.R. Brann*, T.I. Bonner* and W.S. Young (SPON: M. Freed) Lab. of Cell Biology NIMH, Bethesda, MD 20892.

Substance P (SP), met-enkephalin (ENK) and dynorphin (DYN) are synthesized from the larger precursors preprotachykinin, preproenkephalin A, and preprodynorphin in distinct neuronal systems in the striatum, ventral striatum (olfactory tubercle) and nucleus accumbens. These forebrain structures are densely innervated by dopamine (DA)-containing neurons, and the peptide neuronal systems have been postulated to play a role in DA-mediated behavior. Many neurons within the striatum which synthesize ENK project to the globus pallidus and many neurons which synthesize SP and DYN project to the substantia nigra. However, the precise anatomy of these peptide systems is complex and only partly understood. To examine the interaction of mesencephalic DA neurons and these neuropeptide systems, we made unilateral lesions of mesencephalic DA neurons with 6-OH-DA, and localized and quantitated levels of mRNAs which encode ENK, SP and DYN in the forebrain *via in situ* hybridization histochemistry (Young *et al., Neurosci Lett.*, 1986).

Male sprague-dawley rats were injected with 8 µg of 6-OH-DA or vehicle into their left substantia nigrae. Two weeks later the rats were sacrificed and 12 µm frozen sections were obtained from several levels of the forebrain and substantia nigra. These sections were hybridized with probes to SP (complementary to the base sequence encoding the first 16 amino acids of exon 3 in rat, Bonner *et al.*, in preparation), ENK (complementary to bases 388-435, Yoshikawa *et al., JBC*, 1985), DYN (complementary to bases 862-909, Civelli *et al., PNAS*, 1985), and tyrosine hydroxylase (complementary to bases 1441-1488, Grima *et al., PNAS*, 1985). The probes were labeled by tailing the 3' end with alpha ^{35}S-dATP and terminal deoxynucleotidyl transferase.

The pattern of distribution of mRNAs encoding SP, ENK and DYN in the striatum, ventral striatum and nucleus accumbens was strikingly similar to the distribution of dopamine-D2 receptors. Animals with complete 6-OH-DA lesions were devoid of mesencephalic neurons which express tyrosine hydroxylase mRNA on the injected side. In these animals, the striatum, ventral striatum and nucleus accumbens on the lesioned side expressed lower levels of SP mRNA and higher levels of ENK mRNA than the contralateral side. These effects reflected changes in the amount of mRNA expressed by individual cells and not in the number of detectable cells. The effect on DYN mRNA was less robust. Initial studies revealed an increase only in the rostral striatum.

These data complement and extend the work of others examining the influence of DA on levels of neuropeptides and neuropeptide mRNAs. Our data suggest that SP and ENK neurons in the forebrain are regulated in an opposite fashion by mesencephalic DA neurons. Currently, we are examining the characteristics of the DA receptors which modulate the neuropeptide mRNAs.

210.6 CHARACTERIZATION OF A PURIFIED PAIRED BASIC RESIDUE-SPECIFIC, PROHORMONE CONVERTING ENZYME FROM BOVINE PITUITARY INTERMEDIATE AND NEURAL LOBE SECRETORY VESICLES

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Peptide hormones are synthesized from larger prohormones by limited proteolysis, generally at pairs of basic residues which flank the biologically active peptide sequences. Cell biological studies have indicated that the prohormones are converted to active hormones within secretory vesicles and therefore the processing enzymes should be localized in these organelles. We have recently purified from bovine intermediate and neural lobe secretory vesicles an endopeptidase that is specific for paired basic residues of prohormones. The prohormone converting enzyme (PCE) from both lobes was shown to cleave pro-opiomelanocortin to yield adrenocorticotropin, β-lipotropin and β-endorphin; and pro-insulin to yield the A and B chains of insulin. Neural lobe derived PCE cleaved pro-vasopressin to yield vasopressin-Gly-Lys-Arg. PCE from both lobes are glycoproteins of ~70,000 mol. wt.; have an acidic pH optimum, and an inhibition profile characteristic of aspartyl proteases. The similarity of the properties of PCE from both lobes suggests that they may be the same or closely related enzymes. The ability of PCE to process different prohormones raises the possibility that a family of enzymes similar to PCE may exist in other endocrine cells and peptidergic neurons for peptide precursor processing.

210.7 NONCOORDINATE POSTTRANSLATIONAL PROCESSING OF ALPHA-MELANOTROPINS AND BETA-ENDORPHINS IN PERIFUSED RAT NEUROINTERMEDIATE LOBE CELLS.

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We have used cell perifusion, reverse-phase high performance liquid chromatography, radiolabelling, and immunoassay to study the biosynthesis and acetylation of β-endorphins and α-melanotropins (α-MSH's), independently, in perifused rat neurointermediate lobe (NIL) cells immediately (0-5 hr) after denervation from the hypothalamus. We found that after 5 hr of steady labelling with either ^{35}S-methionine or ^{3}H-acetic acid mature α-MSH-related products were produced while only very small amounts of putative β-endorphin(1-31)-related precursors were produced during the same period. The molar ratio of α-MSH's to β-endorphins synthesized in 5 hr was 260:1. The molar ratio of α-MSH's acetylated to α-MSH's synthesized was 8:1. After 5 hr of labelling, 90% of the newly processed ^{35}S-containing α-MSH's eluted with desacetyl α-MSH, while 70% of the ^{3}H-acetylated material eluted with diacetyl α-MSH. Virtually no newly synthesized ACTH(1-39) was detectable. The pattern of newly synthesized or acetylated α-MSH's appears well before synthesized acetylated β-endorphin(1-31)-related molecules. However, newly synthesized α-MSH's contribute negligibly to available stored, or secreted pools of peptide hormone. The fact that the majority of the ^{35}S CPM eluted with desacetyl α-MSH and the majority of ^{3}H CPM counts co-eluted with diacetyl α-MSH implies that two pathways are at work. One, the biosynthetic pathway in which the newly synthesized α-melanotropins are cleaved from the precursor and acetylated and amidated. And, another where old, stored monoacetylated (α-N-acetylated), α-melanotropins are further diacetylated, presumably at the serine position 1 of ACTH(1-13)NH_2 through the CoA pathway. Further studies will be required to determine at which site the ^{3}H-acetylated α-MSH's were labelled. It is possible that newly synthesized and cleaved α-MSH-sized precursors do not have the same access to the acetyltransferase enzyme as other stored pools of α-N-acetylated α-MSH. β-endorphins were never acetylated after 5 hr of labelling using ^{3}H-acetic acid. In 5 hr of steady labelling of freshly dispersed NIL cells, the first cleavage products derived from POMC are α-MSH's and not β-LPH and ACTH. (Supported by NIH Grant # NS19397-02)

210.8 BRAIN ENDO-OLIGOPEPTIDASE A, A PUTATIVE ENKEPHALIN CONVERTING ENZYME.

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Biologically active peptides are initially synthesised as part of large inactive precursors and subsequently generated by proteolytic cleavages.

The observation that many biologically active peptides are bracketed by pairs of basic aminoacids has led to the proposal that these peptides are liberated by the action of a trypsin-like endopeptidase followed by a carboxypeptidase B-like exopeptidase. However, in this study, we provide evidence that this combination of trypsin-like and carboxypeptidase B-like enzymes may not be the only mechanisms for liberating enkephalin from low molecular weight precursors. Our data demonstrates that brain endo-oligopeptidase A can produce, by a single cleavage, Leucine^{5}-enkephalin (LE) and Methionine^{5}-enkephalin (ME) from small enkephalin-containing peptides.

Endo-oligopeptidase A has been highly purified from the cytosol of bovine brain. This thiol peptidase, known to cleave the Phe^{5}-Ser^{6} bond of bradykinin and the Arg^{5}-Arg^{6} bond of neurotensin has been shown to produce by a single cleavage LE from Dynorphin B (DYN-B). The specificity constants (Kcat/Km) obtained for hydrolysis of bradykinin, neurotensin and DYN-B were of the same order, indicating that the substrate aminoacid sequence is not the only factor determining the cleavage site of this enzyme. The quantity of LE released was equal to the amount of DYN-B hydrolysed during the reaction. LE formation was inhibited in a concentration dependent manner by the alternative substrate bradykinin. Similar results were obtained for DYN-A_{1-8}, α-neoendorphin (NEO) and β-NEO. The related peptides DYN-A_{1-13}, DYN-A_{1-17} and DYN-A_{1-6} were not detectably cleaved while DYN-A_{1-7} was hydrolysed to form Tyr-Gly-Gly-Phe but not LE. This general pattern of size dependent cleavage was also observed with proenkephalin derived peptides. Hydrolysis of ME-Arg^{6}-Gly^{7} and ME-Arg^{6}-Phe^{7} liberated only Tyr-Gly-Gly-Phe whereas the octapeptide ME-Arg^{6}-Gly^{7}-Leu^{8} released both Tyr-Gly-Gly-Phe and ME in a 1:2,5 ratio. When the octapeptide was extended to form ME-Arg^{6}-Gly^{7}-Leu^{8}-Lys^{9} the major product was ME and using BAM 12P as substrate this was the only product formed. The hexapeptide ME-Arg^{6} was resistant to cleavage as were the larger peptides E, F, B and BAM 18P as well as the higher molecular weight (8.6-23.3kDalton) enkephalin-containing peptides purified from bovine adrenal medulla. From these results the optimum substrate size for hydrolysis by endo-oligopeptidase A seems to be between 8 and 13 amino acid residues, with enkephalin the only product released from precursors where this sequence is immediately followed by a pair of basic residues.

210.9 EFFECT OF CYSTEAMINE ON THE BIOSYNTHESIS AND DEGRADATION OF RAT HYPOTHALAMIC L-[35S]CYS-VASOPRESSIN, -OXYTOCIN, AND -SOMATOSTATIN, MEASURED AFTER S-CARBOXYMETHYLATION. R.E. Franco-Bourland* and A.O. Rodríguez-Hernández* (SPON: J. Villarreal). Dept. of Biochemistry, Inst. Nal. Nutrición, SZ, México, D.F. CONACYT-MEXICO: PCCBBNA-001716.

The first reports on the in vivo effect of cysteamine (CSH) to selectively deplete immunoreactive somatostatin (SRIF) from various tissues, and adenohypophyseal prolactin (PRL), suggested, based on in vitro analysis, that the mechanism of action did not involve the formation of mixed disulfides between the peptides and the drug. Accordingly, in our initial in vivo studies, we interpreted the absence of L-[35S]Cys-SRIF in the hypothalamic extracts from CSH-treated rats, determined by HPLC, as proof of the inhibition of biosynthesis (Soc Neurosci Abstr 9:746,1983) or the activation of degradation (Soc Neurosci Abstr 10:284,1984) of SRIF, and proposed them as mechanisms for SRIF depletion in rats given CSH. L-[35S]Cys-OT was also absent in the hypothalamic and neurohypophyseal extracts of these rats. Recently, however, Lorenson (Endocrinology 115:728,1984) has shown that, the depletion of immunoreactive PRL is, in fact, due to the in vivo, possibly enzyme-linked, formation of mixed disulfides between PRL and CSH. In the light of Lorenson's data, our afore mentioned results could now also be attributed to the formation of mixed disulfides, unidentifiable in our HPLC system, calibrated, using only native peptide standards. In order to test this possibility, we have repeated our experiments using the methodology described previously to this Society (see above), except that here, we have S-carboxymethylated (S-CM) the pre-purified labeled AVP, OT, and SRIF before HPLC analysis, thereby disrupting any mixed disulfides that might have been formed between the peptides and CSH. The HPLC column was calibrated this time using S-CM-peptide standards. In this way, any alterations in the levels of the labeled peptides in hypothalamic or neurohypophyseal extracts should now accurately reflect changes in their biosynthesis or degradation. After S-CM, S-CM-SRIF was recovered with a 60% yield, and S-CM-AVP and -OT with only a 15% yield. HPLC analysis of the hypothalamic extracts from rats given CSH sc 4h before icv L-[35S]Cys (Exp. I) showed the absence of L-[35S]Cys-S-CM-SRIF, thereby proving the inhibition of SRIF biosynthesis (possibly by inhibiting post-translational processing). In the hypothalamic extracts from rats given the label 4h before the drug (Exp. II), L-[35S]Cys-S-CM-SRIF was clearly demonstrated, disproving an effect of CSH on its degradation. In both Exp. I and II the levels of accumulation of neurohypophyseal L-[35]Cys-S-CM-OT and -AVP were unaffected by CSH. Thus, the absence of L-[35S]Cys-SRIF and -OT in our former experiments, was probably due to the formation of mixed disulfides lost in the HPLC analysis.

210.10 RAPID DEGRADATION OF NEUROTENSIN BY NEUROBLASTOMA CLONE N1E-115. J. A. Gilbert and E. Richelson. Mayo Foundation, Rochester, MN 55905.

Murine neuroblastoma clone N1E-115 possesses receptors which specifically bind the tridecapeptide neurotensin (NT) and mediate the formation of intracellular cyclic GMP and the stimulation of inositol phospholipid hydrolysis. These intact cells also rapidly degraded radiolabeled neurotensin (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu). [Tyrosyl^{3,11}-^{3}H]Neurotensin, at a concentration of 0.1 μM, began degrading at 37°C as soon as 10 sec after exposure to intact N1E-115 cells at a level of 1 x 10^6 cells/ml, and 96% of the radiolabeled peptide was degraded (n=2) after 5 min of exposure. Six major tritiated products were formed over a period of 20 min, with the degradation products formed and the degree of proteolysis of [^{3}H]neurotensin being dependent upon the length of exposure and the number of cells/ml. The tritiated degradation products were analyzed by high pressure liquid chromatography (HPLC) employing a Waters μ-Bondapak C_{18} column and a series of gradients in a range of 10%-40% acetonitrile in 10 mM trifluoroacetic acid. Two of the initial products formed eluted with the same retention times as the standards NT(9-13) and NT(11-13). These products, in turn, were each degraded to 33% of the original level by 5 min and completely degraded upon 20 min of exposure to cells (n=2). The corresponding amino-terminal remnants of the NT structure were also among the first products formed; in particular, a peptide with the elution time of standard NT(1-8) was only slightly degraded at 5 min after formation and was degraded by about 50% at the end of 20 min (n=2). The major radiolabeled product after 20 min of degradation of [^{3}H]neurotensin under these conditions eluted on HPLC with a retention time identical to that of standard tyrosine. Degradation studies of [^{3}H]neurotensin performed in the presence of specific protease inhibitors suggested that at least three peptidases appeared to be involved in a sequential fashion in the degradation of radiolabeled neurotensin by intact N1E-115 cells.

[Tyrosyl^{11}-^{3}H]NT(8-13), the carboxyl-terminal portion of neurotensin for which the neurotensin receptors on this neuronal cell type have a higher affinity and a greater responsiveness, was more rapidly degraded under identical conditions than neurotensin itself. [^{3}H]NT(8-13), at a concentration of 0.1 μM, was degraded to 10% of its original level upon 60 sec of exposure at 37°C to 1 x 10^6 cells/ml (n=2). Two of the three initial radiolabeled products formed eluted from HPLC with the same retention times as the standards NT(9-13) and NT(11-13), and the single, final radiolabeled product found after 5 min of degradation of [^{3}H]NT(8-13) eluted with the same retention time as that of standard tyrosine. (Supported by Mayo Foundation and U.S.P.H.S. grant MH27692).

210.11 HIGH-AFFINITY, SPECIFIC INACTIVATION OF ANGIOTENSIN III BY MEMBRANE-BOUND PEPTIDASES OF RAT BRAIN. Carlos G. Cámara and Joseph W. Harding. Departments of Pharmacology, University of Virginia School of Medicine, Charlottesville, Virginia 22908, and VCAPP, Washington State University, Pullman, Washington 99164.

Angiotensins are rapidly inactivated in a non-specific manner by soluble peptidases present in circulatory fluids and crude tissue extracts. Termination of the angiotensin message in target cells has been assumed to occur in a similar non-specific manner as a result of the action of lysosomal peptidases following endocytosis of the receptor-angiotensin complex. We now report the results of our preliminary characterization of membrane-bound angiotensinases of rat brain. Synaptosomal fractions from the combined hypothalamus, thalamus, septum and anteroventral third ventricle of rat brain were washed twice in isotonic medium and assayed for their ability to metabolize ^{125}I-A III when this was added either alone or together with agonist or antagonist angiotensin analogs, or with various unrelated but biologically active peptides. Degradation of ^{125}I-A III by membrane-bound peptidases was defined as the difference between the degradation produced by the total synaptosomal homogenate and the degradation produced by the 36,000 x g supernatant of that homogenate. All assays were performed at 22°C and initial rate conditions. After quenching, the reaction medium was extracted with acetonitrile and the extracts were taken to dryness, made up in elution buffer, and analyzed by HPLC. The results indicate that ^{125}I-A III is sequentially degraded from the aminoterminus by membrane-bound aminopeptidases. At low substrate concentrations the first reaction in this sequence (^{125}I-A III = Arg + ^{125}I-des Arg A III) is catalyzed by a single enzyme. This angiotensinase is highly specific for A III relative to all other peptides tested and displays a very high affinity for this substrate (Km = 75 nM).

210.12 NEUTRAL METALLOENDOPEPTIDASE (ENKEPHALINASE) ACTIVITY IS LOCALIZED IN ENKEPHALIN-RICH REGIONS OF RAT SPINAL CORD. Stephen A. Back and Charles Gorenstein. Department of Pharmacology, University of California, Irvine CA 92717.

We have examined the distribution of neutral metalloendopeptidase (enkephalinase) in rat spinal cord and brain stem by fluorescent histochemistry. Nitrosalicylaldehyde was used to localize the enzyme's reaction product resulting from cleavage of the synthetic peptide substrate, glutaryl-Ala-Ala-Phe-4-methoxy-2-naphthylamide. Enzyme distribution was examined in spinal cord segments from high and low cervical, thoracic, lumbar, and sacral regions from rats which had received 100 μg of colchicine i.c.v. prior to perfusion. The specificity of the staining pattern observed was demonstrated using the "enkephalinase"-specific inhibitor thiorphan at 30 nM concentration. At this concentration, thiorphan completely inhibited all staining observed. Cellular staining was verified by a fluorescent counterstain with ethidium bromide.

The enzyme was found to be richly and highly localized at all levels of the spinal cord examined. The reaction product was heavily localized within the substantia gelatinosa as a very finely dispersed yellow fluorescent precipitate. Somata lightly filled with fluorescent precipitate were occasionally observed in this area. A very similar pattern of reaction product was observed more rostrally in the medulla, pars caudalis in the homologous spinal tract of the trigeminal nerve. Throughout the cord the enzyme was localized to the central cervical nucleus and to lamina X. Appreciable staining was also observed in the ventral horn. In this region cellular staining was largely associated with interneurons and medium-sized motoneurons, as well as with some selected populations of alpha motoneurons. At thoracic and lumbar levels, staining was associated with the medium-sized cells of lamina IV. Laminae V and VII and the intermediolateral cell column were extensively stained, and the gray-white borders of laminae V and VII were associated with staining of distinctive small-to-medium-sized cells.

The spinal cord constitutes an important region of the nervous system where enkephalins are associated both with central and peripheral pain pathways, as well as with pathways which do not appear to subserve a role in the modulation of nociceptive information. The localization of neutral metalloendopeptidase in the present study to regions of spinal cord shown to contain enkephalin-like immunoreactivity suggests a possible role for the enzyme in modulating the activity of enkephalinergic pathways in spinal cord.

Supported by NIDA DA03031 and NIDA predoctoral PHS fellowship DA05255.

210.13 DIFFERENTIAL CARBOXYPEPTIDASE H ACTIVITY IN NERVE FIBERS AND TERMINAL OF THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM.
V.Y.H. Hook and M. Palkovits*. Dept. of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD., and *Laboratory of Cell Biology, NIMH, Bethesda, MD. 20892.

Carboxypeptidase H (CPH) is one of several processing enzymes required for the conversion of neuropeptide precursors into their smaller biologically active forms. (CPH has been refered to as 'carboxypeptidase B-like' and 'enkephalin convertase'). Recent studies (1) show that CPH released from chromaffin cells by nicotinic receptor stimulation is more active than CPH in secretory granules isolated from bovine adrenal medulla tissue. Nicotinic receptor stimulation of chromaffin cells releases the contents of mature granules containing small enkephalin pentapeptides (2), whereas granules from medulla tissue includes immature granules that also contain precursor forms of enkephalins. These data suggest that CPH may be more active in mature than in immature granules. To test this hypothesis, we have compared the state of activation of CPH in pituitary stalk and posterior pituitary of the rat hypothalamo-neurohypophysial system. CPH in posterior pituitary was six times more active than in pituitary stalk. Pituitary stalk transection nearly abolished CPH activity in posterior pituitary. These data demonstrate that CPH must undergo a process of activation as it is transported along the axons of the pituitary stalk to nerve terminals in posterior pituitary. Thus, maturation of secretory vesicles during axonal transport may include mechanisms for the activation of processing enzyme activitie(s).

(1) V.Y.H. Hook and L.E. Eiden. BBRC 128, 563-570,(1985).
(2) Chaminade et al. J. Physiol 353, 317-320,(1984).

ACTION POTENTIALS AND ION CHANNELS V

211.1 SLOWLY ACTIVATING K^+ CURRENT IN XENOPUS OOCYTES INJECTED WITH POLYA$^+$ RNA FROM RAT UTERUS.
M.B. Boyle, E.M. Azhderian*, N.J. Maclusky*, F. Naftolin*, and L.K. Kaczmarek. Departments of Pharmacology, Physiology, and Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510.

To assay mRNA coding for ion channels from the uterus, we have used the Xenopus oocyte translation system. Estrogen treatment of the adult uterus causes hyperplastic growth and a profound increase in the electrical excitability of the smooth muscle. We have injected polyA$^+$ RNA from the uteri of estrogen-primed rats into Xenopus oocytes and found that they express a new voltage-dependent potassium current. This current is unusual in two ways: 1) it activates very slowly yet does not appear to be activated by calcium entry, and 2) its activation upon depolarization is strongly reduced by pre-hyperpolarization of the membrane potential. PolyA$^+$ RNA was obtained from whole uteri of ovariectomized adult rats implanted with estradiol-filled silastic capsules and sacrified after 3 days. Experimental oocytes were injected with 50 nl of a 4 ug/ul RNA solution and incubated at room temperature for 2 to 3 days. Voltage-clamp depolarizations from a holding potential of -40 mV elicited a voltage- and time-dependent outward current in RNA-injected oocytes (9 out of 12 cells from 5 frogs). Similar currents were never observed in water-injected or noninjected control oocytes (n=21 and 124). Moreover, the currents induced by uterine RNA differed from those seen in cells injected with RNA from other excitable tissues (n=128). Resting potentials and input resistances were near -45 mV and 0.5 MΩ in both RNA- and water-injected oocytes. The uterine-RNA-induced current took more than 2 seconds to activate fully at +50 mV from a holding potential of -40 mV. From a holding potential of -100 mV, the current appeared to activate about 2.5 times more slowly. No inactivation was apparent during 2-second depolarizing steps. A slowly decaying outward tail current of 227 $\pm$ 66 nA (SEM) was seen following a 2-second step to +50 mv from -40 mV; only inward tail currents were observed in control oocytes. The tail currents reversed near E_K; the reversal potential shifted as predicted by the Nernst equation to more positive potentials with elevation of $[K]_o$, but was insensitive to reduction of $[Cl]_o$. The current was reduced by 10-20 mM TEA in the bath (n=2). The current was not reduced (n=3) by up to 15-20 min of exposure to a 0 Ca^{2+}, 0.1 mM EGTA bathing solution (which blocked a Ca^{2+}-influx-dependent component of the endogenous Ca^{2+}-activated Cl^- current), implying that the K^+ current is not directly dependent on Ca^{2+} entry. The simplest explanation for the appearance of the new current in the oocytes is expression of a K^+ channel coded for by the uterine RNA. Such a slowly activating channel may play a role in the slow electrical bursting of the myometrium.

211.2 FOUR DISTINCT CLASSES OF VOLTAGE-DEPENDENT K CHANNELS IN PC-12 CELLS.
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Voltage-dependent K channels in clonal PC-12 cells grown in the absence of nerve growth factor (NGF) were characterized using whole-cell, inside-out, and outside-out configurations of patch clamp technique. The internal solution contained (mM) 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 11 EGTA (10 nM free Ca^{++}), 10 HEPES, pH 7.2 with N-methylglucamine (NMG). The external solution contained (mM) 145 NMGCl, 2.8 KCl, 2.8 $MgCl_2$, 10 HEPES, pH 7.2 with HCl.

At least four types of voltage-dependent K channels could be easily distinguished on the basis of their unitary current amplitudes; (1) a large Ca^{++}-dependent K channel (60 to 80 pS or 180 to 250 pS in symmetrical K solutions), (2) a 17 pS channel, (3) an 11 pS channel, and (4) a 7 pS channel (conductance values are based on the unitary current amplitudes in voltage range of -10 to 60 mV. Open channel I(V)'s are not necessarily linear). In addition, very small outward channels (3 to 4 pS) were occasionally observed. All of the channels are activated by depolarizing pulses. Only the large Ca^{++}-dependent K channel is blocked by charybdotoxin. Tetraethylammonium (TEA) and 4-aminopyridine (4-AP) block all four types of channels.

In response to depolarizing step pulses, the 7 pS channels activate slowly and the probability of being open does not decline during a pulse lasting up to 500 msec or more. However, the channels are typically (but not always) inactivated at holding voltage of -70 mV and require hyperpolarizing prepulses to more negative voltages to remove inactivation. Open time histograms can be fitted by a sum of two exponentials and closed time histograms by a sum of three exponentials.

Channels with 11 pS conductance activate faster than the 7 pS channels. The probability of being open declines during a pulse with a time constant of about 300 msec at +40 mV. Open time histograms can be fitted by a sum of two exponentials and closed time histograms by a sum of three exponentials. Sweeps without openings were observed clustered together in time, suggesting presence of a very slow inactivation process.

The 17 pS channels show the most transient behavior among the four channel types. The probability of being open typically declines with a time constant of 40 msec at 20 mV. The 17 pS channels also show significant steady-state inactivation. However, holding voltages more positive than -20 mV are often required to totally inactivate 17 pS channels. Open time histograms are reasonably well fitted by a single exponential.

Effects of both short- and long-term pharmacological treatments, such as NGF application, on properties of the four classes of K channels are now being investigated (Supported by NS23294 and Searle Scholars Program/Chicago Community Trust to R.W.A. and by American Heart Assoc. Postdoctoral fellowship to T. H.).

211.3 PROPERTIES OF VOLTAGE-ACTIVATED POTASSIUM CURRENTS IN ISOLATED, IDENTIFIED MAMMALIAN CORTICAL NEURONS. J.M. Nerbonne[1] and A. Burkhalter[2], Departments of Pharmacology[1] and Neurosurgery[2], Washington University School of Medicine, St. Louis, MO 63110.

Mechanistic studies of cortical information processing are complicated by the inherent complexity of the cortex itself; it is difficult to identify particular "types" or "classes" of cortical neurons and to evaluate the relative roles of intrinsic membrane properties and synaptic connections in determining the overall properties of cortical circuits. In order to circumvent these difficulties, with the long-term aim of studying defined cortical circuits, we are investigating the physiological properties of isolated, identified cortical neurons.

Neurons are identified (based on their projection targets) in vitro following retrograde labelling in vivo with rhodamine-labelled latex microspheres (beads). In neonatal Long Evans rat pups (3-12 days), bead injections are made into either the primary visual cortex (C), area 17, or the superior colliculus (SC). Following 2-15 days survival, tissue pieces are dissected from the contralateral (C) or ipsilateral (SC) area 17, enzymatically dissociated and cells are plated on monolayers of primary astrocytes. As our previous physiological studies revealed differences in the properties of isolated cortical neurons (Nerbonne et al., Neurosci. Abs., 11:149), we have focussed on potassium (K^+) currents. Using the whole-cell variation of the patch clamp recording technique with 140 mM KCl in the recording pipettes, voltage-activated outward K^+ currents are evoked by depolarizations to potentials positive to -30 mV from holding potentials (hp) -50 to -90 mV; 1 μM Tetrodotoxin and 5 mM Co^{++} (with 1 mM Ca^{++}) are added to the bath to supress inward Na^+ and Ca^{++} currents. In all C-projecting cells, at least two K^+ currents are revealed: a rapid, transient (I_t) and a slower, non-inactivating, steady-state (I_s) K^+ current; these currents can be separated based on differing voltage-dependence and pharmacology. In contrast, I_t does not appear to be present in all SC-projecting neurons. With Ca^{++} in the bath, an additional component of K^+ current (I_c), of variable amplitude in different cells, is revealed. Hyperpolarizations to potentials negative to -90 mV from hp -50 to -90 mV evoke small inward K^+ currents (I_i) in all C- and SC-projecting cells but not in all cortical neurons. Experiments under current clamp reveal differences in the amplitudes and durations of action potentials and the firing patterns of identified cells, perhaps owing to differences in K^+ currents. The effects of a variety of putative cortical neurotransmitters on action potentials and K^+ currents in identified cells are under investigation.
Supported by the Epilepsy Foundation of America.

211.4 EVIDENCE THAT C-CURRENT AND A-CURRENT CONTRIBUTE TO REPOLARIZATION OF THE ACTION POTENTIAL IN CA1 PYRAMIDAL CELLS OF RAT HIPPOCAMPUS. J.F.Storm* (SPON: P.R.Adams). Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Spike repolarization (s.r.) mechanisms are important e.g. for control of repetitive firing and transmitter release. Yet little is known about s.r. mechanisms in the vertebrate brain. In squid axons the delayed rectifier, I_K, is the main repolarizing current, but data from autonomic ganglia suggest that Ca-activated and transient K-currents may be important in vertebrates. The present study aimed at testing whether similar mechanisms operate in the mammalian CNS.

CA1 cells (n=76) were studied in slices (28-37°C). Spikes were elicited by brief (1-3ms) current pulses. As shown before (Storm, Neurosci.Abstr.11:1183) Ca-free medium or the Ca-channel blockers Cd, Co and Mn slowed s.r. and blocked the fast afterhyperpolarization (fAHP). Tetraethylammonium (TEA, 0.5-2mM) or charybdotoxin (CTX, 30nM) had similar effects; and the effects of TEA and Cd (or Mn) showed mutual occlusion. Norepinephrine (NE,5μM) did not affect s.r. or the fAHP, although it blocked the slow Ca-dependent AHP (sAHP). Raising the external K-concentration also slowed s.r. and reduced the fAHP, whereas Cl^--loading of the cell was ineffective. These results suggest that a Ca-activated K-current of the fast, TEA-sensitive kind (I_C) contributes to s.r. and fAHP, whereas the slow NE-sensitive Ca-dependent current (I_{AHP}; Lancaster & Adams, Neurosci.Abstr.10:872) does not.

The transient K-current, I_A, seems also to contribute to s.r., because: (1) 4-aminopyridine (4-AP; 0.1mM), which blocks I_A, slowed s.r.; (2) depolarizing pre-pulses, which inactivate I_A, had a similar effect; (3) hyperpolarizing pre-pulses speeded up s.r.; (4) the effects of 4-AP and pre-pulses resisted Ca-blockade (like I_A); (5) depolarizing pre-pulses reduced the effect of 4-AP; (6) 4-AP or pre-pulses broadened the spike less and in a different way than 0 Ca, Cd, Co, Mn, TEA or CTX; the former broadening was uniform, with little effect on the fAHP, whereas the latter affected mostly the last 2/3 of the s.r. and abolished the fAHP. [In Mn/4-AP-medium, 4-30mM TEA slowed the s.r. further, suggesting that a third current (I_K ?) plays a role when the spike is broadened.]

In addition to outward currents, an inward Ca-current seemed to be active during the falling phase of the spike. Thus, in 2-5mM TEA the spike developed a 'shoulder' (s) which was blocked by Cd or Mn.
(Supported by a Fogarty fellowship and by NIH grant NS18579.)



211.5 MUSCARINIC MODULATION OF IONIC CURRENTS IN ISOLATED MAMMALIAN CENTRAL NEURONS. W.J. Wadman*, R.K.S. Wong and M.J. Gutnick*. (SPON.: D. Eaton). Dept. of Physiol. and Biophysics, UTMB, Galveston, TX 77550.

Neurons were dissociated from brains of adult guinea pigs using the methods described elsewhere (Kay and Wong, J. Neurosci. Meth., 1986). After removing the brain the relevant areas were dissected out (Region CA1 and Fascia Dentate from the hippocampus as well as the medial and lateral Habenula). Dissociation was accomplished using an enzymatic treatment (trypsin) for one and a half hour followed by mechanical dissociation shortly before the cells were used. Dissociated cells retain their proximal dendrites, so that their morphology can be compared to those described in Golgi preparations.

Voltage-clamp recordings were made with patch clamp electrodes (2 MOhm) used in the whole cell configuration. Extracellular medium included 2 μM TTX to block Na currents. We studied the modulation of various inward and outward currents by pressure applied muscarine (concentration range 10^{-6}M to 10^{-4}M). Recording electrodes contained either K-phosphate or Tris-phosphate to examine K and Ca currents respectively.

Depolarization from a holding potential more hyperpolarized than -60 mV elicited a transient outward current (I_A) followed by delayed components with slower kinetics. Muscarine reduced the amplitude of the delayed components; it can, however, both increase and decrease I_A for different cells. Analysis of the tail currents revealed two components that decayed with time constants of about 50 and 300 ms; the latter one has been determined to be calcium dependent. Muscarine suppressed both currents.

When outward currents are blocked by intracellular Tris or Cs, the calcium current can be studied. This Ca current consisted of a transient and a persistent component, present in different ratios in different regions. Both components were suppressed by muscarine (10^{-6}M). This implies that the reduction in outward current by muscarine can, at least in part, be ascribed to its action on the calcium current.

Additional experiments where the calcium current was blocked by 1 mM extracellular Cd showed that muscarine still reduced the delayed outward current. Moreover they revealed that muscarine invariably suppressed the A-current, suggesting that the variability observed in normal solution arose from the summed effect of muscarine on the inward and outward currents.

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211.6 MOLECULAR ANALYSIS OF SHAKER, A GENETIC LOCUS THAT AFFECTS A-CURRENT. D.M. Papazian*, T.L. Schwarz*, B.L. Tempel, Y.N. Jan, and L.Y. Jan. (SPON: Dale Bredesen). Howard Hughes Medical Institute and Department of Physiology, University of California, San Francisco, CA 94143.

Mutations of the Shaker locus in Drosophila melanogaster have been shown to alter a potassium current, the A-current, in nervous tissue and muscle. Over a dozen alleles of this gene have been isolated and analysis by genetic and electrophysiological techniques have indicated that the locus is likely to code for a structural component of the A-channel (Salkoff and Wyman, Nature 293,228; Salkoff, Cold Spring Harbor Symp. Quant. Biol. 48,221; Jan et al., Cold Spring Harbor Symp. Quant. Biol. 48,233).

This gene is located at band 16F on the X chromosome (Tanouye et al., PNAS 78,6548). To clone the locus, we initiated a recombinant λ-phage walk from a piece of cloned DNA that fortuitously hybridized to this band. The walk now covers 200kb of DNA isolated in 14 phage steps. In determining where within this DNA the Shaker locus lies, four chromosomal breakpoint mutations have been crucial. These are T(X,Y)B55, which greatly reduces A-current and marks the distal boundary of the gene, and T(X,Y)W32, T(X,3)Sh^{LC} and Sh^{K82a}, all of which abolish the A-current. Each of these chromosomal rearrangements has been covered by the walk and the position of the breakpoints determined by analysis of Southern blots of the DNA from these mutant flies. In situ hybridization of cloned DNA to salivary gland giant chromosomes has confirmed the position of the breakpoints. They are spread out over approximately 60kb and occur in the order (distal to proximal relative to the centromere): B55, LC, K82a, W32. In one other allele of Shaker an alteration in the DNA has been found which may be responsible for its phenotype: the mutant K83f, a mutant produced in a dysgenic cross, contains an insert of 2kb between the B55 and the LC breakpoints.

In summary, because several mutations map to the region we have cloned, it is likely that we have isolated at least part and perhaps all of the gene encoding the Shaker product.

(The authors would like to thank Dr. B. Ganetsky for generously providing mutant strains K82a and K83f.)

211.7 TRANSCRIPTION FROM THE SHAKER LOCUS. T.L. Schwarz*, B.L. Tempel, D.M. Papazian*, K.J. Lee*, Y.N. Jan and L.Y. Jan. (SPON: K. Buckley). Howard Hughes Medical Institute and Department of Physiology, University of California, San Francisco, CA 94143.

The Shaker locus is thought to encode a structural component of a potassium channel, the A-channel. 200kb of genomic DNA from this region have been cloned and 5 mutations have been mapped (see Papazian et al., this volume). Locations of the DNA rearrangements associated with these mutations, however, are spread out over 60kb of DNA. This might imply that the Shaker gene is large, with widely separated exons. Alternatively, the coding region itself may be compact and the rearrangements in the DNA may have long-range effects that disrupt the function of the gene. One approach that we have taken in order to determine where within this expanse of DNA the regions that code for the Shaker product are, is to identify transcripts with Northern blots of poly-A^+ RNA from larvae and pupae. To do this, 90kb of genomic clones were subcloned in 25 pieces to be used as probes.

The major transcript from the area delineated by the mutations is 1.3kb in length and is present in RNA from both larvae and pupae. The transcript is detected by probes from 7 different regions that are spread over 50kb of the genomic map. The most proximal of these regions is about 10kb proximal to the W32 breakpoint, the most proximal mutation we have mapped. The most distal of the regions that see the transcript is within 20kb of the B55 breakpoint, the distal boundary of the gene. Some, but not all, of the regions implicated by this analysis to be coding regions were predicted to contain exons by the cDNA described below (Tempel et al., this volume). Because the 1.3kb transcript seems to span the W32, LC, and K82a breakpoints, it is consistent with the observation that these mutations lack all A- current.

Other transcripts have also been seen. In both pupal and larval RNA a weaker 1.5kb band is seen by all the subclones that detect the 1.3kb transcript. On longer exposures of these blots, additional transcripts of 0.8, 2.3, and 4kb can be seen. All of the transcripts seem to be related because 1) they are, for the most part, detected by the same fragments of genomic DNA and 2) a single cDNA from this region (Tempel et al., below) detects the entire family on Northerns. We are currently investigating the possibilities that the less prominent bands represent differently spliced products of the major transcript or closely homologous transcripts from elsewhere in the genome.

211.8 ISOLATION OF cDNAs IN THE SHAKER REGION; APPROACHED THROUGH CROSS-HYBRIDIZATION. B.L. Tempel, D.M. Papazian*, T.L. Schwarz*, Y.N. Jan and L.Y. Jan. Howard Hughes Medical Institute and Dept. of Physiology, UCSF, San Francisco, CA. 94143.

Sequence homology has allowed the cloning through cross-hybridization of proteins conserved among different species. DNA sequence comparisons reveal that evolutionary drift occurs throughout the protein with functionally important domains changing less (Boulter, J., et.al., J. Neurosci., 5:2545, 1985). In some cases a single, highly-conserved domain has been used in the cloning of genes with little other sequence homology (e.g. homeo-box genes, Fjose, A., et al., Nature 313:284, 1985). If sequence conservation exists among channel genes (e.g. because of the structural constraints of forming an aqueous pore in a lipid membrane), this conservation might help us locate channel coding sequences in the Shaker walk.

Using fragments derived from the membrane spanning regions 1-3 and the amphipathic region of the mouse AcetylCholine Receptor α-subunit (kindly provided by J. Patrick), we have probed DNA from the phage clones of the Shaker walk and identified two regions of cross-homology. These regions are separated by >20Kb of genomic DNA; one is proximal and the other distal to breakpoints that eliminate A-current (K82a and $T(X;3)Sh^{LC}$, see Papazian, et.al. this volume). We probed Drosophila cDNA libraries (provided by L. Kuvar) with four different probes: two from the mouse AChR cDNA at lower stringency and two from the cross-hybridizing regions of the fly's genomic DNA at normal stringency. Several classes of cDNAs were recovered: 1. cDNAs homologous only to the two fly probes; 2. cDNAs homologous only to the mouse AChR probes; 3. cDNAs homologous to the distal-most fly probe and the membrane passing fragment of the AChR; and 4. cDNAs homologous to the proximal-most fly probe.

We have focused our attention on classes 1 and 3. One cDNA from class 3, c5A, was partially sequenced. It contains a sequence highly homologous to the third membrane passing domain of the mouse α-AChR. Interestingly, although c5A hybridizes to the Shaker walk, the homology is not perfect. In fact, by in situ hybridization it appears that c5A is encoded by a region on the third chromosome.

Based on very high stringency washes, cDNAs from class 1 are encoded at the Shaker locus, near the regions of cross-homology with the mouse AChR. To date, however, no direct homology to the AChR probes has been seen among these cDNAs. Mapping a cDNA of about 700 bp back to the genomic walk revealed that it was encoded by at least 3 exons spanning >45Kb. These exons bracket the two breakpoints mentioned above and lie near a third, T(X;Y)W32, that also eliminates A-current. Northern analysis using these exon-containing genomic fragments confirms that each of these widely spaced fragments hybridizes to the same size message (see Schwarz, et.al., this volume).

We are currently sequencing cDNAs from class 1.

211.9 **SLOW CHANGES IN MEMBRANE POTASSIUM CONDUCTANCE CONTRIBUTE TO POSTINHIBITORY REBOUND AND SPIKE ADAPTATION IN APLYSIA BUCCAL NEURONS.** **B.R. Jones and S.H. Thompson*. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950.**

Isolated neuronal somata from the B-cell group of the buccal ganglia of Aplysia californica were studied using intracellular recording and two-microelectrode voltage clamp. Many of these cells exhibit postinhibitory rebound (PIR) following a hyperpolarizing current injection, spike adaptation during a depolarizing current injection, and postburst hyperpolarization (PBH) at the termination of a depolarizing current injection.

Membrane current responses were measured from tail currents which had been digitally processed to eliminate components with rapid kinetics. Voltage clamp steps between -80 and -20 mV with durations of 1 to 30 sec elicited tail currents of less than 5 nA in most cells studied.

Graphs of tail current magnitude as a function of conditioning potential reflect the voltage dependence of activation of the slow ionic currents. These curves exhibit characteristic shapes which can be divided into three regions. The first region represents the outward tail currents, activated by depolarizing conditioning pulses, which are related to spike adaptation and PBH. The second region represents the inward tail currents, activated by hyperpolarizing conditioning pulses in the physiological range of membrane potentials, which are related to PIR. The third region represents inward tail currents, activated by conditioning pulses to more negative membrane potentials (-70 and -80 mV), which limit the magnitude of physiological hyperpolarizations in these cells.

Several lines of evidence indicate that the slow currents represented by the first two regions are caused by changes in membrane K conductance. This evidence includes: 1) the holding potential dependence of the tail currents, 2) the conductance change and reversal potential associated with the tail currents, 3) the sensitivity of the tail currents to intracellular injection of TEA and Cs ions, and 4) the sensitivity of the tail currents to changes in the extracellular concentration of K, Na, Cl and Ca ions. The Ca dependence of the K-conductance varies from cell to cell, and some cells exhibit distinctly Ca-independent slow K currents. The slow current represented by the third region exhibits more complicated behavior, and is apparently due, in part, to an increase in Na conductance.

In Aplysia buccal B-cells: 1) spike adaptation and PBH are caused by an increase in K conductance activated by depolarization, 2) PIR is caused by a decrease in tonic K conductance resulting from hyperpolarization, and 3) a relatively nonspecific inward current influences the lower limit of the physiological range of potentials. (Support: USPHS NS 14514).

211.10 SEROTONIN-MODULATED MEMBRANE CURRENTS IN APLYSIA TAIL SENSORY NEURONS. D.A. Baxter & J.H. Byrne, Dept. of Physiol. & Cell Biol., Univ. of Texas Med. Sch., Houston, TX 77225.

We have utilized the techniques of two-electrode voltage-clamp and computer subtraction in order to examine the current(s) modulated by 5-HT in the tail sensory neurons of Aplysia. Clusters of sensory neurons were bathed in ASW and TTX at 15°C. Membrane currents were activated by 200 ms duration voltage steps (-30 to 50 mV) from a 25 s prepulse to -70 mV. Cells were held at the resting potential between steps and the voltage steps were separated by 80 s. At least 3 responses at each voltage were averaged before and after 5-HT application (10 to 100 uM). Only cells with stable step and holding currents, membrane potentials greater than -40 mV (mean = -43 $\pm$ 3), and input resistances greater than 15 MΩ (mean = 34 $\pm$ 9) before 5-HT application were analyzed.

The isolated 5-HT current had a complex time- and voltage-dependence. With small depolarizations (up to -10 mV), the isolated 5-HT current appeared to have only one component that was relatively linear with respect to voltage and had a fairly constant time-to-peak of about 130 ms. With greater depolarizations (0 to 50 mV), the isolated 5-HT current began to include other components that were highly voltage-dependent, including: i) a fast component that peaked at about 20 ms and reflected a significant decrease in the outward current early in the voltage step, and ii) a slower component in steps above 20 mV that reflected an increased outward current. The increased outward current was attenuated by Co^{+2} and Ni^{2+} but was relatively unaffected by low concentrations of TEA (0.5, 2 and 5 mM). Both voltage-dependent components were blocked by 100 mM TEA. The 5-HT modulated current remaining in 100 mM TEA was similar to the current modulated at small depolarizations (see above). While other interpretations are possible, the component blocked by 100 mM TEA may represent a slowing of the activation and inactivation kinetics of the delayed K^+ current ($I_{K,V}$) by 5-HT.

Application of forskolin (100 uM) did not mimic the 5-HT response. For example, forskolin reliably reduced the fast K^+ current ($I_{K,A}$) (Baxter and Byrne, 1985), but 5-HT appeared to reduce $I_{K,A}$ in only 6 of 35 preparations.

The component of 5-HT modulated current remaining in 100 mM TEA has properties consistent with previous descriptions of the S-current (Klein et al, 1982) while the other component(s) has not been described previously. These results and others (Boyle et al, 1984; Walsh and Byrne, 1985), indicate that the effects of 5-HT/cAMP on modulating cellular properties of these neurons are rather complex. While complex ,all the effects may act synergistically to modify synaptic transmission at the sensory-motor neuron synapse. Supported by AFOSR grant 84-0213.

211.11 PHARMACOLOGICAL CHARACTERIZATION OF THE S POTASSIUM CHANNEL OF APLYSIA SENSORY NEURONS. M.J. Shuster* and S.A. Siegelbaum (SPON: L. Simmons). Howard Hughes Medical Instit., Dept. of Pharmacology, Ctr. for Neurobiol. & Behav., Columbia Univ., College of P&S, and NYS Psychiat. Instit., New York, NY 10032.

Serotonin (5-HT) closes a specific potassium channel ('S' channel) in Aplysia sensory neurons through cyclic AMP-mediated protein phosphorylation, resulting in a slow depolarization and increase in action potential duration. We have used the patch clamp technique to characterize the pharmacological properties of the S channel to allow comparison of this channel with other known K channels and to assess the role of S channel closure in the overall response of the sensory neurons to serotonin.

External 4-aminopyridine, barium or cobalt (10 mM) have no effect on S channel activity when applied to outside-out patches (N=4). External 5-HT (100 uM) also has no effect on S channels in outside-out patches, confirming the importance of intracellular messengers for the action of this transmitter (N=3). Tetraethylammonium ions (TEA) reduce the apparent single S channel unitary current, i, in a dose-dependent manner when applied to the outside (K_d = 80 mM; N=6) or inside (K_d = 40 mM; N=3) of the membrane. The block by TEA shows little or no voltage dependence, indicating that there are two distinct TEA binding sites located near the internal and external mouths of the S channel pore. The blockade by TEA is consistent with a model where the drug rapidly binds to and dissociates from a site within the channel pore on a microsecond time scale, leading to the apparent decrease in open channel current.

TEA, at both internal and external binding sites, is a selective open channel blocker, binding to open channels 10-100 fold more strongly than to closed channels. This suggests a model for S channel gating that involves a global change in channel structure, rather than a localized movement of a discrete gate. As a result of the selective open channel block by TEA, the fractional reduction (F) in mean current flow through the S channels, $\langle I \rangle$, depends on the initial S channel open probability, p_o, according to the relation: $F=1/(1+p_o \cdot (TEA)/K_d)$. Since TEA, at concentrations several times K_d, has little effect on the macroscopic S current (which is proportional to $\langle I \rangle$), the S channels appear to be primarily in a low open probability mode of gating in the intact cell. The relative insensitivity of the S channel to external TEA, Ba, and 4-AP is consistent with the pharmacological properties of the macroscopic S current obtained from voltage clamp experiments (Klein et al., 1982, PNAS 79:5713).

211.12 FMRFamide DEPRESSES TRANSMITTER RELEASE AND INCREASES THE OPENING PROBABILITY OF THE S K^+ CHANNEL IN APLYSIA SENSORY NEURONS. F. Belardetti*, T.W. Abrams, V.F. Castellucci, E.R. Kandel and S.A. Siegelbaum. Howard Hughes Med. Instit., Ctr. for Neurobiol. & Behav., Columbia Univ., College of P&S, and NYS Psychiat. Instit., New York, NY 10032.

By activating a cAMP cascade, serotonin produces prolonged all-or-none closures of the S potassium channels in Aplysia sensory neurons (SN), resulting in an apparent decrease in the number of functional S channels (Nf) in the SN membrane (Siegelbaum et al., 1982, Nature 299:413). This slows the repolarization of the AP and contributes to facilitation of transmitter release. It has recently been reported that a macroscopic K^+ current, similar to the S current, present in several identified Aplysia neurons, is enhanced by the endogenous tetrapeptide FMRFamide (Erxleben et al., 1985; Soc. Neurosci. Abstr. 11:710). Since the same peptide shortens the duration of AP in the SN (Abrams et al., 1984, PNAS 812:7956), we have investigated the actions of FMRFamide on transmitter output and S channel activity in Aplysia SN.

Bath application of FMRFamide (1-100 uM) leads to three electrophysiological changes: 1) hyperpolarization of the resting potential and a decrease in input resistance in the SN; 2) shortening of the spike duration of the SN; and 3) depression of the EPSP in the postsynaptic motor neuron in response to stimulation of the presynaptic SN. To study the mechanism of action of the peptide, whole-cell patch-clamp recordings were obtained from dissociated SN in culture. FMRFamide produces changes in both inward and outward currents. We have here only focused on a slow transient outward current that FMRFamide elicits at holding potentials (Vh) between -50 and 0 mV. This current reverses direction at a Vh of around -80 mV, consistent with an increased K^+ conductance. In some cells this current is followed by a slow inward current.

To test if S channel modulation plays a role in the action of FMRFamide, single S channel currents were recorded from cell-attached patches. When applied to the bath, FMRFamide (2-20 uM) gradually increased the average current ($\langle I \rangle$) carried by the S channels (in six out of seven experiments), consistent with the observed increase in outward current in the macroscopic records. Binomial analysis of single channel records shows that this effect is due to an increase in the probability of a channel being open (p) and is not due to an increase in Nf or in single channel conductance. On average p increases by 475 $\pm$ 214% (s.e.m., N=6) after addition of the peptide. Since bath application of FMRFamide opens S channels under the patch pipette, the peptide probably acts through a second messenger. However, FMRFamide must recruit a second messenger system distinct from cAMP, since the peptide does not affect either the level of cAMP (Ocorr & Byrne, 1985, Neurosci. Lett. 55:113) or the cyclase activity in the SN. Moreover, a depression of cAMP would be expected to increase Nf (the converse of serotonin's action) rather than to increase p.

CELL LINEAGE, DIFFERENTIATION AND DEVELOPMENT II

212.1 ISLET CELLS OF MOUSE AND RAT PANCREAS CONTAIN PHENYLETHANOLAMINE N-METHYLTRANSFERASE. G. Teitelman, M. Evinger, J.K. Lee, T.H. Joh and D.J. Reis. Dept. of Neurology, Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

We have previously reported that cells transiently expressing tyrosine hydroxylase (TH), the first enzyme of the catecholamine (CA) biosynthetic pathway, are present in pancreas of mouse embryos from prenatal day 11 (E11). At E12 some of the TH cells also contain glucagon while at E14.5 some TH cells contain insulin. In postnatal and adult mice all islets contain few TH cells and these cells always co-expressed insulin. These findings suggested that, during embryonic development, TH cells of pancreas are the precursors of glucagon (A) and insulin (B) cells of adults and the TH-insulin cells present in islets after birth may represent a reservoir of B-cell precursors.

In this study we sought to determine whether: 1) islet cells of mouse contain phenylethanolamine N-methltransferase (PNMT), the CA enzyme responsible for the conversion of norepinephrine into epinephrine, during pre and/or postnatal development; 2) PNMT is expressed by the TH cells of the pancreas or by a different population of cells; and 3) PNMT is also expressed by islet cells in rat. Finally, we used molecular biological techniques to confirm the presence of PNMT mRNA in the mouse pancreas.

Pancreata were removed from E17 to adult mice and from E14 to adult rats, sectioned and processed for immunocytochemical staining using newly developed antibodies against PNMT and an improved PAP complex. In contrast to previous reports (Teitelman et al., P.N.A.S. 78: 5225, 1981) few PNMT cells were seen in mouse pancreas at all stages examined. In adults, 20% of all islet cells contained PNMT. These cells were localized in the periphery of the islets and were lightly stained. Using immunohistochemical techniques to label two antigens in the same histological section, TH and PNMT were localized in different populations of pancreatic islet cells of adult mouse. Cells containing PNMT were also found in rat pancreas at all stages examined. The distribution and number of PNMT cells in rat islets was similar to mouse.

Additional confirmation that the pancreatic antigen recognized by immunohistochemistry is PNMT was provided by the detection of PNMT mRNA in poly A^+ RNA prepared from mouse pancreas. PNMT antiserum immunoprecipitated PNMT following translation in vitro of pancreatic poly A^+ RNA. Furthermore, the full-length PNMT cDNA probe (Baetge et al., PNAS, in press) hybridized to a single RNA band of approximately the same size (1100 ntd) as adrenal PNMT mRNA.

In conclusion, we have shown that different populations of islet cells of mouse and rat pancreas express TH and PNMT. These findings raise the possibility that, while islet cell precursors expressing TH give rise to the A and B cells of pancreas, other islet cells originate from precursor cells containing PNMT.

212.2 EXPRESSION OF BRAIN SPECTRIN SUBTYPES DURING MAMMALIAN BRAIN DEVELOPMENT. B.M. Riederer*, I.S. Zagon and S.R. Goodman* (SPON: R.B. Hamilton). Depts. of Physiology and Anatomy, M. S. Hershey Med. Ctr., Penn. State Univ., Hershey, PA 17033.

Mouse brain contains at least two spectrin subtypes which both consist of two 240 kDal and 235 kDal subunits. They are detected with two different antibodies, one raised against synaptosomal brain spectrin and the other against red blood cell spectrin (Riederer et al., J.Cell Biol., in press). In this study we investigated the appearance of these two subtypes in the brain at embryonal day 15 and 18, birth, and throughout postnatal development. The brain spectrin antibody reacts with the 240 kDal subunit of brain spectrin(240/235). This subtype is present in embryonic brain tissue and increases gradually throughout ontogeny. From birth to adult, brain spectrin(240/235) concentration doubles as estimated by quantitative immunodot assay and immunoblots. This subtype is expressed in germinal cells and is present in many neural processes and cell bodies, as detected by indirect immunofluorescence. Later in ontogeny the immunofluorescent staining decreases in the soma but is prominent in axonal processes. The erythrocyte spectrin antibody reacts with brain spectrin(240/235E) subtype and is more specific for the 235 kDal subunit. This subtype is not found in embryonal tissue. After the first postnatal week it appears in neurons and glial cells and increases gradually into adulthood. It is found in cell bodies and dendrites during differentiation. A similar onset of immunostaining in most brain regions indicates that the protein is expressed at the same time in the majority of neural elements. The most intense staining was seen in granule cells of the cerebellum and hippocampus. The distinct appearance of these two mammalian spectrin subtypes and their different distribution within neural cell structures throughout development suggests their involvement in different cellular functions. This work was supported by National Institutes of Health Grants NS-19357 and HL-26059 to S.R.G and NS-21246 to I.S.Z. and S.R.G.

212.3 INTERCLONAL VARIATION IN OLIGODENDROCYTE DIFFERENTIATION. M. Dubois-Dalcq. Department of Zoology, University College London, WC1E 6BT

The sequential events leading to the morphological and antigenic differentiation of oligodendrocytes, the myelin-forming cell of CNS, can be studied in a microculture system where individual clones can be followed. A single bipolar progenitor cell from 7 day old rat optic nerve (that was identified by antibodies to surface gangliosides A_2B_5) can generate a clone of multipolar oligodendrocytes within 8-10 days when cultured in defined medium (with 0.5% serum) and in the presence of a mitogen provided by astrocytes type I (Temple and Raff, Cell, in press). Clonal size was followed for up to 25 days and cell population analysed by immunolabelling for the cell specific markers A_2B_5, GC (galactocerebroside, a marker for differentiated oligodendrocytes), GFA (an astrocyte specific intermediate filament protein) and O_4, an oligodendrocyte specific sulfatide that appears before GC (Sommer and Schachner, Dev.Biol. 83, 311, 1981). Two types of clones were identified: (1) the first group contained only GC + $A_2B_5^-$ oligodendrocytes. These had been generated during the second week in culture. Subsequently such clones showed a decrease in size due to the restricted lifespan showed by oligodendrocytes formed in vitro (3-12 days). (2) The second group of clones showed no decrease, or a moderate increase in clonal size during the third week in vitro (i.e. 31→50, 56→88,79→90). The majority of the cells in such clones are identical to those seen in the first type of clones and consist of GC^+, $O4^+$, GFA^-, $A_2B_5^-$oligodendrocytes. However the remaining 2 to 38% of oligodendrocyte-like cells are $A_2B_5^+$, $O4^+$, GFA^- but strikingly GC negative. Thus, a bipolar neonatal progenitor cell of optic nerve can generate cells with an intermediate phenotype along the pathway of oligodendrocyte differentiation. These $A_2B_5^+$, $O4^+$, GC^- cells closely resemble the progenitor cell found in adult rat optic nerve (ffrench-Constant and Raff, Nature 319, 499, 1986) in their antigenic phenotype and morphology; such cells have a lower proliferation and differentiation ability than the neonatal progenitor cell (Wolswyk and Noble, personal communication) . Whether these cells have the potential to repair myelin after a demyelinating process remains to be determined.

212.4 GENERATION OF IMMUNOCYTOCHEMICALLY IDENTIFIED CHOLINERGIC NEURONS IN RAT CERVICAL SPINAL CORD. P.E. Phelps, R.P. Barber*, and J.E. Vaughn, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Previous immunocytochemical studies identified the following four groups of choline acetyltransferase-positive (ChAT+) neurons in the developing cervical spinal cord: motoneurons, partition cells that divide dorsal and ventral gray matter, small neurons scattered in laminae III-VI of the dorsal horn, and central canal cluster cells (Phelps et al., J. Comp. Neurol. 229:347-361). The existence of four different ChAT+ populations that varied in both somal size and location within the spinal cord provided an opportunity to examine whether neurons with the same transmitter phenotype would be generated simultaneously or, conversely, according to previously described general gradients of spinal cord neurogenesis. The methods of ^{3}H-thymidine autoradiography were combined with those of ChAT immunocytochemistry to examine the generation of these populations. Pregnant rats were given a single intraperitoneal injection of ^{3}H-thymidine on one of the following embryonic days: E10-16. Sections of spinal cords (C6-8) from isotopically labeled progeny were processed first for ChAT localization and then for autoradiography. CHAT+ neurons with distinct nuclear profiles were identified, counted, and classified as to the amount of silver grains overlying their nuclei.

Embryonic day 11 was the earliest time that neurons within the cervical enlargement were produced. Large and small ChAT+ motoneurons were generated during the E11-12 period, with the majority of both populations being formed on E11. ChAT+ partition cells were generated from E11-13, with the peak occurring on E12. Approximately three quarters of both ChAT+ central canal cluster cells and immunoreactive neurons in the dorsal horn were generated on E13, with the remainder of each of these populations being born on E14. ChAT+ neurons appeared to be among the earliest cells generated within the ventral, intermediate and dorsal subdivisions of spinal cord. The salient finding of this study was that all neurons of the cholinergic phenotype are not generated simultaneously. Rather, they appeared to follow the general ventrodorsal sequence of neuron formation previously described for spinal cord. Although ChAT+ neurons with larger somal sizes generally originated before those with smaller somata, large and small motoneurons were simultaneously generated in similar proportions. In combination, these findings suggest that location within the spinal cord is more closely associated with the relative timing of cell generation than either somal size or transmitter phenotype. Supported by NSF grant BNS-8219831.

212.5 MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) IMMUNOREACTIVITY IN HUMAN FETAL NEOCORTEX. K.B.Sims*, J.E. Crandall, K.S. Kosik* and R.S.Williams. E.K.Shriver Center, Waltham, MA and Department of Neurology, Harvard Medical School, Boston, MA

In mature neurons MAP2 is concentrated selectively in dendrites and perikaryal cytoplasm. We investigated the distribution of monoclonal MAP2 immunoreactivity in human fetal cortex (19-22 wks gestation). Tissue from frontal and motor regions was immersion fixed in 2% PLP 30-90 minutes post mortem. Coronal 20-40um vibratome sections were processed for immunocytochemistry using a peroxidase-conjugated secondary antibody and diaminobenzidine as chromagen. The morphology and distribution of MAP2-immunoreactive neurons was compared in companion sections stained with Nissl, NADPH-diaphorase and rapid Golgi methods.

Neuronal somata and dendrites in all cortical layers and in the intermediate zone were stained with MAP2 antibody. Cajal-Retzius cells of layer I, large pyramidal neurons of the inner cortical plate and interstitial neurons in the intermediate zone were most strongly immunoreactive. Companion Golgi material confirmed that the dendritic morphology of the large neurons in the inner cortical plate was like that of pyramidal cells in future layer V, and that the dendrites of subplate and interstitial neurons were highly varicose. Apical dendrites of these pyramidal neurons fasciculated into prominent bundles that coursed radially through the cortical plate and terminated as finely beaded tufts in layer I. A dense coarsely globular staining pattern in the subplate resulted presumably from staining of the dendritic varicosities of neurons in the subplate and underlying intermediate zone, and perhaps the basal dendrites of pyramidal neurons in the adjacent cortical plate. MAP2-immunoreactive interstitial neurons were most prevalent in the outer half of the intermediate zone. In general interstitial neurons were smaller and more densely packed, and bipolar forms predominated superficially near the subplate. Larger pyramidal and polymorphic neurons were scattered more deeply. Pyramidal and bipolar neurons usually were aligned radially, but neurons with oblique or horizontal dendrites also were seen. The stout dendrites of the larger deep neurons were often exceptionally long, extending for several hundred micrometers. The NADPH-d method stained only a small subgroup of interstitial neurons, which included all morphological subtypes but predominantly those closer to the subplate.

In summary, most if not all neurons in the cerebral cortex of the 19-22 wk human fetus are MAP2 immunoreactive. MAP2 immunocytochemistry permits an incomparable survey of the temporal and spatial gradients of dendritic differentiation among cortical neurons. It also allows a better assessment of the morphological diversity, density and regional distribution of interstitial neurons in the intermediate zone.

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212.6 CHOLINERGIC AND OTHER NEURONAL CHARACTERISTICS OF PERMANENT CELL LINES DERIVED FROM THE SEPTAL REGION. D.N. Hammond, B.H. Wainer and J.H. Tonsgard.* The University of Chicago, Chicago, IL 60637.

Cholinergic neurons of the mammalian basal forebrain play an important role in learning and memory, and are prominently affected in Alzheimer's disease. In order to more fully study the properties of these cells, we generated clonal hybrid lines derived from cells of the septal region which constitutively express properties typical of cholinergic neurons.

Septal cells from E-14 C57BL/6 mouse embryos were fused in a monolayer using polyethylene glycol with cells of the murine neuroblastoma, N18TG2. The latter cell line does not express a cholinergic phenotype and is deficient in hypoxanthine-guanine phosphoribosyl transferase (HGPRT). The fusion products were plated in medium containing hypoxanthine, aminopterin, and thymidine (HAT), which selects against HGPRT-deficient cells. Individual hybrid colonies were isolated using cloning cylinders, subcultured, and expanded in medium without HAT. Primary septal cells or N18TG2 cells alone were treated in a similar manner and produced no viable colonies. Nine cell lines were isolated and screened for choline acetyltransferase (ChAT) activity by a radiochemical assay. Two lines, SN5 and SN6, showed significant ChAT activity (100-200 pmol acetylcholine formed/min/mg protein), and were further evaluated. The hybrid nature of these cholinergic lines was verified by karyologic and isoenzyme analysis. In addition to ChAT activity, SN5 and SN6 cells display other characteristics typical of septal cholinergic neurons, but not of the neuroblastoma parent. In monolayer culture, the cells elaborate neuritic processes which stain immunocytochemically for neurofilament protein, using antibody TA.51. The cells also express ChAT immunoreactivity, but do not visualize with an antibody against the glial astrocytic marker, GFAP. Finally, SN6, and to a lesser extent SN5, aggregate in rotation-mediated culture, a feature also expressed by normal septal cells, but not by N18TG2 cells.

The nine cell lines isolated from the fusion are the first permanent lines to be derived from a specific region of the basal forebrain. SN5 and SN6 are especially interesting because they express properties characteristic of a particular neuronal subset of that region, and could therefore be used to study the factors influencing septal cholinergic neuron development, selective synapse formation and maintenance. (Supported by grants NS-07195, NS-17661, HD-04583, and MH-28942.)

212.7 REGULATION OF CELL PRODUCTION IN DEVELOPING RETINA. T. Reh
Dept. Medical Physiology, Univ. Calgary, Calgary, AB Canada T2N 1N4

During the development of the vertebrate CNS a large number of different types of neurons are generated in precise ratios. We have previously postulated the existence of a feedback mechanism from already differentiated neurons that regulates the production of new neurons. Evidence for such a regulatory feedback role comes from experiments in which dopamine containing amacrine cells, ablated in the developing retina by 6-hydroxydopamine, (6-OH-DA) were up-regulated in their production. To determine if this is a general phenomenon of the developing retina, other cell types were also examined for their ability to respond to selective ablation byup-regulation in their production. To this end, the neurotoxin kainic acid, known to produce selective lesions of certain cell types in other species, was injected intraocularly (1-2 µl of 1 x 10^{-2} M) in midlarval staged Rana pipiens tadpoles. After periods of 1-21 days, the animals received I.P. injections of H^3-thymidine (1 µl/body wt of 20 Ci/mmole, 1 µCi/µl). Animals were then allowed to survive for periods of up to three weeks and were then fixed in Bouin's solution, the eyes embedded in plastic, sectioned at 3 µm, and processed for autoradiography by standard methods.

The kainic acid (KA) produced a cell-specific lesion in R. pipiens similar to that observed in other species. At the dosage used there was no decline in the density of cells in the outer nuclear layer (ONL) or retinal ganglion cell layer (RGC), but there was a dramatic decline in the cell density and total thickness of the inner nuclear layer (INL). In addition, in animals fixed within 4 days of the KA administration, numerous pycnotic nuclei were observed in the INL.

The H^3-thy allowed us to detect any changes in the numbers of new cells added to the retina after the KA lesion. Within the first week after the KA injection, there was a marked decrease in the number of H^3-thymidine labelled cells in the INL of the lesioned eye as compared with the control retina. However, this decrease in the number of H^3-thy labelled cells was not observed in the other RGC or ONL. This suggests that INL neuroblasts acquire kainate receptors prior to their final division.

Following one week, the pattern of H^3-thy labelling changed: in animals that received H^3-thy injections more than one week after the KA lesion, the number of labelled cells in the INL was significantly increased over that in control eyes, while the number of labelled cells in the ONL and RGC was not. Thus, one week following the loss of INL cells, the production of new cells in this layer was specifically increased. This is similar to the up-regulation observed for DA-amacrine cells following 6-OH-DA treatment, and indicates that feedback regulation of neuron production is a general phenomenon of retinal histogenesis.

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212.8 IN VITRO DIFFERENTIATION OF QUAIL NEURAL CREST CELLS INTO SENSORY-LIKE NEUROBLASTS. M. Sieber-Blum[1], S. R. Patel*[2], and D.A. Riley[1]. 1) Dept. of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226, and 2) The Johns Hopkins University, Baltimore, MD 21205.

The neural crest of the vertebrate embryo gives rise to most of the neurons and nerve supporting cells of autonomic and sensory ganglia, to pigment cells, to endocrine cells, and to cephalic mesectodermal derivatives of the mature organism. So far, the differentiation of neuroblasts in vitro has been limited to the autonomic phenotype. In the present study, we report the differentiation of cultured quail neural crest cells into sensory-like neuroblasts. Their identity has been established by the following criteria: 1. Morphological features: Sensory-like neuroblasts were rounded, dipolar or pseudounipolar with large diameter long processes that lacked varicosities. 2. Neurotransmitter content: Cells with the sensory-like morphology reacted with antibodies directed against the peptide transmitters, vasoactive intestinal polypeptide (VIP) and substance P (SP). Some multipolar neuroblasts of the autonomic type that were present in the same cultures also bound anti-VIP or anti-SP antibodies. 3. Histochemical staining for carbonic anhydrase (CA): A number of the cells with the typical morphologic appearance of sensory neuroblasts contained CA activity; all other neural crest cells were non-reactive. We verified with aldehyde perfused material from adult quails that in this species a subpopulation of spinal sensory neurons has CA activity in vivo, whereas ventral root axons and neurons in sympathetic ganglia are negative. 4. Neurofilament content: Sensory-type neuroblasts bound antibodies against the 68K neurofilament polypeptide. 5. Sensitivity to nerve growth factor (NGF): Addition of NGF to the culture medium caused an increase in the number of sensory-like neuroblasts with SP-like immunoreactivity. The sensory neuron-like cell type characterized in this study should prove useful in the future analysis of the interrelationship of progenitor cells of the autonomic and sensory cell lineages.

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212.9 PATHWAYS FOLLOWED BY CREST CELLS MIGRATING FROM DEVELOPING QUAIL GUT BACK-GRAFTED INTO YOUNGER CHICK EMBRYOS: COMPARISON OF VAGAL AND TRUNCAL GRAFTS. I. P. Rothman, N.M. Le Douarin*, and M.D. Gershon. Dept. of Anat. and Cell Biol., Columbia Univ. P&S and l'Inst. d'Embryol., CNRS and Coll. de France, Nogent-sur-Marne, France.

The enteric nervous system (ENS) is the largest and most complex portion of the PNS. It is formed by precursor cells that migrate to the bowel from "vagal" and sacral regions of the neural crest. Following its colonization the gut retains cells that are able to regain their ability to emigrate when the gut is transplanted into a neural crest migration pathway of a younger host embryo (back-graft). When a back-graft of embryonic quail bowel that contains crest cells (4 day) is made into the trunk region, both neural and mesenchymal structures of the host embryo become populated by donor cells from the back-grafted gut. The fate of cells leaving a back-grafted bowel can be followed when quail gut is transplanted into chick embryos because the quail cells of the donor are stably marked by a distinct clump of nucleolar-associated heterochromatin. Migration of cells from back-grafted bowel that has been colonized by neural crest is more extensive than from control hindgut transplanted prior to colonization. Nevertheless, cells emigrate even from back-grafts of aneuronal gut; however, in the absence of crest only mesenchymal structures are colonized by cells from the donor's bowel. Back-grafted bowel containing crest will, when placed into truncal pathways, colonize sympathetic ganglia, the adrenal medulla, as well as spinal roots and peripheral nerves. In contrast, truncal back-grafts never provide emigres that migrate to enteric ganglia, although donor cells may be found in the vagus nerves. On the other hand, if "vagal" crest (level of somites 5-6) is replaced at stage 11, when migration of crest cells from this region has begun, quail cells do reach the host gut. The donor cells extend from just above the proventriculus to the cloaca, but they are restricted in the enteric wall to the the myenteric and submucosal plexuses. In addition to the gut, these "vagal" back-grafts also colonize the petrosal ganglion and contribute large numbers of Schwann cells to cranial nerves IX-XII. In contradistinction to truncal back-grafts, "vagal" back-grafts do not colonize sympathetic ganglia or the adrenal medulla. These experiments confirm that even after the colonization of the early bowel and the beginning of the formation of enteric ganglia the gut contains precursor cells that can migrate along pathways in younger embryos normally traversed by crest cells. Moreover, the route taken by these precursor cells is appropriate for the host location into which a back-graft of gut is placed, rather than for the enteric origin of the migrating cells. Thus, previous migration of cells to the gut does not ensure they will again migrate there; instead cells from the bowel will migrate along pathways defined by the host embryo into which they are placed.

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212.10 PERTURBATION OF CRANIAL NEURAL CREST DEVELOPMENT IN VIVO BY ANTIBODIES TO CELL SURFACE MACROMOLECULES. Marianne Bronner-Fraser, Developmental Biology Center and Department of Developmental and Cell Biology, University of California, Irvine, Ca. 92717

Previous studies from this laboratory (Bronner-Fraser, J. Cell Biol, 101: 610, 1985) have demonstrated that an antibody to a cell surface receptor complex caused alterations in avian neural crest cell migration. Here, these observations are extended using two monoclonal antibodies: the CSAT antibody, which recognizes a cell surface receptor for fibronectin and laminin, and the HNK-1 antibody, which recognizes a carbohydrate moiety on the neural crest cell surface. Either CSAT or HNK-1 antibodies were injected lateral to the mesencephalic neural tube at the onset of cranial neural crest migration. Injected antibody molecules did not cross the midline, but appeared to diffuse throughout the injected half of the mesencephalon, where they remained detectable by immunocytochemistry for about 22 hours. The CSAT antibody appeared to bind to all cells on the injected side, whereas HNK-1 antibody was observed primarily around the neural tube and on neural crest cells.

CSAT treated embryos were examined either during neural crest migration (up to 24 hours after injection) or after formation of neural-crest derived structures (36 - 48 hours after injection). In those embryo fixed within the first 24 hours, the major defects were: a reduction in the neural crest cell number on the injected side, a build up of neural crest cells within the lumen of the neural tube, and ectopically localized neural crest cells. These effects appeared to be dose-dependent. In embryos allowed to survive for 36 to 48 hours after injection, the neural crest derivatives appeared normal on both the injected and control side, suggesting that the embryos compensated for the reduction in neural crest cell number on the injected side. However, the embryos often had severely deformed neural tubes and ectopic aggregates of neural crest cells.

Embryos injected with the HNK-1 antibody were examined during the period of neural crest migration (up to 24 hours after injection). The HNK-1 treated embryos often had similar defects to those observed after injection of the CSAT antibody. Some embryos had reduced numbers of neural crest cells on the injected side as well as ectopic neural crest cells either external to or within the neural tube. The percentage of embryos with ectopic neural crest cells was, however, considerably higher for HNK-1 treated than CSAT treated embryos. In contrast, several control antibodies had no effect. These findings suggest that both the CSAT receptor complex and the HNK-1 antigen are important in neural crest morphogenesis.

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212.11 A COMBINED GENETIC AND PHYSIOLOGICAL STUDY OF NEURONAL SPECIFICITY IN MOUSE: DOES PURKINJE CELL LINEAGE PLAY A ROLE IN ESTABLISHING PROJECTION PATTERNS IN CEREBELLAR CORTEX? K. Herrup and J. Bower. Yale Med. Sch., New Haven; Caltech, Pasadena. (Spon: R.J. Mullen)

Increasing attention is being paid to the role that intrinsic (cell autonomous) factors play in the establishment of the specificity of neuronal connections within the CNS. We have investigated this issue as it applies to the establishment of the tactile projections to the cerebellar granule cell layer using extracellular recordings from chimeric mice. The spatial organization of the mossy fiber tactile projections have been well described (Shambes et al., Brain Behav. Evol. 15, 94). The map of these projections is highly disjunctive such that sharp boundaries between projections from different body regions can be defined. Despite this patchy, mosaic organization, the resulting map is remarkably invariant from animal to animal.

Several developmental observations suggest that the arriving mossy fiber system might use intrinsic features of the overlying PCs to help to establish their patterns of connections. The mossy fibers are present early in postnatal development in the rodent, at a time when the Purkinje cells are present, but the granule cells are still being generated. Further, recent results by Mason & Gregory (J. Neurosci. 4, 1715) have shown that these fibers make transient connections directly with the overlying PCs. To determine if there is a relationship between the physiologically defined map and the lineage of the PCs, we have mapped the tactile projections to the surface of Crus II in several mouse chimeras and reconstructed a "lineage map" of the PCs overlying these inputs. β-glucuronidase histochemistry was used to reveal the genotype of the cells, and a computerized microscope was used to reconstruct the two-dimensional map. As reported by others, there is intermingling of PCs of the two genotypes in the chimera which gives the impression of overall randomness, especially when viewed in single sections. Serial reconstructions, however, reveal regions where the ratio of the PC genotypes is strikingly different from the ratio in surrounding areas. Furthermore, we have found that the location of these regions is similar from one chimera to the next. Finally, the location of one such large region in Crus IIa consistently corresponds, at least roughly, to the physiologically defined map position of the large Crus IIa upper lip projection. This apparent correlation between a physiologically defined projection area and PC lineage in the adult animal suggests that information of a cell-autonomous nature may be used to help establish the pattern of connections in this system.

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212.12 'REGULATED' VISUOTECTAL PROJECTIONS IN YOUNG ADULT XENOPUS RAISED FROM EMBRYONIC STAGES WITH A GENETICALLY MARKED 'COMPOUND EYE'. R.K. Hunt, J. Kilty and S. O'Gorman (SPON: F. Crick). The Salk Institute, La Jolla, CA 92037.

When embryonic half-eye rudiments are grafted together (in non-complementary pairings) in 20% 'rearing' saline at stages 31-36 in Xenopus, the resulting adult eyes usually show a (normal or 'double-nasal' twinned) retinotectal projection whose polarity and topographic order do not match the starting polarities of the grafted half-eye (Hunt and Frank: Science 189:563, 1975; Gaze and Straznicky: J. Embryol. Exp. Morph. 55:143, 1980; O'Rourke and Fraser, Develop. Biol., in press). We prepared four types of these so-called 'compound eyes', by replacing the posterior half of the right eye of albino X. laevis host embryos with a half-eye graft from pigmented X. borealis (or X. borealis/X. laevis hybrid) donor embryos: grafted half-eyes included the posterior half of the donor's right eye (Type I) or left eye (Type II); the dorsal half of the donor's right eye (Type III), or the anterior half of the donor's left eye (Type IV). The pigment marker enabled us to photograph graft and host fragments during healing, and their derivatives at regular intervals through metamorphosis. At 1-3 months after metamorphosis, detailed (80-100 point; 100-125 μm resolution) electrophysiologic maps were made of the visual field projections of 4-6 individuals with each type of 'compound eye'. The eye was then removed and fixed in Carnoy's; and 6 or 8 μm serial wax sections were cut in a plane perpendicular to the optic axis and stained with quinacrine. X. borealis neurons were readily identifiable in all layers of the neural retina; and graft and host territories were digitized with an Apple computer to make reconstructions of the ganglion cell layer. All Type I (control) and Type III eyes (and a minority of Type II eyes) formed a single, continuous normal visuotectal projection; in each case, the posterior retina showed a coherent sector territory, some 120-220 degrees in angular extent in individual cases, of graft-derived neurons in all three retinal layers. All Type IV and most Type III eyes formed a twinned 'double-nasal' (occasionally 'double-nasoventral') projection, with each half-field projecting over most of the tectal surface. In each case, the posterior retina showed a coherent sector territory similar in size and form to those described earlier. We infer that the 'regulated' maps do not reflect loss of the graft (and its covert replacement by regenerated host tissue). Rather these 'regulated' maps must reflect either a reprogramming of positional labels on ganglion cell axons (perhaps confined to new ganglion cells arising on the eye rim at later larval stages), or an altered deployment of these labels in the search-and-find interactions by which the growing fibers select their axonal neighbors and tectal targets. Supported by NSF PCM-83 11082 to R.K.H.; NIH 5F32 EY-05853 to S. O'G.

TRANSMITTER CYTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY

213.1 HYPERINNERVATION OF THE RAT THALAMUS FOLLOWING NEONATAL 6-HYDROXYDOPAMINE TREATMENT, DEMONSTRATED BY DOPAMINE-BETA-HYDROXYLASE IMMUNOHISTOCHEMISTRY. E.L. Gustafson and R.Y. Moore. Depts. of Neurology, Neurobiology and Behavior and Psychology, SUNY at Stony Brook, Stony Brook, NY 11794.

Neonatal administration of the noradrenergic (NA) neurotoxin 6-OHDA results in dramatic changes in the NA innervation of the brain. The most striking of these are a total denervation of neocortex and hippocampus and a concurrent hyperinnervation of the cerebellum and some brainstem nuclei (Sachs and Jonsson, 1974; Schmidt and Bhatnagar, 1979; Levitt and Moore, 1980). This has been interpreted as a "pruning" effect but this explanation is uncertain because of a lack of information concerning the nature and time course of the initial events following neonatal 6-OHDA administration and about the effects of the toxin on diencephalic NA innervation. The present study was performed to resolve these issues. On the day of birth (P1) and P2, rat pups were injected subcutaneously with 100 mg/kg of 6-OHDA HCl, with an equal number of littermates injected with the same volume of saline. Animals were perfused at P5, P10, P20 and P180 and brains were processed for DBH immunohistochemistry using a modified avidin-biotin procedure.

The effects of neonatal 6-OHDA administration observed in the adult brain are as follows. There is a total loss of NA innervation in neocortex and hippocampus and a near total loss in the remaining basal forebrain. In contrast, there is a striking hyperinnervation of the dorsal thalamus, primarily affecting the anterior, lateral, ventral and medial nuclear groups, and the lateral and medial geniculate nuclei. Each nucleus is innervated in proportion to its normal innervation, moreover the pattern of hyperinnervation is identical to that of the normal innervation. The hypothalamus exhibits no alteration in NA innervation. At P5, following neonatal 6-OHDA administration, NA fibers are lost from all areas of the brain back to major fiber bundles with the exception of the hypothalamus where the innervation is largely unaffected. By P10 hyperinnervation of the thalamus is apparent and this is largely complete by P20.

The present study indicates that the effect of neonatal 6-OHDA is a "dying-back" of NA axonal arborizations in all areas except the hypothalamus. As the axons grow and re-innervate terminal areas they preferentially enter thalamus, brainstem and cerebellum. The resultant innervation in these areas is increased, indicating a "pruning" effect. Since the innervation is normal in pattern in each area, this effect must represent the interaction of two developmental processes, the genetically determined capacity of NA neurons to produce an extensive but limited amount of terminal and axonal arborization and the capacity of the areas being innervated to determine the pattern of innervation. Supported by NS-16304.

213.2 HISTAMINE-IMMUNOREACTIVE NEURONAL SYSTEMS IN THE HYPOTHALAMUS OF NORMAL AND BRATTLEBORO RATS. P. Panula, R.K. Tuominen*, T. Watanabe* and L. Tuomisto*. Dept. Anatomy, Univ. Helsinki, 00170 Helsinki, Finland; Dept. Pharmacology and Toxicology, Univ. Helsinki, 00170 Helsinki; Dept. Pharmacology II, Osaka University, Osaka 533, Japan; Dept. Pharmacology and Toxicology, Univ. Kuopio, Kuopio, Finland.

Biochemical, pharmacological and immunohistochemical studies have indicated that histamine may act as neurotransmitter in the hypothalamus. Neuronal cell bodies and fibers have been localized in several nuclei using antibodies against histamine and its synthesizing enzyme, L-histidine decarboxylase (HDC). Histamine may be involved in the regulation of vasopressin release, and histamine concentrations in the hypothalamus of vasopressin-deficient homozygous Brattleboro rats (BB rats) are considerably higher than those of normal Long-Evans rats (LE rats).

LE and BB rats were perfused with 4% paraformaldehyde and 30 μm thick cryostat sections of the brains were incubated with antisera against HDC or histamine. Some rats had received a single intracerebroventricular injection of colchicine to inhibit the axonal transport. The caudal magnocellular nucleus (CMC) of 15 normal rats was lesioned with a radio frequency lesion generator 14 days before killing. All operations were carried out under sodium pentobarbital anesthesia. The peroxidase-antiperoxidase method was used in these studies.

Distribution of HDC- and histamine-immunoreactive neuronal cell bodies was restricted to the same nuclei in the posterior hypothalamus in LE and BB rats. Cells were seen in the tuberal, caudal and posterior caudal magnocellular nucleus. The density of histamine-immunoreactive nerve fibers and terminals in the SO of BB rats was considerably higher than that of LE rats. Lesions of the CMC caused almost total disappearance of histamine-immunoreactive nerve fibers and terminals in ipsilateral SO. The results suggest that the histamine-immunoreactive fibers in the SO originate at least in part from the CMC, and that the lack of vasopressin in BB rats is associated with an increase in histamine-immunoreactive fibers in the supraoptic nucleus (SO).

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213.3 CENTRAL CHOLINERGIC INNERVATION OF THE LOCUS COERULEUS. B.K. Hartman, C. Cozzari[1]*, A. Berod[2]*, S.J. Kalmbach*, P.L. Faris*. Dept. of Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110, [1]Istituto Di Biologia Cellulare, Roma, Italy, [2]Inserm U 171, Lyon, France.

A major hypothesis of this laboratory has been that the central noradrenergic system represents a part of a intra-axial autonomic system serving the same function as the sympathetic system in the periphery. An important correllary of this hypothesis is that cholinergic afferents should provide an important input to this system analgous to the cholinergic preganglionic neurons. Although indirect evidence for innervation of noradrenergic neurons of the locus coeruleus (LC) exists (i.e., LC neurons are stimulated by cholinergic agonists and the nucleus exhibits high levels of cholinesterase and choline acetyltransferase activities) no direct anatomical demonstration of cholinergic innervation has been reported.

A double label immunohistochemical technique was developed for the simultaneous visualization of tyrosine hydroxylase (TH) and choline acetyltransferase (CAT) in rat brain. Brains were pressure perfused with 2% paraformaldehyde/0.03% gluteraldehyde at two different pHs (6.5 followed by 8.5). First, CAT localization was accomplished using the peroxidase-antiperoxidase (PAP) technique with nickel-cobalt enhanced diaminobenzadine reaction product. Sections were then processed for TH localization using PAP with alpha-napthol as substrate and differentiated with the dye pyronin-B. The jet-black CAT positive perikarya and axon fibers contrasted well with the bright magenta staining of TH-positive soma and dendrites of the LC neurons making resolution of interactions between these two transmitter systems feasible at the light microscopic level.

The cell bodies of the rostral LC were observed to overlap with the caudal extent of the ponto-mesencephalic cholinergic group of neurons. Furthermore, cholinergic neurons from this group were dispersed deep within the LC. In coronal sections CAT-positive axons were observed coursing dorsal-medially into the dendritic field of the LC. Intensely stained black varicosities were in apparent contact with many red, TH-immunolabeled dendrites. Though less frequent in number, CAT-containing varicosities were observed in clear association with TH-positive soma. In some cases cholinergic axon fibers could be observed outlining the surface of TH-containing the surface of TH-containing dendritic profiles. This pattern of staining is interpreted as axo-dendritic and axo-somatic contacts between these two neuronal systems and may provide an anatomical basis for the hypothesized cholinergic control of central noradrenergic function.

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213.4 CHOLINERGIC INNERVATION OF HIPPOCAMPAL COMMISSURAL NEURONS IMMUNOREACTIVE FOR GAD AND SOMATOSTATIN (SS): EM DOUBLE IMMUNOSTAINING COMBINED WITH RETROGRADE TRACER TECHNIQUE C.Leranth* and M.Frotscher* (SPON: L.Heimer) Yale University, School of Medicine, Section of Neuroanatomy and Dept. of Obstetrics and Gynecology, New Haven, CT. and M.F. Institute of Anatomy, Johann Wolfgang Goethe University, Frankfurt/M.F.R.G.

We have hypothesized that there is a functional connection between the septo-hippocampal cholinergic afferents and the hippocampal GABA and ST interneurons as well as commissural GABA and ST neurons, based on the following observations: 1. Fornix transection and/or hippocampal commissurotomy alter spatial discrimination and working memory processes. 2. Alzheimer's disease is associated with a loss of septal cholinergic neurons, and a marked decrease in hippocampal SS and GABA levels. 3. Our own observations have demonstrated that cholinergic axons synapse on hippocampal non-principal neurons, in addition to pyramidal or granule cells. 4. A population of hippocampal commissural neurons are immunoreactive for SS and GAD.

This hypothesis was tested in rats using a combination of EM double immunostaining and retrograde HRP tracer technique. Two days prior to sacrifice HRP was injected into the dentate hilar area. The retrogradely transported HRP was visualized by a glucose oxidase reaction in Vibratome sections prepared from the contralateral hippocampus. Sections were than double immunostained for choline acetyltransferase (ChAT) and GAD, or for ChAT and SS using immunoperoxidase and avidinated ferritin as contrasting electron-dense immunolabels. Immunoreactivity for ChAT was labeled with immunoperoxidase while GAD or SS neurons were immunolabeled with avidinated ferritin. EM analysis of the dentate hilar area Amaral's zone 4 revealed symmetric synaptic contacts of immunoperoxidase labeled ChAT axons on avidin ferritin immunolabeled GAD and SS interneurons,and on GAD and SS commissural neurons containing retrogradely transported HRP granules. Asymmetric synaptic connections were observed between mossy axon collaterals and the complex spines of the commissural GAD and SS neurons.

Based on these observations, on previous reports of the co-existence of GAD and SS in hippocampal neurons and on the suggested disinhibitory action of acetylcholine on hippocampal principal neurons, we propose that: 1) cholinergic action could lead to disinhibition of both ipsi - and contralateral hippocampal neurons by inhibiting local and commissural GABA neurons; 2) This disinhibition is antagonized by mossy axon collaterals of the ipsilateral excitatory granule cells.

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213.5 PRELIMINARY DATA ON THE ULTRASTRUCTURAL FEATURES OF DOPAMINE TERMINALS IN ADULT RAT CEREBRAL CORTEX. Philippe Séguéla*, Kenneth C. Watkins* and Laurent Descarries. Centre de recherche en sciences neurologiques (Dép. physiologie), Université de Montréal, Montréal (Qué.), Canada H3C 3J7.

In contrast with the detailed knowledge of its topographic distribution, little is known about the ultrastructural features of the dopamine (DA) innervation in cerebral cortex. We therefore used a highly specitic antiserum directed against DA-glutaraldehyde-lysyl-protein conjugate (Geffard et al., Brain Res. 294:161, 1984) to identify and examine DA terminals (axonal varicosities) in two regions of adult rat cortex known to receive a relatively dense DA input. The tissue was fixed by perfusion with 3.5% glutaraldehyde, immunostained in the presence of 0.025% saponin using the peroxidase-antiperoxidase technique, postfixed with osmic acid and embedded in Epon. Ultrathin sections were obtained from layers V and VI of the anteromedian "prefrontal" cortex and layers IV to VI of the dorsal insular agranular cortex (suprarhinal DA field). Immunoreactive profiles of DA varicosities were defined as such by the presence of aggregated synaptic vesicles. In both regions, the DA terminals showed mainly small and clear, round synaptic vesicles (30-50 nm) occasionally associated with several larger electron lucent vesicles (90-150 nm). The mean diameter (average of length and breadth ± SEM) of the immunostained profiles measured 0.72 ± 0.16 µm in the anteromedian cortex and 0.74 ± 0.19 µm in the suprarhinal cortex. In anteromedian cortex, 19 of 53 profiles (36%) from randomly selected immunostained varicosities exhibited a junctional complex of morphologically defined synaptic differentiation. These synapses were made on dendritic branchlets or spines. None were found on neuronal perikarya. The junctional complexes averaged 0.27 ± 0.07 µm in width, and all but one appeared to be symmetrical. In the suprarhinal cortex, the immunopositive DA terminals showed similar vesicular content and membranous specializations. The width of their junctional complexes was 0.31 ± 0.06 µm. However, the latter were found on only 16% of examined profiles (8/49). Considering the size of these varicosities, the width of the observed junctions and the section thickness, the probability of seeing the junctional complex in single thin sections could be estimated at 37% and 39% for the anteromedian and the suprarhinal cortex, respectively. From these data, two main conclusions could be drawn: 1) the DA innervation of the anteromedian "prefrontal" cortex is essentially junctional; 2) less than half of DA terminals in the suprarhinal DA field appear to be engaged in synaptic contact. Examination of other innervation fields complemented by serial sectioning should reveal the extent of interregional variations in the incidence of synaptic junctions made by cortical DA varicosities. (Supported by MRC and FRSQ).

213.6 IMMUNOCYTOCHEMICAL LOCALIZATION OF ARPP-21, A SUBSTRATE FOR cAMP-DEPENDENT PROTEIN KINASE ENRICHED IN DOPAMINE-INNERVATED BRAIN REGIONS C.C. Ouimet, H.C. Hemmings, Jr. and P. Greengard. Laboratory of Molecular and Cellular Neuroscience. The Rockefeller University, 1230 York Avenue, New York, NY 10021

ARPP-21 (cAMP-regulated phosphoprotein M_r-21,000) is a neuronal phosphoprotein with an apparent molecular weight of 21 kDa that is phosphorylated by cAMP-dependent protein kinase. It has been identified in homogenates of the rat and bovine basal ganglia and purified to homogeneity from bovine caudate nucleus cytosol (Hemmings et al., see accompanying abstract). Monoclonal antibodies specific for ARPP-21 have been prepared and used in the present study for immunocytochemical localization of ARPP-21 in the rat brain. Immunoreactivity for ARPP-21 was observed in neuronal cell bodies, dendrites, spines, axons and terminals of labeled neurons. This is consistent with the observation that ARPP-21 is a cytosolic protein. Strongly labeled neuronal somata and dendrites were present throughout the caudatoputamen, nucleus accumbens, and olfactory tubercle. Within these regions, labeling was most intense in patches in the nucleus accumbens and along the medial edge of the caudatoputamen. Immunolabeled nerve terminals may also have been present in these neostriatal regions but were difficult to observe against a background of heavily labeled neuropil. At the electron microscopic level, several immunolabeled nerve terminals were found and these were usually in contact with labeled neurons, suggesting that they may have been recurrent collaterals. Both light and electron microscopy demonstrated that ARPP-21 is present in medium spiny neurons in the nucleus accumbens and in the caudatoputamen. In the regions that receive the neostriatal projection (the globus pallidus, entopeduncular nucleus, ventral pallidum and substantia nigra), labeled nerve terminals were observed. Labeled axons were present along the routes taken by the neostriatal fibers to their targets in the pallidum and substantia nigra. Because ARPP-21 is present in medium spiny neurons and because these neurons contain D-1 receptors (which generate cAMP in response to DA activation), it is likely that ARPP-21 is a third messenger for dopamine. It is also likely that ARPP-21 coexists with DARPP-32, a third messenger for dopamine (Walaas et al., Nature 301:69, 1983) that has been shown to be a potent protein phosphatase inhibitor in its phosphorylated form (Hemmings et al., Nature 310:503, 1984).

213.7 CORRELATED LOCALIZATION OF CHOLINE ACETYLTRANSFERASE AND GLUTAMIC ACID DECARBOXYLASE IMMUNOREACTIVITY IN RAT CEREBRAL CORTEX. D.R. Brady and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Although the neurotransmitter synthesizing enzymes choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) previously have been localized in a variety of CNS regions, their respective immunocytochemical distributions have not been correlated. In this study, the laminar distribution of ChAT and GAD immunoreactivity was compared in 17 different cytoarchitectonic areas comprising limbic and nonlimbic regions of rat cerebral cortex. The specificity of the ChAT antibody and GAD antiserum along with the general immunocytochemical methods have been reported previously. The relative immunoreactive intensities within the cortical mantle were assessed qualitatively, and measured quantitatively with dark field optics and an image analysis system.

This study revealed distinct distribution patterns for ChAT and GAD immunoreactivity in the cerebrum. In general, limbic cortical regions displayed greater intensity of both ChAT and GAD immunoreactivity than neocortex. For example, ChAT immunoreactivity in piriform and retrosplenial cortices was 25% and 14% greater respectively than in neocortex, and GAD immunoreactivity in the same cortical areas was 4.5% and 19% greater, respectively. In addition to these general differences, there were marked variations in the laminar distribution of immunoreactivity specific for these two neurotransmitter synthesizing enzymes. The laminar pattern and relative intensities of ChAT and GAD immunostaining were similar in layers of entorhinal cortex and hippocampus, but the dentate gyrus, subiculum and retrosplenial cortex showed varying degrees of reciprocity in their distributions of ChAT and GAD. For example in retrosplenial cortex, ChAT immunoreactivity in layers II/III was 13% less intense than that in layers I and IV/V, whereas GAD-positive staining in layers II/III was 5-9% more intense than in layers Ib and IV/V. In summary, the highest cortical levels of both ChAT and GAD are found in specific layers of limbic cortex, and these regions have been implicated in the linking of affective and memory functions with environmental cues of neocortical and subcortical origin.

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213.8 THE IMMUNOCYTOCHEMICAL DEMONSTRATION OF SMALL TRANSMITTER MOLECULES: VISUALIZATION OF GLYCINE IN INHIBITORY NEURONS. J. Storm-Mathisen*, O.P. Ottersen* and S. Davanger* (SPON: ENA). Anat. Inst., Univ. of Oslo, Karl Johansgt. 47, N-0162 Oslo 1, Norway.

Glycine (Gly) is thought to be an important inhibitory transmitter. A method for direct visualization of putative glycinergic neurons is clearly needed. We have previously shown that specific antibodies can be produced that recognize transmitter amino acids in tissue fixed by glutaraldehyde (G) (Storm-Mathisen et al. 1983 Nature 301:517-20). The reaction of G with amino acid yields complexes in which several amino acid residues are linked together side by side radiating from a pyridinium core (Hardy et al. 1976 J.Chem.Soc. Perkin Trans. 9:958-62). This makes it posible to envisage how even such a small molecule as Gly could give rise to characteristic antigens against which specific antibodies could be raised.

Gly was conjugated to carrier proteins by G and injected in rabbits. The carrier was changed (Seguela et al. 1984 PNAS 81:3888-92) for each immunization, 7 different carriers being used. The crude serum was absorbed with Sepharose-BSA-G, -BSA-G-glutamate and -carriers-G. The antibodies were tested for specificity against various compounds fixed by G to a total macromolecular extract from rat brain. The conjugates were spotted on to 13mm cellulose ester filters and processed together with the tissue sections (Ottersen & Storm-Mathisen 1984 J.comp.Neurol. 229:374-92). At serum concentrations giving strong staining of the Gly spots there was no significant staining of other spots, comprising those of GABA, Tau, B-Ala, phosphoethanolamine, Ala, Val, Leu, Pro, Ser, Lys, Asp, Glu, Gln, GSH, carnosine, spermine and "O" (i.e. macromolecules fixed with G alone). Solid phase absorption of the serum with Gly fixed by G to ovalbumin or Lys abolished staining of test spots and tissue sections, and so did absorption with 100uM Gly-G complexes (Gly:G = 1:2, excess ethanolamine added before mixing with serum), whereas G complexes of B-Ala, GABA, Tau, GSH, Asp, Glu, Gln or Ser (300uM) did not.

Gly-like immunoreactivity (Gly-LI) was studied in G perfused rat, cat and monkey in 3 regions known to be rich in Gly and likely to contain Gly-ergic neurons: In cerebellum Gly-LI was localized in Golgi cells, and in fibres and nerve terminal-like dots. Golgi cells are inhibitory and were the only neurons to be stained. Stained dots formed rosettes resembling Golgi terminals in glomeruli. This distribution was distinct from those of GABA-LI, Tau-LI, Asp-LI, Glu-LI and Gln-LI. GABA-LI was abundant in cells and terminals in the molecular layer and in baskets around Purkinje cells, but like Gly-LI also ocurred in Golgi cells and in rosettes. Staining alternate 7um sections (facing surfaces) revealed that some Golgi cells contained GABA-LI as well as Gly-LI. In autoradiograms of slices incubated with (^{3}H)Gly or (^{3}H)GABA the distributions of labelled terminals corresponded to those in sections stained for Gly-LI and GABA-LI, respectively. In retina Gly-LI was localized in cells restricted to the inner nuclear layer and in dots in the inner plexiform layer (IPL) forming 2 dark subzones. GABA-LI occurred in cell bodies on both sides of the IPL; IPL had 3 dark subzones. In the spinal cord the localization of Gly-LI again contrasted with that of GABA-LI and suggested Gly to be concentrated in propriospinal neurons. (Pourcho & Goebel (1985 Brain Res. 348:339-42), using modifications of our methods, have also raised antibodies to Gly.)

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213.9 GLYCINE IMMUNOREACTIVE NEURONS AND PRESYNAPTIC BOUTONS IN THE SPINAL CORD. A.N. van den Pol and T.Gorcs* Sect.Neurosurgery, Yale Univ. Sch. Med., New Haven, Ct. 06510 and U.S.-Japan Biomed. Res.Cntr., Tulane Univ., Belle Chasse, LA, 70037.

Glycine has been postulated as a major inhibitory neurotransmitter in the spinal cord. To study the localization of glycine-like immunoreactivity, antisera were raised in rabbits against glycine conjugated with glutaraldehyde to either thyroglobulin or keyhole limpet hemocyanin. Antiserum specificity was tested with amino acids conjugated to bovine serum albumin on nitrocellulose blots with peroxidase histochemistry, and on 96- well plastic plates with ELISA. Antisera bound to immobilized glycine selectively, with little apparent cross-reactivity with other amino acids, including serine, glutamate, aspartate, taurine, and glutamine. A potential cross-reactivity of the antiserum with GABA and alanine was reduced by affinity chromatography purification.

Immunocytochemical staining of aldehyde-fixed rat cervical and lumbar spinal cord with fluorescent, gold, and silver intensified gold immunolabels revealed glycine immunoreactive axons throughout all laminae of the cord, with large numbers in the deeper layers of the dorsal horn. Glycine immunoreactive neurons were found in both dorsal and ventral horns. Control staining of the hippocampus with glycine antiserum showed little immunoreactivity compared with the spinal cord, while GABA antiserum stained large numbers of axons in both cord and hippocampus. The anatomical overlap of glycine and GABA immunoreactivity in the cord suggests a convergence of the two substances; the possibility of co-localization of the two amino acids requires further analysis. Incubation of the glycine antiserum with glycine conjugated to BSA blocked specific immunoreactivity.

Ultrastructurally, glycine immunoreactive boutons labeled with colloidal gold were found to make synaptic contact with many dendrites and perikarya in both dorsal and ventral gray matter, including large non-immunoreactive motor neurons in the ventral horn. Presynaptic glycine immunoreactive boutons contained predominantly small clear vesicles; regions of high vesicle density were strongly immunolabeled.

Taken together with previous electrophysiological and pharmacological work, these immunocytochemical data suggest that if glycine is released from loci containing immunoreactive terminals, the amino acid may have widespread functions in the spinal cord, related both to control of movement and to modulation of sensory input from the periphery.

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213.10 RECYCLING OF VESICLES AT SYNAPTIC SITES IN LEECH NEURONS IN CULTURE. D.P. Kuffler and P. Drapeau. Dept. ob Pharmacology, Biocenter, Univ. of Basel, 4056 Basel, Switzerland.

Well-defined synapses develop between pairs of Retzius cells and P sensory cells in culture, with clusters of clear vesicles apposed to thickenings of the presynaptic membrane. These clear vesicles are capped by a layer of dense core vesicles that have no contact with the presynaptic membrane. Antibody to serotonin labelled with colloidal cold showed serotonin, the transmitter of the Retzius cell (Henderson, 1983), to be localized in the dense core vesicles. 95% of the Retzius dense core vesicles were labelled with 1 to 20 gold particles. Studies were made to determine whether vesicles at the synaptic sites were recycled during synaptic transmission. Pairs of synaptically coupled cells were stimulated by high K in the presence of extracellular markers. Colloidal gold coated with protein A, was taken up by coated vesicles adjacent to the synaptic sites. Gold was also found in both the dense core and clear vesicles at the synaptic sites. Ferritin was found in cisternae, dense core and clear vesicles. HRP was detected in cisternae and clear vesicles.

These studies show that transmitter release leads to turnover of dense and clear vesicles at serotonergic synapses (Henderson, L., 1983, J. Physiol. (Lond.) 339: 311-326).

214.1 TOPOLOGY OF THE SEMICIRCULAR CANAL CUPULA IN THE GUITARFISH. S.L. Guth*[1] and D.P. O'Leary. Dept. of Otolaryngology - Head & Neck Surgery, University of Southern California, School of Medicine, Los Angeles, CA, 90033.

The cupula of the semicircular canal is a transparent structure which couples the hydrodynamic forces generated by rotation of the semicircular duct to the hair cells in the sensory epithelium (crista). The shape, mode of deformation, and elasticity of the cupula are major elements in this transduction process. The purpose of this study is to construct topographic maps of cupulae from the guitarfish, Rhinobatos productus.

The unfixed ampulla was excised, and oriented in a bath. Microscopic glass reflective beads (diameter 20 microns) were positioned on the cupular surface. Preparations were not used when the circumferential attachment between the cupula and the ampullar wall was observed to be ruptured. Damage was indicated if the cupula either oscillated as a "swinging gate" or was displaced by gravity.

A series of photomicrographs was taken of the beads lying on the cupula with the bath containing the preparation tilted across the Y axis at several angles (aprox 8° increments) . The Z coordinate (depth) was calculated from the angle of tilt and the translation of the images of individual beads orthogonal to the tilt axis (X coordinate). Topographic and 3-dimensional plots of the surface of the cupula were generated and displayed with a microcomputer.

Preliminary results indicate that the curvature of the cupula at the base of the horizontal crista is shallow, concave and symmetric. The curvature becomes deeper and narrower toward the far wall of the ampulla. These quantitative, 3-dimensional results establish necessary boundary conditions for determining mechanical properties of the cupula.
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214.2 PERSISTENCE OF PRESYNAPTIC BODIES IN SACCULAR HAIR CELLS OF AGED C57BL/6NNia MICE. J.C. Park and A.D. Woods*. Dept. of Biological Sciences, Florida Institute of Technology, Melbourne, FL 32901.

Presynaptic bodies, composed of an electron dense form surrounded by clear vesicles, are characteristic of sensory cells in many species. Because of their presumed role in synaptic transmission, and lack of information on the contribution of the peripheral vestibular organs to age-related functional decline in equilibrium, the organization and ultrastructure of presynaptic bodies was examined in saccules of young and very old C57BL/6NNia mice.

Both saccules from six, 6-8 week old and six, 29-32 month old C57BL/6NNia mice were locally perfused with glutaraldehyde, dissected free from the bone, routinely prepared for electron microscopy, and serially sectioned.

Unexpectedly, synaptic bodies were found in equal abundance in young and old mice. The two hair cell types contained distinctly different forms. The typical shape of those in young, Type I hair cells was an elongated bar. Bars were also present in the Type I hair cells of old mice, but many crescent or round profiles were seen. Regardless of orientation to the plasma membrane, only one end of the bar was involved with the synaptic membrane specialization frequently found associated with it. Both young and old Type I hair cells commonly had one, two, or three presynaptic bodies in a set. Although previously thought to be peculiar to developing hair cells, clusters of five or more were not uncommon even in very old mice. When presynaptic bodies were clustered, each body had a separate halo of vesicles. In Type II hair cells of young and old mice, the presynaptic bodies were smaller, spherical and always found singly.

The persistence of presynaptic bodies suggests that functional interaction between synaptic bodies and synaptic membranes does not change with age in the saccules of C57BL/6NNia mice.

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214.3 RESPONSE OF VESTIBULAR HAIR CELLS TO ROTATIONAL STIMULI BEFORE AND AFTER TTX ADMINISTRATION. H.P.Wit* and B.J.Tideman* (SPON: A.B.A.Kroese). Dept.of Exp.Otorhinolaryngology, Univ.of Groningen, Postbox 30.001, 9700 RB Groningen, The Netherlands.

High frequency (acoustical) stimulation through the ear canal evokes a vestibular microphonic potential in cochlea deprived pigeons. The amplitude of this AC potential is modulated by rotational stimulation of the animal. The potential is measured with differential thin wire electrodes. One electrode contacts perilymph through a small hole in the top of the horizontal ampulla; the other is placed in a hole in the vestibulum. Acceleration induced changes can well be described with a model that incorporates an asymmetric hair cell input-output curve and a simple saturating relation between acceleration and hair bundle deviation. The input-output curve for the hair cell has been given a similar shape as the curve measured by Hudspeth and Corey (*Proc.Natl.Acad.Sci. U.S.A.*, 74:2407, 1977) for hair cells in the sacculus of the bull frog. Experimental results are in good agreement with Ewald's second law. Application of tetrodotoxin (TTX) to the perilymphatic space hardly changed the amplitude of the response to the high frequency (300 Hz) stimulus. However, the modulation of this amplitude by rotational stimuli changed drastically. This result can tentatively be explained by assuming efferent control of the vestibular hair cell transduction process.

214.4 ADENOSINE HAS A NEUROMODULATORY ROLE IN AFFERENT TRANSMISSION IN THE SEMICIRCULAR CANAL OF THE FROG. P.S. Guth, G.M. Bryant, G.C. Sprague*, and C.H. Norris. Dept. of Pharmacology, Tulane Medical School, New Orleans, LA 70112.

Adenosine has been implicated in neuromodulation in the central nervous system (Snyder, S., Ann. Rev. Neurosci. 8:103, 1985). Its mechamism of action is thought to be by presynaptic inhibition of transmitter release, a receptor mediated phenomenon. The presynaptic adenosine receptor can be blocked by μM concentrations of theophylline (or other alkyl xanthines) resulting in an increase in transmitter release and concomitant increase in firing rate. To assess adenosine's role as a neuromodulator in the vestibular periphery, an in vitro whole labyrinth preparation (Valli, et al., Brain Research 330:1, 1985) was used in which spontaneous activity of the afferent fibers in the ampullar nerve of the posterior semicircular canal can be measured. Afferent firing has been shown to depend on transmitter release by the hair cells (Valli, et al., Acta Otolaryngol. 84: 344, 1977). Antagonists were applied by bath substitution and agonists by close injection. A dose response curve for theophylline showed that the drug caused a 23% increase in spontaneous activity at a concentration of 500μM ($p<0.01$). There was a 22% increase in the firing rate at 50μM theophylline although it was not significant. The application of adenosine caused a maximal decrease of 28% in the firing rate ($p<0.01$). This maximal effect was achieved with 6μl of a 5 mM solution injected into a 15 ml bath. Other agonists are under investigation. To test whether endogenous adenosine is released and exerts a neuromodulatory effect, adenosine deaminase was applied, again by close injection; 0.22 enzyme units caused a 100% increase in spontaneous activity. The effect of adenosine deanimase inhibitors are also being studied as well as the effect of the breakdown products of the reaction, inosine and NH_3. Dipyridamole, an adenosine uptake blocker decreased the spontaneous activity and potentiated the effect of exogenous adenosine. Experiments are underway to measure the amount of adenosine released into the bath fluid during evoked responses of an isolated canal preparation.

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214.5 RESPONSE PROPERTIES OF HORIZONTAL SEMICIRCULAR CANAL AFFERENTS IN THE RABBIT. John S. Stahl* and John I. Simpson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., NY, NY 10016

The rabbit is a popular animal for the study of vestibular-ocular coordination, but to date no quantification of the properties of its semicircular canal afferents has been available. We recorded from eighth nerve axons in six anesthetized, pigmented rabbits. Responses were obtained during sinusoidally varying angular acceleration and velocity steps about the vertical axis. The head was positioned to minimize stimulation of the vertical canals. The population (N=41) histogram of velocity sensitivities to sinusoidal stimuli of 0.3 Hz, ±10 deg. suggested the presence of two response classes - one clustered at low sensitivities and the other spread more broadly at higher sensitivities. This distinction correlated with the Bode plots. Units from the low sensitivity class had virtually flat sensitivity-frequency relations in the range .05 - 0.3 Hz, while those from the other class had more variable sensitivities, generally increasing with stimulus frequency. Accordingly, a classification was defined on the basis of sensitivity at 0.3 Hz, with a demarcation criterion of 0.9 spikes/sec/deg/sec (sps/dps). The cells falling below the criterion (LO cells, N=32) had a sensitivity of .37 ± .15 sps/dps (all values are averages ± SD). Those above the criterion (HI cells, N=9) had a sensitivity of 2.01 ± .76 sps/dps. These values are significantly different ($p < .001$). Spontaneous discharge rates are not significantly different for the two classes (LO=78 ± 23 sps, HI=63 ± 26; $p < 0.2$). Variability of discharge (CV, measured by spontaneous rate SD/spontaneous rate) also did not differ significantly, (LO class CV=.09 ± .06, N=26; HI class CV=.23 ± .18, N=8; $p < 0.1$). Time constants were measured by fitting single exponential curves to the responses to velocity steps of 12-20 deg/sec. For the LO class, the time constant of the decay from excitation was 4.0 ± 2.0 secs (N=9). For the HI class the value was 2.8 ± .9 secs (N=4). For the recovery from suppression, the LO and HI class time constants were 3.8 ± 1.1 secs (N=10) and 2.8 ± 0.5 secs (N=3), respectively. Combining the LO and HI classes yielded an excitation time constant of 3.6 ± 1.8 secs, and a suppression time constant of 3.5 ± 1.0 secs. For many cells the exponential fits were good, particularly for those of the HI class. Nevertheless, for any one cell the time constant varied between trials; even those with the best fits gave estimates differing by 12-50% of the average. Furthermore, for some cells in some trials, the value of the time constant was large enough so that the response to the velocity step appeared more linear than exponential. Supported by NS-13742.

214.6 RELATION BETWEEN THE MORPHOLOGY AND PHYSIOLOGY OF SEMICIRCULAR CANAL AFFERENTS IN THE CHINCHILLA. J. M. Goldberg, C. Fernandez, G. Desmadryl* and R. A. Baird. Dept. Pharm. & Physiol. Sci., Univ. of Chicago, Chicago IL, 60637.

There are three kinds of axons ending within the cristae (Soc. Neurosci. Abstr. 9: 738). Some fibers give rise only to calyces; others only to boutons; and still others, so-called dimorphic units, to both kinds of endings. In the present study, we have related the physiological properties and peripheral terminations of 50 fibers that were injected with HRP. Axons could be classified as calyx or dimorphic units. Only one bouton unit was recovered. Calyx units were found in the central zone of the cristae; the bouton unit was in the peripheral zone. Dimorphic units were found in all parts of the epithelium.

Units were characterized in terms of their discharge regularity, their sensitivity to galvanic currents, and the phase and gain of their response to 2 Hz sinusoidal head rotations. Discharge regularity: All calyx units were irregular. The bouton unit was regular. Dimorphic units could be regular or irregular. There was a tendency for the regularity of dimorphic units to be related to their location in the cristae: the proportion of regular units was higher in the peripheral zone than in the central zone. Galvanic sensitivity was related to discharge regularity and not to the morphology of the terminal per se. Thus, regular dimorphic units and the regular bouton unit had low sensitivities, whereas irregular calyx and dimorphic units had high sensitivities. Response phase: The response dynamics should be reflected by the phase of the response to sinusoidal rotations. Response dynamics were related to discharge regularity and not to morphology as such. The responses of regular units were close to being in phase with head velocity. Irregular dimorphic and calyx units both had phase leads near 30°. Response gain: Irregular dimorphic units had gains that were 3x higher than those of calyx units, which, in turn, had gains that were only slightly higher than those of regular units.

The findings can be interpreted as follows. The similarity of the irregular dimorphic and calyx units in their discharge regularity and galvanic sensitivity is consistent with the conclusion that these discharge characteristics are largely determined by properties of the postsynaptic axon. The similarity in the response dynamics of the two groups of irregular fibers implicates presynaptic factors other than the kinds of hair cells innervated. The afferents innervating the cristae can be divided into three classes: 1) regular, low-gain units corresponding to dimorphic (and bouton) fibers; 2) irregular, high-gain dimorphic units; and 3) irregular low-gain calyx units. Another interpretation is that the first two classes form a continuum ranging from regular, low-gain units to irregular, high-gain units. The calyx units, since they are distinctive in both their physiology and morphology, definitely form a separate group. Type I hair cells and calyx endings are a relatively late phylogenetic acquisition. This suggests that these structures play a distinctive role in vestibular function. One notion is that the calyx unit, because it has a low gain, will have an extended dynamic range and not become saturated by the large angular velocities characterizing the head movements made by terrestial vertebrates.

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214.7 DETERMINATION OF PRIMARY VESTIBULAR AFFERENT INPUTS TO THE VESTIBULO-OCULAR REFLEX. L.B. Minor and J.M. Goldberg. Dept. Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

We have developed a paradigm that can be used to study the discharge of neurons monosynaptically or polysynaptically related to the vestibular nerve as well as to investigate the afferent input to entire reflex pathways. The method is based on the differences in the electrical sensitivity of irregularly (I) and regularly (R) discharging afferents (Brain Res. 252: 156). I afferents have a mean sensitivity to dc currents of 3 spikes·s^{-1}/μA. The sensitivity of R afferents averages 0.3 spikes·s^{-1}/μA. R afferents in the monkey have a resting discharge of around 100 spikes/s whereas that of I afferents may be somewhat lower. An anodal (inhibitory) current of 100 μA is presented to both ears. This current should silence I afferents to the extent that they no longer respond to natural stimulation. The background discharge of R afferents will be reduced by the current, but R afferents should not be silenced and should still respond to head rotations. In short, the currents should produce a selective and reversible ablation of I afferents.

The horizontal and vertical vestibulo-ocular reflex (VOR) were studied in three alert squirrel monkeys. Labyrinthine stimulating electrodes were implanted bilaterally. In each ear, one electrode was placed in the perilymphatic space of the vestibule and a second electrode was placed in the middle ear. A scleral search coil technique was used to monitor eye movements. Anodal currents delivered through the perilymphatic electrode in either ear evoked a horizontal nystagmus with a slow phase velocity near 25°/s. Bilateral anodal galvanic stimulation, as expected, reduced the slow phase velocity to 0-5°/s. Remarkably, the same bilateral galvanic stimulation caused, at most, a small reduction in the gain of the VORs evoked by sinusoidal or by transient head rotations. The results indicate that it is mainly R afferents that contribute to VOR pathways. This conclusion is in agreement with the results of unilateral galvanic stimulation. Were the horizontal VOR entirely due to an R input, the horizontal nystagmus predicted from the responses of vestibular afferents to galvanic and rotational stimuli would have a slow phase velocity near 30°/s, close to the value that we observed. Alternatively, an exclusive I input should lead to a slow phase velocity of approximately 200°/s.

Evidence that the currents are acting in the way we have predicted has been obtained by recording field potentials in the vestibular nerve and vestibular nuclei. The thresholds of fields evoked by 100-μsec cathodal shocks delivered to the ipsilateral labyrinth ranged from 50-100 μA. Superimposing a 100 μA anodal dc current raised the field potential threshold by a factor of 4x. Since the cathodal shock thresholds of the most sensitive R afferents are 4x greater than those of the most sensitive I afferents, this electrophysiological result is entirely consistent with the notion that the anodal currents result in a selective ablation of I afferents.

There are two limitations to this polarization paradigm. First, the paradigm cannot distinguish the contributions of I afferents to direct and indirect VOR pathways. Second, I afferents from all five vestibular end organs will be functionally ablated by the current. This may not be serious since there is evidence that the horizontal VOR is mainly influenced by the horizontal semicircular canals and that there is a similar relation between the vertical VOR and the vertical canals.

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214.8 ACTIVITY IN THE VESTIBULAR NUCLEI DURING OFF-VERTICAL AXIS ROTATION (OVAR). H. Reisine, T. Raphan & B. Cohen. Depts. of Neurology, Mt. Sinai, N.Y., N.Y. & CIS Brooklyn College, Brooklyn, N.Y.

OVAR in darkness induces continuous horizontal nystagmus with superimposed oscillations in slow phase velocity related to head position. There are also oscillating vertical and roll eye position changes. The otoliths play the key role in generating nystagmus during OVAR, but little is known about how it is produced centrally. Extracellular activity was recorded in the vestibular nuclei of 2 rhesus monkeys during OVAR. Eye position was monitored with frontal plane and roll scleral search coils. Slow phase velocity was obtained by removing saccades and digitally differentiating eye position. Impulse rates were obtained by counting the number of spikes in a 192 msec window and updating the count every 1.6 msec. With the head fixed animals were given OVAR at 20 deg tilt and a constant velocity of 60 deg/sec. Seventeen units had responses to yaw rotation about a space vertical axis, but not to pitching or rolling about a horizontal axis. They all responded during horizontal OKN and OKAN. Eight of the 17 neurons responded primarily to horizontal angular head acceleration (Vest. units). The other 9 had components in their impulse rates related to oculomotor activity (Om-Vest. units). Of the 8 Vest. units 7 increased their discharge rate during OVAR when the yaw was in the excitatory direction for rotation about a vertical axis. When tested, the average rates decreased during OVAR in the opposite direction. All 8 neurons were modulated in phase with head position. Two Om-Vest. units had responses that were difficult to assess due to strong eye position signals. Of the other 7 Om-Vest. units with weak or no eye position signals, 5 increased their average discharge rate during OVAR in the excitatory direction and 5 were modulated with head position. For both Vest. and Om-Vest. units the increase in impulse rate paralleled the increase in slow phase velocity of the induced nystagmus. If the axis of rotation was made vertical during OVAR, impulse rates decreased to spontaneous levels with a time course similar to that of the decrease in slow phase velocity during OKAN, relating them to velocity storage. Thus, central vestibular neurons that respond during yaw rotation about a vertical axis are not only activated by optokinetic stimuli but also by OVAR. Many of these neurons are also modulated in association with changes in head position indicating an otolith related component in their impulse rates. Presumably, they are part of the network that produces OVAR nystagmus. The data also show that the central vestibular system utilizes velocity storage as a focus for combining information from a wide variety of sensory systems about motion.

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214.9 **A PATTERN ANALYSIS APPROACH TO MODELLING VELOCITY GENERATION DURING OFF-VERTICAL AXIS ROTATION (OVAR).** C. Schnabolk* and T. Raphan. Dept. of CIS, Brooklyn College, Brooklyn, New York 11210.

Rotation about an off-vertical axis (OVAR) causes continuous unidirectional horizontal nystagmus in darkness (Guedry, 1965). A mathematical model has been developed which shows how the continuous nystagmus during OVAR might be generated by estimating the velocity of the head from changing patterns of otolith excitation and inhibition. The estimation of velocity is based on a "template matching" algorithm. It is assumed that a signal arising in each cell of the macula is delayed by a certain time (T). Thus, as the head rotates in the gravitational field a delayed pattern representing a previous position of the head is available as a "template" that can be compared to the pattern associated with the present position of the head. Because the gravity vector sequentially activates the cells of the otolith macula, the delayed signal level for each cell can be approximated from the present pattern by a spatial extrapolation in pattern space using information from the given cell and an adjacent one. The value of the displacement that minimizes the mean square error between the extrapolated and the delayed signal values over all cells gives a best estimate of head rotation (d) in time T. By using a linear spatial extrapolation function and assuming a uniform distribution of polarization vectors over 360 degrees, sinusoidal spatial patterns are obtained. The formula for the estimate of head velocity ($\hat{\omega}$) reduces to a sinusoidal function of angular head velocity (w) and delay time (T). For T=1.15 seconds, the model predicts that the steady state estimate of head velocity will rise as a function of stimulus velocity (w) to a peak value at $\hat{\omega}$=50 deg/sec. It then declines for larger values of stimulus velocity (w). This type of behavior is observed in the slow phase velocity characteristics of OVAR in monkeys (Raphan et al., 1981). In addition, the 1.15 sec delay predicts that the estimate of head velocity for a step of rotation rises linearly for the first 1.15 sec. If such a function activates the velocity storage integrator the response would be parabolic in nature. This would account for the slow convex buildup during the initial 1-2 seconds when animals are tilted after prolonged rotation about a vertical axis. Once a steady state estimate of head velocity has been established, the rise in slow phase velocity is consistent with the charging of the velocity storage integrator in response to a step in input velocity. If the axis of rotation is in the plane of the macula the model generates a velocity estimate equal to zero and predicts that there would be no unidirectional horizontal nystagmus. Thus, the model suggests a simple mechanism whereby the vestibular system can estimate velocity from position information from the otoliths and predicts many of the steady state slow phase velocity characteristics of horizontal nystagmus during OVAR.

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214.10 ADAPTATION OF VESTIBULO-OCULAR REFLEX (VOR) DIRECTION IN CATS IS SPECIFIC TO ORIENTATION WITH RESPECT TO GRAVITY. J.F. Baker, C.R. Wickland*, R. Harrison, and B.W. Peterson. Dept. Physiology, Northwestern University Medical Ctr., Chicago, IL 60611.

The VOR can adapt its amplitude and direction to reduce retinal image slippage that occurs during head motion. Schultheis and Robinson (NYAS 374:501, 1981) demonstrated that pitch vertical rotation could be made to produce horizontal VOR eye movements after pairing it with horizontal optokinetic motion. Recently, we have shown that the dynamics of this cross-axis coupling of vestibular stimulation to eye movements differ depending on whether or not otoliths are stimulated by the rotations during adaptation. In this abstract we extend that finding and also describe the dependence of adaptive VOR direction change on the orientation of the cat during the testing after adaptation.

Four cats were exposed to two hours of 0.25 Hz rotation coupled with optokinetic motion in an orthogonal direction under four conditions. Upright sagittal (US): the animal in an upright position, rotated in a pitch vertical plane, horizontal image slip. On side sagittal (SS): the animal lying on its side, rotated in a horizontal plane (cat's sagittal plane), horizontal image slip (re cat). Upright horizontal (UH): the animal upright, horizontal plane rotation, vertical image slip. On side horizontal (SH): the animal on its side, pitch rotation (cat's horizontal plane), vertical image motion (re cat). We made horizontal and vertical electrooculographic recordings during rotations in the dark at frequencies from 0.02 to 2.5 Hz, before and after adaptation, in upright and on side testing positions. Saccades were removed from records and adaptive VOR was taken to be the vectorial difference in VOR gains and phases before and after adaptation.

All four conditions produced adaptive VOR in the direction of retinal slip. SS and UH conditions, which stimulated vertical and horizontal semicircular canals, respectively, produced adaptation with peak gain and compensatory phase at the 0.25 Hz adaptation frequency. US and SH conditions, which stimulated canals and otoliths, produced adaptive VOR that had increasing gain at frequencies below 0.25 Hz. In all four conditions, adaptive VOR gain to the same canal stimulus was reduced when tested in the orientation of the cat not used for adaptation. The reduction was about 50%, with a greater reduction at low frequencies when the change in orientation removed rotational stimulation of otoliths. When tested on the side opposite the orientation during adaptation, gain was less than that measured on the adaptation side or upright.

These results show that adaptive change in VOR direction can occur under several stimulus conditions, that otoliths probably participate in VOR adaptation whenever rotation modulates position with respect to gravity, and that adaptation in VOR direction is in part specific to the orientation of the animal during adaptation.

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214.11 DIFFERENTIAL CANAL-CANAL CONVERGENCE IN SECOND-ORDER VESTIBULO-OCULOMOTOR NEURONS IN THE CAT. W. Graf, J.F. Baker, B.W. Peterson and C.R. Wickland*. The Rockefeller University, New York, NY 10021, and Dept. Physiology, Northwestern University, Medical School, Chicago, IL 60611.

Coordinate transformation from vestibular into oculomotor reference frames is required to produce compensatory eye movements in light of the differing, although similar, peripheral geometry of the semicircular canals and the extraocular muscles. This transformation requires convergence of canal-specific signals at some level in the vestibulo-ocular reflex arc. In order to investigate the possible points of such convergence, we recorded intra-axonally from second-order vestibulo-oculomotor neurons in the ascending MLFs of precollicularly decerebrate cats. Neurons were identified by their monosynaptic potentials following electrical labyrinthine stimulation. Responses to natural vestibular stimulation were recorded during rotations in the horizontal plane and 6-20 vertical planes. In addition, selected neurons were stained intracellularly with horseradish peroxidase (HRP) for single cell morphology.

Anterior (AC) and posterior canal (PC) related neurons fell into two classes of single canal and convergent-canal responses. In the group of contralaterally projecting neurons, 11/26 had single canal responses and 15/26 (nine AC and seventeen PC) had convergent vertical canal input which shifted their best response planes toward the ipsilateral or contralateral oblique eye muscle planes. Of four ipsilaterally projecting neurons, three had single canal plane responses (AC and PC), and one AC neuron had its response plane shifted towards the ipsilateral vertical recti plane. Our results demonstrate single canal responses and specific canal-canal convergence within the population of vertical vestibulo-oculomotor neurons. This convergence may participate in the coordinate transformation from vestibular into oculomotor reference frames within the characteristic geometry of the vestibulo-ocular reflex system. At this point, the data suggest a coordinate transformation to occur in at least two stages: at the level of second-order vestibular neurons by converging canal signals, and at the level of oculomotor neurons via axon collaterals of second-order vestibular neurons to non-principal eye movers as demonstrated by HRP-labelled single cell morphology.

In addition to vertical canal convergence, 12/30 AC and PC neurons also received horizontal canal (HC) input sufficient to shift their best response planes towards the horizontal plane by 10-30 deg. Positive and negative HC responses tended to average out, suggesting that the HC input to the vertical system may not normally be expressed but instead may provide a neuronal substrate for adaptive changes in vestibulo-ocular reflex directionality.

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214.12 **ROLL OKN AND OKAN: EFFECTS OF HEAD POSITION ON VELOCITY STORAGE IN THE MONKEY.** D. Schiff*, B. Cohen and T. Raphan. Departments of Neurology, Mount Sinai School of Medicine, N.Y., N.Y. and Computer & Information Science, Brooklyn College, N.Y.

Torsional, or roll eye position can be recorded by implanting a magnetic search coil on top of the eye, in approximately the animal's horizontal plane. The coil is fixed to the sclera behind the attachment of Tenon's capsule, and its posterior portion lies under the superior rectus muscle. A second coil lying in the frontal plane records horizontal and vertical eye position. These coils were used to study torsional optokinetic nystagmus (OKN) and optokinetic after nystagmus (OKAN). With animals upright, OKN induced by full field movement about the roll axis was weak, and there was little OKAN. In contrast, when animals were supine or prone, roll OKN and OKAN were strong. At the onset of stimulation there was a small rapid rise in slow phase velocity, and the rise to the steady state level was slow. Peak steady state velocities were in the range of 40 to 60 deg/sec. After stimulation there was typical OKAN, with a slow decline in slow phase velocity to zero. Roll OKAN could last for several minutes in darkness in naive monkeys and was symmetrical for visual field movement in either direction. Roll OKAN was frequently followed by secondary after nystagmus. If animals were exposed to a stationary visual surround during OKAN or post-rotatory nystagmus, eye velocities decreased more rapidly than in darkness. Static positions optimal for producing roll OKN and OKAN were tested by giving the animal steps of roll surround velocity while in different initial positions. If animals were more than 30 deg from the supine toward the 90 deg roll position or toward the upright, there was a steep fall-off in the peak velocity of OKN and OKAN. Thus, there was a relatively narrow range of positions with regard to gravity in which roll OKAN could be induced. There was powerful coupling from horizontal OKAN to roll nystagmus under appropriate circumstances. With animals supine if horizontal OKN was to the right, a counterclockwise component of OKAN was induced, followed by secondary clockwise nystagmus. The reverse was true if the OKN and OKAN were to the left. The cross coupling tended to bring the after nystagmus to the earth horizontal plane. In conclusion, there do not appear to be significant pathways for rapid changes in torsional slow phase eye velocity from the visual system in the monkey, and probably little or no roll pursuit. It is likely that the main processing of roll OKN and OKAN is through the vestibular nuclei and the velocity storage mechanism. The data shows that roll OKN and OKAN is strongly dependent on head position with regard to gravity. They support the idea that velocity storage has a 3 dimensional representation.

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215.1 EFFECTS OF TRANSIENT ISCHEMIA ON DYNORPHIN IMMUNOREACTIVITY AND PRODYNORPHIN mRNA LEVELS IN HIPPOCAMPUS T. S. Nowak, Jr.*, C. J. Molineaux, D. R. Schoenberg* and B. M. Cox, Lab. of Neuropathology and Neuroanat. Sci., NINCDS, NIH, Bethesda, MD 20892; Dept. of Pharmacology, Mt. Sinai School of Medicine, New York, NY 10029; and Department of Pharmacology, USUHS, Bethesda, MD 20814.

Recent studies have demonstrated a lasting depletion of hippocampal dynorphin following transient ischemia in the gerbil (Nowak, T. S., Jr. and Fried, R. L. (1986) Trans. Am. Soc. Neurochem. 17, 290). Dynorphin A immunoreactivity was reduced by 30 % at 1 and 6 hr recirculation, returned to control levels by 24 hr, and subsequently declined to 60 % of control levels by 2-4 days. Met-enkephalin immunoreactivity tended to increase after 12 hr. Similar changes in hippocampal peptides have been described by others following a series of electroconvulsive shocks or chemically induced seizures. Ischemia and prolonged seizures result in similar patterns of cell loss in hippocampus, although the dynorphin-containing dentate granule cells remain intact.

These results can be interpreted to reflect initial depletion due to increased release and a biosynthetic response leading to transient recovery of peptide levels, suggesting an increase in dynorphin turnover in hippocampus after ischemia. To the extent that this reflects increased activity of dentate granule cells, these results suggest a role for this component of the intrinsic hippocampal circuitry in proposed "excitotoxic" mechanisms of cell death for hippocampal neurons.

An increase in prodynorphin mRNA levels would be expected to precede the intermediate recovery of peptide levels. Recent results have shown that the transient recovery of dynorphin immunoreactivity is not consistently observed. Since peptide level reflects a precise balance between precursor synthesis/processing and peptide release/degradation, mRNA induction may provide a more reliable index of increased peptide turnover.

Female gerbils (3 mo old, 50-70 gm) under halothane/nitrous oxide anesthesia were subjected to 5 min bilateral common carotid artery occlusion. Hippocampal mRNA was prepared from control animals and after 6 hr and 24 hr recirculation (n=4/group), separated by gel electrophoresis, transferred to nitrocellulose, and hybridized with a ^{32}P-labelled plasmid containing sequences complementary to rat prodynorphin mRNA. Preliminary results have failed to reliably detect hybridizable sequences in the gerbil RNA preparations. This may simply reflect inadequate levels of prodynorphin mRNA in the experimental samples. Alternatively, there may be problems with the use of a heterologous probe, since comparative studies have shown little conservation of prodynorphin sequence outside of the opioid peptide coding regions. Continuing efforts are expected to resolve these difficulties.

215.2 **EFFECTS OF CHRONIC NALTREXONE ADMINISTRATION AND WITHDRAWAL ON BRAIN GLUCOSE METABOLISM.** Roy D. Vingan*, Diana L. Dow-Edwards, and Thomas H. Milhorat. Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Health Sciences Center at Brooklyn, Brooklyn, NY 11203.

Chronic administration of the opiate antagonist, naltrexone, has been shown to upregulate central opiate receptors by about 100% (Zukin et al. Br Res 245:285, 1982). Withdrawal of the opiate antagonist has been shown to return opiate receptors to baseline levels at 6 days and then increase them 40% at 10 days (Tempel et al. Lif Sci 31:1401, 1982) The functional consequences of this upregulation are not fully understood. Previous studies have shown that these receptor changes are reflected in specific behavioral and neurochemical changes. In order to determine which specific brain structures might mediate these changes, the deoxyglucose method of Sokoloff was carried out on animals during naltrexone pellet implantation as well as following pellet removal.

Twenty adult female Sprague-Dawley rats weighing between 175 and 249 g were housed under standard laboratory conditions and underwent chloropent anesthesia (0.2ml/100g body weight) for the insertion of naltrexone (2X30 mg) or placebo pellets. Eight days later half of the animals underwent the deoxyglucose (DG) procedure with the pellets in place and the remainder of the animals had the pellets removed. This second group underwent the DG method 10 days later. The night prior to the DG method, the animals were fasted. In the morning, the animals were anesthetized with a mixture of halothane, N_2O and O_2 for the placement of arterial and venous catheters. Following a 2 hour recovery period, 125 uCi/kg body weight of [^{14}C] deoxyglucose were infused and timed arterial blood samples taken for the next 45 minutes. The animals were killed with pentobarbital and the brains removed and processed for quantitative autoradiography.

The results will be discussed in terms of the alterations in behavior and neurochemistry reported by others.

215.3 FOCAL BRAIN STIMULATION ATTENUATES MORPHINE WITHDRAWAL BEHAVIORS. B.E. Thorn and C.L. Beaulieu*. Ohio State University, Columbus, Ohio 43210.

In the present study, we investigated whether FBS in a site implicated in a descending opiatergic pain inhibitory system could attenuate some of the behaviors associated with naloxone-elicited morphine withdrawal. Male albino rats were stereotaxically implanted with a bipolar stimulating electrode in the periaqueductal gray and also received a subcutaneous surgical implantation of a 72 mg morphine or a placebo pellet. Seventy-two hours following pellet implantation, naloxone precipitated withdrawal was induced. Opiate withdrawal behaviors were observed and quantified using a behavioral checklist which integrated the observations of previous investigators. Animals then received focal brain stimulation for 30 minutes after which they were again observed for opiate withdrawal behaviors. The data suggest that focal brain stimulation attenuates morphine withdrawal behaviors, specifically some recessive behaviors associated with autonomic changes. Attenuation of withdrawal behaviors may depend on the timing and duration and intensity of stimulation. These findings are consistent with other studies which speculated that more than one system is involved in the mediation of opioid dependence and analgesia.

215.4 EFFECTS OF BENZOMORPHAN ENANTIOMERS ON EEG, EEG POWER SPECTRA AND BEHAVIOR IN THE RAT. G. A. Young, D. Calligaro*, O. Hong* and N. Khazan. Univ. of Maryland Sch. of Pharm., Baltimore, MD 21201.

(+)-Cyclazocine, (+)-SKF-10,047 (N-allyl-normetazocine) and (+)-pentazocine at doses of 2, 2.5 and 5 mg/kg, i.v., respectively, but neither (+)-ketocyclazocine nor (+)-ethylketocyclazocine (EKC) at a dose of 2.5 mg/kg, i.v., produced desynchronized EEG and an aroused or "psychotomimetic" state for 20-30 min that was not blocked by naltrexone pretreatment.

(-)-Ketocyclazocine and (-)-EKC at a dose of 2.5 mg/kg, i.v. produced high-voltage EEG slow-wave bursts that were associated with dose-related increases in spectral power in the 5-8 Hz band. These EEG slow-wave bursts were correlated with behavioral stupor lasting for 30-45 min, followed by EEG and behavioral arousal for another 30-45 min. (-)-Cyclazocine, (-)-SKF-10,047 and (-)-pentazocine at doses of 2, 2.5 and 2 mg/kg, i.v., respectively, produced 20-30 min of desynchronized EEG and behavioral arousal followed by the delayed emergence of cortical EEG slow-wave bursts associated with both predominant spectral peaks in the 5-8 Hz band and behavioral stupor for another one to two hrs. The delayed emergence of EEG slow-wave bursts may be due to a masking psychotogenic effect of the (-)-enantiomers. For example, when naltrexone treatment preceded (-)-cyclazocine administration, the "psychotomimetic" behavioral profile emerged that was similar to that produced by (+)-cyclazocine.

Benzomorphan-induced EEG slow-wave bursts and associated behavioral stupor are produced only by the (-)-enantiomers. EEG power spectra derived from these EEG slow-wave bursts are qualitatively different from those produced by morphine or methadone (mu opioid agonists). Naltrexone pretreatment blocked the appearance of these benzomorphan-induced EEG slow-wave bursts, but higher doses were needed than in the case of morphine or methadone. Thus, benzomorphan-induced EEG slow-wave bursts represent kappa agonistic effects. Furthermore, "psychotomimetic" behaviors were produced by some of the (+)-enantiomers, and were not blocked by naltrexone pretreatment. These behaviors represent sigma agonistic effects. Therefore, it may be assumed that differential pharmacological profiles of the benzomorphans are dependent on the relative kappa and sigma potencies and kinetics of the enantiomers.

(Supported by NIDA grant DA-01050.)

215.5 ENDOGENOUS OPIOID PEPTIDE-LIKE ANTICONVULSANT SUBSTANCE: PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF ITS PROPERTIES. F.C. Tortella, J.B. Long and D.R. Liston, Neuropharm. Branch, Div. of Neuropsychiatry, Walter Reed Army Inst. Res., Washington, DC 20307 and Dept. Chem., Univ. Oregon, Eugene, OR 97403.

A unique pathophysiological consequence of a seizure is its ability to arrest spontaneously and abruptly, and to maintain a prolonged period of postictal refractoriness. Since opioid peptides appear to be activated by seizures and display marked anticonvulsant activity, Tortella and coworkers (Life Sci. 29 & Eur. J. Pharmacol. 76, 1981) proposed that seizure-activated endogenous opioid systems decrease the probability or severity of subsequent seizures. This proposal has been supported by recent studies from several groups demonstrating that endogenous opioids play a selective role postictally in seizure arrest and refractoriness.

Recently, it was observed that cerebrospinal fluid (CSF) collected from the cisternal space of rats following an MES seizure and injected icv to naive recipient rats increased the seizure threshold to flurothyl convulsions in the recipients (Tortella & Long, Science 228, 1985). The anticonvulsant activity of the CSF was antagonized by prior treatment of recipient rats with naloxone or the selective delta opioid receptor antagonist ICI 174,864, and was enhanced by the peptidase inhibitors aprotinin and bacitracin. These results suggest that the anticonvulsant substance may be an opioid peptide. In the present study , we define additional physiological and molecular characteristics of this substance, providing supportive evidence of its opioid peptide-like nature.

The anticonvulsant substance is heat (90°C) labile and is inactivated by trypsin. It appears to be unrelated to the enkephalins or dynorphin A (mws of 574 and 2148, respectively) as its activity is unaffected by thiorphan and bestatin, inhibitors of enzymes principally involved in the degradation of these molecules. Consistent with this, the anticonvulsant substance fails to pass thru Amicon ultrafiltration membranes with 1K and 5K mw cut-offs, while complete anticonvulsant activity is recovered following ultrafiltration thru 10K mw membranes, indicating that bioactivity is associated with a higher mw opioid. Measurements of opioid peptide-like immunoreactivity in CSF following an MES seizure in rats have revealed marked increases in beta-endorphin-like, but not dynorphin-, met- or leu-enkephalin-like immunoreactivity. Met-enkephalin-like immunoreactivity is increased however following exposure of the CSF to trypsin and carboxypeptidase B, suggesting the seizure-induced release into the CSF of a larger mw form of met-enkephalin not recognized immunologically prior to digestion. Preliminary results using gel exclusion chromatography (Sephadex G-75) reveal a mw of 8700 daltons for the major species yielding met-enkephalin-like immunoreactivity.

These results indicate that the anticonvulsant substance is heat labile, sensitive to peptidase degradation and exhibits specific opioid receptor activity. Collectively, the results of the immunoreactivity, ultrafiltration and chromatography experiments suggest that the anticonvulsant substance is an opioid peptide with a mw between 5K and 10K daltons.

215.6 MICROINFUSION OF MORPHINE ONTO THE AREA IN THE DEEP PREPIRIFORM CORTEX INDUCES ANALGESIA IN THE RAT. M.Massotti, Lab. di Farmacologia, Istituto Superiore di Sanità, 00161 Roma, Italy.

Pharmacological studies have clearly evidenced a functional interaction between opioid and GABA systems in several brain areas, i.e., sensorimotor cortex (Spillantini and Massotti, Pharm.Biochem.Behav.,25:1,1986), periductal grey area (Zambotti et al.,Naun-Schm.Arch.Pharmacol.,318:368,1982) and substantia nigra (Garant and Gale,J.P.E.T.,234:45,1985). An area in the deep prepiriform cortex (DPC) has been recently identified, which is sensitive to very low doses of GABAmimetic drugs (Piredda and Gale,Nature,317:623,1985). This prompted us to explore whether DPC was also sensitive to manipulation of opioid system. To examine this possibility, morphine sulfate (MS) was injected directly onto DPC of rats.

Deep cannulas were stereotaxically implanted unilaterally under Na pentobarbital anesthesia using the following coordinates: A-P,+4.0mm from bregma; M-L,+3.5 mm from longitudinal suture; D-V,-6.5mm from dura. Solutions of bicuculline methiodide (BM) and of MS were infused onto DPC by slow speed (7 nl/min,for 5 min). Twelve hours after surgery, the animals were challenged with infusion of BM (30 ng/rat). The animal showed clonic convulsions (score 1-5, according to Piredda and Gale, 1985).

At least 6 hours later, the animals were tested with MS. At various times after the end of MS infusion, the hot plate (55.4° C) test was carried out, measuring separately the reaction times of the right and left forepaws.

The dose of 1 ng/rat of MS (5 rats) resulted in a significant ($p<0.01$) increase in the latency to respond to the hot plate stimulus on the side contralated to injection (17.4 sec) in respect to the ipsilateral, which remained at the control values (7.4 sec). This effect was present at the 1st and 5th min and disappeared at the 15th min. The dose of 10 ng/rat of MS (10 rats) increased the latency in the contralateral side (24.5 sec; $p< 0.001$) from the 1st to the 30th min. This effect disappeared at the 60th min. The animals were sedated with a mild catalepsy of the contralateral limbs throughout the period of analgesia. At the end of the experiments, gross histology confirmed the right localization of the tip of the cannula.

Neither BM-induced convulsions nor MS-induced analgesia were noticed when the drugs were infused 1.2 mm more rostrally or caudally from DPC (9 rats).

These data suggest that also DPC contains both GABA and opioid receptors.

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215.7 CLONIDINE BLOCKS THE EFFECT OF MORPHINE ON INTRACRANIAL SELF-STIMULATION RATES FROM THE MEDIAL FOREBRAIN BUNDLE AND THE LOCUS COERULEUS. Gimino, F.; Villegas, F.; Murphy, P.; Ocheret, D.; Bashir, T.; Alvarez, R.; Abrue, J.; Steiner, S.S.*; and Ellman, S.J.* Dept. of Psychology, City University of New York, N.Y. 10031.

Intracranial self-stimulation (ICSS) behavior is believed to be mediated by both the catecholamines and the endogenous opiate neuropeptides. The effects of ethanol, amphetamine, morphine, chlorodiazipoxide, and pentobarbital on ICSS can be blocked by injections of the opiate antagonist naloxone. The present study investigated if the alpha-2 adrenergic agonist clonidine would alter morphine's effect on ICSS response rates.

Nineteen male albino Sprague-Dawley rats were implanted with pairs of stimulating electrodes aimed at the medial forebrain bundle (MFB), the locus coeruleus (LC), K2 fields of Forel (FF), and the cru cerebri (CC). After Ss demonstrated stabilized rates of lever pressing for intracranial stimulation, they were administered a series of drug injections which consisted of either .9 percent saline, clonidine NCL (0.5, .1, or .5 mpk), or morphine sulphate (1.25 mpk) alone or in combination with clonidine. Subjects received these drugs under two ICSS conditions. In one condition electrical stimulation was delivered solely to one ICSS area, while in a second condition the stimulation was "split" between two ICSS sites.

Clonidine produced a systematic decrease in ICSS response rates in animals with LC and MFB electrode placements. This drug acted like naloxone in that it blocked the effects of morphine on ICSS. Clonidine also decreased ICSS response rates in the "split" pulse condition. This effect was again only observed in those animals with LC and MFB placements. Clonidine also blocked the effect of morphine on ICSS in the "split" pulse condition, but only when the locus coeruleus and not the medial forebrain bundle was stimulated first. Animals with electrodes localized in the FF and CC showed no reliable effect to clonidine in either ICSS condition.

The data from the present study suggested a noradrenergic mediation of morphine's effects on ICSS response rates. It further indicates that the adrenergic and noradrenergic systems interact systematically with the endogenous opiod neuropeptides.

215.8 MULTIPLE OPIOID MODULATION OF THE HYPOTHALAMIC-PITUTARY- ADRENOCORTICAL (HPA) AXIS: SUB-CHRONIC TOLERANCE STUDIES OF ENDOGENOUS OPIOID PEPTIDES. S. Iyengar, H.S. Kim and P.L. Wood. Neuroscience Research, Pharmaceuticals Div.,Ciba-Geigy Corp., Summit, NJ 07901

We recently showed using various opiate agonist drugs that the HPA axis is under central stimulatory control of multiple opioid receptors and that kappa opioid agonists stimulate this system potently. Opiate antagonists, naloxone and WIN 44441-3, potently inhibited this axis in opiate-naive animals suggesting a role of endogenous opioid peptides on plasma corticosterone release. We now report further studies with icv administration of endogenous peptides in opiate-naive and sub-chronic tolerated rats. Dynorphin (1-13) (DYN), B-endorphin (END) and DADLE potently released corticosterone in a dose-dependent, naloxone-reversible manner. END was the most potent and efficacious of the peptides, while DYN also increased corticosterone profoundly. These peptides were then injected into animals made subchronically tolerant to mu ,delta and kappa agonists, to characterize their in vivo actions on the HPA axis. Tolerance was achieved by injecting either morphine or the kappa agonist, U50488H, every 12 hours with 10,15,23,34,55 and 71 mg/kg, i.p. of either drug and treated acutely 12 hours later. Morphine tolerant animals showed tolerance to acute morphine and U50488H tolerant animals to acute U50488H. There was no cross tolerance between morphine and U50488H, indicating that the two drugs were acting at independent receptors. The effects of DADLE and DYN were not inhibited in the morphine tolerant rat while those of END were partially inhibited. In U50488H tolerant rats END and DADLE still elevated corticosterone levels while the actions of DYN were completely inhibited. These data confirmed endogenous kappa opioid stimulation of the HPA axis at an independent receptor site and that END had effects both at a mu opioid site as well as possibly a separate site. The effect of DADLE also seems to be at a site separate from that of morphine. In experiments where animals were made tolerant to both morphine and DADLE the effects of both acute morphine and DADLE were fully inhibited, END was partially inhibited and DYN was not inhibited at all. We thus confirm independent mu, delta and kappa opioid modulation of the HPA axis. Besides having effects at mu and delta opioid sites, there may be END modulation at a uniquely separate "epsilon" opioid site.

215.9 OPIOIDS DO NOT MODULATE BASAL AND NOREPINEPHRINE-INDUCED RELEASE OF GnRH FROM RAT HYPOTHALAMIC CELL CULTURES. F. Petraglia, A.T.W. Lim and W.Vale (SPON: P.M.Plotsky). The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

We have recently shown that norepinephrine (NE) and adenylate-cyclase-cAMP system are involved in the release of immunoreactive (ir) GnRH from cultures of rat hypothalamic cells. In vivo evidence suggest that endogenous opioid peptides (EOP) exert an inhibitory action on LH secretion, and that this effect may be due by opposing the stimulatory action of NE on GnRH. However, it remains unclear whether the EOP exert the negative effect directly or indirectly on GnRH producing neurons. In the present study we evaluated the effects of EOP and various opiate receptor agonists and antagonists on basal and NE-induced ir-GnRH release in vitro. Primary cultures of hypothalamic cells were prepared from 2-3 days old Sprague-Dawley rats. Following enzymatic dispersion, cells were resuspended in 10% fetal calf serum enriched B-PJ medium and plated at $5x10^6$ cells/well, for ten days. Cultures were pretreated 24 h with forskolin (10^{-6} M) and estradiol (10^{-8} M) prior the stimulation. Ir-GnRH release was measured by double-antibody RIA (using an N-terminal directed antiserum), in media collected following 1 hour incubation with various secretagogues. Acute stimulation with NE (from 10^{-9} M to 10^{-5} M) and forskolin (from 10^{-7} M to 10^{-4} M) consistantly induced a dose-related secretion of ir GnRH (from 10.5+2.3 to 27.7+3.9 pg/well and from 9.7+1.9 to 23.7+3.5 pg/well, n=4, mean+s.e.m., respectively; control 6.2+1.5 pg/well). The EOP (beta-endorphin, dynorphin or met-enkephalin), the opiate receptor agonists (morphine and FK33-284, on mu/epsilon subtypes, or U-50,488H, on kappa subtypes), or antagonists (naloxone, on mu/epsilon, Mr1452 BS, on kappa, or ICI 154,129, on delta)(from 10^{-9} M to 10^{-5} M) failed to modify both the basal secretion and the dose-related release of ir-GnRH induced by NE or forskolin. These results, showing the lack of direct effect of EOP, opiate receptor agonists or antagonists on NE-induced ir-GnRH release from hypothalamic neurons in vitro, suggest that in vivo EOP may act indirectly on GnRH neurons, probably through suppressing the activity of NE neurons.

215.10 NOVEL FRAGMENTS OF PROENKEPHALIN A STIMULATE MOTILITY IN THE SMALL INTESTINE. T.P. Davis, T.J. Gillespie*, J.E. Shook*, and T.F. Burks. Dept. of Pharmacology, Univ. of Arizona, College of Medicine, Tucson, AZ, 85724.

Peptide E is a 25 amino acid opioid peptide derived from proenkephalin A which contains met-enkephalin (met-E) at its amino (N) terminus and leu-enkephalin (leu-E) at its carboxyl(C) terminus. Based on in vitro binding studies, the N-terminal tyrosine is very important in opiate peptide-receptor interactions. Previously we reported that peptide E, and sequential fragments, BAM-22P and BAM-12P induce naloxone sensitive changes in intestinal motility (Hirning, L.D., et al., Gastroenterology 86:1351, 1984). We now report that a new fragment without an N-terminal tyrosine shows pronounced opioid activity. The purpose of these experiments were (1) to determine if a selective difference exists in opioid activity between N- and C-terminal extended, processing fragments of peptide E and (2) to test whether the response noted is dependent on the presence of an N-terminal tyrosine. Peptide E 1-18 contains met-E at its N-terminus and peptide E 2-18 is the des-Tyr metabolite. Peptide E 15-25 contains leu-E at its C-terminus but does not contain an N-terminal tyrosine. These fragments were synthesized in our laboratory by solid phase methodology. Purification, amino acid analysis, and fast atom bombardment/mass spectrometry (FAB/MS) confirmation were completed prior to testing. The effects of these peptides on intestinal motility were examined in isolated, vascularly perfused segments of canine small intestine (Davis, T.P., et al., J. Pharmacol. Exp. Ther. 227: 499, 1983). Peptides were administered by intraarterial bolus. Contractile activity was measured as increases in intraluminal pressure (mm Hg) above background, and was recorded from a latex balloon secured in the lumen. Peptide E 1-18 (0.1-50 nmol) induced intestinal contractions ranging from 16-56 mm Hg, which were antagonized by naloxone. Perfusion with des-Tyr 1-18 did not cause an increase in small intestine motility at any dose tested (0.1-50 nmol). Perfusion of peptide E 15-25 (0.3-25 nmol) caused a significant increase in contractile activity ranging from 6 to 28 mm Hg and was antagonized by naloxone. Since peptide E 15-25 does not contain met-E or an N-terminal tyrosine, opioid activity was confirmed using the classical mouse vas deferens (MVD) and guinea pig ileum (GPI) assays. The MVD-IC50 was 589 nM versus 10,000 nM in the GPI assay (effect was naloxone reversible) yielding a 17 fold delta selectivity based on the GPI (mu) to MVD (delta) ratio. Although peptide E 15-25 lacks tyrosine and met-E at the N-terminus it does possess opioid activity which is more delta receptor selective. (Supported by NIH Grant AM3628901 from NIADDK.)

215.11 COUPLING OF THE SYNTHESIS AND RELEASE OF METHIONINE-ENKEPHALIN IN THE GUINEA PIG ENTERIC NERVOUS SYSTEM. A.R. Gintzler, W.C. Chan* and J. Glass*. Dept. of Biochemistry, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

The in vitro release of methionine-enkephalin (met-enkephalin) from 2 longitudinal muscle myenteric plexus (LMMP) strips from guinea pig ileum has been obtained during continuous superfusion and quantitated directly using a radioimmunoassay specific for this opioid peptide. Electrical stimulation (5-80 Hz) produced a significant increase in the rate of release of met-enkephalin the magnitude of which was not dependent on the frequency of stimulation; release was inversely proportional to the frequency of stimulation. Electrically evoked release (40 Hz) of met-enkephalin was reduced by greater than 80% by substituting $CoCl_2$ for $CaCl_2$ or by pretreatment with tetrodotoxin (TTx; 1 ug/ml for 15 min). Evoked release was also substantially reduced by pretreatment with morphine (1 uM, 3.0 min). Alternatively, pretreatment of naive LMMP strips with the opiate antagonist (-) naloxone caused a significant increase in the rate of met-enkephalin release in the absence of electrical stimulation. In contrast, (+)-naloxone was devoid of any activity. These data, in combination with indirect pharmacological experiments, strongly indicate that met-enkephalin functions as a neurotransmitter in the enteric nervous system. Moreover, the activity of neurons that transmit via met-enkephalin appear to be under tonic opioid regulation.

This laboratory recently reported that perturbations that increase that rate at which enkephalin is released from myenteric neurons also increase the content of this opioid peptide in the myenteric plexus (Glass, et al., Brain Res. in press). Thus, as has been demonstrated for the conventional, small molecule neurotransmitters the synthesis and release of enkephalin appear to be events that are closely coupled and coordinated with each other. (Supported by grant DA 02893 to A.R.G. from the National Institute on Drugs of Abuse.

216.1 MOSSBAUER MEASUREMENT OF MIDDLE EAR FUNCTION IN THE CHINCHILLA. M.A. Ruggero, J.A. Costalupes and N.C. Rich*. Dept. of Otolaryngology, Univ. of Minnesota, 2630 University Ave. S.E., Minneapolis, MN 55414.

The chinchilla basilar membrane (BM) response has been recorded at a site 3.5 mm from the round window as a function of the acoustic pressure at the external auditory meatus [L. Robles et al., in: Peripheral Auditory Mechanisms, edited by J. B. Allen et al., Springer Verlag, Berlin, 1986]. In order to distinguish between cochlea-specific properties and properties of the middle ear it is necessary to determine the response of the ossicular chain as a function of the acoustic stimulus. In addition, it is of interest to explore the effect of opening the bulla on middle ear function in order to relate primary afferent responses, which are usually recorded under closed-bulla conditions, with BM behavior, which must be studied with the bulla widely open. Therefore, we have applied the Mössbauer technique to the measurement of the vibration of the malleus at its attachment to the umbo of the tympanic membrane. Under open-bulla conditions, the malleus velocity response at a sound pressure level of 100 dB is relatively invariant at 1-2 mm/sec for frequencies from 100 Hz to at least 8 kHz, thus approximating the velocity of air particles (2.5 mm/sec). At frequencies lower than 100 Hz the malleus velocity response rolls off at a rate of about 8 dB/octave. Under closed-bulla conditions, approximated by venting the bulla by means of a 25-cm length of 0.3-mm-inner-diameter tubing, the velocity response is similar to that for open bulla for frequencies above 500 Hz. As stimulus frequency is decreased below 500 Hz, however, the response rapidly diminishes so that at 200 Hz the closed-bulla response is smaller than the open-bulla vibration by some 15 dB. This 15-dB difference persists relatively unchanged for frequencies as low as 30 Hz. The malleus response phases for open- and closed-bulla conditions are similar below 100 Hz and above 2 kHz but differ substantially in the intermediate frequency range. At the lowest stimulus frequencies (30-60 Hz) the malleus moves nearly in phase with rarefaction in the ear canal. As stimulus frequency is raised, the malleus response progressively lags air rarefaction so that, at 8 kHz, it lags by some 200 degrees, irrespective of whether the bulla is open or closed. At frequencies between 100 Hz and 2 kHz the open-bulla malleus response lags the closed-bulla response by as much as 90 degrees (at 700-800 Hz). Comparison of the response phases of the malleus with those of the BM reveals different behavior at frequencies below and above 125 Hz. At frequencies 100 Hz and below, the BM response is uniformly out-of-phase (180 degrees) with the malleus; at frequencies between 150 Hz and 1 kHz the BM leads the malleus by a constant amount of about 90 degrees (i.e., it is in phase with malleus velocity).

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216.2 MECHANICAL RELAXATION OF THE HAIR BUNDLE CORRELATES WITH ADAPTATION IN THE MECHANOELECTRICAL TRANSDUCTION PROCESS OF A HAIR CELL. J. Howard* and A. J. Hudspeth. Department of Physiology, University of California, San Francisco, CA 94143-0444.

Hair cells in the bullfrog's sacculus can detect oscillatory accelerations as small as 100 $\mu m/s^2$ in the frequency band 50-250 Hz, while largely rejecting a static gravitational input 10^4 times as large. This differential sensitivity comes about through the activity of an adaptation mechanism that continuously resets the sensitive range of the hair bundle to accord with the position in which the bundle is held by tonic stimulation (R. A. Eatock *et al.*, *Soc. Neurosci. Abstr.* 5: 19, 1979).

To test the possibility that adaptation ensues from mechanical rearrangements in the hair bundle, we made measurements of the bundle's mechanical properties with sufficient temporal resolution to detect transient changes in stiffness. In each experiment, a single hair bundle in the isolated saccular epithelium was deflected with the tip of a flexible glass fiber roughly 250 nm in diameter and 80 μm in length. The horizontal motion of the fiber's tip was monitored by projecting its image onto a dual photodiode; the root-mean-square background noise in the 5-1000 Hz frequency band was 300 pm.

The steady-state stiffness of the bullfrog's hair bundle is approximately 600 $\mu N/m$. The stiffness is time-dependent: a force stimulus produces an immediate displacement roughly 70% of the steady-state value, to which the displacement then relaxes with a time constant of 11 to 77 ms. The hair bundle, in other words, is initially about 50% stiffer than it becomes in the steady state. This relaxation occurs only along the bundle's axis of morphological symmetry; orthogonal stimuli elicit negligible relaxation.

The rate of mechanical relaxation of the hair bundle correlates well with the rate of adaptation in the same cell's mechanoelectrical transduction process. Whether measured directly in two-electrode voltage-clamp experiments or inferred from single-electrode voltage recordings, the transduction current decays from its value shortly after the onset of a stimulus with a time constant similar to that of the bundle's mechanical relaxation.

Because the stiffness of the hair bundle is correlated in orientation and time course with the transduction current, it is likely that relaxation of the bundle is associated, perhaps causally, with adaptation of the mechanoelectrical transduction process. More specifically, the polarity of the mechanical relaxation is consistent with the idea that adaptation to a positive stimulus results from a reduction in the tension in an elastic element that gates the transduction channel.

This research was supported by grant NS20429 from the National Institutes of Health and by the System Development Foundation.

216.3 THE VOLTAGE DEPENDENCE OF HAIR-CELL TRANSDUCTION. T. Holton and A. J. Hudspeth. Department of Physiology, University of California School of Medicine, San Francisco, CA 94143-0444.

Recent theoretical (Weiss, T. F., *Hearing Res.*, 7:353, 1982) and experimental (Crawford, A. C. and Fettiplace, R., *J. Physiol.*, 364:359, 1985) results suggest that there is a reciprocal coupling between the receptor potential generated in a hair cell and the force on the cell's hair bundle, implying the existence of a voltage-dependent force-generating mechanism in the cell. We have tested the possiblity that the transduction channel, which is gated by mechanical stimulus, itself shows voltage sensitivity.

Using the whole-cell voltage-clamp technique, we previously measured the receptor current evoked from single hair cells in the bullfrog sacculus by stimulating their hair bundles (Holton, T. and Hudspeth, A. J., *J. Physiol.*, in press). These results show that the relation between the equilibrium receptor-current amplitude (I) and hair-bundle displacement (x) at a membrane potential (V_m) of -73 mV is well fit by the function:

$$I(x) = N\gamma(V_m - V_{REV})p_{MAX}/[1+e^{(G-Zx)/kT}],$$

where N is the number of transduction channels per cell, γ is the single-channel conductance, V_{REV} is the reversal potential of the transduction current, p_{MAX} is the maximum probability of a channel's being open, and G and Z are respectively the displacement-independent and -dependent free energies of gating the channel. Ensemble-variance analysis of the receptor-current fluctuations shows that N can be as large as 280, γ is about 13 pS at 10°C, and p_{MAX} is approximately one. The reversal potential (V_{REV}) is near 0 mV, consistent with the view that the channel is a relatively non-selective aqueous pore.

We have now employed similar techniques to examine the equilibrium dependence of I upon V_m. When recorded with external and internal solutions of similar conductivity, the relation between receptor-current magnitude and V_m is essentially linear for values of potential ranging from -90 to +40 mV. Analysis of receptor-current fluctuations recorded at various values of V_m shows that N, γ, and p_{MAX} are not voltage-dependent. Fits of the function $I(x)$ to receptor-current data indicate that G and Z are also essentially independent of V_m.

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216.4 CORRELATION BETWEEN THE KINETIC PROPERTIES OF ION CHANNELS AND THE FREQUENCY OF MEMBRANE-POTENTIAL RESONANCE IN HAIR CELLS OF THE BULLFROG. W. M. Roberts, L. Robles* and A. J. Hudspeth. Department of Physiology, University of California School of Medicine, San Francisco, CA 94143-0444.

Hair cells of bullfrog auditory and vestibular organs exhibit an electrical resonance that contributes to their frequency selectivity. This resonance persists in enzymatically isolated cells. It arises from the interaction between a voltage-gated calcium current and a calcium-activated potassium (K_{Ca}) current (Lewis and Hudspeth, Nature, 304:538, 1983). In order to investigate how different cells are tuned to stimuli of different frequencies, we have compared kinetic parameters of currents in hair cells from the sacculus, which respond to low frequency substrate vibrations, to those in the amphibian papilla, which respond to higher frequency auditory stimuli.

Whole-cell recordings were obtained from cells isolated from Rana catesbeiana by papain digestion. Electrodes were filled with 152 mM K^+, 77 uM Ca^{2+}, 2.27 mM Mg^{2+}, 60 mM SO_4^{2-}, 10 mM Cl^-, 1 mM ATP, 10 mM EGTA and 10 mM HEPES. All recordings were made in the presence of 10 mM extracellular 4-aminopyridine to block transient K^+ currents. Ca^{2+} currents and K_{Ca} currents were separated by subtraction of voltage-clamp records obtained before and after addition of 10 mM tetraethylammonium to the bath.

Cells from both organs displayed damped membrane-potential oscillations at the onset of depolarizing current steps. Papillary cells oscillated at higher frequencies than saccular cells and displayed a wider range of resonant frequencies. Ca^{2+} channels had the same current-voltage relation in all cells studied, but they activated more rapidly in papillary than saccular cells at all potentials. Evidently, Ca^{2+} channels in cells from the two organs differ. K_{Ca} currents likewise activated more rapidly in papillary than saccular cells, and also showed a faster decline of tail currents. These differences could reflect differences in the K_{Ca} channels themselves, or in the kinetics of intracelular Ca^{2+} movement. Several factors are therefore involved in determining a cell's resonant frequency. These results are consistent with the predictions of a model that incorporates channel kinetics, Ca^{2+} buffering and cell capacitance.

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216.5 ULTRASTRUCTURAL OBSERVATIONS OF A PREVIOUSLY UNIDENTIFIED SPECIFIC SURFACE COAT ON COCHLEAR HAIR CELLS. P.A. Santi, C.B. Anderson*, and B.N. Lakhani*. Dept. of Otolaryngology, Univ. of Minnesota Med. School, Minneapolis, MN 55455.

The anatomy of hair cell stereocilia has recently received considerable attention due to physiological evidence which suggests that the stereocilia are the primary site of auditory transduction. Recent anatomical studies have shown that the stereocilia consists of a core of actin filaments. While sparse, discrete strands of extracellular material have been observed on their plasmalemma, little or no surface coat has been observed using conventional transmission electron microscope (TEM) methods. Unfortunately, complex carbohydrates such as those associated with the glycoconjugates (e.g., glycoproteins, glycolipids and proteoglycans) are poorly preserved and stained by routine TEM methods. In other tissues, it has been shown that these carbohydrates, which have a strongly anionic character, can be simultaneously stabilized, fixed and stained if the primary fixative contains a cationic agent. In order to determine whether a significant surface coat exists on cochlear cells, we fixed normal chinchilla cochleas with a mixture of glutaraldehyde and the cationic dye Alcian Blue (AB). AB consistently stained the tectorial membrane, the outer hair cell (OHC) apical plasmalemma, and a portion of the basilar membrane. The most striking finding was that of a surface coat on the endolymphatic surface of the OHCs. This coating was approximately 90 nm in thickness, homogeneous and occurred on the apical plasmalemma of the OHCs and along the length of the stereocilia. A similar surface coat occurred on the inner hair cells (IHCs) in the middle and apical portions of the cochlea. No surface coat appeared on adjacent supporting cells. In some cochleas a prominent band of acellular, AB stained material separated the pillar/OHC region from the IHC/internal sulcus region of the organ of Corti. Preliminary observations of this band of material suggest a fluid separation (by the lack of AB staining) in the subtectorial space between the IHCs and OHCs in the basal turn of the cochlea. We cannot yet identify the type of glycoconjugate which constitutes the surface coat. However, previous biochemical studies have revealed a significant quantity of sulfated proteoglycans in cochlear tissues. Several functions may be served by the glycoconjugates in cochlear tissues. In other tissues, these molecules provide mechanical functions and have been linked to actin. They also sequester cations and influence the chemical environment surrounding cells. Now that a specific surface coat has been identified on cochlear hair cells, its possible role in sensory cell transduction, ototoxicity and sound-induced hearing dysfunction may be investigated.

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216.6 AXON MORPHOLOGY OF TYPE II SPIRAL GANGLION CELLS IN CATS. D.K. Ryugo, L.W. Dodds* and N.Y.S. Kiang. Dept. of Anatomy and Cellular Biology, Harvard Med. School, Boston, MA 02115, and Eaton-Peabody Lab., Mass. Eye & Ear Infirmary, Boston, MA 02114.

The cochlea of cats contains two types of receptor cells and two types of spiral ganglion neurons. The large, usually bipolar (type I) ganglion cells contact inner hair cells (IHCs) and the small, usually pseudomonopolar (type II) ganglion cells contact outer hair cells (OHCs, Kiang et al., Science, 217:175, 1982). These observations establish that the two hair cell systems have separate afferent pathways. Most of our anatomical and physiological knowledge of auditory nerve fibers are of type I neurons.

In the present study, extracellular injections of horseradish peroxidase (HRP) into the auditory nerve (2-36 hr survival) labelled thick (2-4 um), thin (~0.3 um), and very thin (~0.1 um) fibers. Although labelled fibers could be traced both centrally and peripherally, no labelled fiber could be traced through the injection site. Thick fibers were shown to arise from type I neurons. Very thin fibers branched, had frequent varicosities, and were assumed to be of autonomic origin. Thin fibers (n=40) were reconstructed peripherally through serial sections of the nerve and found to be relatively uniform in diameter, unbranched, and almost always without varicosities. These thin fibers all arose from cell bodies that were typical of type II neurons; that is, the cell bodies were small in size and pseudomonopolar in shape. Some (n=9) were even traced out to their terminations under OHCs. These observations imply that the thin, unbranched fibers of the auditory nerve are the axons of type II ganglion cells.

Central to the injection site, thin fibers were traced through the nerve and into the cochlear nucleus (n=18). Two thin fibers examined with the electron microscope were found to be unmyelinated. Thin fibers branched upon entering the cochlear nucleus but the degree of branching varied greatly. The HRP reaction product did not stain these fibers beyond 2 mm into the nucleus, so data are available only for the region surrounding the nerve root. Collaterals were nearly always thinner in diameter than the parent fiber and could be long or short, branched or unbranched. Darkly stained branches terminated with a swelling at the tip. En passant and terminal swellings were mostly found in neuropil. In this region of the cochlear nucleus, nearly half of the thin fibers were associated with capillaries. The possibility that type II neurons can influence blood vessels makes it essential to establish the validity of these preliminary observations.

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216.7 ONTOGENY OF SINGLE UNIT RESPONSES IN THE AUDITORY NERVE OF THE MONGOLIAN GERBIL: NORMAL DEVELOPMENT. Nigel K. Woolf and Allen F. Ryan*. Division of Otolaryngology, Department of Surgery, UCSD Medical School, San Diego, CA 92093, and Veterans Administration Medical Center, La Jolla, CA 92161.

Single auditory nerve fibers of mongolian gerbils (Meriones unguiculatus) were recorded at postnatal ages 10, 12, 14, 16, 18, 30 and 90 days after birth (DAB). This range of ages includes the onset of neonatal hearing through achievement of mature auditory system characteristics.

At 10 DAB none of the VIIIth nerve fibers encountered were responsive to auditory stimuli. At 12 DAB only a small percentage of the neurons isolated were responsive. For subjects 14 DAB, or older, the great majority of neurons were responsive to acoustic stimulation. Based upon data taken from responsive neurons, it was evident that with increasing age: first, neural thresholds rapidly and monotonically decreased; second, the absolute frequency range of the neural characteristic frequency values increased; and third, mean spontaneous discharge rate increased. Most of the neural parameters examined achieved adult characteristics by 18 DAB.

In contrast with the results of previous single unit studies of the early development of auditory function in other mammals and birds, gerbil auditory nerve fibers were found to have sustained responses to long duration, gated tone bursts, throughout the stimulus period. No evidence of "phasic" or "bursting" temporal patterns within the PST histograms were observed. Even at 12 DAB, the earliest age at which responsive auditory nerve fibers were isolated, units did not exhibit signs of habituation or intermittent responsiveness during 500 msec tone bursts.

Regardless of age, all of the auditory nerve fibers recorded exhibited primary-like post-stimulus-time (PST) histograms. However, while PST histograms for mature subjects exhibited well defined onset peaks, the great majority of PSTs for neonates did not. This reflected greater variability in initial spike latency and a less pronounced initial rate of firing for neonatal responses than were characteristic of mature subject responses.

The maturation of VIIIth nerve fiber discharge patterns is well correlated with our previous reports on the ontogeny of the endocochlear potential, cochlear microphonics, N1 compound action potentials, auditory brainstem responses and ventral cochlear nucleus single unit responses in this species.

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216.8 A DUAL EFFERENT GABAERGIC PROJECTION TO THE RAT COCHLEA. D.E. Vetter, J.C. Adams, and E. Mugnaini. Lab. of Neuromorphology, Univ. of Connecticut, Storrs, CT 06268 and ENT Dept., Medical University of South Carolina, Charleston, SC 29425.

Recently, it has been shown that the efferent olivo-cochlear system is not homogeneous and contains both cholinergic and GABAergic components. GABAergic terminals have been observed immunocytochemically in the guinea pig inner spiral bundle and in relation to outer hair cells (Fex et al., 1984, 1986). Also, uptake of exogenous tritiated GABA has been demonstrated in the organ of Corti by several authors. Using single and double labeling procedures, we have analyzed the peripheral cochlear GABAergic projection and the origin of such fibers in adult rats.

GABAergic boutons and cell bodies were detected immunocytochemically using a sheep antiserum to glutamic acid decarboxylase (GAD) (Oertel et al., 1981). In order to identify efferent GABAergic projection neurons, the round and oval windows were unilaterally exposed and opened. The cochlea was then perfused with 5-15 μl of 10% WGA-HRP through the round window, after which both windows were sealed. The animals were transcardially perfused 48 hrs later with a zinc-aldehyde fixative, pH 6.5. Serial frozen sections of the acoustic brainstem were cut and reacted for the presence of retrogradely transported HRP using either cobalt-nickel intensified DAB or 4-chloro-1-naphthol as the chromogen. The sections were then processed for immunolocalization of GAD with DAB as the chromogen.

Double labeled neurons, with HRP positive (dark) granula in a GAD positive (diffuse brown) cytoplasm, were observed in the lateral superior olive (LSO) ipsilateral to the injection and in the ventral nucleus of the trapezoid body (VNTB) bilaterally. Non-GABAergic cells that were labeled by HRP granula alone and possibly representing cholinergic efferent neurons, were present ipsilaterally in LSO and VNTB, and bilaterally in a region of the olivary complex medial to MSO and in the trapezoid body. More of these HRP labeled neurons were present ipsilaterally than contralaterally.

GAD immunoreactive terminals were located in the organ of Corti of untreated animals. The GABAergic boutons were localized at the base of the inner hair cells and at the level of the nucleus of the outer hair cells. Cholinergic boutons, as seen by immunostaining with anti-calcitonin gene-related peptide (CGRP), represent a separate population of efferent boutons.

Thus, these studies demonstrate that in rat the efferent cochlear bundle is not homogeneous. Moreover, GABAergic boutons (presumably inhibitory) are associated with both inner and outer hair cells and derive from at least two major pools of GABAergic neurons. Preliminary comparative studies suggest that a dual GABAergic efferent cochlear system is present also in gerbil, guinea pig, and cat. [Supported by PHS grants NS9904 and NS21307.]

216.9 ORIGINS OF INPUTS TO CHOLINERGIC LATERAL OLIVOCOCHLEAR SYSTEM. J.C. Adams. Dept. of Otolaryngology and Communicative Sciences, Med. Univ. of S.C., Charleston, SC 29425.

GABAergic efferent terminals in the cochlea (as shown by immunostaining against glutamate decarboxylase (GAD)) form a population of terminals separate from cholinergic terminals and both have their origins in the superior olivary complex (Vetter et al., this meeting). In cat the locations of the cholinergic olivocochlear neurons that are origins of terminals beneath inner hair cells are sufficiently distinct from those of GABAergic neurons to permit observations on the neuropil surrounding many of the cholinergic cells. A high concentration of these cells is consistently found along the lateral border of the dorsal hilus of the lateral superior olive. These cells have been recognized by a number of investigators using acetylcholinesterase, anti-cholineacetyltransferase, anti-enkephalins, and anti-calcitonin gene-related peptide (CGRP). This investigation describes possible inputs to these cells by visualizing terminal plexuses that surround them. A plexus along the lateral border of the hilus is seen with cholinesterase stains and with antisera to enkephalins and CGRP. A similar plexus is demonstrated by all three methods. It is possible that the plexus, at least in part, consists of recurrent collaterals of axons that project to the cochlea. One or perhaps several other plexuses at the same location can be visualized with antisera to GAD, substance P, and neuropeptide Y. The source(s) of these fibers can at present only be inferred from the locations of cell bodies that are stained with the antisera. If it is assumed that the fibers arise from auditory structures then the possible sources becomes quite limited for the case of the peptides. To date cell bodies that are immunostained against substance P and neuropeptide Y have been located only in the external portions of the inferior colliculus. GAD immunostained cells are also found there, so it is possible that antisera to substance P, neuropeptide Y and GAD are staining one terminal plexus. There are many other possible origins of the GAD immunostained fibers, however, including collaterals of GABAergic olivocochlear cells. The present results suggest that cholinergic lateral olivocochlear cells have complex innervation and that inputs may include recurrent collaterals from that system, descending inputs from the inferior colliculus, and possibly recurrent collaterals from the GABAergic lateral efferent system.
Supported by PHS grant NS21307.

216.10 AN ANTI-GABA ANTIBODY LABELS SUBPOPULATIONS OF AXONAL TERMINALS AND NEURONS IN THE GERBIL COCHLEAR NUCLEUS AND SUPERIOR OLIVARY COMPLEX. I.R. Schwartz and S.-M. Yu*, Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Antibodies, produced in rabbit against GABA conjugated to bovine serum albumin by glutaraldehyde and affinity purified against a number of amino acids (Wenthold et al., Brain Res. 1986), have been utilized to identify presumptively GABA containing cell bodies, terminals and processes in the cochlear nucleus (CN) and superior olivary complex (SOC) in frozen sections of mixed aldehyde fixed brains of Mongolian gerbils (Meriones unguiculatus) ranging from 7 days to 15 months in age.

At the light microscopic level consistent labeling patterns were observed over a range of fixation and incubation conditions. Findings in the gerbil were similar to those reported in the guinea pig (Thompson et al., Brain Res. 1985; Wenthold et al., Brain Res. 1986). In young adult animals the most intensely stained neurons were observed in the CN. Three cell groups were heavily stained: small oval neurons (L=20.0um, s=4.3, W=13.4um, s=4.3) in the molecular and fusiform cell layers of the dorsal CN (DCN); larger, more irregular neurons (L=30.0um, s=5.3, W=18.4um, s=3.5) in the deep DCN; and neurons in the superficial layer of the anteroventral CN (AVCN). Another group of neurons in the DCN molecular and fusiform layers larger and rounder (L=24.2um, s=4.7, W=17.7um, s=5.2) than the small oval neurons were moderately stained. Prominent terminal staining was observed in the DCN molecular layer, the posteroventral CN (PVCN), and around spherical cells in the AVCN. Very marked terminal labeling was observed along the ventral surface of the brainstem in the fiber tracts passing through the ventral and lateral nuclei of the trapezoid body. Many neurons in this region were also moderately stained and a few were heavily stained. A few neurons labeled with varying intensity were also observed between the medial nucleus of the trapezoid body and the medial superior olive (MSO), between the MSO and lateral superior olive (LSO), and around the LSO. Neither prominent neuronal nor terminal labeling were observed in the MSO or LSO, although labeled fibers were observed passing through both nuclei. Within neuronal somata in the MSO and LSO, moderately stained bodies were observed which became more prominent with increasing age. Electron microscopic analysis is underway to identify the ultrastructural characteristics of labeled elements.

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216.11 NIPECOTIC ACID: PREFERENTIAL RETROGRADE LABELING OF A SUBPOPULATION OF OLIVOCOCHLEAR NEURONS FOLLOWING ACCUMULATION BY GABA UPTAKE SYSTEMS IN THE COCHLEA. A.F. Ryan*, I.R. Schwartz and R.H. Helfert (SPON: E. Keithley). Division of Otolaryngology, VA Medical Center & UCSD School of Medicine, La Jolla, CA 92093 and Division of Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Two populations of efferents endings in the gerbil cochlea can be differentiated based upon their amino acid uptake characteristics. One population preferentially accumulates aspartic acid, and can be selectively labeled with 3H-d-aspartic acid (D-ASP). A second population can be selectively labeled by 3H-GABA. We have shown previously that 3H-D-ASP taken up by efferents in the cochlea is retrogradely transported to the brainstem and selectively labels the fibers, collaterals and cell bodies of a subpopulation of the olivocochlear efferents. The cell bodies of these neurons are primarily ipsilateral to the injected cochlea, and are located within the cellular region of the lateral superior olivary nucleus (LSO). This corresponds to the location of lateral system efferents in the gerbil. However, following 3H-GABA uptake in the cochlea, label is only weakly transported to the brainstem, presumably because the GABA is metabolized and the label is lost.

Nipecotic acid (NIP) is a GABA analog which appears to be accumulated by the same uptake systems as GABA, and to be much more slowly metabolized in neural tissue. The uptake of NIP by GABA uptake systems in the cochlea and its retrograde transport to brainstem were evaluated using autoradiography. 3H-NIP perfused into the cochlea at probe concentrations was rapidly and selectively accumulated with a pattern identical to that seen with probe concentrations of 3H-GABA. This included a subpopulation of the cochlear efferents. When 3H-NIP was perfused into the cochlea and animals were allowed to survive for 6 hours, labeling of olivocochlear fibers in brainstem was noted. After 24 hours post-perfusion survival, labeling of olivocochlear fibers and cell bodies in the superior olivary complex was observed. Labeled cell bodies were located in the lateral and ventral nuclei of the trapezoid body, and in the region between the medial nucleus of the trapezoid body and the medial superior olivary nucleus, the majority contralateral. This corresponds to the location of medial system efferents in the gerbil. No neurons were labeled in the cellular region of the LSO. In some NIP animals, cells were also labeled in the neuropil surrounding the LSO, suggesting that some lateral system efferents may accumulate NIP.

The results indicate that NIP can be used to selectively label by retrograde transport neurons whose terminals preferentially accumulate GABA.

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216.12 A SYSTEM TO RECORD FROM FREELY MOVING ANIMALS IN A CONTROLLED ACOUSTIC ENVIRONMENT. K.M. Mudry, Departments of Biomedical and Electrical Engineering, The University of Akron, Akron, OH 44325.

Processing of sensory information throughout central nervous system pathways can be affected by many factors including anesthesia and attention. In order to study higher level sensory processing, recording techniques using awake and freely-moving animals have been developed for some systems, including the visual system (Borchers, J. Theoret. Neurobiol. 1, 1982). Auditory neurophysiological studies have been reported on awake restrained animals. The complex technical problems associaed with controlling and/or calibrating auditory stimuli at an animal moving about an arena have delayed the development of similar experimental paradigms for auditory neurophysiological studies.

A system has been developed to record from freely-moving anurans, weighing between 20 to 40 grams. Since stereotaxic placement of electrodes is not possible, chronic microdrives weighing less than 3 grams were designed to allow the placement of electrodes visually. Differential recordings are obtained as the animal moves about a semi-anechoic room. The chamber is nonreflective over the audio range above about 200 Hz. However, since the sound pressure in a room does not exactly decrease by 6dB with a doubling of the distance from the source, to determine the sound intensity in the room the chamber was calibrated. Over a .1m square grid pattern the intensity of tones was measured at 1/3 octave frequencies between 100 and 5000 Hz for each of the two speakers located at opposite ends of the room. A best fit plane is used to interpolate the sound intensity between any four points on the grid. The animal's location in the room is tracked. Knowing the animal's location, an auditory signal of a specified intensity can be generated at the animal. Alternatively, the sound intensity at the animal can be determined when the signal from the source is held constant. Since the sound synthesis equipment is under computer control during the experiment the test signals can be randomized and changed very rapidly. Audiograms can also be obtained and signals from the two speakers can be alternated for sound discrimination experiments.

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217.1 THE PITUITARY GLAND MODULATES THE RELEASE OF ATRIAL NATRIURETIC PEPTIDES FROM THE CARDIAC ATRIA. N. Zamir*, M. Haass*, J.R. Dave* and Z. Zukowska-Grojec*. (SPON: P.L. Kornblith) Hypertension-Endocrine Branch, NHLBI, NINCDS and NIAAA, DICBR, NIH, Bethesda, MD 20892.

Evidence suggests that the mammilian atrium is an endocrine organ that may be involved in the control of blood pressure and extracellular fluid volume. A group of peptides, which appear to be associated with specific atrial secretory granules have potent natriuretic, di uretic and smooth muscle relaxant activities. The atrial peptides are termed atrial natriuretic peptides (ANP). Little is known about the exact stimuli regulating the secretion of ANP into circulation. The present study examines the role of the pituitary gland in basal and stimulated (acute volume expansion) release of ANP in the rat.

Blood samples were obtained from rats with indwelling catheters and aliquots of plasma were extracted for immunoreactive (ir)-ANP with an 80% recovery. Blood pressure and heart rate were monitored continuously throughout each experiment. Basal plasma concentrations of ir-ANP in male Sprague-Dawley rats were 80 ± 15 pg/ml (mean ± SEM, n=10). Acute volume expansion (intravenous infusion of 20 ml of 5% glucose/kg body weight over 1 min) increased ir-ANP 2-3 fold in plasma within 2 minutes. In the pithed rat preparation, which produced a complete cardiac denervation and removed humoral influences of brain origin, the stimulated release of ANP was blocked. The results suggest that humoral and/or neuronal influences play a role in stimulated release of ANP. Bilateral carotid artery occlusion in pentobarbital anesthetized rats attenuated markedly (more than 50%) stimulated release of ANP further supporting the involvement of humoral factors. In addition, hypophysectomy in rats (2 and 8 days after operation) markedly reduced basal and stimulated release of ANP. In diverse experimental groups studied, alterations in heart rate and mean arterial pressure were not correlated in any consistent directional manner with changes in ANP release.

It seems that pituitary gland-derived hormones provide a physiological milieu at the secretory atrial myocytes for secretion of ANP.

217.2 EFFECTS OF BARORECEPTOR DENERVATION ON GLUCOSE UTILIZATION IN THE SUBFORNICAL ORGAN DURING ADMINISTRATION OF ANGIOTENSIN II. M. Kadekaro, M. Creel,* M.L. Terrell,* H.M. Eisenberg. Div. of Neurosurgery, University of Texas Medical Branch, Galveston 77550.

Neural regulation of body fluid homeostasis is mediated over a circuit of cerebral structures located primarily in the forebrain near the ventricular system. This circuit includes the subfornical organ (SFO) and its connections with the septum, preoptic area, hypothalamus, organum vasculosum of the lamina terminalis and the pituitary neural lobe (NL). The activity of structures within this circuit is stimulated by plasma and cerebrospinal fluid hyperosmolality, high plasma levels of angiotensin II (AII) and decreased input from baro- and volume-receptors. There is evidence suggesting that the SFO is the site of integration of stimuli arising from circulating AII, osmoreceptors and volume- and baroreceptors. We showed previously that glucose metabolism of the SFO is closely associated with circulating levels of AII. Intravenous infusion of AII induced pressure and drinking responses and stimulated glucose utilization in the SFO (+52%) and NL (+81%) compared to saline-infused animals. Because AII increases arterial blood pressure, we investigated the influence of baroreceptors on the glucose metabolic response of SFO to AII, with the quantitative [^{14}C]deoxyglucose (2-DG) method.

Adult male Sprague-Dawley rats were anesthetized with Equithesin and the sino-aortic baroreceptors denervated. Sham-operated animals had the same surgical incision but the baroreceptors were left intact. The 2-DG experiments were performed 7-10 days after surgery. Four groups of animals were studied, two sham and two with baroreceptor denervation; each group was intravenously infused with saline or AII. Mean arterial blood pressure but not heart rate was significantly higher in baroreceptor-denervated rats than in sham-operated animals. AII induced a significant bradycardia in sham-operated rats but had no effect on baroreceptor-denervated rats. AII stimulated drinking in sham-operated and denervated rats with latencies and total amount of water consumed that were not statistically significantly different (latencies of 4.3±0.9 and 4.7±0.7 min and water intakes of 4.1±0.7 and 5.0±0.8 ml, respectively, for sham and denervated rats). Accordingly, the rates of glucose utilization (umoles/100g/min, means±SEM) in the SFO and NL in response to AII were not different in these animals, as shown in the table below.

	Sham + Saline (n=9)	Sham + AII (n=8)	Deaff. + Saline (n=6)	Deaff. + AII (n=8)
SFO	36 ± 2	48 ± 6	37 ± 4	59 ± 3
NL	26 ± 2	49 ± 3	30 ± 5	58 ± 7

These results suggest that the influence of baroreceptors on the functional activity of the SFO is too small to be detected by the 2-DG method.

217.3 NALOXONE DOES NOT PREVENT GABA-INDUCED INHIBITION OF THE RELEASE OF VASOPRESSIN AND OXYTOCIN BY ANGIOTENSIN II. J.Y. Summy-Long, L.M Rosella-Dampman and R.D, Hartman*, M.S. Hershey Medical Center, Dept. Pharmacology, The Pennsylvania State University, Hershey, PA 17033.

Endogenous opioid peptides (Summy-Long et al., J. Pharmacol. Exp. Ther. 217: 619, 1981) and gamma-aminobutyric acid (GABA, Unger et al., Eur. J. Pharmacol. 90:1, 1983) inhibit three of the central actions of angiotensin II (AII) to raise blood pressure and affect hydration (drinking, release of vasopressin). An interaction between GABAergic and opioid peptide systems in the CNS has also been documented (Wood et al. Neuroscience 4: 1479, 1979; Sawynok and Labella, Eur. J. Pharmacol. 70: 103, 1981). We therefore investigated if the inhibition of AII-stimulated release of vasopressin (VP) and oxytocin (OT) by GABA could be prevented by an opiate receptor antagonist, naloxone (NAL). Adult male Sprague Dawley rats were anesthetized with pentobarbital and the lateral cerebroventricle was cannulated. Three days later animals received three injections, one every 5 min: first, saline (SAL; 1 ml/kg) or NAL (5 mg/kg), sc.; second, artificial cerebrospinal fluid (CSF; 5 ul) or GABA (500 µg/5ul), intracerebroventricularly (IVT); and third, CSF (5 ul) or AII (100 ng/5 ul), IVT. Rats were decapitated 1 min later. VP and OT were extracted from plasma and quantified by RIA. Data, expressed as pg/ml (mean ± SEM) were analyzed by 3-way AOV. AII (SAL-CSF-CSF vs SAL-CSF-AII) elevated ($p < 0.05$) plasma [VP] (1 ± 0.5 vs 64 ± 5) and [OT] (9 ± 2 vs 65 ± 9). GABA attenuated ($p < 0.05$) the AII (SAL-CSF-AII vs SAL-GABA-AII) stimulated rise in [VP] (64 ± 5 vs 2 ± 0.6) and [OT] (65 ± 9 vs 23 ± 4) in plasma. Endogenous opioid peptides inhibited release of OT by AII as naloxone (SAL-CSF-AII vs NAL-CSF-AII) elevated ($p<0.05$) [OT] (65 ± 9 vs 367 ± 51) but not [VP] (64 ± 5 vs 57 ± 8) in plasma. However, GABA IVT in the presence of naloxone inhibited ($p<0.05$) AII-induced (NAL-CSF-AII vs NAL-GABA-AII) release of VP (57 ± 8 vs 2.5 ± 0.2) and OT (367 ± 51 vs 50 ± 10). Naloxone also increased ($p<0.05$) plasma [OT] (CSF-CSF: 9 ± 2 vs 23 ± 6; GABA-CSF: 14 ± 4 vs 26 ± 3; CSF-AII: 65 ± 9 vs 367 ± 50; GABA-AII: 23 ± 4 vs 50 ± 10) but not [VP] in plasma of all animals regardless of treatment. Therefore, opioid peptides do not appear to mediate GABA-induced inhibition of AII-stimulated release of VP and OT from the hypothalamo-neurohypophysial system. (supported by grant R01 HL32826).

217.4 NEUROHYPOPHYSEAL BLOOD FLOW AUTOREGULATORY RESPONSE TO HYPOTENSION. D.A. Wilson, D.F. Hanley and R.J. Traystman (SPON: R.L. Schnaar). Department of Anesthesiology and Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Arterial hypotension is a potent stimulus for the release of arginine vasopressin (AVP). The entry rate of this peptide into the systemic circulation is dependent in part, upon the neurosecretory rate of the magnocelluar neurons of the supraoptic nucleus and in part, upon neurohypophyseal blood flow (NHBF). In other brain regions it is clear that blood flow is autoregulated, provided arterial blood pressure (AP) does not fall outside of the lower limits of the autoregulatory curve. However, little is known about neurohypophyseal pressure-flow relationships. In this study we have examined the interelationships between AP, NHBF, and AVP.

Regional cerebral blood flow (rCBF) and regional neurohypophyseal blood flow (NHBF) was measured by the radiolabelled microsphere technique in 5 sodium pentobarbital anesthetized dogs. Microspheres were injected under control conditions and at 3,5,10, 15 and 30 minutes after reducing AP to 80 mmHg. Similar measurements were made in 5 animals treated with 10 ug/kg d$(CH_2)_5$ Tyr (Me) AVP. Plasma AVP levels were determined by radioimmunoassay. In no cerebral region (cerebrum, caudate nucleus, white matter, hypothalamus, cerebellum and brain stem) was there a change from control blood flow levels at anytime during hypotension. However, within the first three minutes, neural lobe blood flow (NLBF) increased from 5.9 ml/min/gm to 18 ml/min/gm. Following the initial spike, NLBF returned to its control level. Comparison of the NLBF and AVP transients demonstrated that NLBF was increasing as AVP was being rapidly secreted into the systemic circulation. Whereas AVP was 2 pg/ml in the control state and 52 pg/ml after 30 minutes of sustained hypotension, NLBF was similar at the two AP levels. Thus, NLBF and AVP were not correlated in the steady state. AVP receptor blockade had no effect on either the relative height of NLBF spike or the time course of the NLBF response to hypotension.

We conclude that NHBF is autoregulated. The phasic characteristics of the compensatory adjustments appear to reflect simultaneously induced changes in AVP neurosecretory rate. It does not appear that V1 vascular receptors are involved in the non steady state NLBF response or that the NLBF response is the result of local metabolic events. The transient nature of the response suggests involvement of a neural mechanism.

217.5 DECREASED VASOPRESSIN AND OXYTOCIN CONTENT AND RELEASE IN CULTURED HYPOTHALAMIC EXPLANTS FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR).

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The paraventricular (PVN) and supraoptic (SON) nuclei contain neurons which synthesize the peptide hormones, vasopressin (VP) and oxytocin (OT). There are various theories linking alterations in central neuropeptide secretion with the presence of hypertension. These experiments were designed to investigate the CNS changes in the SHR using tissue culture methodology, monitoring neurochemical and morphological parameters. Thirty day old SHR and Wistar-Kyoto (WKY) rats were sacrificed and microdissected nuclear regions were collected. Four PVN or SON explants were placed in each tissue culture well. Peptide secretion into the media was assayed at 3 and 7 days over a 24 hour period. Results show that both VP and OT content and release were decreased in the SHR at both time points. Table 1 shows that VP secretion from the PVN is significantly lower in SHR at both 3 and 7 days. Although not shown, SON VP release is also significantly reduced in the SHR.

TABLE 1-VP Release (pgs/well)

	SHR	WKY
3 d.	10.7±4.5*	37.3±7.8
7 d.	5.1±1.2*	32.6±7.7

n=8;* = p < .03

TABLE 2-VP Content(pg/ug protein)

	SHR	WKY
0 d.	21.6±9.2	33.7±5.7
7 d.	9.8±2.1*	27.1±8.4

n=4;* = p < .02

Content of VP and OT in tissue was measured at 0 time and after 7 days incubation (Table 2). The difference in VP content was seen at 0 time and was significantly reduced after 7 days in culture. Similar results were found for OT secretion and content. Initial OT content is significantly lower, as is release, but that effect is more pronounced after 7 days of culture. Immunocytochemical (ICC) data were obtained from 13 day hypothalamic cultures of SHR and WKY neonates (3-5 day). ICC staining for neurophysin showed that there were many more positive fibers and cells in the SHR hypothalamic cultures than in the WKY. This would infer that there is a difference in the manner in which the peptide precursors are processed in vitro between SHR and WKY rats.

In summary, the PVN and SON of SHR rats contain and secrete less VP and OT in vitro than do WKY rats at the time points we examined. These findings confirm previous work in which differences were found in central peptidergic systems in the SHR and also reveal that these changes are present even after long term culture.

217.6 ACTH RESPONSE TO STRESS AFTER CONTINUOUS INTRAVENTRICULAR ADMINISTRATION OF CORTICOSTERONE ANTISERUM.

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The present study attempts to characterize the role of glucocorticoid negative feedback at suprapituitary sites in the regulation of ACTH secretion. Continuous removal of free corticosterone (CS) from the brain by corticosterone antiserum (anti-CS) serves as a model of site-specific adrenalectomy and alters ACTH secretion presumably without direct changes in either corticotrope responsiveness or other glucocorticoid actions. Cerebral cannulae were stereotaxically implanted into the lateral ventricle of female Sprague-Dawley rats (230±5(SEM) g) and joined to subcutaneous osmotic minipumps filled with anti-CS or normal rabbit serum (NRS) (mean fill volume=250.0±1.9 mg, flow rate=0.46 µl/hr). The jugular vein was cannulated for repeated blood sampling from freely-moving, acclimatized rats. On days 1, 3 and 7 after surgery, blood samples were taken prior to (t=0), 10 and 40 min after the onset of intermittent footshock (1 min, 1mA) stress. Serum ACTH measured by RIA.

Pre- and Post Footshock ACTH (pg/ml), ±SEM, (n)

Treatment	day 1 t=0	day 1 t=10	day 1 t=40	day 3 t=0	day 3 t=10	day 3 t=40	day 7 t=0	day 7 t=10	day 7 t=40
NRS	157.3 ±39.0 (9)	286.9 ±51.2 (7)	223.6 ±28.9 (9)	47.9 ±8.5 (7)	331.2** ±68.3 (8)	92.5 ±17.5 (8)	66.0 ±17.9 (7)	294.5* ±82.4 (8)	95.6 ±24.1 (7)
anti-CS	127.4 ±30.4 (7)	401.4** ±69.1 (8)	157.6 ±31.0 (7)	52.4 ±7.8 (8)	188.9***++ ±33.9 (8)	56.9 ±6.6 (8)	144.7 ±49.2 (6)	604.0***++ ±245.4 (3)	172.4 ±73.6 (3)

*=(p<0.05); **=(p<0.01) differs from t=0 within treatment group; +=(p<0.05); ++=(p<0.01) differs from NRS controls

On day 1 footshock produced significant ACTH response in anti-CS but not in NRS rats, while the day 3 response was significantly lower in anti-CS than in controls. Basal ACTH levels returned to normal by day 3 in both groups but by day 7 tended to be higher (p=0.06) in anti-CS than in NRS rats. Similarly, the stress response at 10 min was higher (p<0.01) on day 7 than on day 3 in anti-CS rats but did not differ in controls. In summary, the continuous removal of free CS by anti-CS tended to increase basal ACTH levels and markedly increased the stress response on day 7. Presumably the lack of glucocorticoid inhibition at suprapituitary sites led to increased CRF output in anti-CS rats. However, because these rats had adrenals, increased secretion of CS in response to the previously elevated ACTH on day 1 acted to decrease the ACTH response to stress at the corticotrope which may have caused the decreased responsiveness seen on day 3. By day 7 the disinhibition-induced increase in CRF may have overcome the glucocorticoid negative feeback action on the pituitary and may have produced the resulting higher basal and stimulated ACTH secretion.

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217.7 ADRENERGIC AND NEUROPEPTIDE Y SYSTEMS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS AFFECT CIRCULATING LEVELS OF CORTICOSTERONE AND GLUCOSE.

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Monoamine and peptide systems in the paraventricular nucleus (PVN) and other hypothalamic areas were examined with respect to their role in the control of circulating levels of corticosterone (CORT) and glucose (GLUC). Sprague-Dawley male rats (350g) were stereotaxically implanted with Khavari-type swivel cannulas aimed at specific hypothalamic sites. The rats were adapted to the test paradigm, which involved the attachment of a PE-20 tubing containing fresh solution to the implanted swivel and, two hours later, a slow remote drug infusion into the hypothalamus (0.5ul/30sec),with no disturbance to the animal. On the actual test day (with food and water absent), this procedure was performed with either drug or saline vehicle,and 15 min after infusion, the rats were rapidly sacrificed and trunk blood collected within 40 sec of the initial disturbance to the animal. Serum CORT was measured via radioimmunoassay, and glucose assayed via a YSI Glucose Analyzer.

Relative to the baseline levels (approximately 3.5ug% for CORT and 135mg/dL for GLUC) observed after saline administration, norepinephrine (NE) injection directly into the PVN dose-dependently increased serum CORT levels, up to 11.0±1.2ug% (p<0.001) with an NE dose of 40nmoles. A simultaneous increase in GLUC levels (150± 6.2mg/dL, p<0.05) also occurred. A similar effect of CORT levels was observed with NE injection into the dorsomedial nucleus. However, little or no effect was seen after NE stimulation of either the ventromedial,posterior or supraoptic nuclei or the perifornical lateral hypothalamus. PVN infusion of serotonin (40nmoles)produced a small increase in serum CORT levels (to 7.0±0.6ug%, p<0.05), whereas dopamine (40nmoles) had no effect. Epinephrine (EPI, 40 nmoles) in the PVN was even more potent than NE, in releasing CORT (to 18.9±3.2ug%,p<0.001)and inducing a rise in serum GLUC levels (to 210±26.2 mg/dL, p<0.01).

Neuropeptide Y (NPY,0.156nmoles) infused directly into the PVN altered CORT levels in a similar manner to NE and EPI, with which NPY is known to coexist endogeneously. Serum CORT was raised to 14.1±1.7ug% (p<0.001) within 15 min of infusion. However, in contrast to NE and EPI, NPY had no effect on serum GLUC levels in these same animals (139±2.7 mg/dL).

Hence, we propose that the adrenergic and neuropeptide Y systems within the PVN, where corticotrophin-releasing factor cell bodies are known to be particularly dense, have a potent stimulatory role in the control of CORT release. In light of their similar effects after administration into the PVN, it is possible that endogeneous catecholamines(NE and EPI) and NPY,known to coexist within the same PVN neurons,may interact in a positive manner in this process.

217.8 EFFECTS OF ORCHIDECTOMY ON THE PULSATILE RELEASE OF CORTISOL IN ADULT MALE RHESUS MACAQUES.

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Ultradian patterns of serum cortisol were assessed in adult male Rhesus macaques (Macaca mulatta) at the time of orchidectomy and compared with cortisol patterns in controls and animals that experienced sham surgery. Sequential blood samples were drawn every 15' for 28h(hours) beginning at 0800h and serum cortisol concentrations were determined by RIA. Orchidectomies and sham surgeries were performed 4.0-6.0 hours after the first blood samples were collected. For purposes of data analyses, group cortisol measurements were collapsed into seven blocks representing mean cortisol concentrations across 4 hour time intervals. These data were submitted to split-plot factorial ANOVA, and pairwise comparisons between means were determined using non-directional t-tests. The pulse frequency of cortisol release across the 28h sampling period was determined for each subject utilizing PULSAR analysis.

A diurnal pattern of cortisol secretion was documented in the control and sham surgery groups. Cortisol concentrations peaked during the early morning hours (0400-0800h) and reached a nadir in late afternoon (1600-2000h) with mean frequency of cortisol pulses equal 20/28h vs 17/24h. No significant differences between the controls and those animals undergoing anesthesia and surgical cutdown (sham surgery) were detected throughout the 28h sampling period. However, significant but short-lived elevations in cortisol levels were observed immediately in animals subjected to castration. Mean circulating levels of cortisol were six-fold greater (12.9 ug/100ml) than levels displayed by intact animals (2.27 ug/100ml) and two-fold greater than levels displayed by the sham surgery group (4.96 ug/100ml) from 1200-1945h. Cortisol elevations returned to normal in the castrate group at 2000h and remained at baseline throughout the remainder of the sampling period. The pulse frequency of cortisol released throughout the sampling period did not differ between control, sham, and castrate groups.

These findings concerning the effects of sham surgery and orchidectomy on the pulsatile release of cortisol in the Rhesus macaque demonstrate: 1)that the actual removal of the testes results in a significant elevation in serum cortisol levels compared to that observed in shams, and 2) the acute rise in cortisol in the castrate group is transient, i.e., only lasts 8h. These data are consistent with previous findings that suggest the existence of a direct neural connection between the testes and the central nervous system.

217.9 MATURATION OF THE ACTH SECRETORY RESPONSE TO CENTRAL NICOTINIC CHOLINERGIC STIMULATION. S.G. MATTA*, H.S. BEYER*, K. MC ALLEN* AND B. SHARP* (Spon.: R. Phillips). Minneapolis Medical Research Foundation and Dept. of Medicine, Hennepin County Medical Center, University of Minnesota, Minneapolis, Minnesota 55415.

Nicotine is a potent stimulator of ACTH release in the adult rat. The present study was undertaken to assess the development of responsiveness to nicotine and to determine if the effect is mediated by central or peripheral mechanisms. Nicotine (N) (1 or 2 mg/kg BW, free base, IP) or saline were administered to neonatal rats and trunk blood for ACTH by RIA was collected 7.5 min after the final injection. All comparisons were by ANOVA. Nicotine significantly elevated ($p < 0.05$) plasma ACTH (pg/ml, mean $\pm$ se) at all ages and the response increased markedly with age ($p < 0.01$):

	S	N 1.0	N 2.0
d1	29 $\pm$ 4	62 $\pm$ 5	65 $\pm$ 6
d4	23 $\pm$ 6	71 $\pm$ 6	103 $\pm$ 14
d7	27 $\pm$ 4	125 $\pm$ 26	160 $\pm$ 16
d15	98 $\pm$ 13	712 $\pm$ 104	900 $\pm$ 153
d25	51 $\pm$ 6	---	1172 $\pm$ 80

By 15d, ACTH levels were similar to those in adults. To investigate nicotine's site of action, 26d rats were injected ip with 0.25 mg/kg BW N 10 min after the administration of saline (S) or 2.0 mg/kg BE nicotinic cholinergic antagonist: Hexamethonium (H) which does not cross the blood-brain barrier, or mecamylamine (M), which does. ACTH levels were: S/S 84 $\pm$ 8; S/N 738 $\pm$ 87; M/N 136 $\pm$ 22; H/N 519 $\pm$ 51 (S/S = M/N < H/N = S/N, $p < 0.01$). Other experiments compared equimolar concentrations of N with cytisine (C), a potent agonist which does not cross the blood brain barrier, administered iv in the same adult animal on separate days via chronic jugular cannulae. Neither C nor 3x C affected ACTH at any time point, whereas N elicited peak elevation by 7 min (562 $\pm$ 124 vs baseline: 28 $\pm$ 7). Studies on the effect of centrally administered nicotine were conducted on adult rats with ICV cannulae. On separate days in each animal, 2.5 ul buffer (pH 7.3) or 10 ug N in buffer was injected over 30s into IIIV and blood was drawn via jugular cannulae. Four out of five rats responded with peak ACTH levels by 7 min ($p < 0.01$), in the absence of visible behavioral change. Thus, it appears that a nicotinic cholinergic mechanism for ACTH secretion is present on d1 and rapidly matures, reaching levels similar to adult by d15. The selective blockade by mecamylamine in contrast to hexamethonium, the lack of responsiveness to cytisine and the efficacy of ICV nicotine indicate that a central mechanism is operative. (Supported by DA03977).

217.10 EFFECTS OF CHRONIC BEHAVIORAL STRESS ON CORTICOTROPIN-RELEASING HORMONE (CRF) AND ITS CO-EXPRESSION WITH OXYTOCIN (OT) IN THE RAT PARAVENTRICULAR NUCLEUS (PVN). A. Hou-Yu, D.D. Kelly+ and A-J. Silverman. Departments of Anatomy & Cell Biol., and Psychiatry+, Columbia Univ., N.Y., N.Y. and NYS Psychiatric Inst+. 10032

The 41 amino acid peptide, CRF, is the major regulator of ACTH release. In addition both OT and vasopressin (VP) can potentiate the action of CRF and have been found in cells with CRF. The present experiments address the question of the effect of chronic behavioral stress on the CRF neurons of the PVN and their ability to co-express neurohypophysial peptides. Adult male Wistar rats were exposed to 72 hr or 10 days (n=3 per group) of alternating 30-min sessions of 92 db white noise and of conditioned emotional response training which consisted of intermitent pairings of auditory warning stimuli and brief inescapable footshocks. Normal, no stress controls (n=3) remained in their individual home cages. All animals were perfused under pentobarbital anesthesia with paraformaldehyde fixative, brains post-fixed at rm temp for 24 hr and 25 um vibratome sections. Sections through PVN were first stained for CRF using a rabbit antiserum (gift of C. Hollander), an avidin-biotin-HRP kit and DAB as the chromagen. Alternate sections were then double stained with a specific monoclonal antibody to either OT or VP which was visualized with an avidin-fluorescein marker.

Following extended exposure to the stress paradigm several reliable changes occurred in the PVN neurons of these physiologically intact, non-colchicine treated subjects. (1) After 72 hrs of stress there was a significant increase in the number of neurons expressing both CRF immunoreactivity (CRF-ir) and OT. In the unstressed animal CRF-ir was found predominately in the medial aspect of the parvocellular sub-population of the middle PVN and also in large cells in the rostral and middle PVN that fluoresced intensely for OT. Co-expressing cells represented 12% of all CRF-ir neurons in the normal PVN. This increased to 27% ($p=0.032$) following 72 hr stress and the increase occurred predominately in the middle PVN. We saw no evidence for co-expression of CRF and OT in parvicellular neurons in non-colchicine treated animals. Nor did we observe CRF/VP coexpression in the PVN. (2) After 10 days of stress there was an increase in the number of cells making CRF ($p=.06$). CRF neurons also increased in size (102.7 um vs 183.6 um, $p<.001$) and in staining intensity (Chi sq. 6, $p=.0137$). These data illustrate that an organism's prior history of exposure to stress can induce plastic changes in neurosecretory neurons responsible for the control of the pituitary-adrenal axis.

217.11 PERMANENCE OF CHANGES IN CORTICOTROPIN RELEASING HORMONE (CRF) NEURONS IN THE RAT PARAVENTRICULAR NUCLEUS (PVN) IN RESPONSE TO CHRONIC BEHAVIORAL STRESS. A.J. Silverman, A. Hou-Yu and D.D. Kelly+, Depts of Anat & Cell Biol, and Psychiatry+, Columbia Univ., N.Y., N.Y. and NYS Psychiatric Inst+., NY NY, 10032 .

We have found that following 10 days of exposure to chronic behavioral stress CRF neurons in the PVN of intact rats increased in number, in size and in staining intensity, parameters indicative of increased neurosecretory activity. To determine the relative permanence of these changes, adult male rats were stressed for 10 days and then returned to their home cages (vacation) for 21 additional days before preparing the brains for immunocytochemical detection of CRF. The stress paradigm consisted of alternating 30-min exposure to loud white noise and to conditioned emotional stimuli. No stress control subjects remained in their individual home cages and were maintained on a 12:12 LD. 21 days following the conclusion of the stress paradigm, all animals were perfused under pentobarbital anesthesia with 4% paraformaldehyde, the brains post-fixed at 4C for 24 hr and 75 um frozen sections cut. Sections through the rostral-caudal length of the PVN were stained for CRF using a rabbit antiserum (gift of C. Hollander), an avidin- biotin-HRP kit and DAB as the chromagen. Cell size was measured with the aid of a computerized image analysis system. Staining intensity was rated on a psychophysical scale by an observor who was uninformed as to the behavioral history of the subjects.

CRF cells in previously stressed animals remained significantly larger than controls (two tailed 't' test, $p<0.001$). Moreover, the staining intensity of CRF neurons in these previously stressed but vacationed animals was enhanced in the same manner as in subjects sacrificed immediately following 10 days of stress exposure. Staining intensity in the vacationed subjects differed significantly from corresponding controls on a non parametric median test (Chi sq. 6.0, $p=0.0137$). These experiments demonstrate that plastic changes in CRF neurons induced by stress are maintained for at least 3 wks after exposure to the stressor has ended. We acknowledge that whether the increased size and peptide content reflect a continued hypersecretion of CRF cannot be ascertained from our data. Nevertheless, these quantitative anatomical observations raise the possibility that long after an extended exposure to stress the hypothalamus of once-stressed animals might remain capable of responding in a vigorous or exaggerated fashion to novel stressors.

217.12 BEHAVIORAL CORRELATES OF A CHRONIC STRESS PARADIGM THAT ALTERS HYPOTHALAMIC CRF NEURONS. Dennis D. Kelly (a), Ann-Judith Silverman and Anna Hou-Yu. New York State Psychiatric Institute (a), and Departments of Psychiatry (a) and Anatomy & Cell Biology, Columbia University, New York, NY 10032.

We have observed that when a physiologically normal rat is stressed for 72 hrs by exposure to alternating 30-min periods of white noise and of conditioned emotional stimuli, the number of cells in the hypothalamic paraventricular nucleus that co-express both corticotropin releasing hormone (CRF) and oxytocin increases. After 10 days of similar exposure, the number, size and staining intensity of CRF neurons also increase, and the latter changes endure for at least three weeks after the environmental demands upon the organism have returned to normal.

To characterize the behavioral consequences of exposure to the same chronic stress paradigm, 48 adult male rats were exposed in weight- and age-matched groups (n=6 each) to 72 hrs or 240 hrs of stress, or to corresponding no-stress control conditions. Nociceptive tail-flick thresholds were systematically tracked in half of the groups before, during and after stress exposure. In the other half acoustic startle responsivity was monitored. Daily water intake and body weight were measured in all subjects.

The temporal pattern of adaptation to continuous stress exposure differed markedly among the behaviors. Water intake decreased by almost 50 % during the first 24 hrs of stress exposure, which reflected principally a decline in prandial drinking. Recovery of normal consummatory behavior in most subjects was rapid, returning to normal within the 72-hr paradigm. Weight decreased steadily throughout the 72-hr stress paradigm and through the initial 5-7 days of the 10-day paradigm, recovering slowly thereafter. Nociceptive thresholds: While acute exposure to neither stressor alone (noise or CS-US pairings) induced analgesia, chronic alternating exposures to both resulted in a slow developing, yet profound analgesic state which peaked at 24-32 hrs after stress onset and which was maintained throughout the 72-hr paradigm. Startle Responsivity: In contrast to the decline in nociceptive reflex sensitivity, prolonged cycles of hyperresponsivity to the acoustic startle stimulus also accompanied chronic stress exposure. This suggests that the decreased nociceptive thresholds did not result from a general motor deficit. Most interesting in light of the relative permanence of the stress-induced changes in hypothalamic CRF cells described in the preceding abstract, the cyclic periods of enhanced startle responsivity that developed during 10-day stress exposure were maintained in many subjects throughout the 21-day post-stress behavioral vacation. Whether the stress-induced plasticity displayed by hypothalamic neurosecretory neurons is related to the regulation of behavior under stress remains to be determined. (USPHS Grant NS 18822)

218.1 POSTNATAL DEVELOPMENT OF AXONAL BIFURCATION IN THE AFFERENTS TO THE VISUAL AREAS OF THE TWO CEREBRAL HEMISPHERES OF THE RAT. J. Bullier*,C. Dehay* and B. Dreher* (SPON: European Neuroscience Association) INSERM U94, 69500 Bron, France, and Dept. of Anatomy, Univ. of Sydney, Australia.

It is known that, during the first two weeks of postnatal life of the rat, many neurons in the parietal cortex send one axonal branch in the ipsilateral cortex and another in the contralateral hemisphere (Ivy,G.O. and Killackey, H.P., J. Neurosci., 2:735-743, 1982). The number of such neurons with bilateral connections decreases dramatically during the postnatal development. We have tested the generality of the retraction of bilateral cortical afferents by injecting the visual cortex of each hemisphere with one of two different retrograde fluorescent tracers. These injections were performed at different ages and we estimated the proportions of doubly labelled neurons in the afferent structures. An extensive injection of the fluorescent tracer diamidino yellow was placed in the visual areas of one hemisphere while a small injection of another fluorescent dye, fast blue, was made in area 17 of the other hemisphere. In the thalamus, the labelling was strictly ipsilateral at all ages except for a small amount of mixing of the two populations of labelled cells in the midline nuclei of animals aged 10 days or less. The claustrum contained a mixture of ipsi and contralaterally labelled neurons and a small percentage (about 1%) of bilaterally labelled neurons. The percentage of such neurons with bilateral projections did not change with age. In the cortex, all areas contained some doubly labelled neurons. The proportions of bilaterally projecting neurons with respect to ipsilaterally projecting cells varied between 8 and 30% for different cortical areas in the adult animal. This proportion was unchanged with age in area 8 whereas in other cortical areas (18a, 18b, 29, 35) it was higher (50-60%) in animals aged 10 days or less than in the adult. These results demonstrate that, in certain visual cortical areas, the proportion of bilaterally projecting neurons decreases after 10 days of age, suggesting that the reduction of callosal connectivity known to occur at that age involves the retraction of axon collaterals. On the other hand, in structures such as area 8, the claustrum or the thalamus, the laterality of the corticopetal connections appears to be already established at birth.

218.2 POSTNATAL DEVELOPMENT OF CORTICO-CORTICAL CONNECTIONS. T.A. COOGAN* AND A. BURKHALTER. Dept. Neurosurgery and the McDonnell Center for Studies of Higher Brain Function, Washington University Medical School, St. Louis, MO 63110.

It is well known that different types of cortico-cortical connections originate from different layers with different compositions of pyramidal and nonpyramidal neurons. To provide a clearer understanding of the processes that underlie the formation of this organization, we began to study the postnatal development of the projection from primary (area 17) to secondary (area 18a) visual cortex in the hooded rat.

Injections of rhodamine labelled beads were made into area 18a at postnatal day P1, P6, P16 and P30. Animals injected at P1, P6 and P16 survived until P20, when migration of cortical cells is complete. Young adult animals survived for one week. The brains were sectioned and the laminar distribution of retrogradely labelled cells in area 17 analyzed and compared.

After injections at P1, the majority (80%) of labelled striate cortical cells are located in lower layers, 5 and 6. In contrast, the proportion of labelled layer 4 (9%), layer 2/3 (6%) and layer 1 (5%) cells was low. In P6 animals the percentage of labelled cells in granular (19%) and supragranular layers (18%) is increased, while the percentage of cells in layers 5 and 6 was decreased (63%). The projection in P16 animals resembled that of adults, in that it originated predominately from layers 2/3 (52%) and the contribution of layers 4 (8%), 5 and 6 (40%) was diminished. In order to quantitate the changes within a single layer we calculated the density of labelled cells. Between P1 and P6 the density increased in layers 2-6. This trend continued in layers 2/3 between P6 and P16. In contrast, between P6 and P16 the density of layer 4 and upper layer 6 labelling decreased. A similar decrease was found in layer 1 labelling between P1 and P6. The density of labelled cells in layer 5, however, did not change appreciably.

To investigate possible explanations which could account for these developmental changes, we performed double-labelling experiments. Area 18a was injected with beads at P6, and the same site was reinjected with Fast Blue (FB) at P16. In such cases, most FB labelled cells were found in layers 2/3 where bead labelling was sparse. Conversely, bead labelling was restricted to layers 4 to 6, where FB cells were rare. Double labelled cells were found in layers 5 and 6 only. This indicates that the fibers from layer 2/3 develop after P6 and destruction of fibers cannot account for the absence of labelling at P6. In addition, cells in layer 4 do not die or migrate into upper layers, rather they withdraw their axons.

These results provide for two conclusions: 1) The layers in area 17 are labelled in the inside-out order they are generated, thus, axons arrive in area 18a in the order in which the cells of origin are born, and; 2) The mature pattern of connections is produced by retraction of axons from their initial targets. (Supported by NIH grant EY05935).

218.3 **THE POSTNATAL DEVELOPMENT OF THE POSTERO-MEDIAL SUPRASYLVIAN AREA OF THE CAT; A QUANTITATIVE ELECTROPHYSIOLOGICAL STUDY.** Colin Blakemore, D.J. Price* and T.J. Zumbroich*. University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Although there have been a number of studies of the normal postnatal maturation of the primary visual cortex of the cat, little is known about the time-course of development of extrastriate areas. In the adult cat one such area , the postero-medial lateral suprasylvian area (PMLS), contains a particularly high proportion of cells that are selective for the direction of stimulus motion, and which generally have low spatial resolution compared to neurons in area 17. We were interested in studying the time-course of development of different response properties in PMLS.

We have recorded from PMLS in kittens aged between 9 days and 8 weeks as well as in adult cats. Amongst the properties we assessed qualitatively were response strength, ocular dominance and degree of habituation to repeated visual stimulation. For quantitative measurements, high-contrast drifting or stationary, flashed sine-wave gratings were presented on a display screen and spikes collected under the control of a computer to assess the selectivity for the direction, the orientation, the spatial frequency and the temporal frequency of the stimulus (using a statistical response criterion).

At 9 days many cells were visually unresponsive, but at 12 days units responded preferentially to moving stimuli and 25 % of the cells showed clear direction selectivity, but were more broadly tuned (mean half-width at half amplitude: 32.8^{o}) than in adult PMLS. In the 12-day-old kitten 77% of neurons showed rapid habituation to repeated visual stimulation, but this percentage decreased with age to 13 % in adult cats. The majority of responsive units at all ages could be driven independently from either eye.

At the end of the 3rd week of postnatal life, direction selectivity seemed to have matured completely: 79 % of units were clearly direction selective (the same proportion as in adult cat) and the mean half-width was 23.9^{o} (in adult animals: 23.1^{o}). In all animals older than 9 days, tests with stationary flashed gratings of varying orientation revealed that a substantial proportion of cells was orientation-selective. The major improvement of selectivity for spatial and temporal frequency also occurred during the first 3 weeks of postnatal life.

A comparison of our data from PMLS with those of previous studies on striate cortex suggests that development of receptive field properties is comparable in many respects to that in striate cortex, but, if anything, PMLS matures more rapidly.

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218.4 **BINOCULAR INTERACTION IN YOUNG KITTENS.** R.D. Freeman and I. Ohzawa*, Neurobiology Group, 360 Minor Hall, University of California, Berkeley, CA 94720.

Nearly all neurons in the striate cortex of the cat exhibit some degree of binocular interaction. Suggestions have been made that these cells signal different binocular disparities and hence form the substrate for depth discrimination. These neurons have also been examined in young kittens in relation to questions of normal and abnormal development, and results appear to be somewhat conflicting. It is important to establish if visual connections are functional during the substantial changes that occur in early postnatal weeks of the kitten. In the study reported here, we have focused on binocular function.

We use a robust technique to study binocular interaction in single cells of visual cortex. Two sinusoidal gratings, under independent control, are presented to left and right eyes, respectively, such that one stimulus is phase-shifted with respect to the other. A sequence of phase varying stimuli is used to test a given cell, after optimal parameters of orientation, spatial frequency and velocity have been determined monocularly. All stimulus presentation and data acquisition is under computer control.

We studied kittens at 2,3, and 4 weeks postnatal. A surprising degree of binocular specificity was found in all three age groups. At 2 weeks, around half the cells exhibit phase-specific binocular interaction. For some relative phases, responses are substantially larger than either monocular response and for other phases, response was suppressed markedly. This kind of binocular interaction is seen even for cells which appear to be monocular, when tested alternately through either eye. A much smaller proportion of cells exhibited non-phase specific binocular interaction. Only about 25% displayed no interaction and were apparently monocular.

By 3 weeks, a large majority of cells show phase-specific binocular interaction of the type described above. Once again, relatively small proportions exhibit non-phase specific binocular interaction. The proportions of cells which were monocular and displayed no interaction were relatively small, around 15%. An additional group was observed for which a suppressive influence is seen from an eye that appears silent based on monocular tests. At 4 weeks, specificity is very close to that found in adults.

These results indicate a highly developed binocular organization of receptive fields of striate cortical cells in very young kittens, in spite of the considerable changes in interocular alignment that occur in early postnatal weeks. (EY01175)

218.5 BINOCULAR DEPTH PERCEPTION AND PUPILLOGRAPHIC CHANGES IN KITTENS REARED WITH INTEROCULAR TORSIONAL DISPARITY. Paul G. Shinkman, Brian Timney, and Michael R. Isley*. Univ. North Carolina, Chapel Hill, NC 27514, and Univ. Western Ontario, London, Canada N6A 5C2.

We studied the relation between the physiological and behavioral effects of rearing kittens with interocular torsional disparities. During the first several weeks of life kittens wore goggles fitted with prisms that introduced rotational disparities between the images in the left and right eyes. Each kitten experienced one of three disparity values: 16°, 32°, or 0° (control). We have reported previously the physiological effects of these rearing procedures. With respect to visual cortical binocularity, large (32°) disparities produce severe disruption while small (16°) disparities do not. These findings were verified for kittens in the present experiments at the conclusion of behavioral testing.

Sixteen kittens wore goggles for several hours daily between the ages of 4-12 weeks and were kept otherwise in the dark. At age 3 months the kittens were sent to the University of Western Ontario for behavioral testing. Using the jumping stand procedure, all kittens were tested for binocular and monocular depth perception, and three were also tested for acuity.

Behavioral results from the 0° and 32° groups were relatively straightforward. 32° kittens had poor binocular depth perception, consistent with the lack of binocular neurons, whereas control (0°) kittens, with a single exception, exhibited good binocular depth perception; that is, performance under binocular testing conditions was distinctly superior to monocular performance. This finding was not due to differences in acuity, since kittens in both groups showed acuity in the low normal range. The 16° group yielded mixed results. Three kittens showed clear superiority of binocular over monocular depth perception, and five did not. This within-group difference appeared to be due to pupillographic changes that occurred in some kittens as measured by vergence ratios. Three kittens in the 16° condition and one in the 0° condition that did not perform well were tested after an interval of several weeks in the light, during which time gradual changes in eye position occurred and the kittens became esotropic. The other two 16° kittens that failed to show binocular depth perception were found to have abnormal ocular alignment at the end of goggle rearing, before behavioral testing was initiated.

We conclude that binocular depth perception is disrupted in goggle-reared kittens either by large interocular torsional disparity leading to a loss of binocular neurons in visual cortex, or by changes in ocular position consequent upon visual deprivation followed by return to a normal visual environment. Small rotational disparity experienced early in life, however, does not by itself interfere with the normal development of binocular depth perception. (Supported by USPHS grant HD-03110 and MRC grant MA7125)

218.6 EFFECT OF DARK REARING ON DEVELOPMENT OF MYELINATION IN CAT VISUAL CORTEX. N.W. Daw, Dept. of Cell Biology, Washington Univ. Med. School, St. Louis, MO 63110.

Myelination in the cat visual cortex develops over the first four months of life (Haug, H., Kölln, M. and Rast, A., Cell Tiss. Res. 167: 265, 1976). This corresponds to the critical period for the development of the visual cortex in cats. Rearing cats in the dark prolongs this critical period (Cynader, M. and Mitchell, D.E., J. Neurophysiol. 43: 1026, 1980). We therefore decided to investigate whether dark rearing would also prolong the development of myelination.

Two litters of kittens (born three days apart) were reared, one in the light and one in the dark. Kittens from each litter were sacrificed at 64, 84, 105 and 168 days of age, and perfused with 10% formalin. Their cortices were sectioned and stained with a silver stain (Gallyas, F. Neurol. Res. 1: 203, 1979). Tissues from animals of the same age were processed at the same time. A distinct difference was noticed in the 84d animals. There was less myelination in layers IV, V and VI of the visual cortex of the dark reared animal. Comparing the cortices of the 105d animals, two horizontal bands were noticeable in area 17 of the light reared animal but not in area 17 of the dark reared animal. Myelination in the visual cortex of the 64d dark-reared animal was also less prominent than in the light reared animal of the same age.

As a control, sections were also taken from auditory, somatosensory and motor cortices. There was little difference in the myelination of light and dark reared animals in these areas: if anything, the myelination was heavier in the dark-reared animals.

In addition, an 86d animal with binocular eyelid suture was compared with an 87d normal littermate. Myelination in the normal animal was heavier than that in the binocularly deprived animal.

We conclude that dark rearing and binocular deprivation delay the myelination of visual cortex, and that the effect is not the result of a general delay in myelination, since it is specific to the visual system.

218.7 **PROGRESSIVE EFFECTS OF DARK-REARING ON THE RESPONSE PROPERTIES OF CELLS IN THE VISUAL CORTEX OF THE CAT.** A.S. Ramoa* and R.D. Freeman (SPON: T.E. Cohn). Neurobiology Group, 360 Minor Hall, University of California, Berkeley, CA 94720.

Cats that are deprived of light during early postnatal development, exhibit severe visual abnormalities. In the visual cortex of these animals, e.g., substantial proportions of neurons are unselective for orientation or are unresponsive to visual stimulation. The abnormal visual properties could be due to a developmental failure or a progressive degeneration of normal pathways, or a combination of both processes. In an attempt to separate these possibilities, we have studied two groups of cats that were dark-reared from birth. One group was studied as kittens (1 month postnatal) and the other as adults. Our questions were as follows. First, do the relative proportions of cells which are unresponsive or weakly responsive increase in long-term dark-reared cats? Second, are there unusual or abnormal response properties that are expressed in one group but not the other? To attempt to answer these questions, we used standard extracellular recording techniques coupled with a microiontophoretic method. This latter procedure allows us to study cells which are first classed as visually unresponsive, by application of an excitatory amino-acid (DL-Homocystate or DLH). DLH raises neuronal activity of these cells to above threshold.

Our results are as follows. At 3 weeks postnatal, properties of cortical cells are generally comparable in normal and deprived kittens, as shown in previous work. The same comparison in adults, normal or dark-reared, shows gross differences, as expected, and the following distinctive features in dark-reared adults as compared to kittens. **1)** Adults exhibit greater proportions of cells which are visually unresponsive. **2)** With application of DLH, some of these visually unresponsive cells displayed only suppression of maintained discharge in response to visual stimulation. This type of response was never found in kittens, normal or dark-reared, and it may represent a group of neurons receiving relatively strong inhibitory input. **3)** A greater proportion of orientation unselective cells. **4)** Some of these cells displayed a very strong, orientation unselective response from a large area of the visual field. Again, this was only found in adult cats.

These results show that considerable deterioration occurs progressively in dark-reared cats. There is an apparent degeneration of cortical connections, which probably accounts for the greater proportions of visually unresponsive cells in dark-reared adults. In addition, there appears to be an unbalanced development of excitatory and inhibitory connections, causing, in some cases, a predominance of inhibitory (finding 2) or excitatory (finding 4) responses. (EY01175)

218.8 SPLIT BRAIN INDUCES MONOCULAR DOMINANCE OF CELLS IN THE VISUAL CORTEX OF ADULT CATS. U. Yinon*, M. Chen* and A. Hammer* (SPON: Z. Wollberg). Physiol. Lab., Goldschleger Eye Inst., Tel-Aviv Univ. Sackler Fac. of Med., Sheba Med. Ctr., Tel Hashomer, 52621, Israel.

Our previous study (Yinon and Hammer, Exp. Brain Res., 58: 552, 1985) showed that cancellation of the direct thalamocortical input following surgical chiasm split prevents the appearance of binocularity in the majority of the cortical cells. The present study was carried out in order to find a possible supplementary effect resulting from interruption of the callosal input, assuming that it has also an effect on binocularity of cortical cells.

Single unit recording was made in cortical areae 17,18 boundary, the callosal projection zone, following optic chiasm and corpus callosum sagittal transection both in acute and in chronic adult animals (6 cats; 311 cells). In these experimental cats 98.2% of the cells recorded in both hemispheres were ipsilaterally driven and 1.8% were binocularly driven. In similarly studied split chiasm controls (10 cats; 550 cells), similar proportions of ipsilaterally (95.7%) and binocularly (2.8%) driven cells were found. When only the corpus callosum was sectioned in such cats for comparison (14 cats; 653 cells), the proportion of ipsilaterally driven cells was 29.7%; 43.8% of the cells were binocularly driven. In the normal controls (16 cats; 625 cells) the proportion of the ipsilaterally driven cells was 14.1%; 72.7% of the cells were binocularly driven.

It is concluded that from point of view of the visual cortex, elimination of the direct thalamocortical and indirect callosal inputs simulate a condition of split brain. This is mainly expressed by the almost complete disappearance of interhemispheric interaction in the experimental cats.

218.9 **CHEMICAL AND SURGICAL LESIONS OF THE ANTERIOR INTRALAMINAR THALAMUS YIELD DIFFERENT EFFECTS ON OCULAR DOMINANCE PLASTICITY IN KITTEN STRIATE CORTEX.** M.F. Bear, A. Kleinschmidt* and W. Singer. Max-Planck-Institut für Hirnforschung, 6000 Frankfurt 71, FRG.

Cat visual cortex receives a direct projection from the anterior intralaminar thalamus, including the central lateral (CL), para-central (PC) and central (CE) nuclei. Electrical activation of this region elicits conjugate saccadic eye movements and enhances the potentials evoked in visual cortex by stimulation of the optic nerves. Previous work in this laboratory has shown that electrical stimulation of the intralaminar thalamus will also facilitate ocular dominance modifications in anesthetized kittens (*Exp. Brain Res.* (1982) **47**: 223-233). Moreover, the surgical removal of the anterior diencephalon was shown to severely retard ocular dominance (OD) plasticity, even after periods of monocular deprivation (MD) in excess of 6 months (*Exp. Brain Res.* (1982) **47**: 209-222). Together, these data suggested that the intralaminar projections to striate cortex play an essential role in modulating visual cortical plasticity. However, a viable alternative interpretation is that both effects were due to an involvement of fibers passing through the thalamus. To examine this possibility, we produced lesions in the anterior dorsal thalamus by injecting the excitotoxin N-methyl aspartate (NMA), which destroys neurons but spares axons of passage, and then tested for a deficit in OD plasticity.

At approximately 4 weeks of age, 3 light-reared and 3 dark-reared kittens received unilateral thalamic injections of 50-75 µg of NMA through a microliter syringe. The needle tip was positioned stereotaxically to lie in the internal medullary lamina (A7, L3, D12.5). At the same time, one eyelid was sutured closed. 7 to 10 days later, we performed a standard OD assay in area 17. This revealed an apparently normal OD shift to the open eye in all cases. Subsequent histology confirmed that the anterior intralaminar nuclei had been destroyed. The lesions involved all of CL, PC and CE as well as portions of the medial dorsal, reticular, anterior medial, ventral lateral, ventral medial, lateral posterior and habenular nuclei. Therefore, the NMA lesions included all the structures that had been removed in the previous study.

Thus, while the intralaminar projections to area 17 may contribute to the modulation of cortical plasticity, they apparently are not *essential* for synaptic modifications to occur. These data suggest that the surgical lesions were effective in preventing normal OD plasticity because they interrupted pathways that traverse the thalamus.

218.10 **INFUSION OF AN NMDA RECEPTOR ANTAGONIST DISRUPTS OCULAR DOMINANCE PLASTICITY IN KITTEN STRIATE CORTEX.** W. Singer, A. Kleinschmidt* and M.F. Bear (SPON: L.N Cooper). Max-Planck-Institut für Hirnforschung, 6000 Frankfurt 71, FRG.

Glutamic acid or a closely related substance is thought to be the transmitter of excitatory axo-spinous synapses at many locations in the forebrain, including the visual cortex. It appears that two broad categories of glutamate receptor coexist postsynaptically: "NMDA" (A1) receptors and "non-NMDA" (A2/A3) receptors. A2/A3 receptors are thought to mediate "classical" synaptic excitation. A1 receptors, on the other hand, appear to be linked to non-selective cation channels which pass Ca^{++}, but only when the membrane is depolarized. Thus, Ca^{++} entry through A1-linked channels requires contingent pre- and post-synaptic activity. Previous work in this laboratory has suggested that pre- and post-synaptic coactivation is also a necessary condition for synaptic modification to occur in the striate cortex. Our present working hypothesis is that the experience-dependent modification of cortical synapses depends on A1 receptor activation. To test this hypothesis, we infused the A1 receptor blocker D,L-2-amino-5-phosphonovaleric acid (APV) into the striate cortex of monocularly deprived kittens, and then tested for a deficit in the ocular dominance (OD) shift.

Osmotic minipumps were connected to cannulae that were inserted in the striate cortex of 4-5 week old kittens. One hemisphere was infused with 50 mM APV at a rate of 1µl per hour; the other hemisphere received vehicle solution (saline). At the time of implantation, one eyelid was sutured closed. 7 days later we performed a standard OD assay in striate cortex near the cannula site. As expected, neurons in area 17 of the control hemispheres were tuned normally for stimulus orientation and their OD was shifted strongly to the non-deprived eye. In contrast, neurons in the APV-infused cortex displayed a striking decrease in orientation selectivity and their OD appeared to be unaffected by the MD. In some animals the pumps and cannulae were left *in situ* during recording. Visual cortical neurons still responded vigorously to light under these conditions, thus indicating that the APV had not caused an overt disruption of excitatory transmission.

These data are consistent with the hypothesis that A1 receptor activation is necessary for the expression of OD plasticity in the visual cortex. We suggest that the effects of APV on OD plasticity relate specifically to a blockade of postsynaptic Ca^{++} entry through gates linked to the A1 receptor. These results support the notion that changes in the efficacy of excitatory synapses in area 17 depend upon the postsynaptic activation of cortical neurons.

218.11 THE EFFECT ON MONOCULAR DEPRIVATION ON THE ULTRASTRUCTURE OF FUNCTIONALLY IDENTIFIED GENICULOCORTICAL CIRCUITRY IN THE CAT. M.J. Friedlander, K.A.C. Martin and V. Alones Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL and Dept. of Experimental Psychology, Oxford University, Oxford, U.K.

The neurons of the visual cortex of the cat undergo profound changes in their receptive field properties due to unequal visual input (as in monocular eyelid suture) to the two eyes during the critical period of postnatal development. The most striking change is the reduction in neurons activated by visual stimulation of the previously sutured eye. At least 2 hypotheses have been suggested to explain this finding: 1) The geniculocortical axon arborizations from the LGN_d layers innervated by the eye which was sutured make fewer and/or less potent synaptic connections with cortical neurons, 2) These synaptic connections are present in near normal degree and function, but are subjected to an intracortical inhibition. This effect may result from an increased innervation of GABAergic cortical neurons by geniculocortical axons from the non-deprived eye (that subsequently reduce the effectiveness of synaptic inputs from axons innervated from the deprived eye). We have begun a series of studies to examine these hypotheses. Individual geniculocortical axons in cats reared to one year of age with monocular eyelid suture (suturing done at 6-9 days of age) were physiologically classified as to cell type (X- or Y-), receptive field location and organization, normalcy of response to visual stimuli and ocular dominance by recording in the optic radiations with HRP-filled micropipettes. Axons were intracellularly filled by ionophoresis of HRP. Perfusion was with 2.0% Gluteraldehyde and 1.0% paraformaldehyde. Sections were vibratomed at 80um. The visual cortices were processed for HRP histochemistry (modified Hanker-Yates reaction), osmicated and embedded in plastic (Durcupan). Ultrathin sections were taken for analysis of divergence patterns and structure of synaptic boutons of geniculocortical axons innervated by the deprived and nondeprived eye. Semithin sections were treated with GABA-antiserum (provided by Prof. P. Somogyi and P. Gabbott, Oxford University) with the post-imbedding method of Somogyi. Individual synaptic boutons from axons innervated by the deprived eye are smaller than normal and have a lower than normal synaptic density. To date, no differences have been noted in innervation of GABAergic cortical neurons by axons innervated by the deprived or nondeprived eyes when compared to axons in normal animals.

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218.12 THE CONDITIONS REQUIRED FOR OPTIMAL RECOVERY FROM THE BEHAVIORAL EFFECTS OF MONOCULAR DEPRIVATION IN KITTENS, D.E. Mitchell, K.M. Murphy*, H. Dzioba* and J.A. Horne*. Psychology Dept., Dalhousie Univ., Halifax, N.S. B3H 4J1 Canada.

Current procedures for inducing visual recovery from the severe visual deficits caused by an early extended period of monocular occlusion in kittens are unsatisfactory. The effects they promote upon the vision of the deprived eye are neither complete nor permanent, and frequently they cause deleterious changes in the vision of the other eye. We describe a procedure that will, in certain conditions, induce complete and long-lasting recovery of vision of the initially deprived eye of monocularly deprived kittens without impact upon the vision of the other eye. The procedure combines two commonly investigated recovery conditions, namely reverse occlusion where the formerly nondeprived eye is occluded, and the situation where both eyes are open following the period of deprivation.

Thirteen kittens were monocularly deprived until 6 weeks of age at which time various regimens of part-time reverse occlusion were imposed for 6 weeks. During the latter period, and for the next 5 weeks, the kittens were housed in complete darkness for all but 7 hours each day. The initially nondeprived eye was occluded by an opaque mask that was worn for either 0,1,2,3.5,5,6 or 7 hours from the beginning of the 7 hours of daily visual exposure. The mask was removed to allow visual input to both eyes for the rest of the exposure period. Measurement of the visual acuities of the two eyes for square-wave grating were made at frequent intervals by use of a jumping stand.

Kittens that received 0,1 or 2 hours of daily occlusion showed only very limited recovery of vision in their initially deprived eye. The substantial recovery exhibited by the animals that received either 6 or 7 hours daily occlusion was merely temporary and virtually disappeared within a month following cessation of the 6 weeks of daily occlusion. By contrast, those kittens that received daily occlusion for either 3.5 or 5 hours (50% or 70% of each day), while exhibiting less recovery during the 6 week occlusion regimen, continued to improve afterward and eventually attained normal grating and vernier acuities as well as contrast sensitivity functions in both eyes. Control experiments performed in kittens that wore prisms (that introduced a binocular vertical disparity of 10 prism diopters) during the daily periods of binocular visual exposure revealed that similar substantial recovery could occur in this situation, a finding which suggests that the visual input to the two eyes need not be concordant. These various findings have important implications for patching therapy in certain forms of amblyopia.

219.1 AN IN VITRO ANALYSIS OF PROCESSING OF THE NGF PRECURSOR. R.H. Edwards*, M.J. Selby* and W.J. Rutter* (SPON:J. Day). Hormone Res. Institute & Dept of Biochemistry & Biophysics, Univ of California, San Francisco, CA 94143

The mature NGF polypeptide has a molecular weight of 12,500 and results from the processing of a larger precursor protein. We have shown that several distinct NGF precursors are predicted from cDNA clones divergent at their 5' ends. To study the biological significance of these different precursors, we have used an in vitro system to examine prohormone processing.

The NGF gene encodes several mRNA transcripts. The two major transcripts differ as a result of alternative RNA splicing. The larger transcript, which predominates in the mouse salivary gland, predicts a 34 kd precursor, with a single, internal hydrophobic domain. The shorter transcript, predominant in virtually all other tissues, predicts a 27 kd precursor, with the initiator methionine located just upstream of the hydrophobic domain. We thus first inquired whether translation of these two transcripts would result in precursors with different cellular localization.

To study the question of a different fate within the cell for the two predicted precursors, we have used an in vitro coupled translation-translocation system. The two cDNAs (representing the long and short transcripts) were cloned into the SP6 vector and SP6 polymerase used to generate the appropriate RNAs. In vitro translation produced the corresponding precursor proteins. Addition of dog pancreas microsomes to the mixture has resulted in full translocation of both precursors, without concomitant proteolytic cleavage of the putative signal peptide. Additionally, the short precursor was glycosylated in the microsomes, while the long was not. We then inquired whether proteolytic processing also differed in the two precursors.

The short precursor lacks 60 amino acids at the NH-terminus of the long prohormone. Several sets of basic residues elsewhere in the precursor suggest potential sites for proteolytic cleavage. Cleavage of the short precursor would thus yield a significantly truncated NH-terminal peptide. To study proteolytic processing, we added the gamma-subunit of NGF, a known protease and able at least partially to process an NGF precursor, to the translation mixture. Increasing amounts of gamma-NGF release several peptides from the NH-terminus of the precursor, with the expected difference in size between the long and short. We have shown that these proteins are not related to mature NGF (located at the C-terminus of the precursor) by 2D gel electrophoresis; and by using truncated SP6 RNAs that yield only the NH-terminus of the precursor. Antibody to NGF also does not recognize these polypeptides. Gamma-NGF thus appears able to process the non-NGF moiety of the NGF precursor and the long and short precursors therefore generate different NH-terminal peptides.

219.2 Cortisone, Aldosterone, and Testosterone Reduce Levels of mRNA Coding For Nerve Growth Factor (NGF) in Cultured L 929 Fibroblasts K. Siminoski, P. Rennert*, R.A. Murphy, and G. Heinrich*. Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115, and Howard Hughes Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Nerve growth factor (NGF) is produced in peripheral target tissues, in brain, and in mouse submandibular glands. NGF production is regulated by multiple factors including density of innervation and hormones, but the molecular mechanisms that underlie regulation of NGF gene expression are largely unknown. Fibroblasts in culture produce NGF and may be an appropriate model in which to investigate the regulation of NGF gene expression. Previous studies from our laboratory indicate that cortisone, aldosterone, and testosterone reduce NGF concentrations in medium conditioned by L 929 fibroblasts in culture (Endocrin. 118:1417, 1986). To determine the levels at which inhibition of NGF production occurs, we examined the effects of hormones on levels of the mRNA that encodes NGF. Confluent cultures of L 929 cells were incubated three days in serum-free medium in the presence or absence of hormones. NGF and protein concentrations were measured in conditioned medium. Total RNA was extracted and analyzed for NGF mRNA content by Northern blot hybridization using a ^{32}P labeled NGF cRNA as the hybridization probe.

Cortisone and testosterone decreased NGF concentrations in conditioned medium with ED_{50} of 7×10^{-9} M and 4×10^{-9} M respectively. Aldosterone (10^{-6} M) had similar effects while T_3 and progesterone (10^{-6} M) had no effect. Estradiol reduced NGF concentrations by 50% (10^{-6} M). NGF mRNA was reduced by up to 90% in cultures treated with cortisone with an ED_{50} of (9×10^{-9} M) and by up to 80% in cultures treated with testosterone with an ED_{50} of 4×10^{-9} M. Aldosterone also decreased NGF mRNA by 70% (10^{-6} M). T_3, progesterone and estradiol had no effect. Further analysis revealed that hydrocortisone reduced NGF mRNA levels within 3 hours of treatment and the effects of a 2-day exposure to the hormone were completely reversed 12 hours after hormone removal. These studies demonstrate that steroid hormones regulate NGF gene expression in fibroblasts at the level of NGF mRNA, and suggest that reductions in NGF levels previously described are mediated in part by regulation of NGF mRNA.

219.3 CONTINUOUS INFUSION OF NGF PREVENTS NON-CHOLINERGIC AS WELL AS CHOLINERGIC NEURONAL DEATH IN THE MEDIAL SEPTUM AFTER FIMBRIA-FORNIX TRANSECTION. L.R. Williams[1], G.M. Peterson[2], S. Varon[2], and F.H. Gage[1]. Depts. of Neurosciences[1] and Biology[2], School of Med. Univ. of Calif. San Diego, La Jolla, CA 92093.

Neurons in the rat medial septum (MS) undergo a rapid cell death after transection of the ipsilateral fimbria-fornix (Gage et al. Neurosci. in press). By one week postaxotomy, there is a loss of 40% of the total, Nissl-stained neurons, 70% of the cholinergic neurons, 20% of the GABAergic neurons, and a virtually complete loss of the substance P-containing neurons compared to the number of neurons surviving on the contralateral, control side. In view of recent reports that the MS cholinergic neurons are responsive to Nerve Growth Factor (NGF), we hypothesized that exogenous NGF might provide trophic support to the MS cholinergic neurons. Exogenous 7S NGF (11µg/ml in artificial cerebrospinal fluid) was infused into the right lateral ventricle through a permanently implanted, preformed cannula device using an Alzet pump (flow rate of 0.5 µl/hr; NGF dose = 1 µg/wk). Cytochrome c was used in the control infusate. Three days after cannula implantation and continuous delivery of experimental infusate, all animals received a complete unilateral aspiration of the fimbria. Two weeks after the fimbria transection and 17 days after the start of the continuous infusion, the animals were prepared 1) according to the method of Butcher (1983) for the visualization of cholinergic, i.e., AChE-positive, cell bodies, or 2) for the immunohistochemical visualization of GAD-positive and substance P-positive cell bodies. Alternate sections were stained with cresyl violet. Total Nissl-stained neuronal numbers, and cholinergic, GABA, and substance P neuronal numbers were determined by direct count in coronal sections of the MS on both the side ipsi- and contralateral to the transection. In control-infused animals, aspiration of the fimbria resulted in the loss of 50% of the total and 60% of the cholinergic neurons. Infusion of NGF reduced these losses to 20% total neurons and 25% cholinergic neurons which represents a sparing of 50% and 60%, respectively (p 0.002). Comparisons of the neuronal numbers sampled in control and NGF-treated animals indicated that non-cholinergic neurons comprise the majority (80%) of the neurons that were spared by the NGF treatment. These results provide a quantitative validation of the neurono-trophic hypothesis with regard to adult mammalian CNS neurons in vivo in that NGF promotes the survival of most of the non-cholinergic and cholinergic neurons in the MS which would die following transection. Work is now in progress to determine if the non-cholinergic neurons spared by NGF treatment include GABAergic and/or substance P neurons.

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219.4 SUSTAINED INCREASE IN NGF-LIKE ACTIVITY IN MEDIUM CONDITIONED BY HIPPOCAMPAL SLICES FROM SEPTAL-LESIONED RATS. F. Collins and K.A. Crutcher, Dept. of Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

We have previously shown that medial septal lesions, which elicit the growth of sympathetic axons into the hippocampal formation in vivo, also result in increased NGF-like activity in medium conditioned by hippocampal slices in vitro (J. Neurosci. 5:2809, '85). In that study growth-promoting activity was elevated at 4 weeks after a septal lesion but longer survival times were not examined. In the present study we measured NGF-like activity in medium conditioned by hippocampal slices taken from rats 4 weeks to a year following a medial septal lesion. Tissue from control (unlesioned) rats was run at the same time. The Wilcoxon matched-pairs signed-rank test was used to determine statistical significance.

Slices which included the dentate gyrus and CA3 region of the hippocampus were incubated for 48 hrs in Ham's F12 medium. The resulting "conditioned medium" was then assayed for sympathetic growth-promoting activity by adding it to embryonic (9-day old) chick sympathetic ganglia and quantifying neurite outgrowth 48 hrs later. The similarity of the activity to NGF was determined by testing conditioned medium in the presence and absence of affinity-purified antibodies to NGF.

Sympathetic growth-promoting activity was greater in medium conditioned by hippocampal slices from animals receiving septal lesions 4-12 weeks earlier when compared with paired control tissue ($p < .01$). In addition, although only a few samples have been run to date, increased activity appears to be present even at longer survival times (24 wks to a year). As found previously, the majority of activity is antagonized by affinity-purifed antibodies to NGF.

These results are particularly interesting in light of the evidence that sympathetic sprouting may continue for several months after a septal lesion (Madison and Davis, Exp. Neurol. 80:167, '83) and that septal cholinergic reinnervation of the hippocampal formation occurs over several months following fimbria-fornix transection (Gage et al., Brain Res. 268:27, '83). Taken together, these observations are consistent with the hypothesis that sympathetic fibers, although responsive to the hippocampal growth signal, may not regulate the level of growth-promoting activity. The fact that sympathohippocampal fibers appear to be restricted to the perivascular region may also be relevant to this suggestion (Crutcher and Marfurt, this meeting). In addition, the prolonged time course of septohippocampal reinnervation may reflect the response of septal cholinergic neurons to the same hippocampal growth signal. (Supported by NIH grant #NS-17131.)

219.5 NERVE GROWTH FACTOR-LIKE IMMUNOREACTIVITY IN THE RAT PITUITARY GLAND. S. Soinila', J. Lakshmanan*'', T. Lahtinen*°,H.Rauvala*°°,O.Häppölä°,H.Päivärinta°,L. Eränkö°.
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''Growth Factor Laboratory, Center for Neurologic Study San Diego, CA 92121.

The nerve growth factor (NGF) is synthesized by the peripheral sympathetic target tissues, as well as by the brain regions innervated by the septal cholinergic neurons. NGF has an essential role as a trophic substance required by developing sympathetic neurons. Its importance for the central neurons under physiologic conditions is not clear. Serum contains NGF, the source of which has not been established. The present study was undertaken to examine the presence of NGF in the endocrine organs of the rat.

Using the indirect immunohistochemical technique of Coons, it was demonstrated that the intermediate lobe of the pituitary gland exhibits intense reactivity to beta-NGF, while the anterior and neural lobes are non-reactive. During the postnatal development, NGF immunoreactivity appears in the intermediate lobe first on day 21 and is fully developed by the age of 60 days. The immunoreactivity was totally inhibited after pre-absorption with 1 μM beta-NGF, but not with 2.5S NGF.

Homogenate of intermediate lobes from 10 rats was driven through SDS-polyacrylamide gel by electrophoresis and transferred on nitrocellulose for immunoblotting. Beta-NGF, but not 2.5S NGF, produced, in addition to the expected 13 kD band, a smaller band at 45 kD. The intermediate lobe did not produce any reactivity at 13 kD. Instead, a band was observed at 45 kD.

Coculture of newborn rat superior cervical ganglia with pieces of the intermediate lobe revealed a marked stimulation of fiber growth, which was totally inhibited by NGF antiserum.

The present results suggest that the rat pituitary intermediate lobe contains a substance that (1) is immunologically closely related to beta-NGF; (2) has the biological effect of NGF, but (3) which may be chemically distinct from the classical NGF molecule.

219.6 PARTIAL PURIFICATION OF A PARASYMPATHETIC NEUROTROPHIC FACTOR. Thomas L. Wallace and Eugene M. Johnson, Jr., Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

Previous work has shown that pig lung, among a number of tissues examined, contained relatively high amounts of survival-promoting and choline acetyltransferase (ChAT)-sustaining activities for parasympathetic, ciliary ganglion neurons from chicken embryos. The bioassay was one in which dissociated embryonic day 9 ciliary ganglia neurons were incubated in tissue culture media containing 10% serum and 35mM KCl on a double layer of air-dried collagen. After 36 to 48 hrs, survival was evaluated microscopically and, on replicate samples, ChAT activity was measured using a radiochemical method. A 25,000 X g supernatant fraction of pig lung had EC_{50}s for these two activities of 55 μg/ml and 50 μg/ml respectively, indicating that they were related. It is reported here that these two activities have been partially purified and shown to coelute from several chromatographic columns, indicating that the activities probably reside in the same molecule. Three species were detected with apparent molecular weights (MW) of over 100 kD, 40 kD, and 3 kD, and the two high MW species converted into the low MW species. All species had both survival-promoting and ChAT-sustaining activity, whereas only the high MW species were associated with neuritic outgrowth. The low MW form was purified over 2,000-fold by Amicon filtration, and gel sieving and cation exchange chromatography. This partially purified form had EC_{50}s for survival-promoting and ChAT-sustaining activity between 100 and 150 ng protein/ml, again indicating that both activities probably reside in the same molecule. The low MW species was heat and acid stable, and resistant to proteases, deoxyribonucleases, ribonucleases, and neuraminidase. As previously found with the 25,000 X g supernatant fraction of pig lung, the trophic effects were specific for parasympathetic neurons; neither sympathetic nor peripheral sensory neurons were affected. In addition, the low MW species did not increase ChAT activity in either sympathetic neurons or PC12 cells. These findings indicate that the pig lung factor(s) has a more specific target spectrum than previously reported ciliary neuron survival factors, suggesting that it may be a novel and important neurotrophic substance.

219.7 ENDOGENOUS OPIOIDS CONTROL NEUROGENESIS AND SYNAPTOGENESIS IN THE CEREBELLUM AND CEREBRAL CORTEX. K.F. Hauser, P.J. McLaughlin and I.S. Zagon. Dept. of Anatomy, The M.S. Hershey Med. Ctr., Penn State Univ., Hershey, PA 17033.

Our laboratory has previously demonstrated that endogenous opioid systems (i.e., endogenous opioids and opioid receptors) regulate somatic and neurobiological development (Science 221:1179, 1983; Life Sci. 35:2057, 1984). For example, continuous blockade of endogenous opioid-opioid receptor interaction results in a dramatic acceleration in body, brain, and behavioral maturation. In the present study we sought to examine whether neuronal structure, particularly as related to synapse concentration and content, is influenced by endogenous opioid systems.

Beginning at birth, rats received daily s.c. injections of 50 mg/kg naltrexone (NTX), a dosage which blocked opioid receptors for the duration of each injection period. At 10 days of age, the brains of rats (males) were impregnated using the Golgi-Kopsch method, and sagittal and coronal sections of the cerebellum and cerebral cortex were examined. The dendrites, soma, and dendritic spines from Purkinje neurons in cerebellar lobule VIII, and from pyramidal cells of layer III of the somatic motor cortex in the frontal lobe, were evaluated. In the cerebellum, the total area of Purkinje cell dendrites was significantly increased (30%) from control levels. This effect appeared to be due to a corresponding increase in the number of terminal spiny branches. The number of Purkinje cell dendritic spines/μm dendrite was comparable in NTX and control rats, although the spines in the NTX group appeared more differentiated. In the cerebral cortex, the most noticeable changes were that the second order, basilar dendrites of pyramidal cells in NTX-treated rats were significantly longer than in control animals, and that the numbers of spines/μm dendrite on the basilar, oblique, and apical dendrites of NTX rats were approximately double that of controls.

These findings, utilizing the naltrexone-induced receptor blockade paradigm, indicate that neurogenesis and synaptogenesis are controlled by endogenous opioid-opioid receptor interaction. Our data suggest that endogenous opioids are trophic factors in neuro-ontogeny. Interestingly, the increase in neuronal development documented in this study is correlated with previous evidence that continuous opioid receptor blockade is of functional importance since 50 mg/kg NTX treated animals have accelerated spontaneous motor and sensorimotor development.

Supported by NIH grant NS-20500.

219.8 MORPHOLOGICAL, METABOLIC AND BEHAVIOURAL EVIDENCE FOR INCREASED DOPAMINERGIC NIGRAL AND STRIATAL FUNCTION FOLLOWING GANGLIOSIDE GM1 TREATMENT IN THE PARTIALLY HEMITRANSECTED RAT. K. Fuxe, L.F. Agnati*, I. Zini*, G. Toffano, M. Zoli*, P. Davalli*, A. Corti*, A. Deciderio*, N. Diemer*, C. Owman*, M. Goldstein. Dept. of Human Physiology, University of Modena, Modena, Italy

Chronic treatment with GM1 (10 mg/kg, i.p.) increases the survival of nigral dopamine (DA) cell bodies with their dendrites, an action, which may be responsible for the enhancement of collateral sprouting in striatum as revealed by image analysis of sections stained for tyrosine hydroxylase (TH) immunoreactivity (IR). The lesion used was a partial unilateral hemitransection. Using the radioactive deoxyglucose (DG) technique to study striatal energy metabolism and radiolabelled iodoantipyrine to study the striatal blood flow, GM1 was found to counteract the imbalance in striatal energy metabolism and in striatal blood flow found between the striata of the lesioned and unlesioned side. GM1 produced excitatory effects on the lesioned side and inhibitory effects on the unlesioned side. Studies on cyclic AMP (cAMP) and Ca^{2+} induced protein phosphorylation processes in the striatal P_2 fraction of experimental rats demonstrated that on the lesioned side the actions of acute and chronic GM1 treatment are to substantially restore cAMP and Ca^{2+} protein phosphorylation on the lesioned side. Biochemical studies on ornithine decarboxylase (ODC) activity and on polyamine levels as well as pharmacological treatment with α-DFMO (α-difluoromethylornithine) have given evidence that increases in ODC activity and in putrescine levels represent a condition necessary but not sufficient for spontaneous or GM1 promoted regeneration of mesostriatal DA neurons. Thus, polyamines represent permissive factors in growth mechanisms. Studies on ipsilateral bias and on rotational behaviour in partially hemitransected rats have given evidence that chronic treatment with the ganglioside GM1 can increase striatal function on the lesioned side. These results are probably related to the ability of GM1 treatment to increase DA synaptic function in the striatum of the lesioned side. Both acute and chronic GM1 treatments were found to improve water intake in partially hemitransected rats. The improvement of water intake observed after chronic treatment may be related to increased outgrowth of hypothalamic afferents or to enhancement of collateral sprouting in the hypothalamus and the basal forebrain. Increases in noradrenaline reinnervation of the hypothalamus were in fact observed after GM1 treatment in partially hemitransected rats. Taken together various approaches demonstrate trophic actions of ganglioside GM1 treatment on DA neurons in mechanically lesioned rats.

219.9 MONOSIALOGANGLIOSIDE GM1 POTENTIATES THE NEURITOGENIC RESPONSES OF PERIPHERAL AND CENTRAL NEURONS TO CONDITIONED MEDIA. P.E. Spoerri* and F.J. Roisen**, Department of Neurosurgery, University of Göttingen, Göttingen, FRG and Department of Anatomy, Rutgers Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854.

Previously we found that gangliosides stimulate the in vitro neuritogenesis of Neuro-2a Neuroblastoma and chick embryonic dorsal root ganglia (DRG). They have been shown also to potentiate the action of Nerve Growth Factor (NGF). In this study, the ability of gangliosides to potentiate non-NGF trophic agents was determined by examining the capacity of exogenous mixtures of bovine brain gangliosides (BBG) and the monosialoganglioside GM1 to enhance the neuritogenic action of conditioned media (CM). CM were prepared with cultures of neonatal mouse glial cells, C6 glioma, chick embryonic skeletal or cardiac muscle, rat L6 myoblasts and RN22 Schwannoma. A variety of chick embryonic neuronal tissues were employed as neuritogenic models including: 8 1/2 day DRG, 11 day ciliary ganglia and 8 day spinal cord explants. The neuritogenic responses of coded randomly selected cultures were evaluated microscopically on the basis of neurite length, number and the degree of non-neuronal radial migration. The neurite promoting action of the factors contained in the various CMs was potentiated by either BBG (150 ug/ml) or GM1 (150ug/ml). BBG or GM1 enhanced the number and length of DRG neurites produced by 48 hr exposure to media conditioned by either cardiac or skeletal muscle. The gangliosides also potentiated the effect of cardiac and skeletal muscle CM on the development of ciliary ganglia. Spinal cord explants grown in the presence of skeletal muscle or RN22 Schwannoma CM and either BBG or GM1 covered 5-6 fold greater areas than explants grown in the absence of gangliosides. These studies demonstrate that gangliosides potentiate the neuritogenic and neuronotrophic actions of several NGF-independent trophic agents. The ability of gangliosides to enhance a variety of trophic agents suggests that they play a fundamental role in neuronal development.
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219.10 MONOCLONAL ANTIBODIES AGAINST GM1 GANGLIOSIDE BLOCK THE NEURITOGENIC ACTION OF CONDITIONED MEDIA ON SENSORY GANGLIA. M.M. Rapport, P.E. Spoerri* and F.J. Roisen** . Division of Neuroscience, NY State Psychiatric Institute, NY, NY, 10032; Department of Neurosurgery, Faculty of Medicine, University of Göttingen, Göttingen, Federal Republic of Germany and the Department of Anatomy, Rutgers Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854.

The monosialoganglioside GM1 potentiates Nerve Growth Factor-mediated neuritogenesis in vitro. Recently, we found that GM1 can also potentiate the neuritogenic activity of media conditioned by neonatal glia, C6 glioma, the rat myogenic line L6 and embryonic chick heart or skeletal muscle. To probe further the neuritogenic activity of conditioned media (CM), five mouse monoclonal antibodies (mAbs) against GM1 designated B6, C3, C4h2, D1 and D3 were incorporated individually into nutrient medium (NM) supplemented with CM prior to incubation with sensory ganglia. 8 1/2 day embryonic chick dorsal root ganglia (DRG) were explanted onto collagen-coated coverslips and incubated at 35 °C for 5 hr in NM supplemented with 150 ug/ml GM1. Following washing with NM, the explants were refed with NM + CM containing 20% mAb and cultured for an additional 48 hr. The resultant neuritogenesis was evaluated microscopically by determining mean neurite length and number of coded randomly mixed cultures. The degree of non-neuronal radial migration was also measured. The five mAbs differed in their capacities to inhibit CM-mediated neuritogenesis of these primed cells. D1 and D3 were most effective in reducing neurite length and number for all sources of the CM. C3 and C4h2 were most effective in blocking non-neuronal migration, while B6 was intermediate. The ability of these mAbs to inhibit neuritogenic activity of CM derived from both primary and established glial and myogenic tissue suggests that gangliosides play a basic role in neuronal development. The differing responses elicited by the individual mAbs may reflect a relationship between the structural complexity of the GM1 molecule and the neuritogenic mechanisms. Since the various CMs may contain several specific growth factors, the potentiation of such factors by regions of the GM1 molecule may further regulate the character of the neuritogenic response.
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219.11 THYROTROPIN RELEASING HORMONE DECREASES NEURONAL ATROPHY FOLLOWING PERIPHERAL NERVE SECTION. R.W. Banda*, P. Fisher* and E.D. Means, Department of Neurology, Veterans Administration Medical Center and University of Cincinnati Medical Center, Cincinnati, Ohio 45267

Central chromatolysis is a regenerative response which follows axotomy to peripherally projecting neurons. This involves heightened protein synthesis to meet the regenerative demands. Failure of this process can lead to neuronal atrophy and death. "Trophic" substances can conceivably prevent this occurrence. Thyrotropin releasing hormone (TRH) was found to exert a neurotrophic effect on ventral horn neurons in culture. Its salutory effects in experimental spinal cord injury have been described. The present study was undertaken to examine a possible role for TRH in preventing neuronal atrophy following axotomy.

Unilateral, proximal sciatic nerve section was performed under Nembutal anesthesia on ten Fischer rats (wt. 200-250 gms); the contralateral sides acting as sham-operated controls. Five animals were given TRH daily (Sigma), 1.6 mgm/kg intraperitoneally. The remainder were given vehicle only. Both groups were allowed food and water ad lib. After 3 weeks they were perfuse-fixed with paraformaldehyde and the spinal cords removed. Cross sections of the appropriate levels were stained with cresyl violet and neuronal size (cross sectional area) was digitized using a Zeiss Videoplan semiautomatic image analyzer. There was a statistically significant difference between the neuronal area on the axotomized side in the TRH group compared to the vehicle controls (192.3 $\pm$ 9.3 vs. 152.7 $\pm$ 6.6, mean $\pm$ SEM respectively, $p < 0.01$). No such difference was found on the sham-operated side (197.5 $\pm$ vs. 203.0 $\pm$ 8.8, $p = .15$) - units expressed in um^2.

These results suggest that TRH plays a role in preventing neuronal atrophy following axotomy by unknown mechanisms. This observation has important implications for the potential therapy of peripheral nerve injury.

219.12 EFFECTS OF AGENTS (PHORBOL ESTERS, ADRIAMYCIN, POLYMIXIN B, ETC.), WHICH ALTER PROTEIN KINASE C ACTIVITY, ON THE SURVIVAL AND GROWTH OF SYMPATHETIC NEURONS MAINTAINED IN CULTURE. A.R. Wakade and T.D. Wakade*. Dept. of Pharmacology. State Univ. of New York, Brooklyn, NY 11203.

Phorbol ester have become powerful tools to investigate a wide variety of cellular functions (Nishizuka, Nature 308: 693, 1984). We have investigated the effects of protein kinase C activator, phorbol 12,13-dibutyrate (Pdbu) and 12-0-tetradecanoylphorbol 13-acetate (TPA) on the survival of sympathetic neurons derived from peripheral ganglia of 10-12-day-old chick embryo in the absence or presence of nerve growth factor (NGF). We demonstrate that Pdbu (3 to 100 nM) and TPA (0.3 to 10 nM) supported a maximum of about 30% of plated neurons in a serum-free culture medium. The number of surviving neurons remained virtually unchanged for over 2 weeks. The effect of TPA and Pdbu was observed in the absence of NGF and presence of antisera to NGF. A nonactivator of protein kinase C, phorbol 13-acetate, (10 to 1000 nM) was totally ineffective in supporting the neurons. A combination of supramaximal concentrations of Pdbu and NGF did not produce additive effects. Neurons initially selected in culture by NGF (or Pdbu) for 2 days were successfully supported by Pdbu (or NGF) for several days without a significant loss in their number. Inactivators of protein kinase C, such as polymixin B, adriamycin, etc. (Wise et al., J. Biol. Chem. 257:8489, 1982) caused a concentration dependent reduction in the number NGF-supported neurons over a 2-day period. Experiments are in progress to determine whether toxic effects of these agents could be prevented by TPA or Pdbu. From the above data it is concluded that protein kinase C may be involved in the survival and neurite outgrowth of chick sympathetic neurons in culture.

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220.1 OPTICAL DETECTION OF SINGLE UNIT ACTIVITY IN THE ISOLATED SPINAL CORD OF THE LAMPREY, PETROMYZON MARINUS. Avis Cohen, Jill London, Lawrence Cohen, and Harry Orbach. Marine Biological Laboratory, Woods Hole, MA; Neurobiology and Behavior, Cornell University, Ithaca, NY; Department of Physiology, Yale University School of Medicine, New Havern, CT.

To understand the origin of any behavior, one should monitor simultaneously the individual activity of a large number of neurons. Through the use of voltage sensitive dyes and optical recording techniques this has been achieved, but only in invertebrate preparations. In this report we describe in a vertebrate preparation optical recordings of individual unit activity.

Pieces of isolated lamprey spinal cord, 12-13 segments long, were stained with either WW376, (0.37mg/ml, for 5-8 hours), or RH155, (1mg/ml for 5 hours). The preparation was kept cool (approx. 10°C) at all times. Neural activity was elicited with electrical stimulation of the cord surface. An image of the area of the cord, 1-3 segments from the stimulation site, viewed through a 32X objective, was focused on a 12X12 photodiode array. Each photodiode recorded absorption changes from a $50\mu^2$ area of spinal cord.

A long (100ms) just threshold pulse evoked a small depolarizing signal ipsilateral to the stimulation site. At the same stimulus strength, a hyperpolarizing signal was detected on the contralateral cord. The hyperpolarization followed the depolarization after a brief delay, suggesting that it was postsynaptic to the ipsilateral excitation. With increasing stimulus strength the response increased in amplitude and became more complex. A brief, large amplitude stimulus triggered a long burst of action potentials that was mainly detected on 2 diodes. We think that this signal represents spike activity in an individual neuron.

The pharmacological effects of the dye RH155 were tested. Twelve segment pieces of the cord were exposed to 2mM D-glutamate, a drug which has been shown to induce fictive swimming (A. Cohen, & P. Wallén, 1980). The cords were then stained for 5 hours and retested for ability to generate fictive swimming. Fictive swimming could be induced in preparations stained with 0.5mg/ml of RH155. It may therefore be possible to monitor the neural activity during fictive swimming in this simple vertebrate.

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220.2 SYNAPTIC CONNECTIONS BETWEEN THE MAUTHNER AXONS AND IDENTIFIED MOTONEURONS AND INTERNEURONS IN THE CAUDAL SPINAL CORD OF GOLDFISH. J.R. Fetcho and D.S. Faber. Div. of Neurobiology, SUNY at Buffalo, N.Y. 14214.

The most dramatic component of the escape response initiated by the Mauthner cell (M-cell) in teleosts is a rapid, forceful tail-flip which is most likely mediated by synaptic connections between the Mauthner axons (M-axons) and spinal motoneurons and interneurons. To study these connections we have recorded intracellularly from one or both M-axons in goldfish, and activated them using current injection while simultaneously recording from postsynaptic spinal neurons that we subsequently filled with HRP for morphological study. We have identified two classes of neurons that receive short latency, excitatory connections from the ipsilateral M-axon, and longer latency inhibition from the contralateral M-axon.

The first class of cells corresponds to the primary motoneurons identified in other studies. The 13 of these we filled with HRP had: 1) large, dorsal cell bodies, near the central canal, 2) rostro-caudal dendrites lateral to the cell body, and 3) a large, ventral process that passed medially around the M-axon, and exited in a ventral root. The mean synaptic delay between the foot of the spike in the ipsilateral M-axon and the EPSP in these cells was .52 msec (s.d.=.19,N=8); the delay of the inhibition from the contralateral M-axon was 1.03msec (s.d.=.46,N=5).

Cells in the second class are interneurons. The 15 of them we filled with HRP had: 1) dorsal cell bodies like primary motoneurons, 2)a few dendrites dorsolateral to the cell bodies, and 3) a large axonal process that ran ventrally to a region near the ipsilateral M-axon, then caudally, branching profusely throughout the ipsilateral motor column and forming synaptic boutons on many probable axial motoneurons spread longitudinally over the equivalent of roughly one body segment. The axon then continued dorsally, and caudally in the dorso-medial white matter. The mean synaptic delay of the excitation of these cells by the ipsilateral M-axon was .43 msec (s.d.=.09,N=8); the inhibitory delay from the contralateral axon was .52 msec (s.d.=.06,N=4). These interneurons were not identified in previous studies of the spinal circuitry of the M-cell, although they are physiologically similar to what were considered recordings from collaterals of primary motoneurons.

These observations require a revision of previous accounts of the M-cell circuitry. They indicate that the latencies of excitation and crossed inhibition of motoneurons are longer than previously thought, and introduce a class of interneurons that may exercise segmental control of axial motoneurons. (Supported by an NIH Postdoctoral Fellowship to J.R. Fetcho and NIH grant NS-15335 to D.S. Faber.)

220.3 COACTIVATION OF TWO MOTOR SYSTEMS IN THE ZEBRAFISH. Dennis Liu* and Monte Westerfield. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Lower vertebrates have two distinct motor systems that are thought to mediate different behaviors. We have studied the function of these systems by correlating the activity of muscles and specific motoneurons.

In the zebrafish two distinct classes of motoneurons, primary and secondary, have been identified. Both classes innervate the white axial muscles used for swimming and the startle response. Individual muscle fibers receive inputs from primary and secondary motoneurons. The primary motoneurons have large axons, large peripheral fields, produce large endplate potentials (EPPs), and probably receive direct input from the Mauthner neuron. Secondary motoneurons have smaller axons, smaller peripheral fields, produce smaller EPPs, and probably do not receive direct input from the Mauthner neuron. These observations suggested that primary motoneurons function in the stereotyped startle response whereas secondary motoneurons are used for non-startle swimming behaviors. We examined this hypothesis by studying muscle activity during swimming.

To distinguish muscle activity (EMGs) produced by primary motoneurons and secondary motoneurons we simultaneously recorded EPPs and EMGs from the same muscle. Zebrafish were restrained and EMGs recorded with fine bipolar electrodes. Nearby muscle fibers were impaled with floating tip glass microelectrodes. We found a correlation between the size of EPPs and EMGs; there were two classes of responses which differed by more than twofold. Previous studies have shown that EPPs produced by primary motoneurons are typically more than twice as large as EPPs produced by secondary motoneurons. This suggests that the large amplitude EMG spikes represent muscle activity evoked by primary motoneurons while smaller spikes occur when secondary motoneurons are active.

Using these criteria we have analyzed EMGs from swimming, tethered fish. Pairwise recording from dorsal and ventral muscles on one side of the same body segment showed that both large and small EMG spikes occurred during a bout of swimming. Spikes in muscles on opposite sides of a segment were antiphasic. Moreover, large EMG spikes were recorded during a variety of swimming modes. These results demonstrate that primary and secondary motoneurons can be simultaneously active during swimming and suggest that the primary motor system is involved in behaviors in addition to the startle response.

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220.4 MOTOR PATTERNS DURING ROSTRAL SCRATCHING AND FORWARD SWIMMING IN THE TURTLE. Paul S.G. Stein and Kathryn M. Johnstone*. Biology Department, Washington University, St. Louis, MO 63130.

The red-eared turtle, Pseudemys scripta elegans, uses its hindlimbs to perform many tasks, including both forward swimming (J. Neurophysiol. 40: 768-778, 1977) and rostral scratching (J. Neurophysiol. 53: 1501-1534, 1985). We compare the timing and intensity of motor activity during these two behaviors using electromyographic (EMG) recordings from three hindlimb muscles: VP-HP (ventral puboischiofemoralis internus; a hip protractor), HR-KF (flexor tibialis internus; a hip retractor), and FT-KE (femorotibialis; a knee extensor). We use two preparations: an intact turtle, and a spinal turtle with a complete transection of the spinal cord just posterior to the forelimb enlargement. An intact turtle produces forward swimming spontaneously; electrical stimulation applied with constant frequency to a site in the dorsolateral funiculus produces a forward swimming motor pattern in a spinal turtle. Tactile stimulation of the shell bridge evokes a rostral scratch in both preparations.

The relative timing of muscle activity is similiar for forward swimming and rostral scratching. For both behaviors, VP-HP and HR-KF display rhythmic alternation of activity. The termination of FT-KE activation occurs near the end of VP-HP activity or near the beginning of HR-KF activity.

The amplitude (peak-to-peak voltage) of muscle activity is different for each behavior. The amplitude of VP-HP during the rostral scratch is always larger than during the forward swim. In some forward swim cycles, VP-HP activity is absent. The amplitude of FT-KE during the rostral scratch is usually larger than during the forward swim. The amplitude of HR-KF displays considerable variation; in general, during the forward swim its amplitude is larger than during the rostral scratch. In some rostral scratch cycles, HR-KF activity is absent.

These amplitude differences are understood by considering which phase of the movement cycle is the "powerstroke" of each behavior. VP-HP and FT-KE are active during the rub phase of the rostral scratch, i.e., the "powerstroke" of the scratch. HR-KF is active during the hip retraction phase of the forward swim, i.e., the "powerstroke" of the swim.

In our earlier work, three different scratch forms were distinguished according to the relative timing of muscle activity patterns. In the present study, two different behaviors that show similar relative timings of muscle activation are distinguished according to the relative intensity of muscle activation.

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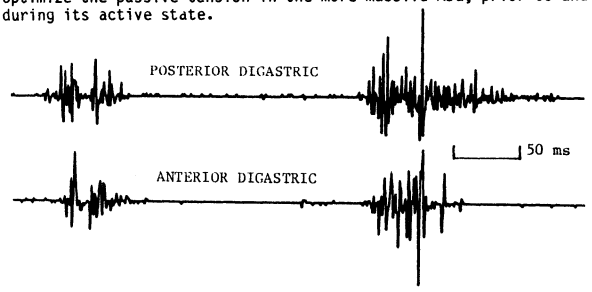
220.5 THE MOTONEURON POPULATION AND THE ACTIVITY PATTERNS OF THE GUINEA PIG DIGASTRIC MUSCLE. M. Tal and A. Lev-Tov, Dept. of Anatomy, The Hebrew University Medical School, Jerusalem, Israel.

The digastric (DG) is a jaw opener muscle, comprised of two bellies which are joined by a central tendon. The present study was aimed at characterizing the organization of the motoneuron pools and the activity patterns of the anterior and posterior DG bellies (ADG and PDG).

Experiments were performed on ketamine anesthetized male albino guinea pigs weighing about 500 gr. Injections of HRP to the mechanically separated DG bellies revealed two different motoneuron pools in the brainstem. The pool of ADG contained a maximum of 68 motoneurons with somatic diameters of 16-52 μm. This pool was located within the motor nucleus of the trigeminal (V) nerve. The pool of PDG contained a maximum of 54 motoneurons with somatic diameters of 16-53μm, and was located within the motor nucleus of the facial (VII) nerve.

The activity pattern of DG was studied using simultaneous EMG recordings from its anterior and posterior bellies. We found that during spontaneous rhythmic jaw movement (spontaneous RJM), and during RJM evoked by repetitive stimulation of the masticatory area of the cerebral cortex, the activity in ADG and PDG was highly synchronized, with relatively few differential bursts. Despite the synchronism, the detailed temporal activity patterns of the two bellies exhibited significant differences. The activity in PDG preceded that of ADG and outlasted it in most of the rhythmic cycles, as demonstrated in the figure below. The detailed mechanisms and the functional implications of these differences are not known at the moment. It is tempting however, to consider the early activity in PDG as a factor which may optimize the passive tension in the more massive ADG, prior to and during its active state.



220.6 SEPARATE GENERATION OF OSCILLATION AND MOTOR BURST PATTERN FOR MAMMALIAN RESPIRATION: *IN VITRO* STUDIES. J.L. Feldman & J.C. Smith*, Dept. of Physiology, Northwestern University, Chicago, IL 60611, Dept. of Kinesiology, UCLA, Los Angeles, CA 90024

We have hypothesized a *timer model* for respiratory pattern generation in mammals (Feldman, Fed.Proc.40:2384,1981). In this model, separate neural networks generate: i) the oscillatory command ("timer") and; ii) the motor burst patterns for each phase. Considerable evidence obtained *in vivo* is consistent with this hypothesis (Feldman,In: Handbook of Physiology,1986). We have exploited a novel *in vitro* mammalian brainstem-spinal cord preparation [Smith & Feldman, Soc. Neurosci. Abs. 11:24, 1985] to test the role of Cl^--dependent synaptic inhibition in pattern generation; the results provide further support for our model. Motoneuronal activity *in vitro* was recorded from spinal ventral and cranial nerve roots. The *in vitro* bath was partitioned at the spinomedullary junction so that we could selectively change the medium bathing the brainstem from a mock brain extracellular fluid (ECF) to a Cl^--free ECF, or to ECF containing Cl^- channel blockers/antagonists to the inhibitory neurotransmitters gamma-aminobutyric acid (GABA) or glycine (Gly). Concentrations of antagonists used [picrotoxin (0.1-1mM), benzylpenicillin (3-5mM), bicuculline (0.1-1mM) and strychnine (0.1-1mM)] were sufficient to block inhibitory actions of bath-applied GABA and Gly. None of the antagonists, nor Cl^--free fluid disrupted the rhythm of spontaneous respiratory motor bursts recorded on cranial nerves (IX,X,XII) and spinal cervical (C4, C5) and thoracic ventral roots. However, blocking synaptic inhibition increased burst durations and amplitudes. These results indicate that Cl^--dependent inhibition is not essential for the generation of respiratory rhythm in the *in vitro* mammalian neonatal brainstem-spinal cord, but contributes to the shaping of the motor burst pattern. The results are consistent with our hypothesis that the generation of rhythm and burst pattern are functionally [*ibid.*] and anatomically [Speck & Feldman, J. Neurosci. 2: 744, 1982; Feldman et al., J. Physiol. 347: 241,1984.] separate. Although our results obtained *in vitro* pertain to Cl^--dependent inhibition, we hypothesize that inhibitory interactions are not necessary for generation of respiratory rhythm; termination of phase bursting by disfacilitation is sufficient for rhythmogenesis *in vitro*. (The role of inhibition in controlling burst duration may be to assure burst termination in intact animals where ill-timed sensory inputs might lead to spurious phase reactivation). If this latter hypothesis were correct, neural pacemaker activity should underlie mammalian respiratory rhythmogenesis. SUPPORTED BY NIH GRANT HL-23820. JCS IS A P.B. FRANCIS FOUNDATION FELLOW.

220.7 OCTOPAMINE STIMULATES A CENTRAL PATTERN GENERATOR FOR FEEDING IN LIMULUS. H.M.Lee* and G.A.Wyse. Dept. of Zoology, Univ. of Massachusetts, Amherst, MA 01003.

The neural bases of centrally generated rhythmic behavior patterns have been characterized in many species; in several of these cases biogenic amines and peptides may modulate the generation of the behavioral rhythm. We present evidence for a neuromodulatory role of the monoamine octopamine in the generation of centrally-patterned rhythmic feeding in the horseshoe crab, Limulus polyphemus.

Limulus is a chelicerate arthropod that lacks jaws and specialized feeding appendages. The basal (coxal) segments of the walking legs bear inwardly-directed gnathobase spines that surround the mouth; rhythmic transverse movements of the coxae are used to shred and ingest food. The pattern of coxal muscle activity during feeding has been described (Wyse and Dwyer, Biol. Bull. 144: 567-579, 1973). Adjacent legs are adducted with a phase delay of 0.5 (out of phase); opposite legs are adducted in phase. These phase relationships are distinctly different from those in swimming and walking. Because impulse bursts in entocoxal nerves (to coxal adductor muscles) parallel coxal adduction, we can reliably identify feeding patterns of motor output in the isolated Limulus CNS. We recorded with suction electrodes from anterior and posterior entocoxal nerves in isolated preparations of the prosomal (cephalothoracic) CNS. The isolated CNS normally generates only unpatterned activity. Perfusion of octopamine (1 to 10 μM) elicited rhythmic motor activity in the entocoxal nerves. The phase relationships within a leg segment and between leg segments and the frequency of the induced rhythmic activity, were consistent with and characteristic of the feeding pattern in intact animals.

We have also explored the pharmacology of octopamine's stimulation of the feeding central pattern generator (CPG). Alpha-adrenergic agonists such as clonidine, naphazoline and tolazoline had no effect on the feeding CPG. Dopamine and norepinephrine stimulated the feeding CPG, but at concentrations ca. ten times greater than octopamine. (These catecholamines are structurally similar to octopamine and may stimulate the same receptors.) Alpha-adrenergic antagonists such as clozapine, metoclopramide and phentolamine weakly antagonized octopamine's stimulation of the CPG. The octopamine agonist NC-5 and the diterpene forskolin were as effective as octopamine in stimulating feeding motor output.

We have preliminary immunocytochemical evidence indicating the presence of paired clusters of octopaminergic cells in the ventral portions of the circumesophageal ring of fused thoracic ganglia. We suggest that such cells may release octopamine onto cells involved in generating the rhythmic motor output, the octopamine action presumably being mediated by an adenylate cyclase.

220.8 SELECTIVE AND INDEPENDENT ACTIVATION OR SUPPRESSION OF SUBUNITS OF THE CENTRAL PATTERN GENERATOR UNDERLYING FEEDING IN HELISOMA. A. D. Murphy. Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60680.

Feeding in Helisoma, like many other rhythmic behaviors, is largely timed and driven by a group of neurons called a central pattern generator (CPG). In the 1970s Kater and colleagues described the basic behavior, many of the muscles and motor neurons, and some aspects of the CPG underlying feeding. Kater described a 2-phase protraction/retraction feeding cycle and showed that a network of electrically coupled interneurons could generate a series of bursts of action potentials following a brief (i.e. a few msecs) depolarizing pulse into a single interneuron. These interneurons evoked suprathreshold excitatory post-synaptic potentials in "retractor" motor neurons and, simultaneously, evoked inhibitory post-synaptic potentials in "protractor" motor neurons. Following the synaptic barrage the retractor motor neurons would become silent and the protractor motor neurons would generate a burst of action potentials from post-inhibitory rebound. This was correct insofar as it went, but as Kater reported (Am. Zool. 14, 1017-1036, 1974) feeding behavior in Helisoma is variable and complex, and his analysis was an over-simplification. Nonetheless, recent reviews of gastropod feeding have indicated that the behavior in Helisoma is simpler than in most other gastropods, which typically have at least three phases of synaptic activity per feeding cycle. In fact, the patterned motor activity (PMA) in Helisoma is remarkably similar to that of another basommatophoran snail, Lymnaea. Each cycle can be divided into three major barrages of synaptic activity (N1, N2, and N3) and a fourth phase which, for most effector neurons, represents an inactive period (I). Furthermore, while the N1, N2, and N3 phases of activity in Helisoma are often phase-linked, the N3 phase, (i.e. the N3 subunit of the CPG) is relatively independent of the other two. The N1 and N2 phases may be obligatorily locked together, but either the N1/N2 phases or the N3 phase can occur independently. Also, both the onset and duration of N3 synaptic activity during PMA is quite variable and this variability profoundly influences the action potential burst configuration in particular identified effector neurons. In addition, stimulation of small cardioactive peptide B immunoreactive neuron VB1 in quiescent ganglia can elicit N3-like synaptic activity in identified follower neurons, and hyperpolarization of neuron VB1 during PMA can eliminate the N3 phase of synaptic activity. Finally, stimulation of an identified inhibitory neuron, which is normally inactive during PMA, can selectively eliminate the N3 synaptic activity. Thus, subunits of a CPG underlying relatively stereotyped, but still variable, PMA and, hence, oral behaviors, can be selectively and independently activated or suppressed.

220.9 EVIDENCE FOR AN EXCITATORY EFFECT OF GABA ON FEEDING PATTERNED MOTOR ACTIVITY (PMA) OF HELISOMA TRIVOLVIS. J.E. Richmond, A.D. Murphy, A.G.M. Bulloch and K. Lukowiak. Dept. Physiology, University of Calgary, HSC, 3330 Hospital Dr. N.W., Calgary, AB, Canada T2N 4N1.

The feeding system of Helisoma trivolvis is one of a few systems in which we can begin to examine the neural basis of a discrete behavior at the level of identified neurons. The cyclical patterned motor activity (PMA) of buccal neurons is subject to variability presumably reflecting that seen in the feeding behavior of the intact animal. Our observations lead us to postulate that the regulation of PMA is under the control of several distributed systems involving a number of neurotransmitters/ neuromodulators. These include serotonin, dopamine, FMRFamide, SCP_B and AVT. Here we present both immunohistochemical and electrophysiological evidence that GABA is involved in the regulation of PMA in Helisoma.

Using a GABA antibody we have visualized a network of irGABA neurons within the CNS consisting of five pairs of neurons in the buccal ganglia and several clusters in the cerebral ganglia with many communicating irGABA fibers in the cerebrobuccal connectives. There are no irGABA fibres in any of the other buccal nerve trunks implying a CNS role for GABA. An additional group of 4 neurons is present in each pedal ganglion.

Buccal neurons B5 (which innervates the gut) and B19 (a motor neuron) were impaled in order to assess the effects of bath application of GABA. With the central ring ganglia attached, GABA at concentrations of $5X10^{-3}M$ activates the CPG for PMA in quiescent preparations, and increases the rate of PMA in active preparations.

Iontophoresis of GABA onto neuron B19 in low Ca^{2+}/high Mg^{2+} saline produced a hyperpolarization which was reversed by Cl^- injection. This direct inhibitory action on neuron B19 was blocked by picrotoxin at a concentration of $10^{-4}M$. These results indicate that the GABA hyperpolarizing response in B19 is mediated by Cl^- channel activation. Given that GABA has been demonstrated to inhibit B19, it is possible that it brings about an increase in PMA by disinhibiting the CPG. Supported by AHFMR and MRC (Canada).

220.10 MODULATION OF NEUROGLANDULAR ACTIVITY BY PEPTIDES IN HELISOMA. A.G.M. Bulloch and A.D. Murphy. Department of Physiology, University of Calgary, H.S.C., Calgary, Alberta, Canada, T2N 4N1.

The present study is part of a series in which the role of peptides in regulating feeding behavior of Helisoma is being investigated. Here we focus on a neuroglandular synapse and investigate the mechanisms by which the molluscan peptides FMRFamide and SCP_B can modulate neuroglandular activity. A previous study has characterized the buccal ganglion - salivary gland synapse as follows: the magnitude of the gland EPSP is proportional to the spike width of the innervating neuron (buccal neuron 4), and both can be reduced by hyperpolarization of the neuron 4 soma. Furthermore, bath application of SCP_B and FMRFamide to the buccal ganglion/salivary gland preparation can modulate the efficacy of the gland synapse by modulating the membrane potential of neuron 4 (Coates and Bulloch, 1985, J. Neurosci. 5: 2677-2684). The present study asks the following questions: (1) Are irSCP and irFMRFamide fibers present upon the salivary gland? (2) Can iontophoretic application of peptides at two potential sites of action (i.e., near the neuron 4 soma and near its terminals on the gland) modulate neuroglandular activity?

The Helisoma buccal ganglia/salivary gland preparation was examined for neurons and fibers immunoreactive to SCP_B and FMRFamide. Extensive networks of beaded immunoreactive fibers were revealed upon the gland by both antisera. In the case of SCP_B, the fibers originated from buccal neuron 4 whose soma and axon are also immunoreactive for SCP_B. The source of the irFMRFamide fibers has not as yet been identified. Given that immunoreactive SCP_B and FMRFamide fibers are present both within the ganglion neuropil and upon the salivary gland, both of these sites are potential loci for action of the peptides.

SCP_B and FMRFamide were iontophoresed either onto gland cells or onto the soma of neuron 4. Iontophoresis of FMRFamide upon the neuron 4 soma caused a substantial hyperpolarization which was due to a conductance increase. This hyperpolarization shunted ongoing rhythmic synaptic input and virtually eliminated rhythmic bursting in neuron 4 and concurrent activity in gland cells. In contrast, iontophoresis of SCP_B caused neuron 4 to depolarize which increased its firing frequency during ongoing activity and consequently increased spiking in the salivary gland. Peripheral application of the peptides to gland cells modulated the neuron 4 gland EPSP, FMRFamide causing reduction and SCP_B enhancement respectively. It is concluded that release of peptides at both potential sites of action, i.e., within the ganglion and the terminals of neuron 4, can modulate the efficacy of neuroglandular activation. Supported by MRC (Canada).

220.11 PATTERNS OF REGENERATIVE ACTIVITY IN COMB PLATES DURING THE UNILATERAL* FEEDING REVERSAL IN THE CTENOPHORE. A. G. Moss and S. L. Tamm. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

Feeding in the cydippid ctenophore Pleurobrachia is effected by a unilateral reversal of comb plate beat direction on the side of the prey-catching tentacle (Tamm and Moss, J. Exp. Biol. 114:443, 1985). Reversal of the power stroke direction is associated with regenerative activity (Moss and Tamm, J. Comp. Physiol. 158:in press, 1986) in reduced preparations for intracellular recording. Because of the unique size of the plate, electrodes can be attached extracellularly directly to the cilia (Moss and Tamm, submitted). This method allows recording of in vivo comb plate electrical activity during feeding or evoked unilateral reversal. Electrodes were attached to comb plates of 11 animals on the stimulated or catching side. Intact animals displayed two types of postsynaptic activity in response to pulse train stimulation. A through-conducted response of small postsynaptic potentials followed the stimulus artifact in a one-for-one manner as seen in the reduced preparation and was often associated with an increased normal beat rate. Reversal of effective stroke was associated with volleys of action potentials which occurred at a rate (~3 Hz) independent of the stimulus frequency (4-6 Hz). Subtentacular (st) rows had very similar electrical activity along their lengths and between adjacent rows, and action potentials occurred nearly simultaneously at all sites. The lag between individual postsynaptic events in any two locations on the same row or between adjacent st rows was less than 60 ms; action potentials in adjacent st rows recorded at the same oral-aboral placement had a lag of less than 10 ms. Although subsagittal rows responded with reversal volleys in about half of the cases, individual events in such volleys occurred at the same rate in near synchrony with activity in the st rows. In either row, the length of the volley was directly related to the length of time reversal persisted in that plate or region of that row. However, plates near the oral end of the row were more likely to reverse beat and began reverse beating earlier than more aborally-positioned plates despite very similar electrical activity, thus providing an explanation for oral-end initiation of the reverse metachronal wave in the apparent absence of mechanical stimulation. Preliminary results suggest that the endogenous oscillation of the reversal volley is dependent upon the presence of a critical amount of the ectodermal nerve net.
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220.12 A NOVEL ROLE FOR CRUSTACEAN RED PIGMENT CONCENTRATING HORMONE: NEUROMODULATION OF THE PYLORIC CPG IN THE CRAB CANCER BOREALIS. M.P. Nusbaum & E. Marder. Biology, Brandeis Univ., Waltham, MA 02254.

Crustacean Red Pigment Concentrating Hormone (RPCH) causes the aggregation of pigment molecules in crustacean chromatophores and pigment-containing ommatidial cells (Rao, 1985, The Biology of Crustacea, 9:395). RPCH has been localized previously to a neurohaemal organ, the sinus gland, and to the CNS (Rao, 1985). We now report immunocytochemical localization of RPCH or an RPCH-like antigen to the stomatogastric nervous system, and excitatory effects of bath-applied synthetic RPCH on the pyloric central pattern generator in the isolated stomatogastric ganglion (STG) of the crab Cancer borealis.

Anti-RPCH antiserum (gift of Madsen & Elde: Madsen et al., 1985, Soc Neurosci Abstr 11:941) labels fine neuropilar processes in the STG and several fibers in the stomatogastric nerve, the nerve by which the STG interacts with the rest of the CNS. Two somata in the unpaired oesophageal ganglion (OG), as well as neuropilar processes and approximately ten somata in each of the paired commissural ganglia, also label. Preincubation of the anti-RPCH antibody with $10^{-8}M$ RPCH completely blocked staining. In contrast, preincubation with either $10^{-4}M$ adipokinetic hormone (AKH), a structurally similar arthropod hormone, or thyroglobulin (1mg/ml), to which RPCH was conjugated in generating the antiserum, did not block labeling. The RPCH-like antigen colocalizes with a FMRFamide-like molecule in the two stained OG somata. Labeling of these somata was not blocked by preincubation of the anti-RPCH antibody with $10^{-4}M$ FMRFamide, or preincubation of an anti-FMRFamide antibody (Marder, Calabrese, Nusbaum & Trimmer, In Preparation) with $10^{-4}M$ RPCH.

Bath-application of synthetic RPCH to the isolated STG initiated rhythmic pyloric activity in quiescent preparations (10^{-8}-$10^{-6}M$) and increased the pyloric frequency in already cycling preparations ($>10^{-10}M$). Frequency increases were maximal with $10^{-7}M$ RPCH, although $10^{-6}M$ RPCH often more strongly affected the waveforms and impulse bursts of the component pyloric CPG neurons. $10^{-6}M$ RPCH sometimes caused periodic interruptions in the pyloric rhythm which were apparently due to activation of the gastric mill CPG, also located in the STG.

Bath-applied AKH ($10^{-6}M$) mimicked the effects of $10^{-6}M$ RPCH on the pyloric CPG. Proctolin and FMRFamide also increase the frequency of the pyloric rhythm (Hooper & Marder (1984), Br Res 305:186), but the pattern of activity expressed with RPCH application was different from the patterns produced by the other two neuropeptides. Possible correlations between the hormonal and neuromodulatory roles of RPCH are being studied.
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221.1 EVIDENCE FOR A PHYSIOLOGICAL ROLE OF ATRIAL NATRIURETIC FACTOR AND ANGIOTENSIN II IN THE CENTRAL REGULATION OF DRINKING. A. Negro-Vilar and C. Masotto (SPON: J.E. Wilson). Reproductive Neuroendocrinology Section, Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

The central regulation of thirst and drinking behavior is complex and multifactorial. Among the many factors involved, different peptides have been shown to affect drinking behavior in several species. Angiotensin II (AII) has been shown to be a very potent dipsogenic agent, and evidence has been presented that indicates that AII neurons and receptors are located in brain areas known to be involved in the regulation of thirst. Recently, mapping of atrial natriuretic factor (ANF) neurons also showed a distribution consistent with a role of these peptides in the central regulation of fluid homeostasis. We have presented evidence (Brain Res. Bull. 15: 523,1985) that central administration of atriopeptin III results in an inhibition of spontaneous water drinking in the rat and, moreover, that atriopeptin III given intraventricularly (ivt) inhibited the stimulatory effect of AII on water ingestion. The present experiments were designed to determine whether the endogenous AII and ANF brain neuronal systems are physiologically involved in the regulation of water drinking in the rat. Adult male rats bearing chronic lateral ventricular cannula were used for all experiments. After implantation of the cannula, rats were allowed to recover and water intake was measured daily. Specific, high titer antibodies against AII (Ab E-44), ANF (Ab R4B16, a gift from Dr. De Bold) and arginine vasopressin (Ab E-291) were administered ivt, at the dose of 10 µl infused over several minutes. After administration of the different antibodies, late in the afternoon, water intake was monitored at different times during the following 24 h. Administration of ANF Ab ivt induced a significant increase in water ingestion at 30 min, 3 h and 24 h. Conversely, ivt administration of AII Ab, drastically suppressed water intake and a reduced water ingestion was still apparent 24 h later. On the other hand, ivt administration of vasopressin Ab in a similar regimen did not affect water drinking when compared with appropriate controls. In an additional experiment, ANF-Ab was given ivt followed 6 min later by a dose of AII (25 ng) which produces submaximal increases in water intake. The ANF-Ab greatly potentiated the AII-stimulated water intake within the first 15 min. The results of these experiments clearly indicate that endogenous AII and ANF systems are involved in the physiological regulation of thirst mechanisms, evoking stimulatory or inhibitory drinking behaviors, respectively. Lastly, an opposite interaction between the AII and ANF systems, as suggested by our earlier studies, is further supported by these data.

221.2 CATECHOLAMINE METABOLISM IN THE LATERAL HYPOTHALAMUS IS RELATED TO SERUM OSMOLALITY AND NOT TO VASOPRESSIN SECRETION. P.A. Mason, J.A. Durr*, and C.R. Freed, Depts. of Med. and Pharm., Univ. Colo. Health Sciences Center, Denver, CO 80262.

The lateral hypothalamus (LH) plays a role in eating and drinking. We have studied the endogenous release of neurotransmitters from LH during normal drinking behavior. Male Sprague-Dawley rats 300-325 g had carbon paste *in vivo* electrochemical (EC) electrodes implanted in the LH. 72 hr after surgery, animals were water deprived for 24 hr. EC electrodes were scanned with linear sweep voltammetry from -0.2V to +0.5V at a rate of 5 mV/sec. Semiderivative signal processing showed a catecholamine and and indoleamine peak at +0.11 V and +0.23 V, respectively. Rats were then given distilled water or 0.9% NaCl. To control for the act of drinking, other rats were injected i.p. with 10 ml of 5% dextrose, 0.3% NaCl, 0.9% NaCl or 4 ml of 12% NaCl. N = 6, all treatments. Tissue monoamines were measured directly in animals sacrificed after treatments as described above. Blood was assayed for osmolality (OSM) and vasopressin (VP). LH was assayed for norepinephrine (NE), dopamine (DA), DOPAC, serotonin (5-HT), and 5-HIAA using HPLC with EC detection. These studies were repeated in male homozygous Brattleboro rats 325-350 g. These animals lack VP but preserve normal serum OSM with prodigious drinking, and so provide a way to dissociate neurotransmitter changes associated with VP release from changes related to OSM. Water deprivation persisted for only 9 hr in the Brattleboro rats and then the animals had 10 hr of free access to water. *In vivo* EC recordings were made before, during and after water deprivation. Results in the Sprague-Dawley rats are shown in the table below:

Treatment	% Baseline EC	Osmolality	Vasopressin	NE(nmole/gm)
Hyd. + 12% NaCl	--	324 ± 1	115	11.2±0.6
Dehyd. Control	100	301 ± 1	80 ± 10	13.9±0.7
0.9% NaCl i.p.	110 ± 2	294 ± 2	--	--
0.9% NaCl p.o.	120 ± 9	301 ± 3	34 ± 3	15.1±1.3
0.3% NaCl i.p.	140 ± 6	293 ± 2	--	--
D5W i.p.	165 ± 10	281 ± 2	--	--
Distilled H2O	200 ± 15	288 ± 3	41 ± 6	16.6±1.5

There was a negative correlation between the concentration of NE and serum OSM and VP. In Brattleboro rats, water deprivation led to a reduction in the EC signal for catecholamines to 40% of baseline. When animals were given distilled water, the catechol signal immediately increased to its pre-water deprivation value. We conclude that changes in the EC peak for catechols and changes in tissue NE in LH are directly related to changes in serum OSM in both normal and VP deficient animals.

221.3 TASTE ACTIVITY IN THE NUCLEUS TRACTUS SOLITARIUS OF CHRONIC DECEREBRATE RATS. G. P. Mark* and T. R. Scott. Dept. of Psychology and Inst. for Neuroscience, Univ. of Delaware, Newark, DE, 19716.

The ingestive behavior of decerebrate rats has been studied extensively, but little is known of its neural substrates. While taste activity proceeds from hindbrain to thalamus and ventral forebrain, this latter region also sends centrifugal fibers to the hindbrain, thereby directly influencing taste activity. We examined the electrophysiological characteristics of taste neurons in the nucleus tractus solitarius (NTS) of chronic decerebrate rats in which this reciprocal communication was disrupted. Wistar rats (350-450 gm) were decerebrated at the supracollicular level as described by Grill and Norgren. After a minimum of one week recovery they were immobilized with flaxedil, administered local anesthetics and prepared for recording. The responses of 50 taste cells were isolated bilaterally from the NTS. Taste stimuli included seven Na-Li salts (including four concentrations of NaCl), three sugars, NaSaccharin (two concentrations), HCl and citric acids and quinine HCl. Mean spontaneous activity was 6.5±5.6 spikes/sec, not significantly lower than the level in intact rats. Cells were broadly sensitive, with a mean breadth-of-tuning metric of 0.80 (vs. 0.77 in intact rats). As with normal rats, most neuron response profiles could be grouped into three loose clusters with peak sensitivities to acid-salt, salt or sugar. An analysis of similarities among stimulus response profiles indicated that Na-Li salts and sweet stimuli tended to be affiliated, as did acids and quinine. In intact animals the primary distinction is between sweets and non-sweets. Intensity-response functions generated from the four NaCl concentrations were similar to those in normal rats. Thus, while the distinction between sweet and salty stimuli may be partially obscured, the overall integrity of the taste system appears to be maintained in decerebrates.

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221.4 ASSOCIATIVE CHANGES IN TASTE REACTIVITY DURING A LITHIUM-INDUCED INTERNAL STATE IN INTACT AND CHRONIC DECEREBRATE RATS. A.C. Spector* & H.J. Grill. Dept. of Psychology, University of Pennsylvania, Phila., PA 19104.

Grill and Norgren (1978) have shown that supracollicullar chronic decerebrate (CD) rats are unable to demonstrate conditioned taste aversions (CTA), even after 8 taste-LiCl pairings, when tested 24 hours following a pairing. In contrast, intact rats require only one taste-lithium pairing to demonstrate a reliable CTA. We have extended these findings by examining how both intact and CD rats respond to intraoral sucrose infusions during the onset and initial phases of a LiCl-induced internal state. Intact rats systematically change their oromotor behavior to sucrose from an ingestive to an aversive profile of responses during the time period immediately following LiCl injection. Intact rats injected with NaCl maintain ingestive responding throughout the session. Results from other experiments suggest that this effect is based on associative processes. At a high dose (3.0 mEq/kg) of LiCl, CD rats similarly decrease their ingestive oromotor responses to sucrose but fail to show an increase in aversive oromotor responses. When CD rats are injected with NaCl they maintain ingestive responding throughout the session. This response profile to sucrose with high LiCl doses in CD rats has been shown by other experiments to be caused by the general suppressive effects of this dose of lithium in this preparation and is not attributed to associative processes. We therefore tested both intact and CD rats with a lower LiCl dose (1.0 mEq/kg). Intact rats gradually changed their oromotor responses to 0.4 ml intraoral sucrose infusions when these infusions were delivered for 30 sec every 5 min during a 30-minute period immediately following LiCl injection. That is, they decreased their ingestive oromotor behavior and increased their aversive responses to sucrose as the test session progressed. In contrast, CD rats maintained a highly ingestive profile throughout the test session. To the extent that the changes in oromotor behavior displayed by the intact rat are based on associative processes, the failure of CD rats to alter their taste reactivity profile to sucrose following low dose LiCl injection suggests that the forebrain is necessary for the formation of CTA in rats. Furthermore, based on these results, the deficits in CTA described by Grill and Norgren cannot be easily explained by a memory deficit in which CD rats do learn CTAs but forget them when tested 24 hours later.

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221.5 LiCl-INDUCED CONDITIONED TASTE AVERSION IS ASSOCIATED WITH CONDITIONED HEART RATE RESPONSES. T. Kosten and R.J. Contreras. Dept. of Psychology, Yale University, New Haven, CT 06520.

In laboratory studies, conditioned taste aversion is an easily-induced, robust phenomenon that reflects the animal's ability to learn to avoid foods associated with illness. In these studies, the taste solution is paired with an agent that presumably causes gastrointestinal malaise; decreased solution intake is usally the sole measure of conditioning. This is problematic because the stimulus is confounded with the response measure, which itself is a decrease in performance. We assumed there must be physiological responses associated with the unconditioned stimulus, the effects of the drug. Certain physiological responses may become conditioned such that the conditioned stimulus, the taste solution, would elicit conditioned responses. We found that change in heart rate was one such response. This is the first report of conditioned, decreased heart rate responses in conditioned taste aversion. Two groups of rats were conditioned with two different drugs: LiCl (50 mg/kg), which lowers heart rate, and scopolamine methyl nitrate (1 mg/kg), which raises heart rate, were studied. A control group had the conditioned and unconditioned stimuli presented in an unpaired manner. The animals were given discrimination training such that a .001 M Na saccharin solution (CS+) was paired with the drug injection and a .001 M HCl solution (CS-) was paired with .15 M NaCl injections. To test for conditioned taste aversion behavior, we measured CS+ preference in a two-bottle drinking test with the CS-. Both the LiCl and scopolamine groups showed a significantly lower saccharin preference compared to controls ($p<.0001$). We also measured heart rate responses. LiCl injections alone elicited an unconditioned, decrease in heart rate ($p<.0001$), while scopolamine injections elicited an unconditioned, increase heart rate ($p<.0001$). Compared to other solutions, a conditioned, decreased heart rate response to the CS+ solution occurred for the LiCl group. Both the control and scopolamine groups showed no conditioned heart rate responses. These results are supported by the significant solution and solution x group effects ($p's<.0001$). Thus, LiCl and scopolamine are both effective in inducing avoidance of saccharin, yet they are associated with different unconditioned and conditioned physiological responses. Studying the physiological responses, which show better discrimination than the behavior, will allow us to better understand the bases of conditioned taste aversion.

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221.6 PERTURBATIONS IN GLUCOSE HOMEOSTASIS INITIATE FEEDING IN RATS: ALTERED TEMPORAL RELATIONSHIPS IN VMH OBESITY. F. J. Smith* and L. A. Campfield (Spon: C. Enroth-Cugell) Depts of Physiology and Biomedical Engineering, Northwestern University, Chicago and Evanston, Il. 60611 and 60201

Our recent studies have shown that transient declines in blood glucose are causally related to meal initiation in free-feeding rats. We have defined the temporal relationships between transient declines in blood glucose and meal initiation in intact rats (Brain Res. Bull. 14; 1985: 16, 1986). In order to test this hypothesis in rats with altered meal pattern, hyperinsulinemia and obesity, continuous monitoring of blood glucose and meal pattern was performed in ventromedial hypothalamic (VMH) lesioned obese rats.

Female Wistar rats that received successful bilateral lesions (transient hyperphagia, increased body weight, and hyperinsulinemia) were implanted with chronic cardiac cannulas 1 week before experimental studies. Experiments were performed 4 weeks post lesion in awake, unstressed rats in their home cages. Studies were conducted in both the light phase and across the light/dark transition. Continuous blood withdrawal (25 μl/min) for blood glucose monitoring was begun 45 min. after IV heparin (200 U).

In 7 experiments with VMH transient declines in blood glucose with similar magnitude (-10%) and time course (19 min) as those seen in intact rats occurred prior to the 10 meals recorded. However, the temporal relationships between the decline in blood glucose and meal initiation was different in VMH lesioned and intact rats: Meal initiation occurred sooner after the nadir of blood glucose (2.6 ± 2.1 vs. 8.7 ± 2.2 min. $p < 0.05$) and the latency to meal initiation was shorter in VMH lesioned rats. Meal size was also increased in VMH lesioned rats (1.8 ± 0.6% vs. 0.7 ± 0.1 gm $p < 0.05$).

The similarities observed in these studies suggest that the causal relationship between transient perturbations in blood glucose homeostasis and meal initiation in intact rats was maintained in obese rats, while the differences in the temporal relationships may, in part, explain the altered meal pattern observed in hypothalamic obesity.

We conclude that these perturbations in glucose homeostasis are among the endogenous cues for meal initiation in both normal and obese states.

221.7 BLOOD GLUCOSE AND MEAL INITIATION: A ROLE FOR INSULIN? L. A. Campfield and F.J. Smith* Depts. of Physiology and Biomedical Engineering, Northwestern University, Chicago and Evanston, Il. 60611 and 60201

In recent studies, we have provided evidence for a causal relationship between transient declines in blood glucose (BG) and meal initiation (MI) in freely behaving rats. We have shown that these transient declines are independent of the presence of food or feeding. The studies reported here represent our initial efforts to determine the mechanisms responsible for the transient declines in BG. We have determined the plasma concentration of insulin prior to and during transient declines in BG.

Six Wistar rats weighing 200-300 gms were implanted with chronic cardiac cannulas and were allowed to recover for 4-7 days. Heparin (250U) was administered ~ 90 min before the next meal was expected (based on previously recorded meal patterns). Blood withdrawal (25 μl/min) using a peristaltic pump was begun ~ 40 min before expected meal. Blood was continuously collected and pooled over 4 min intervals for the next 40-100 min.

Seven experiments were conducted; 4 in the light phase and 3 across the light/dark transition. In two experiments in which feeding did not occur, plasma insulin and glucose remained constant (CV's were 13.8 ± 1.5% and 2.7 ± 0.1% respectively). In 5 of the experiments in which feeding occurred, plasma insulin remained at baseline concentrations (2.2 ± 0.6 ng/ml) until -14 min (prior to the meal), then increased rapidly to a peak (50 ± 7%) at -12.6 ± 1.1 min and then gradually fell reaching baseline or falling slightly below at meal initiation. Plasma glucose remained at baseline concentrations (141 ± 9 mg/dl) until -8 min and then fell to a nadir (-9.8 ± 1.9%) at -3.0 ± 2.4 min before meal initiation. The latencies from the insulin peak and the onset of the fall in glucose to meal initiation were positively correlated ($r = 0.85$) and indicated that in 4 of 5 experiments, the insulin peak occurred (-12.6 ± 1.1 min) before the beginning of the decline (-7.0 ± 2.4 min) or the nadir (-3.0 ± 2.4 min) in glucose.

Therefore, these studies have demonstrated that a consistent, small peak in plasma insulin occurred prior to or at the onset of the transient decline in BG that precedes and signals MI. They suggest that this rise in insulin may be among the factors responsible for the transient decline in BG. The hormonal and/or neural mechanisms underlying the observed rise in insulin and the validation of its suggested role in the origin of transient declines in BG and, thus, in the control of MI must await further experimental studies.

221.8 DECREASED CEREBROSPINAL FLUID INSULIN AND GLUCOSE IN RATS WITH DIETARY-INDUCED OBESITY. A. J. Sipols*, R. Mehiel*, D. P. Figlewicz*, D. M. Dorsa, D. Porte, Jr.* and S. C. Woods. (SPON: E. C. Lotter). Depts. of Psychology, Medicine, and Pharmacology, Univ. of Washington and VA Medical Research Center, Seattle, WA 98195.

Animals offered sweet-tasting calorically rich diets overeat and eventually become obese and have elevated plasma insulin. Since we have found that cerebrospinal fluid (CSF) insulin is correlated with plasma insulin, and since an elevated CSF insulin might be expected to decrease appetite, we determined plasma and CSF insulin in rats fed the starch-derived polysaccharide, Polycose. Male Long Evans rats (N=9) were initially offered a 15% w/v Polycose solution ad lib for 10 days, followed by 10-day blocks with access to 18.3%, 21%, and 25% solutions consecutively; subsequent 10-day blocks were associated with 5% incremental increases up to a final maximum of 65%. They also had ad lib access to laboratory chow. Initial age- and weight-matched controls (N=6) were fed chow ad lib throughout the experiment. At the end of the experiment body weights and overnight-fasted plasma and CSF insulin and glucose levels were measured. Animals fed polysaccharide solution were significantly heavier than controls, but there were no reliable differences of plasma insulin or glucose between the two groups. The levels of CSF insulin and glucose were significantly decreased for the polysaccharide group. These findings are inconsistent with CSF insulin levels seen in genetic obesity, and may help explain the persistent hyperphagia in rats offered the polycose solution.

	Experimental	Control	p
Body Weight (g); Mean (SEM)	471.8 (9.1)	434.5 (15.9)	<.05
Plasma Insulin (μU/ml)	33.1 (7.0)	56.7 (13.0)	n.s.
Plasma Glucose (mg/dl)	117.4 (9.3)	143.3 (20.0)	n.s.
CSF Insulin (μU/ml)	0.35 (0.13)	0.88 (0.23)	<.05
CSF Glucose (mg/dl)	64.4 (4.2)	78.5 (3.5)	<.03

221.9 INTRACEREBROVENTRICULAR INJECTIONS OF INSULIN-LIKE GROWTH FACTOR II, BUT NOT INSULIN-LIKE GROWTH FACTOR I, DECREASE FOOD INTAKE AND BODY WEIGHT IN RATS. T.J. Lauterio*,L. Marson*,W.H. Daughaday*and C.A. Baile. Dept. of Preventive Medicine,Washington Univ. Sch. of Med., St. Louis, MO 63110

A recent study (Tannenbaum et al., Science 220:77, 1983) reported that intracerebroventricular (icv) injections of insulin-like activities (ILAs, 106 ng equiv./rat) reduced food intake by 60% and body weight by 16% in rats over a 24 hour period compared to controls. Since ILAs are crude preparations containing both insulin-like growth factor I (IGF-I) as well as insulin-like growth factor II (IGF-II), it was of interest to determine which IGF, if any, was responsible for the above responses using highly purified hormones. Radioimmunoassay of the IGFs in specific regions of the hypothalamus revealed little or no IGF-I, but IGF-II was present in significant concentrations. Areas rich in IGF-II were the dorsomedial hypothalamus (428 ± 49 pg/ug protein) and supraoptic nucleus (337 ± 86 pg/ug protein). The lowest concentrations were found in the ventromedial hypothalamus (128 ± 28 pg/ug protein). Intermediate levels of IGF-II were present in the lateral and anterior hypothalami and the paraventricular nucleus. Rats (250-300 g) were implanted with cannulae extending into the lateral ventricle under sodium pentobarbital anesthesiaand allowed to recover for 5 days prior to experimentation. Food intake and body weights were recorded between 0930 and 1030 h daily starting 5 days prior to the study, extending through the injection period and 5 days post-injection. Administration of recombinantly produced IGF-I (Amgen) or purified IGF-II (Amano) was given at 100 ng/rat in a 10 ul volume. Controls were given either vehicle (saline) or insulin (100 ng) injections. Cannula placement was verified in the experimental animals after formalin perfusion. Food intake was reduced in IGF-II injected rats by 21% and 19% compared to saline and insulin controls respectively ($P<0.01$) 24 hours post administration. Body weight decreased in IGF-II treated rats by 4% ($P<0.5$) compared to respective weights recorded immediately preceding injections. Neither food intake nor body weight changes in IGF-I injected rats differed from controls. Food consumption was normalized by 48 hours post-injection in the IGF-II treated rats. We propose that IGF-II, but not IGF-I may have a role in the control of food intake and body weight regulation in the rat.

221.10 DEFECTIVE GLUCOSTATIC REGULATION OF ANORECTIC DRUG RECOGNITIOIN SITES AND NA^+K^+ATPase IN GENETICALLY OBESE MICE.

Itzchak Angel*, Mark E. Goldman and Steven M. Paul*, Clinical Neuroscience Branch, National Institute of Mental Health, N.I.H., Bethesda, MD 20892, USA.

According to the glucostatic theory of food-intake, glucose, which is the principal energy source of the brain, is postulated to directly modulate food intake through a central satiety mechanism. In a previous study (Angel et al, Proc. Natl. Acad. Sci. USA, 82, 6320, 1985) we have demonstrated the functional coupling between an anorectic drug recognitiion site and Na^+K^+ATPase, in their stimulation by glucose. The anorectic drug recognition site was labeled in hypothalamic membranes using either (+)[^{3}H]amphetamine or [^{3}H]mazindol. [^{3}H]ouabain binding was used to specifically label the neuronal form of Na^+K^+ATPase. In the present study we have compared genetically obese mice (ob/ob) to their lean littermates (?/+) as to the level and response of [^{3}H]mazindol and [^{3}H]ouabain binding to exogenous glucose. Obese mice were found to have significantly higher levels of [^{3}H]mazindol and [^{3}H]ouabain binding in the hypothalamus and brainstem, which was highly correlated with their blood glucose level. No differences were observed in the cerebellum, striatum or cerebral cortex. Using an in vitro slice preparation, both [^{3}H]ouabain and [^{3}H]mazindol binding were stimulated upon preincubation with physiological concentrations of glucose in the hypothalamus and brainstem of lean mice. Obese mice, which already have elevated levels of binding, failed to respond further following incubation with glucose. Furthermore, in preliminary experiments, ob/ob mice failed to increase food intake following glucoprivic challenge with the glucose antagonist, 2-deoxy-D-glucose; a known stimulator of carbohydrate intake. Our results suggest a defective central glucostat in obese mice which may be involved in the etiology and (or) pathophysiology of their obesity.

221.11 POLYUNSATURATED FAT DIETS INCREASE CARBOHYDRATE PREFERENCE IN RATS. C.E. Greenwood and S.B. Crane*. Dept. Nutritional Sciences, University of Toronto, Toronto, Ontario M5S 1A8.

Dietary fat source modifies certain behaviours such as pain sensitivity and thermoregulation (Pharm.Bio.Behav. 24(6), 1986). Since both behaviours are in part under monoaminergic control, the purpose of this study was to further examine the functional consequences of altered dietary fat intake on monoamine function by examining food selection (protein (PROT) and carbohydrate (CHO), a behaviour mediated by serotonin. Rats selected from two diets containing the same dietary fat (20% w/w lard or soybean oil (SBO) but differing in PROT and CHO content (5% PROT and 67.4% CHO or 55% PROT and 17.4% CHO). While total caloric intake was unaffected (78±7 vs 77±1 kcal/d (mean±SEM,n=10) for lard and SBO, respecitvely), animals selecting from lard-based diets consumed less CHO and more PROT than rats selecting from SBO-based diets (CHO: 7.4±.4 vs 9.0±.2 g/d; PROT: 4.6±.2 vs 2.9±.2 g/d; PROT/CHO ratio: .65±.06 vs .33±.03). That is, the source of dietary fat fed had a marked impact on PROT and CHO intake regulation. A second study was conducted to examine the role of dietary essential fatty acid intake in mediating the behavioural changes observed. SBO is rich in linoleic and linolenic acids while lard is a relatively poor source of both. Therefore we examined the effect of sunflower oil (SFO) since it is high in linoleic yet low in linolenic acid content. Rats selecting from SFO diets chose a PROT/CHO ratio similar to that of SBO fed animals and lower than that of lard fed rats (.64±.12, .33±.04 and .38±.06 for lard, SBO and SFO, respectively). These results support and extend our previous work demonstrating that qualitative changes in dietary fat intake influence certain behaviours and furthermore suggest that linolenic acid intake may not be an important variable mediating the effects of dietary fat on feeding behaviour in rats.

To explore the physiological mechanism whereby dietary fat is exerting its effect on monoamine-mediated behaviours, we measured the activity of the membrane-bound catabolic enzyme, monoamine oxidase (MAO) after 28 days of feeding 20% lard or SBO diets. Vmax of brain mitochondrial MAO was higher in rats fed lard diets compared to those fed SBO diets (19±5 vs 14±4 nmoles/mg prot/20'). Km was unaffected. Although the magnitude of change in MAO activity was insufficient to alter steady state levels of brain stem serotonin or its metabolite, 5-hydroxyindole acetic acid, the results provide evidence that the functioning of the serotonergic neuron is modified by dietary fat source. In conclusion, the results of these studies provide evidence that the source of dietary fat influences 1) feeding behaviour and 2) neuronal function in rats (Human Nutrition Research Council of Ontario and Medical Research Council of Canada).

221.12 INTRAPORTAL ADMINISTRATION OF FATS FAILS TO ELICIT SATIETY. D.Greenberg*, D.C. Becker*, J. Gibbs, and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll. Eating Disorders Institute and Bourne Behavioral Research Laboratory, New York Hospital-Cornell Medical Center, White Plains, NY 10605.

Intraduodenal infusions of a mixture of fats (Intralipid, Kabi Vitrum Inc.,Ca.) at physiological rates rapidly inhibit sham feeding and elicit behaviors typical of satiety in rats (Greenberg et al., Abstr. Soc. Neurosci. 10:531, 1984). To determine if fat-elicited satiety is mediated by preabsorptive intestinal mechanisms or by postabsorptive mechanisms we investigated the satiating potency of intraportal delivery of the same loads of Intralipid that we had previously found elicited satiety when infused intraduodenally.

Method: Nine male Sprague Dawley rats (400-450g) were equipped with chronic Silastic catheters that entered the portal vein and were exteriorized at the intrascapular region. After a 17h overnight food deprivation, rats were permitted to feed a high carbohydrate liquid food (BioServ 40% v:v) for 30 min. Intraportal infusions began 5 min prior to presentation of the liquid diet. Infusions were given at the rate of 1 ml/min. Infusions of Intralipid consisted of 3 total caloric loads of fat (1.25, 5.0, or 10.0 kcal), always at a concentration of 2 kcal/ml, or equivolumetric loads of 0.15M NaCl. Intakes were measured every 5 min.

Results: Intraportal Intralipid failed to inhibit feeding [Table 1, $F=0.87$ (3,21) $p>.4$]. In fact rats ate slightly more when Intralipid was infused than when saline was infused. In our previous study, using intraduodenal delivery of Intralipid, these same caloric loads had a potent inhibitory effect on sham feeding [$F=25.25$ (4,25) $p<.001$, see table 1].

	Mean Percent Inhibition of Intake	
	Portal Infusion (real intake)	Duodenal Infusion (sham intake)
Caloric Load		
1.25 kcal	-9.2	29.9
5.0 kcal	-17.4	67.1
10.0 kcal	-9.3	66.8

(Mean % inhibition calculated from 8-9 rats for portal and 7-8 rats for duodenal infusion; negative % inhibition indicates greater intake when Intralipid rather than saline was infused.)

The failure of portal infusions of fat to elicit satiety is strong evidence that preabsorptive intestinal mechanisms are of primary importance in mediating the satiating effect of fat infused into the intestine.

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221.13 ROLE OF CIRCADIAN TIMING IN HUMAN MEAL ONSET. J. Green*, C.P. Pollak* and G.P. Smith. Wm. Paterson College of N.J., Wayne, N.J. 07470, and Inst. Chronobiology and Eating Disorders Inst., Dept. Psychiatry, The New York Hosp.-Cornell Med. Ctr., Westchester Div., White Plains, N.Y. 10605

The determinants of meal onset are poorly understood. As a periodic behavior linked to activity cycles in animals, meal timing may be regulated by the oscillator that underlies the sleep-wake cycle. In humans studied in time isolation, Aschoff et al. (1984) observed that the length of the waketime-to-lunch interval correlated positively with the sleep-wake period (SWP), suggesting that lunch occurs as part of the architecture of the waking day.

We have explored circadian influences on meal timing in 8 subjects who experienced periods of internal synchrony and desynchrony while free-running in our laboratory. The long sleep-wake periods in free-running desynchrony (FRD, 34±3 hrs) as compared to free-running synchrony (FRS, 25±1 hrs), permitted us to examine the relationship of meal timing to the two distinct temporal frameworks of these conditions and relate them to one of the 2 major oscillators presumed to underlie circadian rhythms in man.

When the SWP lengthened in FRD, the intermeal interval (IMI) increased significantly. In 4 subjects, the increase in IMI was proportional to the increase in SWP. We also found significant increases in pre- and postlunch intermeal intervals during FRD. More than half of the subjects who ate lunch showed increases in these measures that were proportional to SWP, indicating that the lengthening of the IMI during FRD was comparable in early and later parts of subjective day. Caloric analysis demonstrated that the increase in meal spacing in FRD was not due to increased meal size: the total number and size of meals were similar on FRS and FRD "days". As a result of the lengthened IMI, caloric intake declined during FRD and weight loss occurred. The lack of proportional increase of IMI with SWC in 4 subjects could be explained by a metabolic limit on IMI. The deep circadian oscillator did not appear to affect the IMI in any subject.

The findings support an interpretation of dual regulation of meal timing. Proportional increases in IMI and SWP in the face of a caloric deficit suggest that the mechanism that governs the timing of the SWP also governs meal timing. The lack of proportional increase in IMI in some cases suggests that an additional, probably metabolic mechanism limits the intervals between meals.

Aschoff, J., Wever, R., et al., Circadian control of meal timing during temporal isolation. Naturwissenschaften 71: 534-535, 1984.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS I

222.1 NEURODEGENERATIVE LESION OF THE RAT SOMATOSENSORY THALAMUS : BIOCHEMICAL AND METABOLIC ACTIVITY ALTERATIONS. M. Peschanski, S.L. Juliano, J. Weil-Fugazza*, and F. Godefroy* INSERM U 161, 2 rue d'Alesia, Paris 75014, France ; USUHS, Dept of Anatomy, Bethesda, MA 20814.

Kainate injection in the internal capsule at the level of the thalamic ventrobasal complex (VB) produces a complete neuronal depletion in the lateral part of the nucleus. The entire lesioned area shows an extensive glial reaction and gliosis is visible in portions of the somatosensory cortex. Lemniscal afferent fibers terminating in lateral VB exhibit apparently normal electrophysiological activity several months after the excitotoxic injection (Peschanski et al., Neurosc. Lett., 58, 1985). Additionally, morphological changes in the afferent fibers seem to indicate attempts at regeneration (Peschanski et al., CRAS 301, 1985). The present study was undertaken as a preliminary step to analyze long term changes in the somatosensory system induced by VB lesion before attempting transplantation of embryonnic neurons in VB.

To study biochemical changes over time, content in aminergic substances was determined in lesioned (and paired control) VBs using high pressure liquid chromatography and electrodetection. A transitory increase in 5 HIAA and uric acid was observed at d7 but, after d7, the total amount of all substances analyzed (including norepinephrin, serotonin, dopamine, 5 HIAA and uric acid) was unchanged up to d120.

Glucose consumption was analyzed using tritiated 2 desoxyglucose at d7, d30 and d120. At d7 and d30, high glucose consumption was observed in the lesioned area and in parts of the somatosensory cortex, especially in lamina IV ; this increase was apparently due to glial activity. At d120, there was a decrease in metabolic activity in the same structures. Long term changes were analyzed in structures of the somatosensory system related to VB using cytochrome oxydase (CO) histochemistry at d30 and d60. Lamina IV of the somatosensory cortex demonstrated a loss in CO activity in areas topographically related to the extent of the thalamic lesion. Nissl-stained sections verified that this loss was not due to a transsynaptic degeneration of cortical neurons. In parallel, there was a decrease in CO activity in the contralateral dorsal column nuclei. Dorsal column nuclei neurons appeared, however, shrunken but not decreased in number in Nissl-stained sections.

This study confirms the observation that despite a complete neuronal loss in VB leading to decreased activity inthe somatosensory system (as measured by 2 DG and CO), an apparent structural preservation of the afferent circuitry persists (as indicated by biochemical, electrophysiological and anatomical results). This preservation may permit functional recovery with neuronal transplantation.

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222.2 PROJECTIONS FROM THE POSTERIOR NUCLEAR COMPLEX OF THE THALAMUS TO NON-PRIMARY SOMESTHETIC AREAS OF THE CEREBRAL CORTEX IN THE CAT. C. Avendaño and E. Rausell*. Dept. Morfologia, Fac. Medicina, Univ. Autonoma, 28029 Madrid, Spain.

The posterior nuclear complex of the thalamus (PoC) includes a number of nuclear subdivisions, which partially surround the medial geniculate and the ventrobasal complexes. Several components of the PoC receive abundant spinal projections, and their neurons respond in different ways to a variety of somesthetic stimuli. The cortical targets of these 'somesthetic' components of the PoC encompass a number of areas, which could be collectively termed 'non-primary somesthetic cortex', in some of which complete somatotopic maps have been described.

This work was aimed at investigating the organizational plan of the PoC projections to these areas. Both retrograde and anterograde tracing techniques were used. Retrograde techniques consisted of cortical injections of either free or wheat-germ agglutinin-conjugated HRP (WGA-HRP), as well as fluorescent dyes. Anterograde techniques included thalamic injections of WGA-HRP and Phaseolus vulgaris leucoagglutinin. In order to get an accurate picture of the arrangement of these projections, the cytoarchitecture of the cortical regions involved was carefully analyzed, and flat reconstructions of the cortex were obtained, on which either the injections or the terminal labeling were plotted.

Projections from the PoC reach the whole belt of cortex which surrounds caudally SI. From medial to lateral, this belt includes area 5, the anterior suprasylvian area (SsA), SII -a dorsal sector of which could, perhaps, be considered as a separate entity-, and SIV. The divisions of PoC with the largest projection to these areas are: the dorsal and rostral parts of the medial division of the posterior group (PoM); its rostral continuation, the ventrolateral-ventroposterior border zone (VL-VP), and the ventroposteroinferior nucleus (VPi). To a lesser degree, projections also arise from the lateral division of the posterior group (PoL), the caudal sectors of PoM, and the intermediate division of the posterior group (PoI). These projections exhibit a crude topographical arrangement, but, from double labeling studies, it seems that widespread projections from the same neuron into these areas are the exception rather than the rule. Anterograde studies showed, however, that neurons clustered in a small region within a sector of the PoC, may distribute projections to separate loci of the cortex. The densest terminal network concentrated in layer IV and deep III. Projections to layer I depended on the cell groups involved by the injection, and on the cortical area considered.

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222.3 CORTICOFUGAL INFLUENCES OF S1 CORTEX ON VENTROBASAL THALAMIC NEURONS IN THE AWAKE RAT. *B. Yuan, T.J. Morrow, K.L. Casey, Depts. of Neurology & Physiology, Univ. of Mich., and Neurology Research Laboratories (Neurophysiology) VA Medical Center.

In the anesthetized rat, focal suppression of S1 cortex decreases the responses of ventrobasal (VB) thalamic neurons to repetitive somatic or medial lemniscal stimuli (Yuan et al, J. Neurosci. 5: 2971, 1985). A comparison of the effect of cortical suppression on somatic and direct medial lemniscal inputs to VB suggested that corticothalamic facilitation dominated the corticofugal influences. Because general anesthesia affects the activity of cerebral cortical neurons, the responses of 39 ventrobasal (VB) thalamic neurons to repetitive stimuli were studied in awake rats by focally suppressing the evoked and spontaneous electrocortical activity of primary (S1) somatosensory cortex with magnesium or lidocaine plus magnesium. Suppression of S1 cortex reduced the number of spikes discharged by 19 (66%) of 29 VB units in response to each of 25 electrical stimuli delivered to the medial lemniscus; 9 units were unaffected and one showed an increased response to 1 to 10 Hz stimuli. The responses of 6 (38%) of 16 VB units to electrical somatic stimuli were also reduced following S1 cortical suppression; 9 units were unaffected and one showed an increased response to 20 to 40 Hz stimuli. We detected no change in adequate stimulus or receptive fields. A comparison of the reduced responsiveness of 5 units to both medial lemniscal and somatic stimulation did not reveal any additional response attenuation attributable to subthalamic corticofugal influences. We conclude that, in the awake rat, S1 corticothalamic neurons facilitate somatosensory transmission in the VB thalamus and this facilitation appears to predominate over other inhibitory corticofugal influences.

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222.4 NEURONS IMMUNOREACTIVE FOR GLUTAMATE, GABA AND ASPARTATE IN THE RAT SOMATIC SENSORY CORTEX: A SINGLE- AND DOUBLE-LABELING STUDY.
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Using an approach similar to the one introduced by Storm-Mathisen et al. (1983), we have performed a quantitative study of glutamate (Glu)-positive neurons in the somatic sensory cortex of rats (Conti et al., 1985) and monkeys (Conti et al., in preparation). Glu is known to be a precursor of GABA and, therefore, GABA-positive neurons might stain positively for Glu. Recent findings from our and other labs (Campistron et al.,1986) suggest that Aspartate (Asp)-positive neurons have a distribution similar to that of Glu-positive neurons. This finding raises the possibility that Glu and Asp might coexist in the same neocortical neurons.

To investigate these issues, rats were perfused transcardially using a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer; blocks of cortex containing the first somatic sensory cortex were embedded in paraffin and 4 µm adjacent sections were processed immunocytochemically using the avidin-biotin (ABC) procedure: in one series adjacent sections were reacted alternately with Glu and GABA antisera; in another series, sections were alternately stained with Glu and Asp antisera. The antisera were used at the dilutions of 1: 6000 (Glu), 1:4000 (Asp) and 1: 2000 (GABA).

In the series in which Glu and GABA were used, neurons stained by both antisera 1) were < 5% of both populations (and <10% of Glu-positive neurons); 2) were present in all cortical layers and 3) had mean cross-sectional areas similar to those of the largest neurons stained only by GABA. Neurons stained by both Glu and Asp 1) represented about 10% of the total population and about 20% of Glu-positive neurons; 2) were not preferentially distributed throughout the cortical layers and 3) were not distinguishable on the basis of their soma size.

It is postulated that neurons that stain for both Glu and GABA represent a subset of large GABA-positive neurons with particular metabolic and/or functional properties. Neurons stained by both Glu and Asp might use a dipeptide, as recently shown for neurons of rat hippocampus and piriform cortex.

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222.5 LAMINAR POSITION OF CORTICOPONTINE CELLS IN THE BARREL FIELD OF RATS. B.E. Mercier* C.R. Legg* and M. Glickstein, MRC Unit on Neural Mechanisms of Behaviour, 3 Malet Place, London WC1E 7JG, Department of Anatomy, University College London, and Psychology Division, City University, London.

The barrel field of rats provide an opportunity to study the pontine projections from a defined region of somatosensory cortex with known sensory representation from the mystacial vibrissae. We studied efferent projections from the barrel field using orthograde and retrograde transport of horseradish peroxidase. Alternate sections were processed for cytochrome oxidase. Confirming Mihailoff et al. (Mihailoff, G.A., Lee, H., Watt, C.R. and Yates, R. J. Comp. Neurol. 237: 251, 1985) there is a dense projection from the barrel fields to the pontine nuclei. The great majority of these fibers terminate ipsilaterally with a small contralateral projection. Within the barrel fields, cytochrome oxidase staining reveals a striking laminar organization. In the infragranular layers, layer Va stains palely; layer Vb stains densely. Following pontine HRP injection labelled cortical cells were confined entirely to layer Vb of the barrel fields; all layer Vb pyramidal cells within the barrel fields and no other cells were retrogradely labelled. Control injections in the neighboring pontine and medullary reticular formation or in the pyramidal tract produced few or no labelled cells in the barrel field. Results show a highly precise sublaminar origin of efferent fibers to the pons. Since many Vb cells in the barrel fields are also labelled following thalamic injections many, perhaps all, Vb cells must bifurcate.

222.6 EFFECTS OF ACETYLCHOLINE ON NEURONAL RESPONSES TO SOMATIC STIMULI IN CAT SOMATOSENSORY CORTEX R. Metherate, N. Tremblay* and R.W. Dykes Depts. of Physiology, Neurology and Neurosurgery and Surgery, McGill University, Montreal, Quebec, Canada.

Iontophoretically-administered acetylcholine (ACh) has been shown to increase the firing rate of some neurons in cat somatosensory cortex. However, little is known regarding the effects of ACh on neuronal responses to afferent input.

Cats were anesthetized with sodium pentobarbital, a craniotomy was performed over the somatosensory cortex and stimulating electrodes were inserted into the ventroposterior thalamus. Five-barrel micropipettes were pulled after a carbon fiber was inserted into the central, recording barrel. Drug barrels were filled with ACh chloride (1.0M), glutamate (0.5M) to find and study quiescent neurons, atropine sulphate (25mM) to verify the muscarinic nature of the cholinergic effects, and sodium chloride (3.0M) for current controls. Precise tactile stimuli were applied to peripheral receptive fields via a computer-controlled electromechanical device, and qualitative assessments were made using hand-held glass probes. Neuronal responses to somatic stimuli were noted before, during and after iontophoretic application of drugs.

In 26 cats, 233 single neurons were isolated, and ACh applied to 203. Eighty cells (39.4%) were sensitive to ACh (cholinoceptive) in terms of changes in firing rate and/or response to somatic stimuli. These were found at all cortical depths below 0.2 mm. Of the 35 cholinoceptive neurons that were activated from the thalamus, 77.0% responded at latencies less than 3.0 ms, suggesting a monosynaptic connection. The most common result (34 cases) of ACh administration was an increase in a cell's response to a somatic stimulus, accompanied by a decrease in the threshold for activation by the stimulus. Interestingly, while responses to stimuli were enhanced in 26 cells without an increase in firing rate, a further 8 cells were overtly driven by ACh without a change in the evoked response. In 9 cells, a receptive field appeared during ACh application where none existed previously. ACh-driven activity was observed in 16.2% (33/203) of the cells, in agreement with previous studies. Neurons inhibited by ACh were infrequently seen, comprising only 5.4% (11/203) of the population studied.

These data suggest that ACh is a neuromodulator that selectively modifies different characteristics in different cells of the somatosensory cortex. Presumably, selectivity depends on the distribution and types of receptors on these cells. This may also explain why ACh permits long-term changes in neuronal responsiveness in some neurons and not others (Metherate et al., Soc. Neurosci. Abstr. 11:753, 1985). (Funded by MRC, Canada and NIMH)

222.7 THE SECOND SOMATOSENSORY AREA IN PRIMATES: SOMATOTOPIC ORGANIZATION, ARCHITECTURE, AND CONNECTIONS IN MARMOSETS (CALLITHRIX JACCHUS). L.A. Krubitzer and J.H. Kaas, Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240

Microelectrode multiunit mapping methods were used to determine the somatotopic organization and boundaries of the second somatosensory area, S-II, in five marmosets. In the same animals, architectonic fields and cortical and thalamic connections were related to physiologically defined areas.

S-II occupied a portion of the upper bank of the lateral sulcus and extended slightly onto the cortex of the adjoining gyrus to form part of the lateral border of S-I (area 3b) along the representation of the face in S-I. Much of the exposed portion of S-II along the S-I border and the rostral portion of S-II in the lateral sulcus represented the face and head. A large midportion of S-II, largely in the lateral sulcus, was devoted to the hand. The representation of the forelimb adjoined that of the hand deep in the sulcus. The most caudal portion of S-II represented the hindlimb and tail. The upper hindlimb and parts of the trunk projected to cortex deep in the sulcus. Cortex rostral and caudal to S-II was unresponsive to tactile stimulation in the anesthetized marmoset.

Microlesions were placed at physiological boundaries during the recording experiments so that results could be related to cortical architecture. S-I had architectonic features that are characteristic of area 3b of primates. These features included densely packed and deeply stained cells in layers IV and VI, and dense outer and inner bands of myelination. These features changed abruptly at the S-II border so that layer IV and especially layer VI were less densely packed with cells, and the myelination was less dense.

An injection of WGA-HRP restricted to the representation of the hand in S-II resulted in label in the hand region of S-I, cortex deep in the lateral sulcus, very lightly in the lower bank of the lateral sulcus, and in the ventroposterior inferior nucleus of the thalamus.

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222.8 Somatic Activation Of Neurons In The S-II Region Of The Macaque Depends On Postcentral Somatosensory Cortex. T.P. Pons, D.P. Friedman, P.E. Garraghty, J.B. O'Neill*, and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892, Neurosciences Branch, NIDA, Rockville MD 20857, and Section Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Somatic activation of neurons in the second somatosensory area (S-II) has long been thought to depend on a direct projection from the ventroposterior nucleus (VP). Recent anatomical evidence, however, suggests that VP provides little or no input to S-II, raising the question as to what region does constitute S-II's major source of somatic activation. One possibility is the postcentral somatosensory cortex, since it is known that each of the fields comprising this cortex (areas 3a, 3b, 1 and 2) projects densely to layers IV and lower III of S-II. To test this possibility we removed portions of the body representations in the postcentral gyrus and examined the responsiveness of S-II neurons to somatic stimulation. Six to eight weeks following the cortical lesions, single or multiunit activity was recorded with tungsten microelectrodes in the S-II region of 10 hemispheres in 6 adult macaques (4 Macaca mulatta and 2 Macaca fascicularis) anesthetized with a mixture of nitrous oxide and halothane. Electrode tracks were verified histologically as having traversed S-II.

In five unoperated hemispheres, receptive fields of recording sites in S-II included all of the major body parts, with the fields for roughly half of the sites located on the glabrous surface of the hand. By contrast, in over 301 recording sites through the S-II region of three hemispheres in which the postcentral representations of the hand were ablated, no responses were obtained to somatic stimulation of the glabrous surface of the hand, although responses were observed to stimulation of all other body parts. To test the generality of this functional dependency of S-II on postcentral cortex, we examined the effects of two additional lesions. In one, the postcentral representations of all body parts except the hand were removed, and in this case the receptive fields of all recording sites responsive to somatic stimulation in the ipsilateral S-II region were confined to the hand. In the other case, the postcentral representations of the entire body surface were removed and no resonses to stimulation of the body were found in the ipsilateral S-II region. Nissl-stained coronal sections through the thalami ipsilateral to the postcentral lesions revealed virtually total retrograde degeneration within the affected zone of VP, demonstrating the lack of either exclusive or sustaining collateral input from VP neurons to S-II. The findings support the suggestion that postcentral somatosensory cortex contains the essential relay for somatic activation of the S-II region. Funded in part by NIH Grants NS07704 and NS07497.

222.9 COMPARISON OF TACTILE LOCALIZATION CAPACITIES WITH SPATIAL ATTRIBUTES OF RECEPTIVE FIELDS IN THE SOMATOSENSORY CORTEX OF MONKEYS. C.J. Vierck, Jr., B.W. Whitsel and O. Favorov*. Dept. of Neuroscience and Center for Neurobiological Sciences, Univ. of Florida Col. of Med., Gainesville, FL 32610; and Dept. of Physiology, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

Two monkeys were trained on a task requiring identification of stimulated locations on the glabrous skin of the feet. The monkeys pressed a lever to initiate 10 Hz stimulation at one of three sites on either foot. Food reinforcement was presented for releasing the lever and pressing a button located at the appropriate point on a figurine of two feet that faced the monkeys. Pressing any button other than the one corresponding to the stimulated point terminated the trial without reinforcement. With training and testing for months beyond the point of stabilized performance, an average of 24.9% of the trials involving distal stimulation of toe 4 were mislocalized to the tip of toe 2, and 5.5% of these trials were mislocalized to the base of toe 4.

In an attempt to account for the above findings, we have examined the receptive field (RF) properties and the spatial arrangement of single neurons having RFs in the postcentral cerebral cortex (S-I) of anesthetized and awake monkeys. In deeply anesthetized preparations, receptive fields (RFs) on glabrous skin for single S-I neurons are usually quite restricted. For example, digital RFs are typically confined to a single phalanx and rarely include regions on more than one digit. In addition, neurons in cell columns separated by more than 650 um have non-overlapping RFs in anesthetized animals. In contrast, RFs in S-I of awake animals commonly encompass large areas of glabrous skin that include more than one digit. For example, 58.4% of a sample of units that responded to stimulation of the distal forefinger (digit 2) also were sensitive to light contact of the tip of digit 4, and 47.2% of these units were activated by touching the palmar pad at the base of digit 2. In addition, the S-I zone which receives input from a single skin point is extensive (typically 3-8 mm in the mediolateral dimension). Thus, tactile localization of a single point is subserved by neuroelectrical activity patterns which are widely distributed in the S-I cortex. (Supported by NINCDS grants NS 10321, NS 07261 and NS 10865).

222.10 FACTORS INFLUENCING DISCRIMINATION OF DIRECTION OF MOTION ON THE HUMAN HAND. E.P. Gardner and B.F. Sklar* Dept. of Physiology and Biophysics, NYU School of Medicine, New York, NY 10016.

Dynamic tactile displays have been used in psychophysical and electrophysiological studies to simulate motion across the skin. Using a PDP-11 controlled OPTACON stimulator (144 miniature probes arranged in a 6 x 24 matrix), this laboratory has demonstrated that stimulus temporal frequency and spacing have major effects on the firing patterns of SI cortical neurons in monkeys. We now report the roles of (1) spatial frequency, (2) temporal frequency, (3) path length, and (4) total number of stimulated points on the ability of 15 adults and 2 children to discriminate accurately the direction of apparent motion across the fingertip. Single pulses were applied simultaneously to the central 4 columns of a single row on the OPTACON; the active row was then shifted proximally or distally to the immediately adjacent row (1.1 mm), or to rows 2.2 or 4.4 mm away, at interpulse intervals of 10, 20 or 40 ms. In sessions of 256 trials, (a) the path length was kept constant, and the spacing and timing between rows was varied, or (b) the interpulse interval was constant, and the total number and spacing of rows was varied. Subjects used a joystick to indicate the direction of apparent motion on the skin.

Spatial factors proved to be the most important determinants of accurate direction selectivity. Discriminability (d') increased monotonically as the path of motion was lengthened from 4.4 to 22 mm, at all pulse intervals. Increasing the pulse interval from 10 to 40 ms produced only a slight decrease in performance accuracy. However, performance was significantly better with more densely spaced stimuli. At the shortest path lengths (4.4 mm), only stimuli spaced 1.1 mm apart could be discriminated at or above threshold (75% correct), while motion along intermediate paths (8.8 mm) could be discriminated at 90% or better for 1.1 mm intervals, and at threshold at 2.2 mm spacing. Motion along 17.6 mm paths, evoked 99% performance for 1.1 mm spacing, 90% for 2.2 mm spacing, and threshold for 4.4 mm spacing. These changes in performance were related not to the density of stimulation on the skin, nor to the apparent velocity of motion, but rather to the total number of activated rows. d' was random when only two rows were stimulated sequentially at all spacing; threshold occurred when 3-5 rows were activated, and 99% performance was obtained when 8 or more rows were stimulated. Performance was independent of the spatial separation of the activated rows, suggesting that discrimination of direction is a dynamic process, requiring temporal integration of a minimum number of inputs. Although subjects can clearly differentiate long and short path lengths, they require a minimum number of sequential inputs in order to infer correctly the direction of motion. (Supported by USPHS Research Grants NS17973 and NS11862).

223.1 MOLECULAR PROPERTIES OF A_1 ADENOSINE RECEPTORS IN RAT BRAIN.
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A_1 adenosine receptors are linked in an inhibitory manner to adenylate cyclase and mediate the depressive effects of the nucleoside on synaptic transmission in the central and peripheral nervous systems. The relationship, if any, between these biochemical and electrophysiological events is, however, unknown. An important step towards understanding the physiological mechanisms of action of adenosine is the molecular characterisation of adenosine receptor-effector systems. We report here studies on the biochemical properties of A_1 adenosine receptors in the rat cerebral cortex.

The molecular size of the A_1 adenosine receptor has been approached using various methods. High energy irradiation inactivation (target size analysis) allows an estimate to be made of molecular size in situ without prior protein purification. The functional size of the A_1 receptor was measured by the loss of [^{3}H]cyclohexyladenosine (CHA) binding on irradiation with electrons. Inactivation curves were non-linear but showed a linear portion which allowed an estimate to be made of the apparent molecular size of the A_1 receptor. The value obtained was 79,600±6,800 which is approximately twice the size of the subunit obtained by photoaffinity labelling (Choca et al., Biochem. Biophys. Res. Comm. 131, 115-121, 1985; Stiles et al., J.Biol.Chem., 260, 10806-10811, 1985; Klotz et al., J.Biol.Chem., 260, 14659-14664, 1985).

[^{3}H]CHA binding sites can be readily solubilised from cerebral membrane fractions using ionic detergents such as CHAPS or cholate. Gel filtration of cholate-solubilised A_1 receptors on Sephacryl S300 indicated the detergent-receptor complex to have a molecular weight $>5x10^5$. Nonionic detergents such as Triton X100 did not solubilise ligand binding activity. CHAPS- or cholate-solubilised membrane preparations retained their sensitivity to modulation of binding activity by factors such as guanine and adenine nucleotides. Further, magnesium ions, which increased the B_{max} values for the binding of the A_1 receptor agonists, [^{3}H]CHA and [^{125}I]iodohydroxyphenylisopropyladenosine and an antagonist, [^{3}H]diethylphenylxanthine to EDTA-pretreated membrane fractions, increased agonist binding several fold in CHAPS-solubilised membrane extracts. In contrast, the binding sites remaining unsolubilised after CHAPS treatment were not stimulated by magnesium ions. Taken together, these results are consistent with the view of the adenosine receptor as a multimolecular complex.

223.2 IDENTIFICATION AND LOCALIZATION OF ADENOSINE A_1 AND A_2 RECEPTORS IN THE RAT SPINAL CORD. J. I. Choca, H. K. Proudfit, and R. D. Green*. Dept. of Pharmacology, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612.

Differences in the binding characteristics of [^{3}H]-N^6-(L-phenylisopropyl)adenosine ([^{3}H]-L-PIA)—an A_1 selective agonist—and [^{3}H]-N-ethylcarboxamide adenosine ([^{3}H]-NECA)—an agonist with near equal affinities at the A_1 and A_2 sites—suggests the existence of A_1 and A_2 receptors in the rat spinal cord. Briefly, [^{3}H]-NECA binds to twice as many sites as [^{3}H]-L-PIA, while radioligand binding displacement by the A_1 selective agonist, N^6-(cyclopentyl)adenosine (CPA), is monophasic (n_H=0.8) for [^{3}H]-L-PIA, but biphasic (n_H=0.5) for [^{3}H]-NECA (Choca et. al., Neurosci. Abstr. 11: 573, 1985).

We have performed [^{3}H]-NECA:agonist inhibition curves with six A_1 selective agonists to further characterize the two components of [^{3}H]-NECA binding. All curves best fit a two site model when analyzed by LIGAND. L-PIA, CHA, and CPA were the most potent displacers at the first site (IC_{50}=2-5nM), where L-PIA was 20-35 fold more potent than D-PIA. In contrast L-PIA was only 4-6 fold more potent than D-PIA at the second site. This difference in stereoselectivity is consistent with the assignments of A_1 receptor for the first binding component and A_2 receptor for the latter site.

Adenylate cyclase (AC) assays of synaptosomal membranes provided further evidence for A_2 receptors in the ventral and dorsal halves of the cord. NECA maximally stimulated AC 12-15% above basal activity. The adenosine receptor antagonist, 8-phenyltheophylline attenuated the stimulation of AC by NECA. Hence this stimulation is adenosine receptor mediated.

Autoradiograms of [^{3}H]-L-PIA and [^{3}H]-NECA binding in slices indicate three distinct areas of receptor density—listed here in decreasing order: 1) the substantia gelatinosa (SG), 2) a thin area extending along the medial dorsal horn from the SG to the central canal, and 3) the remainder of the ventral and dorsal horns. In all three areas [^{3}H]-NECA binds to approximately twice as many sites as [^{3}H]-L-PIA. We conclude that both the ventral and dorsal halves of the spinal cord contain adenosine A_1 and A_2 receptor subtypes. Since the antinociceptive effect of intrathecally administered NECA may be A_2 mediated (Aran et. al., Neurosci. Abstr. 12) cellular localization of adenosine receptor subtypes may elucidate the role of adenosine in the modulation of pain transmission. (Supported by PHS grant DA03980.)

223.3 SPECIES DIFFERENCES IN STRIATAL ADENOSINE A-2 RECEPTORS. George A. Stone*, Elaine W. Snowhill*, Barbara Weeks* and Michael Williams. Research Department. Pharma.Div., CIBA-GEIGY, Summit, NJ 07901.

Adenosine receptors can be subdivided into A-1 and A-2 subtypes on the basis of their pharmacology. Binding assays for A-1 receptors have shown distinct species differences in the pharmacology of this receptor, especially for the xanthine adenosine antagonists (Murphy and Snyder, Mol. Pharmacol.,22:250 (1982); Ferkany et al., Drug Dev. Res.,in press). Adenosine A-2 receptors in striatal tissue can be labeled by the non-selective agonist ligand [^{3}H]NECA in the presence of the A-1 selective ligand, CPA to block A-1 receptor binding (Bruns et al., Mol. Pharmacol.29:331 (1986)). Analysis of NECA binding in the striatum of six species: rat, mouse, guinea pig, rabbit, man and calf gave evidence for two discrete binding sites, a high affinity site with a Kd value of 1.6 -10 nM and a lower affinity site with a Kd value of 13 - 245 nM. The order of affinity was rat > rabbit > guinea pig > mouse > calf> man. Pharmacological evaluation of the higher affinity site using 2 nM [^{3}H]NECA showed that binding was consistent with the labeling of an A-2 receptor: NECA > 2-CADO > R-PIA > CHA > CPA >> S-PIA. In general, this profile was consistent across the species. With antagonists, PACPX was the most active displacer with 8PT > DPX > 8PST. Marked interspecies differences in activity were observed. Setting the IC_{50} value for PACPX in rabbit (13.6 nM) at unity, the relative values for the other species were: man, 1.1 ; rat, 43; mouse, 35; guinea pig,25 and calf, 4. For NECA (rabbit IC_{50}= 5.4 nM), the relationships were rabbit, 1; man, 2; rat, 2; mouse, 2; guinea pig, 2.5 and calf, 3.6. These data suggest that the choice of species in regard to the evaluation of adenosine antagonists is of paramount importance. Furthermore, they are also suggestive of the existence of teleological differences in the interaction of antagonists with the A-2 receptor. This may have physiological significance.

223.4 ADENOSINE AGONISTS DO NOT INHIBIT THE POTASSIUM-STIMULATED RELEASE OF SUBSTANCE P FROM RAT SPINAL CORD SLICES
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Recent evidence suggests that adenosine may be involved in regulating nociception at the level of the spinal cord. Adenosine receptors have been localized in the dorsal spinal cord. Intrathecal injection of the adenosine agonist, N-ethylcarboxamide adenosine (NECA), produces antinociception. In addition, adenosine agonists reduce calcium conductance in mouse dorsal root ganglia cells grown in culture. Since adenosine may be involved in antinociception and since adenosine has been shown to inhibit neurotransmitter release in various nervous tissue preparations, we studied whether adenosine agonists would affect the potassium (K+)-stimulated release of substance P (SP) from rat spinal cord slices.

Male Sprague-Dawley rats (250-400G) were decapitated and a 2cm segment of lumbosacral spinal cord was removed, chopped into 0.5x 0.5mm pieces, weighed, placed in a perfusion chamber at 37°C and perfused with oxygenated Krebs-bicarbonate solution (0.5ml/min) containing BSA (0.5%), bacitracin (20µM) and dithiothreitol (6µM). To evoke SP release, 50mM KCl was substituted for equimolar NaCl for 8 mins during the perfusion. Drugs were administered starting 4 mins prior to 50mM KCl and throughout the high K+ period. Perfusates were collected every two minutes into tubes containing acetic acid such that the final concentration of acid was 2N. Samples were lyophilized and assayed for SP using radioimmunoassay. The antiserum for SP did not cross-react with numerous other peptides and the assay was not altered by drug manipulations. The immunoreactive substance collected during perfusion co-chromatographed with SP on thin-layer chromatography suggesting that the substance assayed was authentic SP.

Exposure of spinal cord tissue to 50mM KCl for 8 min significantly increased SP release from 0.08 pg/min/mg tissue to 0.24 pg/min/mg tissue ($p<.001$). Adenosine (10^{-3}M), N^6-cyclohexyladenosine (CHA, 10^{-5}M), and NECA 10^{-7}M, 10^{-5}M did not significantly alter the basal or evoked release of SP (defined as release during the 10 min period after exposure to 50mM KCl minus the 10min period prior to 50mM KCl). Evoked release of SP in tissues exposed to 10^{-3}M adenosine, 10^{-5}M NECA and 10^{-5}M CHA was 1.49±0.21, 1.31±0.22 and 1.01±0.24 pg/mg/10min, respectively. Paired-control evoke release values for adenosine, NECA and CHA were 1.27±0.15, 1.65±0.39 and 1.08±0.11 pg/mg/10min, respectively. Unlike adenosine agonists, morphine (10^{-5}M) significantly reduced evoked release of SP from 1.44±0.19 to 0.88±0.11 pg/mg/10min ($p<.01$). This inhibition was unaffected by pretreatment with 10^{-5}M theophylline (evoked release 1.05±0.20 pg/mg/10min). These results suggest that adenosine is not an inhibitor of SP release in the spinal cord and is not involved in opioid-induced inhibition. (Supported by NS21697-01)

223.5 ADENOSINE ANALOGS MODIFY ICTAL AND POSTICTAL EVENTS IN AMYGDALA-KINDLED RATS. K.A. Whitcomb* and R.F. Berman. (SPON: M.M. Kilbey). Dept. of Psychology and Neuroscience Program, Wayne State University, Detroit, Mi. 48202.

The putative neuromodulator, adenosine, is a potent anti-convulsant and sedative which inhibits neuronal firing and the release of neurotransmitters. It has been shown by this lab and others that adenosine agonists shorten afterdischarge (AD) length in amygdala-kindled rats. Recently, this lab has shown that the adenosine agonist L-phenylisopropyl adenosine, modifies postictal events as well by prolonging the duration of postictal depression. The present study extended these findings by (1) examining the effects of N-ethylcarboxamide adenosine (NECA) on both AD and postictal depression duration, and (2) investigating the possible blockade of the actions of endogenously released adenosine by the agonist caffeine.

Male Long-Evans rats, implanted in the amygdala with twisted bipolar electrodes, were stimulated once daily at afterdischarge threshold levels until 3 consecutive, fully kindled, stage 5 seizures were elicited. Drug injections (i.p.) at the following doses were given: NECA at .01 and .1 mg/kg, caffeine at .1, 1, 10, and 32 mg/kg, and NECA at .1 mg/kg along with caffeine at 10 mg/kg. Each animal received each dose; drug injections were randomly ordered. NECA was injected 30 minutes prior to stimulation and caffeine 5 minutes prior to stimulation.

NECA was found to reduce AD duration and prolong postictal depression duration at both .01 and .1 mg/kg. Caffeine prolonged AD duration at 32 mg/kg and shortened postictal depression duration at both 10 and 32 mg/kg. Caffeine antagonized the effects of NECA on both AD and postictal depression durations.

This study supports the hypothesis that adenosine may act as an endogenous anticonvulsant which is released as a result of the seizure state itself.

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223.6 INTRASTRIATAL ADMINISTRATION OF QUINOLINIC ACID BUT NOT 6-HYDROXYDOPAMINE (6-OHDA) REDUCES ADENOSINE RECEPTOR DENSITY. Michael F. Jarvis and Michael Williams. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

Two weeks following unilateral intrastriatal injection of 6-OHDA (12 ug) or quinolinate (QUIN, 150 mmoles), apomorphine (0.5 mg/kg s.c.) caused QUIN-treated rats to turn ipsilateral to the injection site while 6-OHDA animals rotated contralaterally. Analysis of adenosine binding using (^{3}H)CHA to label A-1 receptors and (^{3}H)NECA in the presence of 50 nM CPA to label A-2 receptors in individual lesioned and control striata from animals exhibiting rotational behavior showed no changes in CHA binding following 6-OHDA treatment but a 38% reduction in A-1 receptors following QUIN treatment (control Bmax = 550 fmoles/mg protein; QUIN lesion = 339) without any change in Kd. NECA binding to A-2 receptors was also unaffected by 6-OHDA treatment while a 52% reduction (control Bmax = 596 fmoles/mg protein; QUIN lesion = 287) was observed in Quin lesioned animals. Again the receptor affinity was unchanged. 6-OHDA treatment had no effect on the binding of (^{3}H)nitrobenzylthioinosine (NBI), a putative ligand for adenosine uptake sites, while QUIN lesions reduced binding by 40%. These results indicate that A-1 and A-2 receptors as well as adenosine uptake sites are located on striatal intrinsic neurons rather than on dopaminergic terminals.

223.7 ANTICONVULSANT ACTION OF ENDOGENOUS ADENOSINE IN THE HIPPOCAMPAL FORMATION. B. Ault*, M.A. Olney*, J.L. Joyner*, C.E. Boyer*, F.E. Soroko*, and C.M. Wang. Dept. of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709, U.S.A.

We have previously reported that theophylline (THEO) (30 μM) was capable of increasing the rate of generation of spontaneous epileptiform activity induced in hippocampal slices by the GABA antagonist bicuculline (Ault & Wang, Br. J. Pharmac., 87, 695-703, 1986). In addition, THEO (30 μM) or adenosine deaminase (10 μg/ml) could induce epileptiform activity in the absence of other convulsant agents. In this study we have examined the ability of THEO to enhance the convulsant action of kainic acid (KA) and compared the convulsant effects of THEO and caffeine. Hippocampal slices of approximately 425 μm were superfused with standard Elliott's medium (containing 1.3 mM Ca^{2+} and 3.5 mM K^+), which was warmed to 31°C, at 1.2-1.5 ml/min . Potentials were recorded extracellularly from s. pyramidale in the CA3 or CA1 regions. KA (10 nM) induced spontaneous bursting activity in area CA3 at rates from 3-11/min. When applied in addition to KA, THEO (0.3-30 μM, N=4) and caffeine (1-100 μM, N=4) enhanced the rate of discharge in a dose-related manner such that the highest doses produced approximately a 3-fold elevation in bursting frequency. In the absence of other convulsant treatments, slices superfused with THEO (1-30 μM, N=17) or caffeine (3-100 μM, N=12) displayed bursting in area CA3, and CD_{50} values of 7 μM and 17 μM respectively were calculated from the number of slices bursting at each dose level. In contrast, THEO (100 μM) only induced some secondary firing of CA1 cells in response to synaptic stimulation (N=4). The relevance of these studies to epileptic activity in vivo was investigated in models of partial and generalized seizures. When administered alone, KA (3mg/Kg i.p., N=4) or THEO (50 mg/Kg p.o., N=4) had only weak excitatory effects in rats. Co-administration of KA and THEO, however, induced clear limbic seizures (freezing and staring, facial automatisms, forelimb clonus and rearing) in 4 of 6 animals. Preliminary experiments, though, suggest that THEO (50 mg/kg) does not potentiate the action of Metrazol. These data indicate that endogenous adenosine exerts an anticonvulsant action that may be selective for the limbic system. Compounds that enhance purinergic inhibition may therefore be useful for the treatment of limbic seizures.

223.8 EFFECTS OF CHRONIC ADMINISTRATION OF DIAZEPAM AND RO15-1788 ON ADENOSINE A1 AND A2 RECEPTORS IN THE RAT BRAIN. M.Hawkins*, M.Pravica* and M.Radulovacki (SPON: T.J.Marczynski). Dept. Pharmacology, Univ. of Ill., Chicago, IL 60612

Inhibition of adenosine (ado) uptake by benzo-diazepines (BZD) has been proposed as a central mechanism for some of the actions of these drugs (Philis,J.W., Siemens,R.K., and Wu,P.H., Brit. J. Pharmacol. 70: 341, 1980). RO15-1788, a CNS BZD antagonist, exhibits similar actions to BZD agonists on ado uptake (Morgan,P.F., Lloyd,H.G.E. and Stone,T.W., Neurosc. Lett. 41: 183, 1983). If there is an interaction between BZDs and ado, then we may expect that the chronic administration of a BZD agonist may modify ado A1 and A2 receptors. Thus, the present study was undertaken to determine the effects of chronic administration of diazepam and RO15-1788 on ado receptor binding in different brain regions.

One group of male Sprague Dawley rats (300-350gm) was implanted with a subcutaneous diazepam pellet (5mg/kg/day) whereas another group received RO15-1788 (5mg/kg/day, ip). Animals were sacrificed 10 and 20 days later and ligand binding assays were performed in the cortex, cerebellum, hippocampus and striatum. A1 receptor binding assay was measured with ^{3}H-L-PIA, while A2 receptor binding was estimated by the NECA minus PIA binding assay performed in the presence of saturation concentrations of ^{3}H-L-PIA and ^{3}H-NECA (Yeung and Green, Naunyn-Schmiedeberg's Arch. Pharmacol. 325: 218, 1984). BZD receptor binding assay was measured with ^{3}H-Flunitrazepam. Diazepam decreased A1 receptor binding in the hippocampus (13%) and A2 receptor binding in the striatum (47%) after 10 but not after 20 days. No changes were observed on A1 or BZD receptor binding in the cortex or the cerebellum. RO15-1788 produced no changes on either ado and BZD receptor binding.

These results are in agreement with the evidence suggesting that BZDs increase the levels of ado in the synapse through the inhibition of ado uptake. It appears that chronic stimulation of ado receptors by increasing endogenous ado levels downregulates these receptors. (Supported by NIH fellowship to M.H and grant from AFOSR 85-0349)

223.9 LOW DOSES OF CAFFEINE AFFECT SLEEP COMPOSITION IN A MANNER OPPOSITE TO THAT OF ADENOSINE ANALOGS IN RATS. S.R. Glaum, G.M. Yanik, W. Pan*, P. Hajduk* and M. Radulovacki. Dept. of Pharmacology, U. of Ill. College of Medicine, Chicago, IL 60612.

Recent work from our laboratory has shown that administration of 0.1-0.3 μmol/kg (ip) doses of the adenosine (ado) analogs N^6-phenylisopropyladenosine (L-PIA), N^6-cyclohexyl-adenosine (CHA) and 5'-N-ethylcarboxamide adenosine (NECA) cause a shift in sleep composition from light slow-wave sleep (SWS1) into deep slow-wave sleep (SWS2) without increasing sleep duration in rats (Radulovacki, M., et al., JPET 228: 268-274, 1984). These effects are presumed to occur via drug interaction with ado A_1 and A_2 receptors. In addition, NECA, an agonist with similar potency for A_1 and A_2 receptors, has been shown to decrease the latency to onset of SWS2. Methylxanthines like caffeine induce a biphasic effect on locomotor activity in mice, producing a paradoxical decrease at low doses (Snyder, S. et al., Proc. Natl. Acad. Sci. USA 78: 3260-3264, 1981). Larger doses of caffeine have been shown to antagonize the effects of L-PIA on sleep in rats (Radulovacki, et al., Brain Res. 246: 178-180, 1982). The purpose of this study was to investigate the effects of caffeine over a wide dose range on sleep duration and composition.

Male Sprague-Dawley rats (300 gm) implanted with EEG and EMG electrodes were acclimated to a 12 h light/dark cycle for 1 week post-surgery. Animals were then polygraphically recorded for 6 h during the light cycle following injection of 0.125, 1.25, 12.5 or 25 mg/kg (ip) doses of caffeine. Records were analyzed for duration of waking, SWS1, SWS2, REM and latencies for all sleep stages.

Caffeine at 0.125 and 1.25 mg/kg produced a significant shift in sleep composition, reducing SWS2 and augmenting SWS1 without decreasing total sleep. The 1.25 mg/kg dose also significantly increased the latency of SWS2 onset as compared to control. Conversely, 12.5 mg/kg caffeine decreased total sleep without affecting the ratio of SWS1 to SWS2. The 25 mg/kg dose of caffeine greatly augmented wakefulness and the latency of onset for all sleep stages. These results indicate that low doses of caffeine qualitatively alter sleep composition, such that deep sleep is attenuated both in onset and duration, while the quantity of sleep is unaltered. With increasing doses, caffeine promotes arousal and depresses sleep. These results lend additional support to the concept of a centrally active purinergic tone related to regulation of CNS excitability (Dunwiddie, T. and Worth, T., JPET 220: 70-76, 1982). (Supported by FED-AFOSR grant 85-0349).

223.10 DOWN REGULATION OF ADENOSINE A2 RECEPTORS IS ASSOCIATED WITH AN INCREASE IN DEEP SLOW WAVE SLEEP. N.M. Porter, F.M. Clark*, R. Trifunovic*, R.D. Green*, and M. Radulovacki, Dept. Pharmacology, Univ. Illinois College of Medicine, Chicago, IL 60612.

We have previously shown that chronic intraventricular administration of 5'-N-ethylcarboxamide adenosine (NECA, an adenosine A1/A2 agonist) and deoxycoformycin (dCF, an adenosine deaminase inhibitor) produced a decrease in adenosine A2 binding sites in rat striatum. L-Phenylisopropyladenosine (L-PIA), a selective A1 agonist, did not alter A2 receptor binding. In addition, none of these treatments altered A1 binding in either cortex or striatum. The present study was undertaken to determine the effects of these treatments on sleep.

Male Sprague-Dawley rats were anesthetized and stereotaxically implanted with cannulae into the lateral cerebral ventricle and with electrodes for polygraphic recording of the electroencephalogram (EEG) and electromyogram (EMG). ALZET osmotic pumps, implanted subcutaneously, were connected to the cannulae via PE tubing and one of the following drugs administered chronically: L-PIA (0.50 nmoles/hr for 2 wk), NECA (0.04 nmoles/hr for 2 wk) or dCF (5 nmoles/hr for 1 wk). Each group had its own vehicle control. Twenty-four hours after the termination of the infusions, rats were polygraphically recorded for 8 hours. Records were analyzed for wakefulness (W), light slow-wave sleep (SWS1), deep slow-wave sleep (SWS2) and REM sleep.

NECA and dCF treated animals showed a significant decrease in W with a concomitant increase in SWS2 ($p < 0.05$, ANOVA). The portion of total sleep occupied by SWS2 increased by 38 and 22% in the NECA and dCF groups, respectively. This change was accompanied by a 30 and 10% increase in total sleep. However, sleep-wake patterns in L-PIA treated rats were not altered.

Treatments with NECA and dCF which produced decreases in A2 receptor binding also produced an increase in SWS2. In contrast, L-PIA which did not alter A2 binding sites did not alter sleep-wake patterns. The results suggest that adenosine A2 receptors may be involved in the modulation of the sleep-wake cycle. (Supported by FED-AFOSR grant 85-0349).

223.11 CALCIUM DIFFUSION IN THE RAT CEREBRAL CORTEX. M.E. Rice* and C. Nicholson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The important role Ca^{2+} plays in neuronal and cellular function can in part be attributed to its divalent charge. In addition to its interactions with various calcium-binding proteins, less specific ionic association with polyanionic components of the extracellular matrix has also been suggested as a way in which Ca^{2+} could influence neuronal communication (Nicholson; Neurosci. Res. Prog. Bull. 18: 177, 1980). As a consequence of such charge interaction, the diffusion of Ca^{2+} in the brain might be slowed to a greater extent than that of monovalent ions. Singly charged anions and cations both migrate freely through the extracellular fluid space with diffusion modified only by the restricted space (extracellular volume fraction) and tortuosity (increased path length around cellular elements) of the brain matrix (Nicholson & Phillips; J. Physiol. 321: 225, 1981). Calcium, however, has been variably reported to diffuse at rates much slower than these ions (Morris & Krnjevic; Can. J. Physiol. Pharmacol. 59: 1022, 1981) as well as at similar rates (Nicholson, Fed. Proc. 39: 1519, 1980; Dingledine & Somjen, Brain Res. 207: 218, 1981). This discrepancy may be due to technical difficulties, especially when iontophoresis is used to release the measured Ca^{2+}.

We have developed a procedure using ion selective microelectrodes (ISMs) and pressure ejection from a microelectrode which allows us to measure reliably Ca^{2+} diffusion in the cerebral cortex of the rat. First, the standard values for volume fraction and tortuosity in the cortex were extracted from concentration profiles monitored during iontophoresis of the extracellular marker tetramethylammonium (TMA^+). Then a mixture of Ca^{2+} and TMA^+ was pressure ejected and the concentration profiles for both ions were recorded at ISMs 100-300 micrometers away. Using the volume fraction and tortuosity found earlier, the volume of the ejected drop was determined. From these data, we evaluated the diffusion parameters for Ca^{2+}. For 60 paired measurements in 8 rats, the average volume fraction for TMA^+ was 0.21, while that using Ca^{2+} was 0.26. Tortuosities were 1.5 and 1.6, respectively. From the tortuosity, the apparent in vivo diffusion coefficient can be calculated. For Ca^{2+}, the value of 0.27×10^{-5} cm^2/s is obtained from the solution diffusion coefficient of 0.70×10^{-5} cm^2/s. The slight differences between the Ca^{2+} and TMA^+ parameters can be attributed to experimental variation and to low signal-to-noise ratio of the Ca^{2+} measurements. We conclude, therefore, that Ca^{2+} diffusion in the rat cerebral cortex does not differ significantly from that of monovalent ions. Supported by Grants NS-13742 and NS-07745 from NINCDS.

223.12 **CALMODULIN, PHOSPHOLIPID, CYCLIC AMP AND pH DEPENDENT PHOSPHORYLATION OF Pleurobranchaea CNS PROTEINS.** L. Lipeski*, R. Gillette and M. U. Gillette. (SPON: D.J. Green). Department of Physiology & Biophysics, University of Illinois, Urbana, Illinois 61801

Ion currents in neurons of Pleurobranchaea have been shown to be activated and/or modulated by a variety of intracellular second messenger pathways, including cyclic AMP, Ca^{2+}, and intracellular pH. Pursuant to identifying underlying mechanisms, we have characterized protein substrates in the nervous system which are phosphorylated by endogenous protein kinases. Phosphoproteins were identified by separation on 1-dimensional SDS-PAGE gel electrophoresis after in vitro [γ-^{32}P] ATP labeling. Cyclic AMP notably stimulated phosphorylation of 90, 51 and 29 kD proteins; phosphorylation is suppressed by the specific inhibitory protein of the catalytic subunit of the kinase.

A phosphatidylserine/diolein mixture stimulated appreciable phosphorylation of 168, 148, 135, 90, 76, 57, 16, and 10 kD protein bands; phosphorylation of the 76 kD band was highly specific. The diolein component was replaceable with phorbol-12-myristate-13-acetate with no change in phosphorylation profile. Phospholipid dependent kinase activity was inhibited by trifluoperazine and enhanced by Ca^{2+}.

Ca^{2+} dependent and trifluoperazine inhibited phosphorylation without added phospholipid was prominent in 108, 105, 90, 57 and 56 kD proteins. Addition of exogenous calmodulin was ineffective in stimulating further phosphorylation, suggesting saturating levels of endogenous calmodulin. To remove endogenous calmodulin the protein homogenate was precipitated with 55% $(NH_4)_2SO_4$. Addition of calmodulin to the resuspended pellet notably stimulated phosphorylation in 108, 57 and 56 kD proteins; removal of $(NH_4)_2SO_4$ by dialysis caused appearance of labeling at 105 and 90 kD and disappearance of the band at 108 kD.

Ca^{2+}/calmodulin dependent phosphorylation of the 108 kD protein band was uniquely sensitive to pH, steeply decreasing as pH increased from 7.0 to 8.0. The potential relation of this protein to Ca^{2+}/calmodulin dependent modulation of neuronal excitability by intracellular pH is under study (see Gillette & Green, this meeting).

223.13 VASOPRESSIN NEUROMODULATION IN THE HIPPOCAMPUS: CALCIUM/CALMODULIN OR PROTEIN KINASE C? R.E. Brinton and B.S. McEwen. Lab. of Neuroendocrinology, Rockefeller Univ. 1230 York Ave., New York, NY 10021-6399.

Vasopressin potentiates noradrenergic stimulated adenylate cyclase activity (NASAC) in rat hippocampal slices (Brinton & McEwen, Neurosci. Abs., 1985). The AVP receptor in hippocampus (Biegon et al., Neurosci. Lett., 1984; Brinton et al., PNAS, 1984) is of the V1 type (Stephens & Logan, J. Neurochem., 1986) and very likely mediates, in part, the behavioral effects of AVP (De Weid, Nature, 1971) which are blocked by a V1 antagonist (Koob et al. Reg. Pep., 1981). We therefore tested whether AVP modulation of NASAC was also blocked by the V1 antagonist. Results of these experiments showed that the V1 antagonist d(CH2)5Tyr(Me)AVP was a potent inhibitor of AVP potentiation of NASAC. Further experiments showed that the AVP neuromodulation, but not the NE induced accumulation of c-AMP, was calcium dependent. In the absence of extracellular calcium the neuromodulatory effect of AVP was abolished and was restored in the presence of 0.8 mM extracellular calcium. We then tested whether the calcium dependency was linked to a calcium-calmodulin system. In the presence of 50 uM trifluoperazine, a calmodulin antagonist, AVP potentiation of NASAC was effectively blocked. This result strongly suggested a dependency of the AVP-induced neuromodulation upon calmodulin. Another possibility also existed, however, because trifluoperazine while being primarily a calmodulin antagonist can also antagonize protein kinase C (Nishizuka, Nature, 1984). We therefore tested whether activation of protein kinase C would mimic the potentiation induced by AVP. 4-B-phorbol 12, 13 dibutyrate (PDBU) an activator of protein kinase C did not potentiate accumulation of c-AMP induced by NE. In contrast, the calcium channel activator, Bay K 8644, potentiated NE induced accumulation of c-AMP by very likely increasing the availability of intracellular calcium. These data support our hypothesis that AVP modulation of NASAC is dependent upon calcium/calmodulin. (We thank Dr. M. Manning for generous gifts of AVP & AVP analogues and Dr. A. Scriabine for Bay K 8644. Supported by NRSA MH09224 to R.E.B. and MH41256 to BMc)

223.14 EFFECTS OF PROTEIN KINASE-C ACTIVATION ON PROTEIN PHOSPHORYLATION AND RECEPTOR-MEDIATED CYCLIC AMP ACCUMULATION IN RAT BRAIN TISSUE. S. Shenolikar*, E.W. Karbon* and S.J. Enna (Spon:S.J.Strada) Departments of Pharmacology and Neurobiology and Anatomy, University of Texas Medical School, Houston, Tx. 77025

Effects of phorbol esters and/or protein kinase-C on receptor-mediated cAMP accumulation and on protein phosphorylation in rat brain cerebral cortical tissue were examined. Exposure of cortical slices metabolically labelled with ^{32}P-orthophosphate to phorbol 12-myristate, 13-acetate (PMA) resulted in the increased phosphorylation of a large number of proteins, varying between 10-80%. In control membranes prepared from cortical slices, activation of endogenous protein kinase-C led to a greater increase in protein phosphorylation than observed with membranes from slices previously exposed to PMA.Treatment of control membranes with a partially purified preparation of rat brain protein kinase-C plus PMA enhanced phosphorylation some 2.5 fold, with the majority of phosphate being incorporated in the selfsame proteins which were substrates for endogenous kinase. 15 min exposure of cerebral cortical slices to PMA markedly enhanced cAMP accumulation observed in response to 2-chloroadenosine, while having no effect by itself. However, prolonged (3 hr) exposure of tissue slices to PMA led to a reduction in the ability of the phorbol ester to augment cyclic nucleotide accumulation. The activity of protein kinase-C, assessed by its ability to phosphorylate histone H1 in a Ca^{2+}/phospholipid dependent manner was markedly attenuated when assayed from tissue slices exposed to PMA for 3 hrs as compared to control tissue. Thus, in rat brain cerebral cortex, exposure to phorbol esters, which elicits a phosphoprotein profile similar to that observed with isolated protein kinase-C, results in protein phosphorylation which may mediate the augmenting effect of PMA on 2-chloroadenosine-stimulated cAMP accumulation. In addition, the finding that both protein kinase-C activity and the augmenting response are attenuated following prolonged exposure to PMA is consistent with this notion. Moreover, these latter findings may represent the first demonstration of a "desensitization" of protein kinase-C in an intact tissue preparation. (Supported in part by a grant from the U.S. Air Force).

223.15 CHANGES IN STRIATAL MEMBRANE-BOUND VS CYTOSOLIC DISTRIBUTION OF CaM IN AGED RODENTS. C.C. Loullis, L. Antonian* and J. Sensenbach*. Molecular Neurobiology Group, Dept. of CNS Research, Medical Research Division of American Cyanamid Co., Lederle Laboratories, Pearl River, NY 10965.

Some of the actions of calcium as a second messenger in many physiological processes, including neurotransmission, are mediated by a group of calcium binding proteins, which include calmodulin (CaM). In the brain CaM is distributed in particulate and cytosolic fractions. In striatum the translocation of CaM between these two compartments can modulate the post-receptor signal. This occurs because CaM can affect calcium dependent enzymatic changes in cAMP metabolism. Using specific binding of ^{3}H-CaM to synaptic membranes as a tool, we investigated the distribution of CaM in the striatum of young vs old rats. Redistribution with age was evident and was demonstrated in two ways. First, the heat-treated (90^{0} C, 10 min) supernatants from a 100,000 xg centrifugation of striatal tissue, from young and old animals, were assessed for their ability to displace ^{3}H-CaM from synaptic membranes. The results showed a significantly greater inhibition of ^{3}H-CaM by the supernatants derived from old (31.8±6.2% per 100 µg protein; n=6) as compared to young animals (16.9±1.3% per 100/µg protein; n=6), indicating a higher amount of CaM in the cytosol of aged rats. The assumption that CaM, as opposed to other components in the supernatants, was responsible for the observed inhibition was supported by the fact that CaM can survive heat-treatment and the observation that a component in the supernatants co-eluted with authentic CaM when applied to a molecular sieving column. Second, specific binding of ^{3}H-CaM to membranes from young and old animals (100,000 xg pellet) was assessed. Binding was significantly higher in aged (3.4±0.2 pmoles/mg protein; n=6) as compared to young animals (4.3±0.3 pmoles/mg protein; n=6), indicating a decreased amount of membrane-bound CaM with age. Taken together these findings suggest a translocation of CaM from the membrane to cytosol in the striata of aged animals and indicate that binding of CaM to synaptic membranes may be a useful tool in evaluating changes in brain CaM distribution under various experimental conditions and/or manipulations.

223.16 NEUROTENSIN ALTERS THE PHOSPHORYLATION OF CAUDATE NUCLEUS SYNAPTOSOMAL PROTEINS. S.T. Cain and C.B. Nemeroff. Depts. Psychiat. and Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Neurotensin (NT) is a brain-gut tridecapeptide which is heterogeneously distributed in brain (for a review of NT, see Nemeroff, Psychoneuroendocrinology, 11:15, 1986). NT displays many of the characteristics of neurotransmitter substances such as synaptosomal localization, calcium-dependent release and specific, saturable, high affinity binding to brain membranes. Little is known, however, of the intracellular and membrane events which occur subsequent to NT binding to its receptor, i.e., transduction of the NT signal. In view of its ubiquitous regulatory role in cellular function, we have initiated a series of studies designed to elucidate the potential role of protein phosphorylation/dephosphorylation in mediating the NT signal. Due to the relatively high percentage (30%) of neostriatal NT receptors located on the terminals of nigrostriatal dopamine (DA) neurons, and the potentiation by NT of both the basal and potassium-stimulated release of DA from striatal slices, caudate nucleus synaptosomes would appear to be an ideal preparation in which to study NT-induced changes in protein phosphorylation.

A synaptosomal fraction from rat caudate nucleus was phosphorylated for five minutes in the presence of 500µM ATP. Identical aliquots of the phosphorylated synaptosomes were then incubated for 15 minutes in the presence of NT (1.8µM) or H_2O. Using the same experimental paradigm, we have recently reported (Cain and Nemeroff, N.Y. Acad. Sci., in press) that NT alters the amount of radioactive phosphate incorporated into a TCA-precipitatable protein fraction. In the present experiments, at various intervals after the addition of NT or H_2O, aliquots were removed and processed for SDS polyacrylamide gel electrophoresis. Samples were resolved on 10% gels. Autoradiographs were obtained by exposing the dried gels to X-ray film and radioactive phosphate incorporation into individual proteins was quantitated by microdensitometry.

NT selectively accelerated the rate of dephosphorylation of a 49,000 MW protein. Within the first 2.5 minutes after the addition of NT, the phosphorylation of this protein was decreased 21.4 ± 4.86% relative to control ($p < .01$, n=4). In a separate experiment, it was determined that the phosphorylation of the 49,000 MW protein is calcium-dependent. Protein phosphorylation must now be considered as a candidate mechanism for NT signal transduction. (Supported by NIMH MH-39415 and MH-15177-09.)

223.17 NEUROTRANSMITTER REGULATION OF PHOSPHOTIDYLINOSITOL METABOLISM IN EMBRYONIC SENSORY AND SYMPATHETIC NEURONS O.Harish*, K. Dunlap and L.Role. Anat.& Cell Biol, Ctr Neurobiol.& Behav., Columbia P&S, NY,NY 10032 & Dept.Physiol.,Tufts Med Sch.,Boston, Ma 02111.

The modulation of voltage and chemically gated channels by neurotransmitters can involve the activation of second messenger systems. Recent studies on the modulation of Ca current by norepinerphrine (NE) suggest an involvement of protein kinase C (PKC) activation in embryonic sensory neurons (Rane & Dunlap, PNAS 83: 184 1986). To examine a possible role of phosphotidylinositol (PI) turnover in neurotransmitter-mediated activation of PKC we have measured the generation of the inositol sugars IP, IP_2 and IP_3 in sensory and sympathetic neurons in response to various stimuli.

Whole ganglia or dispersed neurons in culture were pre-labeled with 3H inositol and then incubated with test agents for 15 min in the presence of 10mM LiCl. The cells were then extracted and the labeled sugars separated by column chromatography according to the method of Downes & Michell, Biochem J. 198:133, 1981. Initial experiments indicated 6 fold higher incorporation of ^{3}H-inositol into cultured neurons after 4 days vs 1 day of labeling. Since the percent of this precursor pool that was subsequently converted to IP's was also increased to 10%, we adapted this labeling protocol to permit determination of PI turnover in small quantities of tissue. Similary, intact ganglia were incubated with ^{3}H inositol for 3 hrs or overnight which increased the incorporation by 2 fold.

In 5 of 8 experiments on sensory ganglia and on cultured sensory neurons depolarization with 56mM K+ caused significant increases (2 to 13 fold) in the level of total inositol sugars. Separation of the sugars revealed that this was a selective increase in IP_3. NE had no effect on basal IP's and completely inhibited the depolarization-dependent increase in the generation of the sugars. The K^+ induced increase in IP's was only partly Ca-dependent and NE inhibited both the Ca-dependent and independent components. Thus, NE modulation of Ca current in these neurons may involve activation of PKC independent of PI turnover, perhaps by the hydrolysis of some other phoshoglyceride. NE block of the depolarization-induced increase in IP_3 is consistant with NE's inhibition of secretion from these neurons (see Dunlap et al,this volume).

In 6 of 6 experiments on sympathetic ganglia muscarine increased total IP's by 3.5 ± 0.5 fold reflecting an increase in both IP_2 and IP_3. Substance P also increased IP's by 85%. The effect of muscarine is dose dependent and blocked by atropine. Although VIP had little effect on PI turnover by itself, this peptide greatly potentiated the effects of submaximal concentrations of muscarine. These effects on IPs may underlie the reported modulatory activity of these agents on sympathetic neurons. This work was supported by awards from the Sloan Fdn. (LR), the Klingenstein Fdn. (LR & KD) and NS22061 (LR).

223.PO GUANYL NUCLEOTIDES INDUCE A REVERSIBLE INHIBITION OF HIGH AFFINITY [^{3}H]SPIROPERIDOL BINDING IN HUMAN CAUDATE AND PUTAMEN BY A NOVEL EFFECT VIA LIPID PEROXIDATION. A. C. Andorn*, J. M. Stitts*, and B. R. Bacon* (SPON: V. Rowland). Depts. of Psychiatry and Medicine, Case Western Reserve University School of Medicine and Cleve. Metro. Gen. Hosp., Cleveland, Ohio 44109

GTP (10^{-3}M) in the presence of 0.1 mM ascorbate produces a loss of high affinity (K_D =20 pM) specific [^{3}H]spiroperidol binding in human caudate or putamen. This loss of binding is nearly complete by 60 min at 37°C. The loss of binding can be totally recovered by extensive washing of the particulate membrane fragments previously incubated in GTP and ascorbate. GTP does not cause this loss of binding in the absence of ascorbate. Ascorbate itself (0.1 mM) induces a loss of low affinity (K_D =200 pM) [^{3}H]spiroperidol binding in these tissues, but this loss of binding begins at 60 min and is completed by 120 min. Furthermore, this lost binding cannot be recovered by extensive washing. There is also a time related increase in malonaldehyde (MDA) suggesting that the ascorbate effect may be mediated by lipid peroxidation. Agents that decrease lipid peroxidation (iron chelators, antioxidants) also prevent the ascorbate induced loss of binding. GTP itself causes no lipid peroxidation as measured by MDA production. However, GTP plus ascorbate causes a more rapid increase in MDA production. The ability of various nucleotides to induce MDA production in the presence of ascorbate is GTP > GDP > ITP > IMP = GMP > ATP > cAMP. A similar profile is observed in the ability of these nucleotides to induce the loss of the high affinity binding site. Incubation of particulate membrane fragments from caudate or putamen in GTP plus ascorbate for 120 min results in a complete loss of specific [^{3}H]spiroperidol binding in these tissues. We conclude that binding at the highest affinity site may be inhibited by a product of lipid peroxidation that is formed in the presence of GTP plus ascorbate, but that is not formed in the presence of ascorbate alone. Binding at the lower affinity site, however, appears to be irreversibly lost due to the effects of ascorbate mediated iron induced lipid peroxidation, either directly on the binding site, on the adjacent membrane, or due to the toxicity of one of the products of this reaction.

223.18 THE EXTRINSIC AND INTRINSIC MEMBRANE PROPERTIES OF CALCIUM-DEPENDENT PHOSPHOPROTEINS IN RAT STRIATUM. Y.S. Lau, C. Runice*, F. Dowd* and M. O'Hara*. Depts. of Pharmacology and Medical Microbiology, Creighton University School of Medicine, Omaha, NE 68178.

Calcium (Ca)-calmodulin (CaM)-dependent and Ca-phosphatidylserine (PS)-dependent protein kinases in the brain have been extensively studied. These kinases share many common characteristics including their ubiquitous distribution, Ca requirement and inhibition by the phenothiazine drugs. In rat striatum, we observed that the endogenous substrates for CaM and PS-dependent phosphorylation were different. In this study, the endogenous striatal protein phosphorylation was carried out in a medium containing ^{32}P-γ-ATP (25 μM, 1 μCi), EGTA (0.24 mM), Ca (0.5 mM) and CaM (1 μg) or PS (5 μg). The phosphoproteins were analyzed by SDS-polyacrylamide electrophoresis and autoradiography. Ca-CaM stimulated the phosphorylation of two major proteins at M.W. 40-60 KD. Ca-PS stimulated the phosphorylation of two other proteins at M.W. 14-20 KD, which are identical to the myelin basic proteins shown by other investigators. When the striatal preparation was initially washed 3 times with an EGTA (1.2 mM)-containing buffer, the phosphorylation of Ca-PS-dependent proteins was inhibited whereas the phosphorylation of Ca-CaM-dependent proteins could still be fully demonstrated.

We further studied the characteristics of CaM- and PS-dependent phosphoproteins that were obtained from the non-EGTA treated preparation by using the Triton X-114 phase separation technique (Bordier, C., J. Biol. Chem., 256:1604, 1981). The CaM-dependent phosphoproteins were totally recovered in the detergent (amphiphilic) phase, however the PS-dependent phosphoproteins were not seen in either detergent or aqueous (hydrophilic) phase. Furthermore, when the striatal preparation was first partitioned by phase separation and phosphorylation was then performed with each phase, the CaM-dependent protein phosphorylation could be fully demonstrated in the detergent phase only. The PS-dependent protein phosphorylation was not shown in either phase.

These results suggest that in rat striatum CaM-dependent phosphoproteins are intrinsic (integral) membrane proteins since they are not extracted by chelating agents such as EGTA and they are associated with the amphiphilic phase of Triton X-114. On the other hand, PS-dependent phosphoproteins are extrinsic (loosely bound) membrane proteins. We have previously observed that dopamine receptor up-regulation is associated with an increased sensitivity of CaM-dependent protein phosphorylation in rat striatum (Lau, Y.S., Brain Res., 307:181, 1984; Lau, Y.S. et al., J. Pharmacol. Exp. Ther., 229:32, 1984; Lau, Y.S. and Fung, Y.K., Brain Res., 369:311, 1986), therefore the phosphorylation of these integral membrane proteins by Ca-CaM may have a functional role in the striatum.

224.1 RAPID REGULATION OF [^{3}H]-HEMICHOLINIUM-3 ([^{3}H]HCh-3) BINDING SITES IN VITRO IN RAT STRIATUM. M.D. Saltarelli, P.R. Lowenstein and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Balto., MD 21205.

Studies utilizing both in vivo and in vitro paradigms indicate that the velocity of choline transport is directly related to the immediately antecedent activity of cholinergic neurons. Depolarization of synaptosomes or slices, via elevated [KCl], veratridine-stimulated sodium flux, or direct electrical stimulation have been shown to increase the velocity (V_{max}) of choline transport. [^{3}H]HCh-3, a potent, specific, and reversible inhibitor of choline uptake has recently been utilized to label the choline carrier in brain membrane preparations. Studies in our laboratory reveal that drugs capable of altering cholinergic activity in vivo cause rapid changes in the number of specific [^{3}H]HCh-3 binding sites measurable in cortical and striatal membrane preparations.

We have now examined changes in specific binding of [^{3}H]HCh-3 resulting from exposure of rat striatal tissue to buffers with depolarizing concentrations of KCl in vitro. Striatal slices (300 um) were incubated in Krebs bicarbonate buffer containing various concentrations of KCl (4.2 to 62 mM), after which specific [^{3}H]HCh-3 binding was measured in washed membrane preparations. When slices were incubated in normal Krebs buffer (4.2 mM KCl), specific binding gradually diminished during the initial 20 min from 470 ± 50 to 280 ± 30 fmole/mg protein ($\bar{x}$ ± S.E.M.), a 40% reduction when compared to un-incubated tissue. No significant changes in binding were observed after 60 min additional incubation. Twenty min after high KCl (40 mM) Krebs buffer was added to slices pre-incubated for 20 min in normal Krebs buffer, specific binding increased by 60% over binding measured in slices incubated for 40 min in normal Krebs. Specific binding gradually increased during the 20 min exposure to high K^+, then remained stable for at least 20 min. Additional experiments revealed that these changes in specific binding are directly proportional to [KCl]. Furthermore, changes in [^{3}H]-choline uptake parallel these changes in [^{3}H]HCh-3 binding. Scatchard analysis of saturation isotherms of [^{3}H]HCh-3 binding to membranes prepared under various incubations revealed that these changes result from increases or decreases in the number of binding sites (B_{max}), not alterations in the affinity of the binding site (K_D; 2 nM).

These studies imply that changes in external potassium concentration are capable of rapidly and significantly altering the velocity of choline transport into cholinergic neurons via alterations in the actual number of active or available carrier sites.

224.2 ENRICHMENT OF HIGH AFFINITY [^{3}H]-DIHYDROTETRABENAZINE BINDING IN A SYNAPTIC VESICLE FRACTION FROM BOVINE *CORPUS STRIATUM*. Joseph A. Near and Sassan Meshgin-Azarian*. Medical Sciences Program and Department of Pharmacology, Indiana University School of Medicine, Bloomington IN 47403.

The benzoquinolazines tetrabenazine and dihydrotetrabenazine are potent inhibitors of ATP-dependent catecholamine and serotonin uptake by adrenal chromaffin granules and synaptic vesicles from the CNS. The binding of [^{3}H]-dihydrotetrabenazine to chromaffin granule membranes has been described previously (Scherman, D., Jaudon, P. and Henry, J.P., Proc. Nat. Acad. Sci. U.S. 80:584), and occurs with high affinity. Because [^{3}H]-dihydrotetrabenazine is not a transport substrate, and its binding is not affected by the presence of a transmembrane electrochemical proton gradient, we have investigated its binding to various subsynaptic fractions prepared from the bovine *corpus striatum*.

[^{3}H]-dihydrotetrabenazine binds to a single class of sites in striatal synaptic vesicles with an apparent dissociation constant of 2nM. This is comparable to the inhibitory potency of dihydrotetrabenazine in catecholamine transport assays. In contrast to these results [^{3}H]-dihydrotetrabenazine binds to at least two classes of sites in all other fractions investigated, as revealed by computerized nonlinear least squares curve fitting analysis (Munson, P.J. and Rodbard, D., Anal. Biochem. 107:220). The higher affinity class of sites is comparable in affinity to that of synaptic vesicles, whereas the lower affinity sites exhibit an apparent dissociation constant of 100-300nM. The higher affinity class of sites is most abundant in the synaptic vesicle fraction, followed by a fraction enriched in coated vesicles and a microsomal fraction. Enrichment over homogenate is also observed in synaptosomal and synaptic plasma membrane fractions. Little higher-affinity binding was observed in mitochondrial and myelin fractions. These results are consistent with the expectation that the higher affinity sites are primarily associated with synaptic vesicles and other neuronal entities that are in communication with these organelles.

Lower affinity binding of [^{3}H]-dihydrotetrabenazine is absent in synaptic vesicles, and the greatest enrichment is observed in a microsomal fraction. Although the nature of the lower affinity class of sites is not yet understood, it is unlikely to be associated with the catecholamine and serotonin transporter because of its relatively low affinity and the lack of any evidence for its presence in the synaptic vesicle fraction.

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224.3 EFFECT OF ASCORBIC ACID ON THE SYNAPTOSOMAL UPTAKE OF [^{3}H]MPP^+, [^{3}H]DOPAMINE, AND [^{3}H]GABA. H. Sershen*, E.A. Debler*, and A. Lajtha (SPON: G.M. Lehrer) Center for Neurochemistry, Nathan S. Kline Institute, Ward's Island, NY 10035

The effect of ascorbic acid on the synaptosomal uptake of [^{3}H]MPP^+, [^{3}H]dopamine, and [^{3}H]GABA was examined in an attempt to understand the mechanism of ascorbic acid attenuation of MPTP neurotoxicity (Sershen et al., Neuropharmacology 12:1257, 1985). It has been speculated that the action of ascorbic acid could be related to its antioxidant properties involving hydroxyl radicals. Alternatively, ascorbic acid might inhibit the uptake of MPP^+, the active toxin, by the dopamine terminals. Synaptosomes were prepared from adult BALB/cBy mouse striatum. Tissue was homogenized in 0.32 M sucrose (pH 7.4), and the P_2 pellet was used for the uptake experiments. The uptake of 0.1 uM [^{3}H]MPP^+, 0.1 uM [^{3}H]dopamine, or 2 uM [^{3}H]GABA was defined as total uptake minus the uptake in the presence of 10 uM mazindol for [^{3}H]MPP^+ and [^{3}H]dopamine, and 200 uM GABA for [^{3}H]GABA. Membranes (0.15 mg protein) were incubated for 2 min at 37°C in 50 mM Tris buffer pH 7.4 containing 120 mM NaCl, 4 mM KCl, and 11 mM glucose. Ascorbic acid (0.01-10 mM) was added concurrently with labeled substrate, or to the S_1 supernatant for the washout experiments. The S_1 fraction was incubated with 0.1 or 10 mM ascorbic acid for 2 min at 37°C, and centrifuged at 14,000 g for 10 min. The resulting P_2 pellet was used for uptake. Uptake was terminated by filtration on Whatman glass fiber filters (GF/B). Ascorbic acid inhibited the uptake of [^{3}H]MPP^+ with an IC_{50} = 0.1 mM and of [^{3}H]GABA with an IC_{50} = 10 mM. [^{3}H]Dopamine uptake was increased by 30% by 0.01 mM ascorbic acid, and decreased 65% by 10 mM ascorbic acid. The effect of ascorbic acid on uptake was reduced by a washout step. In the [^{3}H]MPP^+ and [^{3}H]GABA uptake experiments nonspecific uptake was less than 10% of the total uptake in the absence or presence of ascorbic acid. In the [^{3}H]dopamine uptake experiments nonspecific uptake was 30% of total uptake in the absence of ascorbic acid, and 42%, 30%, <5%, <5%, and 27% of the total uptake in the presence of 0.001, 0.01, 0.1, 1.0, and 10 mM ascorbic acid respectively. In the washout experiments, nonspecific uptake increased in the S_1 control membrane (incubation at 37°C without ascorbic acid) to 50% of total uptake, and was 10% of total uptake in the presence of 10 mM ascorbic acid or after ascorbic acid washout. The results suggest that ascorbic acid-induced lipid peroxidation was not involved in the effect on uptake. Although we cannot rule out an effect of ascorbic acid on free radical mediated events related to MPTP neurotoxicity, it is possible that ascorbic acid has a direct effect on dopamine transporter function.

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224.4 METAPHIT: AN IRREVERSIBLE INHIBITOR OF METHYLPHENIDATE BINDING AND DOPAMINE UPTAKE. M.M. Schweri*, A.E. Jacobson*, R.A. Lessor*, and K.C. Rice*. (Sponsor: C.H. Hockman). Mercer U. School of Medicine, Macon, GA 31207 and NIADDK, Bethesda, MD. 20892.

Metaphit (1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine), a derivative of phencyclidine formed by adding an isothiocyanate group to the meta position of the phenyl portion of the parent compound, was originally conceived as a probe which would covalently bind to the phencyclidine/sigma opiate receptor. Indeed, studies using the radioreceptor binding assay for phencyclidine showed that this compound irreversibly binds to phencyclidine receptors in both the striatum and hippocampus (Rafferty et al, 1985).

In view of the fact that phencyclidine is known to reversibly inhibit dopamine transport, it was postulated that the closely-related Metaphit would irreversibly interact with the uptake system for this neurotransmitter. Moreover, it was also predicted that Metaphit would bind covalently to the recognition site for the stimulant drug methylphenidate, which appears to be a part of, or closely associated with, the dopamine transport complex (Schweri et al, 1985). Data showing that micromolar concentrations of Metaphit irreversibly inhibit both synaptosomal dopamine uptake and methylphenidate binding to rat striatal membrane preparations will be presented. These findings suggest that Metaphit and the methylphenidate radioreceptor assay may prove to be useful tools in the study of the structure and function of the dopamine transport complex. Supported by NIH Grant NS-22546

224.5 **PROLACTIN STIMULATES MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC NEURONS: IN VIVO AND IN VITRO STUDIES.** J.C. Chen* and V.D. Ramirez (SPON: A. Feng). Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

Herein we report the effect of oPRL on: (1) the in vivo activity of mesolimbic dopaminergic neurons innervating the nucleus accumbens (NA) in freely moving rats, and (2) the in vitro DA activity from corpus striatal (CS) fragments superfused with or without tetrodotoxin (TTX). For the in vivo study, adult male rats were implanted with push-pull cannulas and perfused within 30 days post-implantation. DOPAC, HVA and 5-HIAA were determined with HPLC every 20 minutes. We previously reported a spontaneous rise in basal DOPAC output during the early afternoon hours reaching plateau levels during the evening hours while HVA and 5-HIAA output remained unchanged. In animals showing this pattern of DOPAC output, inactive PRL (10 ng/ µl, boiled for 30 min) perfused at 1700 h (14 µl/min for 20 min) did not change the release rate of DOPAC from 1720 to 1900 h while native PRL (10 ng/ µl) perfused at 1900 h for 20 min in the same group of rats significantly increased DOPAC output from 1920 to 2040 h ($\bar{x}$ = 174 ± 5% vs. plateau level, $\alpha < 0.05$). HVA and 5-HIAA values remained constant and no special behaviors were recorded during the native or inactive PRL perfusions. These results were observed in animnals bearing PPC in the A9.8 - 9.4 region of the NA (deGroot's Rat Atlas). For the in vitro study, isolated CS fragments were perfused with modified KRP medium containing 0.18% glucose and 10 mM molybdate. After 45 minutes equilibrium time, every 15 minutes samples were assayed by HPLC. oPRL (10^{-7}M, 10^{-6}M and 10^{-5}M perfused for 30 min) significantly increased DA release at 30 minute or 60 minute post-PRL infusions but there was not a dose-dependent relationship. However, with superfusion medium containing TTX (10^{-6}M), oPRL increased DA release in a dose-response manner (10^{-7}M, 5.5 ± 1.4 vs. 7.32 ± 2.3; 10^{-6} M, 4.6 ± 0.7 vs. 10.5 ± 2.6; 10^{-5} M, 2.9 ± 0.5 vs. 17.5 ± 5.7 pg/min/mg tissue, pre- vs. post-PRL infusion, respectively). DOPAC output remained relatively constant during these experiments. These results suggest that interneurons in the corpus striatum can interfere with PRL-elicited DA release from CS fragments.

The PRL-evoked changes in in vivo DOPAC output from the NA and in in vitro DA release from the CS indicate that PRL can activate dopaminergic transmission in both the mesolimbic and nigrostriatal dopaminergic systems.

224.6 EFFECT OF ALPHA-METHYL-P-TYROSINE TREATMENT ON STIMULATION-EVOKED ENDOGENOUS DOPAMINE RELEASE FROM RAT STRIATAL SLICES. L.P. Dwoskin*, G.A. Gerhardt and N.R. Zahniser (SPON: L. Gronke). Depts. Pharmacol. and Psychiat., Univ. Co. Hlth. Sci. Ctr., Denver, CO 80262.

Despite a marked decrease (84%) in striatal dopamine (DA) content following treatment with alpha-methyl-p-tyrosine methyl ester HCl (AMPT; 300 mg/kg, i.p., 16 hr prior to sacrifice), release evoked by electrical stimulation from striatal slices preloaded with ^{3}H-DA was not different in treated and control rats. The question remained whether AMPT pretreatment depleted the endogenous pool of releaseable DA. Therefore, the amount of endogenous DA and dihydroxyphenylacetic acid (DOPAC) were measured in the superfusate from striatal slices from these groups using HPLC-EC. Superfusion chambers contained a single striatal slice (0.5 mm thick) which was superfused at a rate of 1 ml/min with Krebs' buffer. Nomifensine (10 uM) was included in the superfusion medium in half of the slices from control or AMPT treated rats to inhibit the reuptake of DA. Electrical stimulation (60 pulses at 1 Hz or 300 pulses at 5 Hz) occurred after 70 min. One-min sample collection began 2 min prior to and until 10 min after the stimulation. Ascorbic acid oxidase (0.08 mg) was added to each sample, and subsequently 50 ul was injected directly into the HPLC-EC system, which consisted of a 3 micron ODS Ultrasphere reverse phase column and an ESA 5100A Coulochem detector with a model 5011 dual-detector analytical cell. The detection limit was approximately 15 pg/ml superfusate. Complete separations and reequilibration of the system took 4-5 min.

DA was not detected in the superfusate in the absence of nomifensine. In the presence of nomifensine, the amount of DA in the superfusate increased immediately after the stimulus and returned to spontaneous levels (0.15 ng/ml) during the collection period. Lesser amounts of DA were collected in the superfusate when 60 rather than after 300 pulses were delivered (0.6 and 2.4 ng per stimulation, respectively). In both the absence and presence of nomifensine, the stimulus evoked a delayed (2 min) increase in the amount of DOPAC collected. Peak overflow of DOPAC (0.9 ng/ml) occurred 4 min after the 60 pulse stimulation and 6 min after the 300 pulse stimulation and did not return to baseline levels (0.15 ng/ml) during the collection period. AMPT pretreatment did not decrease the amount of endogenous DA collected in the superfusate after 60 pulses of stimulation; however, after 300 pulses overflow of both DA and DOPAC were moderately decreased (38% and 32% during the collection period, respectively). In contrast, after 60 pulses, the presence of endogenous DOPAC in the superfusate during the collection period was markedly decreased (82%). Therefore, the amount of both DA and DOPAC collected must be taken into consideration when quantitating release in this system. Our results emphasize that DOPAC reflects released DA that has been metabolized. Additionally, releaseable pools of DA were not depleted to the same extent as DA content in the striatum following this AMPT treatment. Supported by USPHS grants NS 09199 and AM 07391.

224.7 **A MODEL OF THE DYNAMICS OF DOPAMINE RELEASE, UPTAKE, AND METABOLISM IN RAT NEOSTRIATUM.** J. B. Justice, Jr.,* A. C. Michael, L. C. Nicolaysen and W. H. Church, Department of Chemistry, Emory University, Atlanta, GA 30322.

In vivo voltammetry and on-line microdialysis have been used to examine the dynamics of the dopaminergic nerve terminal. The results are interpreted with a model of the nerve terminal composed of a set of non-linear ordinary differential equations which describe dopamine (DA) synthesis, compartmentation, release, uptake and metabolism. The model is compared with data obtained from experiments on the stimulated release of dopamine. The amplitude and time course of released DA in the extracellular fluid are measured by in vivo voltammetry. Stimulation of the MFB at intervals of 20 minutes (80 µA rms, 100 Hz, bipolar square waves) generates reproducible amounts of released DA. As the interval is shortened to 5, 2, or 1 minutes the released DA decreases over successive stimulations. Alpha-methyl-para-tyrosine also causes a decrease in released dopamine over successive stimulations using a 20 minute interval. The model indicates that released DA is rapidly taken up into the cytosolic pool where it is slowly incorporated into the releasable pool. This recycled DA appears to be a source of releasable DA, in addition to newly synthesized DA, for release by electrical stimulation. At stimulation intervals of 5 minutes, the releasable pool has not had time to be replenished from the cytosolic DA.

The model reproduces the inability of extracellular DA to remain elevated during a 40 second stimulation of the MFB, and the metabolism of DA to DOPAC following stimulated release (Neurosci. Lett., 56, 365-369, 1985).

Data on extracellular dopamine obtained with on-line microdialysis/LCEC indicate an initial rise to 8 times baseline in 40 minutes after 10 mg/kg nomifensine, an uptake blocker. This is followed by a decline to 4 times baseline over the next several hours. The model indicates that the decline in extracellular DA following the initial rise to a maximum tracks a similar decline in cytosolic DA, indicating a rapid communication between released and cytosolic DA.

The model has been used to predict the effect of inhibition of tyrosine hydroxylase, MAO and COMT, the effect of reserpine, and the effect of GBL on compartments of DA and on the metabolites DOPAC, HVA and 3MT. It may also be used to follow the passage of radioactivity through the system, starting from labeled tyrosine.

224.8 OUABAIN STIMULATES DOPAMINE RELEASE FROM TUBEROINFUNDIBULAR NEURONS IN A SODIUM- AND CALCIUM-DEPENDENT MANNER. L.Annunziato, S.Amoroso*, F.Maurano*, G.Kaparos*, M.Taglialatela* and G.F.Di Renzo*. Dept. Pharmacology, 2nd School of Medicine, University of Naples, Via S.Pansini 5, 80131 Naples, Italy.

It is now widely recognized that membrane Na^+-K^+-ATPase inhibition induced by digitalis can affect catecholamine release from central neurons, although the mechanism by which digitalis exerts this action is not yet completely elucidated. These studies were undertaken in order to investigate the membrane processes and the ionic events involved in digitalis action.

Tuberoinfundibular dopaminergic neurons were incubated in 200 ul parallel chambers and superfused at a flow rate of 500 ul/min with a Krebs-Ringer Bicarbonate buffer. Release of ^{3}H-DA "newly taken up" was evaluated in 2 min. fraction eluates. In addition endogenous DA released from the same neurons was studied in a static incubation system and assayed radioenzymatically.

Ouabain (0.1-1 mM) stimulated either endogenous or "newly taken up" DA release. This effect was completely abolished in presence of tetrodotoxin (10 uM), a highly selective blocker of sodium permeability through its specific channels. This finding may indicate that ouabain-induced DA release requires Na^+ entrance into the cells through its specific channels. Furthermore, since Lanthanum (1 mM), a well known blocker of calcium channels and of the anionic binding sites on the surface of cell membrane, markedly suppressed ouabain-induced ^{3}H-DA release, extracellular Ca^{++} influx may be another important ionic event consequent to the inhibition of Na^+-K^+-ATPase in central neurons. The release of calcium from its intracellular stores does not appear to play a relevant role since the putative intracellular Ca^{++}-antagonist 8-(N,N-diethylamino)-octyl-3,4,5-trimethoxybenzoate(TMB-8)(50 uM) failed to prevent ouabain-elicited ^{3}H-DA release. Finally nomifensine (10 uM), a DA transport inhibitor, did not interfere with ouabain stimulated ^{3}H-DA release, suggesting that this efflux is not mediated by a carrier-dependent process.

All together these results seem to suggest that high doses of digitalis stimulate the release of neurotransmitter by causing Na^+ influx and consequently Ca^{++} entry into the cells.

It remains to be clarified whether Ca^{++} entrance elicited by ouabain occurs through Na^+-Ca^{++}-exchange system or its specific Voltage Operated Channels (VOC). (M.P.I. 40 and 60% grants to L.A.)

224.9 VESICULAR UPTAKE OF DOPAMINE AND METABOLISM OF L-DOPA IN THE RAT BRAIN. N.T. Buu and M. Angers*, Clinical Research Institute of Montreal, Montreal, Quebec, Canada (H2W 1R7).

There is still no satisfactory explanation for the absence of change in the brains of rats injected with L-dopa, in concentrations of norepinephrine (NE) and 3-methoxytyramine (3-MT), a marker of dopamine (DA) release. This lack of response occurs despite the manyfold increases in DA concentration in brains following L-dopa administration. Previous studies have shown that neither the turnover rate of DA nor the synthesis rate of NE (Freed and Murphy, 1978) was affected by the expanded L-dopa/DA pool. On the other hand synthesis of NE and release of DA require that DA enters the catecholamine (CA) storage vesicle where dopamine-ß-hydroxylase (DßH) is localized, and from where CA can be released by exocytosis. This study determined the vesicular content of DA in the brain of rats and analysed possible relationship between the vesicular uptake of DA and the central metabolism of L-dopa.

Sprague-Dawley rats (175-200 g) were injected intraperitoneally (i.p.) with L-dopa (100 mg/kg). Others were treated with pargyline (80 mg/kg, i.p.) 1 hour prior to L-dopa. All the rats were killed by decapitation 1 h following L-dopa. The vesicular fraction was prepared from whole brain homogenates. CA and CA metabolites were analyzed by HPLC with electrochemical detection.

In the rats injected with L-dopa there was significantly large increases of DA in all areas of the rat brain but there was no increase in NE, 3-MT and normetanephrine (NMN). The brain vesicular contents (ng/mg protein ± SEM) of DA (1.3 ± 0.2) and NE (4.7 ± 0.4) of the L-dopa-injected rats were similar to those of control saline-injected rats (0.8 ± 0.2 for DA and 4.5 ± 0.3 for NE), indicating that the increased DA pool did not enter the storage vesicles.

In contrast in the brains of rats pretreated with pargyline the large accumulations of DA following L-dopa administration were accompanied by large increases in NE, 3-MT and NMN. At the same time the vesicular fractions from these brain homogenates showed significant increases in DA (27.5 ± 2.9 ng/mg protein) and NE (7.1 ± 0.6 ng/mg protein). There was no detectable 3-MT or NMN in the vesicular fraction.

The data demonstrated that in normal rats the expanded DA pool following L-dopa administration did not enter appreciably into the CA storage vesicles. This fact may account for the absence of any change in NE and 3-MT. On the other hand in pargyline-pretreated rats DA increased significantly in the vesicular fraction. This may explain the increase in NE, 3-MT and NMN following L-dopa. (Supported by MRC of Canada).

224.10 **CHARACTERIZATION OF SEROTONIN UPTAKE SITES WITH [^{3}H]CITALOPRAM AND DIFFERENTIATION OF MULTIPLE [3H]IMIPRAMINE BINDING SITES.** **Brian L. Largent, Robert J. D'Amato*, Adele M. Snowman, and Solomon H. Snyder. Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.**

[^{3}H]Citalopram demonstrates saturable, reversible binding of high affinity (Kd = 0.7 nM) in rat brain membranes. Under the assay conditions used, binding of [^{3}H]citalopram is linear with respect to tissue and radioligand concentration. The binding is sensitive to temperature and pH, and dependent upon salt concentration in a similar fashion to other uptake inhibitors. The drug specificity for [^{3}H]citalopram binding in rat brain membranes for various serotonin uptake inhibitors correlates closely with their ability to inhibit synaptosomal uptake of [^{3}H]serotonin. Inhibition of [^{3}H]citalopram binding by both serotonin and imipramine is consistent with a competitive interaction in both equilibrium and kinetic analyses. Thus, Hill plots for competition curves of imipramine or serotonin vs. [^{3}H]citalopram indicate a Hill coefficient of essentially unity. Equilibrium-saturation analysis of [^{3}H]citalopram binding in the presence of 100 nM imipramine or 200 nM serotonin demonstrates a shift in the apparent Kd for [^{3}H]-citalopram, without an effect on the Bmax, consistent with a competitive interaction. Additionally, imipramine or serotonin, up to concentrations of 50 µM, does not affect the dissociation rate of [^{3}H]citalopram, suggesting a lack of allosteric regulation of [^{3}H]-citalopram binding by imipramine or serotonin.

This apparently competitive interaction of imipramine with serotonin uptake sites, as labeled with [^{3}H]citalopram, contrasts with earlier studies suggesting primarily allosteric interactions of imipramine with serotonin uptake sites. To address this apparent inconsistency, we examined [^{3}H]imipramine binding to directly compare with that of [^{3}H]citalopram. Thus, serotonin only partially inhibits 'specific' binding of [^{3}H]imipramine (defined with a 100 µM imipramine 'blank') - while fully inhibiting specific [^{3}H]citalopram binding - and this amount varies (20-80%) among brain regions. Detailed equilibrium-saturation analysis of [^{3}H]imipramine binding reveals two binding components. At high affinity sites for [^{3}H]imipramine (Kd ≅ 10 nM), which by several criterion (drug specificity and Bmax) seem to represent the same sites labeled with [^{3}H]citalopram, serotonin and citalopram exhibit a Hill coefficient of approximately unity. The serotonin-insensitive portion of binding corresponds to the low affinity binding of [^{3}H]imipramine. No significant effect was seen on the dissociation of [^{3}H]imipramine by infinite dilution in the presence of up to 10 uM citalopram or serotonin. Thus, imipramine appears to bind competitively with serotonin uptake sites, while also binding to lower affinity sites which are insensitive to serotonin and currently uncharacterized.

224.11 REGULATION OF NEUROTENSIN RELEASE FROM CANINE ENTERIC NEURONAL CULTURES. D.L BARBER*, A.M.J. BUCHAN*, S.E. LEEMAN and A.H. SOLL* (SPON: D. Pollen). Dept. of Physiol. Univ. Mass. Med. Ctr., Worcester, MA. Dept. of Physiol. U. British Columbia, Vancouver, BC and CURE, VA Wadsworth Medical Center, Los Angeles, CA.

Neurotensin (NT), although localized within the central nervous system and enteric endocrine cells, has not previously been demonstrated in cells of the enteric nervous system. We have recently identified NT-immunoreactivity within neurons of the canine submucosal plexus. Canine ileum (n=5) was fixed in Bouin's solution, embedded in wax and 5 µm sections immunostained with monoclonal antibodies to NT using the PAP method. At least 60 % of the identified submucosal neurons demonstrated NT-immunoreactivity, however, in the myenteric plexus only occasional cell bodies were observed. We have subsequently developed an enteric ganglion cell culture system to study directly the regulation of NT release. Cells were dispersed from canine ileal submucosa by sequential incubation in collagenase and EDTA. Centrifugal elutriation separated nerve cells from fibroblasts and enriched NT-containing neurons as determined by immunohistochemistry and radioimmunoassay. Cell fractions enriched for NT were cultured in Dulbecco's modified Eagles' medium supplemented with growth factors and 5 % dog serum. After 48 h, enteric submucosal cultures contained 575 ± 30 fmol NT/10^6 cells (mean ± SE, n=5) and released NT in a time dependent manner. High pressure liquid chromatographic analysis of cell extracts characterized NT(1-13) as the primary molecular form in these neuronal cultures. Basal NT release after 60 min was 1.6 ± 0.1 % of NT cell content. Forskolin (10µm), an adenylate cyclase activator, the phorbol ester, ß-TPA (10 nM), and the calcium ionophore A23187 (100 nM) increased NT release to 4.1 ± 0.3 %, 6.3 ± 0.5 %, and 4.8 ± 0.4 %, respectively (n=4). Substance P (SP, 0.1-100 nM) stimulated a dose-dependent increase in NT release with a maximal response of 5.7 ± 0.3 % with 100 nM SP (n=3). Somatostatin (100 nM) inhibited maximum SP-stimulated NT release by 62 % and inhibited NT release in response to a combination of ß-TPA and A23187 from 8.5 ± 0.6 % to 3.3 ± 0.3 % (n=3). In these cell cultures, degradation of added [^{3}H]-NT(1-13) was minimal over 60 min. This primary culture of submucosal ganglion cells provides a model for studying the regulation of enteric neuropeptide release. In culture, NT release was stimulated by receptor-independent mechanisms and by SP and was inhibited by somatostatin.

224.12 RELEASE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) FROM MOUSE CEREBRAL CORTICAL SLICES. P.J. Magistretti and J.L. Martin*, Dept. of Pharmacology, Centre Médical Universitaire, Geneva 1211, Switzerland.

A sensitive radioimmunoassay (RIA) for the detection of small quantities of VIP (lower limit of detection 3 pg/tube, i.e. 1 fmol/ml) has been used to examine the regulation of VIP release from mouse cerebral cortical slices. For such a RIA a commercially available antiserum (Amersham Int. plc N. 1581) was used in combination with a highly purified and characterized molecular form of ^{125}I-VIP, monoiodinated on Tyr^{10} and oxidized to Met-sulfoxyde on Met^{17} (Martin *et al., J. Biol. Chem.* **261**, 5320, 1986). This [mono(^{125}I)iodo-Tyr^{10}, $MetO^{17}$]VIP displays the following characteristics : (1) It possesses a high specific activity (2050 Ci/mmol); (2) It maintains the biological activity of native VIP, and (3) it binds to antibody and membrane recognition sites in a specific, saturable and reversible manner. Two types of experimental paradigms have been used to assess VIP release : (1) static and (2) superfusion conditions. For the release under static conditions, mouse cerebral cortical slices (250 x 250 µm x ~ 1 mm, i.e. cortical thickness, 1-2 mg prot) are incubated for 2 min at 37° in 450 µl of previously oxygenated Krebs-Ringer bicarbonate buffer (KRG) pH 7.4 containing (mM) : NaCl 120; KCl 3; $CaCl_2$ 2.6; $MgSO_4$ 0.67; KH_2PO_4 1.2, $NaHCO_3$ 27.5 and glucose 3 mM. Drugs are added as 50 µl. At the end of incubation, tubes are centrifuged and VIP released is assayed by RIA in the supernatant. VIP tissue content is assayed in the pellet after extraction with 1 M CH_3COOH. The effect of preincubation (P) time on basal and K^+-evoked VIP release has been examined. Results are as follows (VIP release ± SEM expressed as % of tissue content; n=3 separate determinations) : 0 min P : Basal = 3.21± 0.37, 25 mM K^+ = 10.01±2.2; 15 min P : Basal = 0.83±0.14, 25 mM K^+ = 5.71± 2.1; 30 min P : Basal = 0.41±0.03, 25 mM K^+ = 2.68±0.37; 60 min P : Basal = 0.18±0.03, 25 mM K^+ = 1.37±0.15. The decrease in basal and K^+-evoked release as a function of P time is not the consequence of a decrease in VIP tissue content as indicated by the following values (VIP content in pg/mg prot ± SEM; n=17) : 0 min P = 3001.5±110; 15 min P = 3097±137.1; 30 min P = 2876±71.8; 60 min P = 3371±177.5. For further experiments a P time of 30 min has been used. Under these conditions, a 2 min exposure of the slices to 20 or 25 mM K^+ elicits an increase of VIP over basal levels of 367±11 % and 681.7±58 % respectively. Preliminary results indicate that norepinephrine at 100 µM does not affect VIP release. For superfusion conditions, slices are prepared as for static conditions, distributed into individual chambers (1.5 mg prot/chamber) and superfused at a rate of 400 µl/min with KRG containing 30 ug/ml bacitracin and 0.05 % BSA. Slices are equilibrated for 1 hr before stimulation. Fractions (800 µl) are collected every 2 min, lyophilized and VIP released is assayed by RIA. Under these conditions basal VIP release is 4.49±0.31 pg/mg prot (n=9) and is increased by 334.96 % in the presence of 25 mM K^+ for 2 min. This K^+-evoked release is markedly decreased in the absence of Ca^{++} in the medium : Basal VIP release : 4.06±0.17, increased by 183.8 % in the presence of 25 mM K^+. We are currently examining the potential stimulatory or inhibitory effect of cortical neurotransmitters on VIP release.

224.13 MECHANISM OF NOREPINEPHRINE RELEASE ELICITED BY RENAL NERVE STIMULATION, VERATRIDINE AND POTASSIUM IN THE ISOLATED RAT KIDNEY: ROLE OF CALCIUM AND SODIUM. M.M. Mohy El-Din* and K.U. Malik. Dept. of Pharmacol., Univ. of Tenn., Memphis, TN 38163.

Electrical stimulation of postganglionic sympathetic nerves, veratridine (Vt) or potassium chloride (K^+) which cause depolarization of nerve terminal, result in release of norepinephrine (NE) in several tissues. Depolarization of the adrenergic nerve terminal by these stimuli which is associated with increased influx of calcium (Ca^{++}) is considered a key step in release of NE. However, the mechanism by which Ca^{++} enters the nerve terminal in response to various depolarizing stimuli is not well understood. Ca^{++} may enter the nerve terminal through voltage-dependent Ca^{++} channels or by a mechanism involving Na^+-Ca^{++} exchange. To elucidate the contribution of one or more of these mechanisms to release of NE caused by electrical stimulation of sympathetic fibers, Vt and K^+, we have investigated the effect of Ca^{++} depletion, the Ca^{++} channel blocker nifedipine, sodium (Na^+)channel blocker tetrodotoxin and different concentrations of Na^+ in the isolated rat kidney perfused with Tyrode's solution and prelabeled with [^{3}H]NE. Renal nerve stimulation (RNS) (1-4Hz), Vt (10-60μM) or KCl (40-100 mM) produced renal vasoconstriction and increased perfusion pressure and overflow of tritium in a frequency and concentration-dependent manner respectively. Omission of Ca^{++} (1.8 mM) from the perfusion fluid abolished the renal vasoconstriction and the increase in tritium overflow elicited by RNS and K^+ and substantially reduced that caused by Vt. Nifedipine (1.4 μM) attenuated the renal vasoconstriction but enhanced tritium overflow elicited by RNS, Vt and K^+. Tetrodotoxin (0.3 μM) abolished the effect of RNS and Vt but not that of K^+ to produce renal vasoconstriction and to increase tritium overflow. Lowering the Na^+ concentration in the perfusion medium (from 25 to 150 mM) reduced the overflow of tritium and the renal vasoconstriction caused by RNS or Vt ($p<0.05$); the increase in tritium overflow and the renal vasoconstriction in response to these stimuli was positively correlated with extracellular Na^+ (25-150mM). In contrast, K^+-induced tritium overflow and the associated renal vasoconstriction was enhanced by about 3-fold by lowering extracellular Na^+. These data suggest that 1) Ca^{++} is essential for release of NE elicited by RNS, Vt and K^+ whereas Na^+ is required for NE release caused by RNS and Vt but not by K^+, 2) voltage-dependent Ca^{++} channels are not involved in the release of NE elicited by RNS, Vt or K^+ and that 3) RNS and Vt appears to cause release of NE by enhancing entry of Ca^{++} into the nerve terminal consequent to depolarization caused by influx of Na^+ whereas K^+-induced NE release might be due to influx of Ca^{++} through specific K^+-activated Ca^{++} channels. (Supported by USPHS-NIH Grant 19734).

224.14 **EFFECTS OF VESICULAR ACH UPTAKE BLOCKER AH5183 ON ACH RELEASE AT TORPEDO ELECTROPLAQUES.** **M. Murawsky*, R. Manalis and J. Suszkiw. Depts. Physiology & Biophysics, Univ. Cincinnati Coll. of Medicine, Cincinnati, OH 45267 and Biol. Sciences, Indiana Univ.-Purdue Univ. at Fort Wayne, Fort Wayne, IN 46805**

The compound 2-(4 phenylpiperidino)cyclohexanol (AH5183) is a potent inhibitor of ACh uptake into synaptic vesicles isolated from Torpedo electric organ (Anderson, King & Parsons, 1983). We have investigated the effects of AH5183 on vesicular uptake and release of ACh in the electric organs of Torpedo marmorata, stimulated via the attached electromotor nerves. Tissue blocks together with the segments of electromotor nerve were dissected out, perfused with 100 μCi (^{3}H)acetate and 0.1 mM Ch for 2 hrs, and then activated by the application of suprathreshold square wave pulses delivered to the nerve at 1 Hz for 2 min. After additional 2 hrs of perfusion, the tissue was washed for 1 hr with Ringer's solution containing 20 μM paraoxon and then restimulated at 1 Hz for 15 min. Tissue electrical response and (^{3}H)ACh release were measured. After the experiment, tissue was frozen in liquid N_2 for subsequent extraction of synaptic vesicles. Inclusion of 10 μM AH5183 in the perfusion had no effect on tissue response, which declined in a characteristic fashion in both the presence and absence of the drug. The incorporation of (^{3}H)ACh into vesicles and its release were depressed by 90% in the presence of the drug, although total tissue level of (^{3}H)ACh was unaffected. These observations suggest that under the conditions of these experiments (1) the drug did not interfere with the mobilization of preformed vesicular ACh for release, and 2) synthesis and uptake of ACh into so called active vesicles (Suszkiw, Zimmerman and Whittaker, 1978) did not detectably contribute to the pattern of transmitter release. Consequently, the kinetics of ACh release at these synapses reflect primarily the mobilization and depletion of preformed quanta in the depot pool of synaptic vesicles. Supported in part by NIH Grant NS 20786.

224.15 EFFECT OF AH5183 ON ACETYLCHOLINE RELEASE DURING REPETITIVE ACTIVITY AT THE FROG NEUROMUSCULAR JUNCTION. R.S. Manalis and J.B. Suszkiw. Indiana-Purdue Univ., Fort Wayne, IN 46805 and Univ. Cinci. Sch. Med., Cincinnati, OH 45267.

Recent neurochemical studies have shown that 2-(4-phenylpiperidino) cyclohexanol (AH5183) selectively blocks the transport of ACh into synaptic vesicles. In fact, Van der Kloot (Pflugers Arch., 406:83, 1986) has just shown that the quantal size at the neuromuscular junction is reduced by the drug. We have used AH5183 for two reasons. First, to determine if facilitation is dependent on the rate of vesicular transport of ACh. Second, we hoped to gain insight into the relative amount of ACh available for release by noting the total number of transmissions which occur prior to the detection of the initial effect of the drug. Sciatic nerve-sartorius muscle preparations of the frog (Rana pipiens) were bathed in normal Ringer solution containing (in mM): NaCl (111); KCl (2.5); $CaCl_2$ (2.0); tris maleate (4) (pH = 7.1-7.2; temp. = 15° C). Endplate potentials (EPPs) were recorded from curarized preparations ([dTC] = 4.31 μM). Trains of repetitive stimuli were applied to the nerve. For a given train, the stimulus frequency was constant and was within the range of .5-36 Hz. Trains lasted 4 sec each and were applied once every 5-8 min. Changes in ACh release were measured electrophysiologically as variations in EPP amplitudes. Control data obtained at stimulus frequencies of 18 or 36 Hz showed an early and rapid facilitation which was followed first by a rapid decay phase and then by a slow decay phase. The following data were obtained from a single neuromuscular junction at a stimulus frequency of 36 Hz. Three successive measurements of normalized EPP amplitudes gave consistent values for facilitation, the rapid decay time constant, and the inflexion point, as extrapolated to the Y-axis (normalized EPP), between the rapid and slow decay phases. Another stimulus train was not applied until 11 min after the addition of 15 μM AH5183. The facilitation ratio changed slightly from 1.88 to 1.94. However, the rapid decay time constant decreased from 512 to 464 msec and the inflexion point decreased from .43 to .36. AH5183 began to cause this effect immediately following the peak facilitation which corresponds to the 5th EPP in the train. At 32 min into the drug exposure, the time constant was 300 msec and the inflexion point was .19. It is concluded that facilitation is not due to an increase in the rate of transport of ACh into vesicles, that the readily releasable pool of ACh is relatively small, and that this pool is replenished during the rapid decay phase. (AH5183 kindly supplied by Dr. S.M. Parsons.)

224.16 GABA-UPTAKE INHIBITION: PHARMACOLOGICAL CORRELATES E.B. Nielsen, P.H Andersen*, C. Braestrup* and K.H. Wolffbrandt*. Pharmaceuticals R & D, NOVO Industri A/S, Department of Pharmacology, DK-2880, Bagsvaerd, Denmark.

The recent availability of drugs which inhibit the uptake of GABA in synapses has prompted an interest in the potential of such drugs in diseases and states involving GABA-related dysfunction, e.g., epilepsia and anxiety. Examples of such drugs are SKF 100330A and SKF 100561, substituted derivatives of nipecotic acid and homo-beta-proline, respectively.

In the present experiments, we have characterized the effects of these two compounds in a series of in vivo and in vitro tests, predictive for anticonvulsant, anxiolytic and sedative actions. In vitro, with IC_{50} values of 0.35μM and 0.2μM, respectively SKF 100330A and SKF 100561 inhibited GABA-uptake. This inhibitory effect occurred in a non-competitive fashion. In vivo, the compounds were potent inhibitors of sound-induced convulsions in DBA/2 mice with ED_{50} values of 4mg/kg and 3mg/kg (IP, t-30 min), respectively. Some sedation was noted in the rotarod and traction tests. However, consonant with an inhibitory effect on GABA-uptake, where a ceiling level of the net increase in GABAergic function can be expected, the drugs only partially counteracted the convulsions induced by pentylenetetrazol and by bicuculline.

In tests predictive for anxiolytic action, positive results have been obtained in some models.

These results suggest that GABA-uptake inhibitors may exert antiepileptic and possibly anxiolytic action.

224.17 NUCLEOSIDE TRANSPORT IN SYNAPTONEUROSOMES. P.F. MORGAN* AND P.J. MARANGOS. Unit on Neurochemistry, Biological Psychiatry Branch, NIMH, Bethesda, Md. 20892.

Synaptoneurosomes have been employed with much success to study biophysical phenomena, such as the cAMP second messenger system, that require a high degree of physiological integrity (Hollingsworth, E.B. et al, J. Neurosci. 5, 2240, 1985). Nucleoside transport is also such a phenomenon that is dependent on such factors as tissue viability, cellular ultrastructure and the cytoplasmic milieu. We have, therefore, made initial assessment of synaptoneurosomes as a study system for examining nucleoside accumulation vis a vis other particulate preparations, particularly synaptosomal preparations. Adenosine accumulation into rat synaptosomes was studied using [^{3}H]-adenosine as previously described (Morgan, P.F. et al, Eur. J. Pharmacol. 87, 121, 1983). This methodology was also employed to study adenosine accumulation in a whole brain homogenate particulate preparation, unfiltered synaptoneurosomes and filtered synaptoneurosomes prepared as previously described (Hollingsworth, E.B. et al, J. Neurosci. 5, 2240, 1985).

Adenosine accumulation was estimated after a 1 minute exposure to 1 uM [^{3}H]-adenosine. All of the particulate preparations cited above exhibited a similar accumulation of ^{3}H-adenosine per initial minute, namely within the range 6.5-7.5 pmol/initial min./mg protein. The total [^{3}H]-adenosine accumulation in all of the particulate preparations studied was also equally susceptible to inhibition by 10^{-3}M hexobendine (circa 78-83% inhibition in all cases). However, a very salient feature of the data was the extrememly low inter- and intra- experimental variance (as reflected in low standard error of the mean estimations) of the filtered synaptoneurosomes preparation, especially when contrasted to that of the synaptosomal preparation.

Binding of the radionucleotides [^{3}H]-cyclohexyladenosine (^{3}H-CHA) and ^{3}H-Nitrobenzylthioinosine (3H-NBI) to adenosine receptors and nucleoside transporter sites respectively in the above particulate preparations were additionally examined using methodologies previously described (Marangos, P.J. et al, J. Neurochem. 39, 184, 1982). Binding of [^{3}H]-NBI was not significantly different between any of the particulate preparations although [^{3}H]CHA binding was greater to synaptosomal membranes when compared to any of the other particulate preparations ($p's<0.05$, analysis of variance).

It is concluded that the advantages of employing synaptosomes to study neuronal function described are unlikely to be offset by the disadvantages of poor tissue yield, lengthy preparation times and poor tissue viability of the synaptosome preparation compared to the synaptoneurosomes preparation.

224.18 A PURIFIED SYNAPTONEUROSOME PREPARATION FROM RAT NEOCORTEX. I. Reynolds-Niesman and W.T. Greenough Departments of Psychology and Anatomical Sci., University of Illinois, Champaign, IL 61820

To prepare very pure "synaptoneurosomes"; a complex of a presynaptic bouton, a synaptic contact, and a resealed postsynaptic membrane, we modified the method of Hollingsworth et.al. (J. Neurosci. 1985, 5) for rat neocortex. Cerebral cortex was dissected, at 4° C, into blocks with a minimum of white matter. The blocks, approximately 500 mg/brain, were homogenized, with a glass-glass homogenizer, in 7 ml modified Kreb's-Henseliet buffer (KRBS), for 10-12 strokes. The KRBS consisted of 118.5mM NaCl, 4.7mM KCl, 3mM $MgSO_4$, 2.5mM $CaCl_2$, 1.18Mm KH_2PO_4, 24.9mM $NaHCO_3$, and 10mM glucose, and was aerated with O_2/CO_2 (95:5) to pH 7.4. Mg^{+2} concentration was increased from 1.18mM to 3mM to deter aggregation of particulates, simplfying the purification and separation of the homogenate. The homogenate was diluted with 35ml KRBS, and pressure filtered through a prefilter of 3 layers of 20um nylon mesh. Remaining homogenate was again pressure filtered through a 3um 47mm Millipore membrane. The filtered homogenate was divided into 10 tubes and spun at 1000 x g for 15 minutes at 4o C. Pellets were suspended in the appropriate buffer for assays, or fixed in 2.5% gluteraldehyde/KRBS for LM/TEM preparation. Pellets were processed in a routine manner for TEM, and 1um sections through the entire pellet were stained with 1% toluidine blue. Thin sections were cut from the side, middle, and center of each pellet, and viewed on a JEOL 100C.

With a 20um prefiltering mesh, no whole nuclei appeared in 1um sections, and DNA was reduced by 67% from original homogenate to final preparation. (537ug± 14.5 to 175.5ug ±5.0) Remaining DNA is probably mitochondrial or nuclear DNA in solution. Most mitochondria were in bouton-like structures. Free mitochondria were scattered through the preparation. Cytochrome oxidase activity was reduced by 95% from original homogenate to final preparation. (207.6 ±42.7 to 19.7 ±4.4 umol/min/mg protein)

Electron microscopic examination revealed many synaptoneurosome profiles. Sections also contained many free synaptosomes and neurosomes; many with remnants of a synaptic contact-like area. Measured diameters are in close agreement with preparations from guinea pig cortex. (J Neurosci. 1985 5) Synaptoneursome combined diameter: 1.02um ±0.31; bouton: 0.56um ±0.23; neurosome: 0.47 ±0.19; free synaptosomes: 0.66um ±.25. Very few contaiminating elements appear, and are distributed uniformly through the pellet. However, a qualitatively greater concentration of synaptoneurosomes is found near the bottom of the pellet. Therefore, further purification procedures may yet be possible.

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LOCALIZATION: ACETYLCHOLINE, AMINO ACID AND NEUROPEPTIDE RECEPTORS

225.1 IMMUNOHISTOCHEMICAL LOCALIZATION IN RAT BRAIN OF AN ANTIGEN DETECTED WITH A MONOCLONAL ANTIBODY GENERATED AGAINST THE TORPEDO NICOTINIC ACETYLCHOLINE RECEPTOR. J. Holliday*, A.Y. Deutch, R.H. Roth, and E. Hawrot (SPON: C.F. Stevens). Dept. of Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510.

Monoclonal antibodies (mAb) have now been generated against purified Torpedo nicotinic acetylcholine receptor (AChR), and their binding properties have been characterized (1). One such antibody, designated 12.18, was found to cross-react with peripheral ganglia and sciatic nerve in rat (1). These tissues are not known to contain nicotinic AChR of the muscle type; however, they do contain other types of receptors that respond to ACh. An antigen detected by this mAb has now been localized in rat brain using immunoperoxidase techniques.

12.18 immunoreactivity was observed in a number of telencephalic sites, including the olfactory tubercle, the diagonal band complex and the medial septum. A lamina specific pattern of staining occured in several midbrain sites, including the interpeduncular nucleus (especially the lateral subnucleus), the supramammilary nucleus, and the superior colliculus. In the lower brain stem, dense fiber staining was noted in the dorsal parabrachial region. The trigeminal complex, the cochlear nucleus, and the superior olive, as well as the cerebellum were also labeled. The staining pattern obtained with mAb 12.18 was critically dependent upon precise parameters of the immunoperoxidase protocol.

The pattern of mAb 12.18 staining was compared to previously described patterns of ligand binding as revealed by autoradiography. Based on preliminary comparisons with the distribution of binding of various cholinergic ligands, the pattern of immunoreactivity obtained with mAb 12.18 may correspond most closely to the localization of the M2 muscarinic receptor. However, the sensitivity of the different methods employed may be different, and thus some discrepancies may exist.

In order to define the antigen with which mAb 12.18 is reacting, current efforts are being directed towards immunoprecipitating cross-reacting antigen from solubilized ligand-labeled brain regions.

These studies suggest that an antibody generated against the Torpedo nicotinic ACh receptor may recognize a functionally related and pharmacologically distinct brain antigen, such as the M2 muscarinic ACh receptor.

1. Hawrot, E., Holliday, J., Schweitzer, B., and Chun, L.L.Y. (1983). Soc. Neurosci. Abst. 9:577.

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225.2 **ELECTRON MICROSCOPIC LOCALIZATION OF ALPHA BUNGAROTOXIN BINDING SITES IN RAT SUPRACHIASMATIC NUCLEUS.** **M.M.Miller, A. Beaudet and R.B. Billiar.* Departments of Obstetrics and Gynecology, and Neurology and Neurosurgery, McGill University, Montreal, Canada H3A2B4.**

The ultrastructural distribution of labeled alpha bungarotoxin (α-BTX) binding sites was examined by electron microscopic autoradiography in the suprachiasmatic nucleus of the adult female rat. (^{125}I)α-BTX was infused into the third ventricle and 24 hours later the animals were perfused with a mixture of aldehydes and the dorsal-lateral aspect of the SCN, previously shown to contain high levels of α-BTX binding sites (Miller et al, 1982), was processed for autoradiography. In electron microscopic autoradiographs, silver grains overlaid either single (exclusive grains) or multiple (shared grains) neuronal and/or glial profiles. The ultrastructural distribution of silver grains was analysed by line source, direct point count, and 50% probability circle methods. The distribution of grains evaluated by both direct point count and 50% probability circle analyses was compared with the distribution of randomly generated hypothetical grains. The distribution of real grains was significantly different from that of hypothetical grains. Non-specific binding was assessed in rats infused with (^{125}I)α-BTX of a 1000 fold lower specific activity (by addition of radioinert α-BTX). In these later controls, the labeling was not significantly above background levels. In tissues infused with (^{125}I)α-BTX alone line source analysis demonstrated two populations of sources: a population associated with membranes and a second population inside neuronal structures. Probability circle analysis of shared grains indicated that membrane bound radioactive sources were mainly associated with axo-dendritic, axo-somatic, and dendro-dendritic appositions. Axo-dendritic appositions showed the highest frequency of labeling. Only a small proportion of labeled neuronal interfaces, however, exhibited synaptic differentiations in the plane of section. Probability circle analysis of exclusive grains demonstrated that sources that were not linked to neuronal plasma membranes were likely to be associated with nerve cell bodies and dendrites. In conclusion, the present study suggests: (1) that the majority of specifically labeled α-BTX binding sites in the SCN is membrane bound; (2) that, as in sympathetic ganglia, only a small proportion is actually associated with synaptic junctions; and (3) that a significant proportion of the labeling is located inside the soma and dendrites of suprachiasmatic nucleus neurons, suggesting an internalization within these two compartments. Supported by NIH R01- HD 19431 and MRC.

225.3 DISTRIBUTION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE BRAIN OF A NEWBORN RHESUS MONKEY. J.B. O'Neill*, D.P. Friedman, J. Bachevalier and L.G. Ungerleider. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892 and Neurosciences Research Branch, NIDA, Rockville, MD 20857.

The distribution of muscarinic cholinergic binding sites in the brain of a newborn rhesus monkey was studied by in vitro autoradiographic localization of [^{3}H] quinuclidinyl benzilate (QNB). Cryostat-sectioned (25 μm), unfixed, thaw-mounted tissue from the brain of a one-day-old monkey was incubated for 2 hrs at room temperature in 0.01 M phosphate-buffered saline (PBS), pH 7.4 with 0.6 nM [^{3}H] QNB, rinsed 3 times for 5 min each in PBS, and dried in a stream of cool air. Nonspecific binding was determined in the presence of 1 μM atropine sulfate. The sections were then apposed to tritium sensitive LKB film and exposed for 2 weeks at 20°C before being developed. The autoradiographs at selected levels through the newborn brain were compared to those from two adult monkey brains that had been processed in the same way.

As previously described (O'Neill et al., Soc. Neurosci. Abstr., 11:1053, 1985), QNB binding in the neo- and allocortex of the adult brains was characterized by a broad range of density variations, both across cortical fields and across laminae of a given field. These variations were very distinct, indicating the specific localization of muscarinic cholinergic receptors. QNB binding in the newborn brain, by contrast, showed a much narrower range of density variations, without sharp borders either between cortical fields or between laminae within a field. No cortical field in the infant displayed an adult-like pattern of binding, although in many fields, such as the primary visual cortex, the adult pattern appeared to be emerging. Similarly, in the thalamus, the distribution of QNB binding in the newborn brain resembled that in the adult brains, but again density differences between nuclei in the infant were not as distinct as in the adults. Thus, specific localization of muscarinic cholinergic receptors in the cortex and thalamus is not complete at birth but has begun. In contrast to the delayed maturation of muscarinic cholinergic receptor localization in the cortex and thalamus, the pattern of QNB binding in both the hippocampus and striatum of the newborn brain appeared to be identical to that of the adult brains.

The findings suggest that the distribution of muscarinic cholinergic receptors in the macaque brain is adult-like at birth in limbic structures and striatum but is not fully developed in cortical areas. Thus, like many other aspects of neocortical maturation, the distribution of muscarinic cholinergic receptors continues to develop postnatally.

225.4 MUSCARINE RECEPTOR DISTRIBUTIONS WITHIN ARCHITECTONIC SUBREGIONS OF THE PRIMATE CORTEX. D.C. Mash and M-M Mesulam. Harvard Medical School, Boston, MA.

The regional variations in M1 and M2 muscarine receptors in the macaque brain were investigated by in vitro receptor autoradiography. Unfixed, slide-mounted sections were labelled with a saturating concentration of 3H-quinuclidinyl benzilate in the presence of sufficient cold pirenzepine or carbachol to occlude M1 or M2 sites, respectively. In an adjacent series of sections, the M1 subtype was labelled directly with 3H-pirenzepine. Dried, labelled sections were apposed to tritium sensitive LKB Ultrofilm for 4-8 weeks. Alternate sections were stained with thionine for correlation of the receptor patterns with cytoarchitectonics. The results show that M1 and M2 muscarine receptors displayed marked regional heterogeneity which respected architectonic boundaries.

M2 receptor subtype. In the cerebral cortex, regions with the heaviest M2 receptor densities included the visual (area 17, V1), auditory (A1), and somatosensory (3b, S1) primary koniocortical sensory areas, the hippocampal formation, and the parahippocampal region. The premotor and motor regions (areas 6 and 4) had a pattern of labelling of intermediate intensity. In the parahippocampal region, M2 receptors were heaviest in the ventral entorhinal (area 28) and prorhinal cortices, and the parasubiculum. M2 receptors in the hippocampal formation were densest in the CA3 subfield of Ammon's horn and the subiculum.

M1 receptor subtype. M1 receptors were widely distributed throughout most cortical areas. In contrast to the hippocampal distribution of M2 receptors, M1 receptor labelling was most intense over the molecular layer of the dentate gyrus and was markedly reduced in the subiculum. While M2 receptors were heaviest over the deeper cortical layers, M1 receptor labelling was generally more intense in superficial layers. The motor-premotor regions contained relatively low levels of M1 receptors.

These observations demonstrate that putative muscarine receptor subtypes exhibit a heterogeneous distribution in the primate cortex. Therefore, M1 and M2 selective agonists can potentially have effects that are targeted to architectonic subregions of the cerebral cortex.

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225.5 BOVINE BRAIN COATED VESICLES DISPLAY HETEROGENEITY FOR MUSCARINIC ACETYLCHOLINE RECEPTORS. Walter Silva* and Saul Puszkin* (SPON: J. Goldfarb). Departments of Pharmacology and Pathology, Mount Sinai School of Medicine, C.U.N.Y., New York, NY 10029.

Coated vesicles gained recent attention because they participate in the process of receptor-mediated endocytosis. The biological receptors for many ligands have been reported in coated pits and coated vesicles such as insulin, epidermal growth factor, immunoglobulin, mannose-6-phosphate, transferin, asialoglycoproteins, biogenic amines, etc. The experimental evidence so far presented is biochemical and morphological in nature. For other receptor systems like the muscarinic acetylcholine receptor (mAchr) and the β-adrenergic receptor, pharmacological data suggest internalization and recycling since no direct evidence demonstrated the existence of either receptor molecule in coated vesicles.

In the present studies we examined the binding of ^{3}H(-)quinuclidynylbenzilate as the labeling ligand and used atropine as the masking ligand to define specific binding on coated vesicles obtained from bovine brain that were of high purity after being eluted by two passages through Sephacryl S-1000 column chromatography. The coated vesicles' muscarinic receptor displayed a Kd of 25 pM and a Bmax of about 190 fmol/mg protein. To certify that binding activity was ascribed to coated vesicles, immunotitration determinations were performed with polyclonal antibodies to coated vesicle coat proteins bound to heat-inactivated, formaldehyde-fixed Staphylococcus aureus cells and evaluated them morphologically and biochemically. Ultrastructurally, only coated vesicles were observed attached to the cells in the immunoprecipitate which displayed over 80% of the original mAchr binding activity. In addition, increased values of mAchr binding (150-160%) were obtained after removal of the coat proteins from the external surface of coated vesicles.

Competition experiments using atropine, N-methylscopolamine, oxotremorine and carbamylcholine confirmed the muscarinic nature of the binding site. The competition ranking was typical of mAchr with atropine >N-MS >oxotremorine >carbachol. Competition curves for all compounds except atropine displayed Hill slopes significantly different from unity. The presence of 500 μM GTP did not affect the competition curve for oxotremorine. Analysis of data using a two-site model revealed 13% of high affinity sites for oxotremorine and diminished to 9% with GTP. Two-site analysis showed 66% high affinity sites for carbachol. The antagonist N-MS displayed 62% high affinity sites. The data suggest that the coated vesicle population is microheterogeneous and that a portion of coated vesicles may contain an internalized form of the receptor.

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225.6 STRIOSOMAL ORGANIZATION OF HUMAN STRIATUM: RELATIONSHIP BETWEEN PRE- AND POST-SYNAPTIC ELEMENTS OF THE DOPAMINERGIC AND CHOLINERGIC SYSTEMS. J.N. Joyce[1*], P.R. Lowenstein[2], J.T. Coyle[2] and J.F. Marshall[1] (SPON: J. Fernandez Pardal). [1]Department of Psychobiology, Univ. California, Irvine, CA, 92717 and [2]Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

The dopamine (DA) D_2 receptors in the striatum of human post mortem material show a patchy distribution when examined with quantitative autoradiography of [^{3}H]spiroperidol binding (Marshall, Joyce and Sapp, Neurosci. Abstr. 11:207,1985). This patchy pattern appears to bear an important relationship to the striosomal organ- ization of the striatum, since the patches form labyrinthes in the rostral-caudal axis that, in general, align with the acetylcholinesterase (AChE) stained striosomes. We have examined the relationship between dopaminergic and cholinergic systems in more detail by comparing the autoradiographic distribution of both DA receptor subtypes (D_1 and D_2), the DA uptake sites, the high-affinity choline uptake sites, AChE, and the cholinergic M_1 and M_2 receptors in the striatum of human post mortem material.

AChE histochemical staining for definition of striosomes showed AChE-rich and -poor zones aligned with, respectively, D_2 high-density and low-density regions in adjacent serial sections. The D_1 binding ([^{3}H]SCH 23390, Dawson et al, Eur. J. Pharmacol 108:323, 1985) was homogeneous. In general, the AChE/D_2 -rich and -poor zones were also aligned with, respectively, zones rich and poor in [^{3}H]HC-3 binding (cholinergic uptake site, Coyle et al, this meeting) and [^{3}H]mazindol binding (DA uptake site, Javitch et al, J.Neurosci. 5:1513, 1985). The patterning of the M_1 sites, defined using [^{3}H]pirenzepine or 1 nM N-[^{3}H]methylscopolamine plus 100 uM carbachol (Cortés et al, Brain Res 362: 239, 1986), is being examined further.

These findings suggest that there is an important relationship between the organization of striosomes, as defined with cholinergic markers, and markers of both presynaptic and postsynaptic dopaminergic system. Additionally, the distribution of DA D_2 sites is more closely aligned with the density of DA uptake sites than is the patterning of D_1 receptor sites.

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225.7 VISUALIZATION OF CHOLINERGIC PROCESSES IN THE RAT AND MONKEY FOREBRAIN: [^{3}H]HEMICHOLINIUM-3 ([^{3}H]HCH-3) AUTORADIOGRAPHY IN RELATION TO ACHE HISTOCHEMISTRY AND CHAT IMMUNOCYTOCHEMISTRY. J.T. Coyle, P.R. Lowenstein, C. Hohmann, C. Kitt, D. Price and E. DeSouza. Depts. of Neuroscience and Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, and NIDA.

[^{3}H]HCh-3, a potent inhibitor of high affinity choline uptake, labels the high affinity choline carrier on cholinergic neurons. We compared the anatomical distribution of [^{3}H]HCh-3 binding sites with AChE histochemistry and ChAT immunocytochemistry. Standard techniques were used for the autoradiographic visualization of the distribution of [^{3}H]HCh-3 binding sites; a modification of the procedure of Karnovsky and Roots was employed for the AChE histochemistry; and a mouse monoclonal antibody against ChAT was used for the immunocytochemistry.

In the adult rat, [^{3}H]HCh-3 binding to the caudate-putamen presents a lateral to medial gradient, consistent with the AChE histochemical staining but not with the uniform distribution of ChAT positive cell bodies. In the rat thalamus, areas of dense binding included the antero ventral, antero dorsal, reticular, lateral geniculate and habenular nuclei, while the ventrobasal complex was almost devoid of label. This pattern correlated with the intensity of AChE histochemical staining. In the hippocampus, the dentate gyrus had higher levels of [^{3}H]HCh-3 binding than the CA_1-CA_3 regions; and the subiculum had higher levels than the enthorhinal cortex. In the amygdala, different subnuclei were clearly delineated, with higher levels of [^{3}H]HCh-3 binding in the basolateral, central nuclei and the amygdalo-hippocampal area. Cholinergic terminals visualized by CAT immunocytochemistry and AChE histochemistry are concentrated in the basolateral nucleus. The cerebral cortex had a uniform level of binding across different cortical areas, with a slight external to internal gradient and somewhat lower binding to layer IV. Both [^{3}H]HCh-3 autoradiography and AChE histochemistry correlate very well in different regions of the rat brain, including the lateral habenula and the fimbria fornix, where only cholinergic fibers, but no cell bodies, have been described. The same general pattern of distribution of [^{3}H]HCh-3 binding is apparent in the cynomolgus forebrain. In the caudate and putamen, however, [^{3}H]HCh-3 binding is best described as consisting of patches of higher density in a lateral (high) to medial (low) gradient of the surrounding areas. In the lateral geniculate, the magnocellular layers show higher levels of [^{3}H]HCh-3 binding than the parvocellular ones, a pattern also consistent with the AChE histochemical staining.

Our results demonstrate that the [^{3}H]HCh-3 autoradiography is a useful presynaptic marker for visualizing the distribution of cholinergic cell processes in the brain.

225.8 SUBSTANTIA NIGRA DOPAMINERGIC NEURONS MAY RECEIVE A CHOLINERGIC INNERVATION FROM THE PERIBRACHIAL AREA. P.B.S.Clarke, D.W. Hommer, A.Pert and L.R.Skirboll. Biological Psychiatry and Clinical Neuroscience Branches, NIMH, Bethesda, MD 20205.

Dopaminergic (DA) neurons of the substantia nigra pars compacta (SNC) possess high affinity tritiated nicotine binding sites and are excited by nicotine (Clarke and Pert 1985; Clarke et al 1985). The most likely source of cholinergic innervation is the peribrachial region situated in and around the tegmental pedunculopontine nucleus, but such a projection is controversial (Sugimoto and Hattori 1984).

Anatomy Male rats (200g) received unilateral injections of the fluorescent retrograde label Fast Blue centred in the SNC, and were sacrificed several days later. Tegmental brain sections were processed for choline acetyltransferase (ChAT) immunohistochemistry (for method see Skirboll et al 1984) or for histochemical visualisation of NADPH-diaphorase activity (Vincent et al 1986); the latter is a convenient marker for cholinergic neurons in the caudal column. Fast Blue labelled somata formed a diffuse population almost exclusively ipsilateral to the dye injection, and a substantial proportion possessed ChAT immunoreactivity or intense NADPH-diaphorase activity.

Electrophysiology One identified SNC DA neuron was recorded in each chloral hydrate anaesthetised rat (see Clarke et al 1985), and kainic acid (KA) was infused after a baseline period into the peribrachial area in order to produce a local excitation of cell bodies. KA (5 pmol - 1.6 nmol in 0.5 ul), microinjected into the ipsilateral peribrachial area, produced an immediate and dose-related excitation of DA neurons situated several millimetres distant in the SNC. In a second experiment, rats were pretreated intravenously with physiological saline or with the nicotinic antagonist mecamylamine (2 mg/kg) and 5 min later received vehicle or KA (0.5 nmol) into the peribrachial area ipsilateral or contralateral to the recording site. Consistent with our anatomical findings, ipsilateral KA injection again markedly excited SNC DA neurons, whereas contralateral KA injection was ineffective. Intravenous mecamylamine prevented the KA-induced remote excitation of SNC DA neurons.

These results suggest that cholinergic neurons in and around the pedunculopontine nucleus provide an excitatory input to DA neurons of the substantia nigra.

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S.R.Vincent et al (1986) Neurosci 17, 167-182.

225.9 DISTRIBUTION OF GLUTAMATE BINDING IN PRIMATE BRAIN. G.W. Dauth, I. Shimoyama*, Z. Hollingsworth*, J.B. Penney, S. Gilman, J.W. Aldridge and A.B. Young. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109.

Glutamate is thought to be an important neurotransmitter in the cerebral cortex, cerebral corticofugal projections, and the cerebellum. The distribution of glutamate receptors in primate brain is not completely known. We examined the distribution of glutamate receptor binding in selected areas of the normal primate brain.

M. fascicularis monkeys were used. As part of a separate study, the monkeys were injected IV with 14C-2-deoxyglucose prior to death. The deoxyglucose data are not reported here. The animals were killed with an IV overdose of sodium pentobarbital followed with 5cc of saturated KCl. The brain was removed, frozen quickly and stored at -70° C. Sections were cut on a cryostat at 20 µm and thaw mounted on to slides. Sections were processed for glutamate binding as described previously (Greenamyre et al., J. Pharm. Exp. Ther., 223:254, 1985.). The 14C-2-deoxyglucose was effectively removed by preincubation washes in buffered solutions.

Scatchard analyses revealed Bmax values which ranged from 0.3 pmol/mg protein in the fastigial nucleus to 2.5 pmol/mg protein in layers 1 & 2 of the supplementary motor area. Structures were compared at 30 nmol glutamate concentration and portrayed as a percentage of binding in the supplementary motor area (0.1771 pmol/mg protein). When analyzed in this fashion the data could be separated into two groups. In the first group specific binding ranged between 51% - 86% of that in the supplementary motor area: granular layer (51.4%) and molecular layer (64.2%) of cerebellar cortex, putamen (68.8%), caudate (69.7%), and Brodmann's areas 3 (76.1%), 4 (83.8%) and 6 (85.7%) of cerebral cortex. In the second group specific binding was less then 29% of that in the supplementary motor area: fastigial nucleus (4.7%), interposed nuclei (5.9%), red nucleus (6.0%), dentate nucleus (8.1%), subthalamic nucleus (9.2%), internal globus pallidus (9.4%), ventral anterior thalamus (12.2%), substantia nigra pars compacta (13.2%), superior colliculus (14.5%), ventral lateral thalamus (17.2%), external globus pallidus (25.1%) and substantia nigra pars reticulata (28.9%).

The data show a marked heterogeneity in the regional distribution of glutamate receptors in the primate brain. In the cerebral cortex, caudate, putamen and cerebellum the density of receptor distribution parallels that of suspected glutamatergic afferent input. In other regions receptor densities were lower than expected: red nucleus, ventral lateral and ventral anterior thalamus. The reason for this mismatch might be the result of quenching due to high lipid content or to a different than expected distribution of glutamate receptor subtypes. Supported in part by NIH grant No. NS-19613.

225.10 'TRANSMITTER SPECIFIC' INPUTS TO THE MEDIODORSAL THALAMIC NUCLEUS (MD) IN THE RAT. J.P. Ray*, F.T. Russchen*, T.A. Fuller & J.L. Price. Depts. of Anatomy & Neurobiology and Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

^{3}H-D-aspartate (^{3}H-D-Asp) and ^{3}H-gamma-aminobutyric acid (^{3}H-GABA) have been used as 'transmitter specific' retrograde tracers to identify projections to MD that may use glutamate and/or aspartate, or GABA as their neurotransmitter. Following injections of the 'non-specific' tracer WGA-HRP into MD, retrogradely labeled cells are found in a wide variety of forebrain and brainstem areas, including layer VI of the frontal and agranular insular cortex, the thalamic reticular nucleus, the ventral pallidum (including the polymorphic zone of the olfactory tubercle), the deepest layers of the piriform cortex, periamygdaloid cortex and entorhinal cortex, the amygdaloid complex, the superior colliculus, substantia nigra and dorsal raphe nucleus. Following injections of ^{3}H-D-Asp or ^{3}H-GABA, different subsets of these cells are labeled.

Injections of ^{3}H-D-Asp into MD result in substantial numbers of labeled cells in layer VI of the frontal and agranular insular cortex. With injections in the medial part of MD cells are also labeled in the piriform and entorhinal cortices and in the amygdaloid complex, while injections in the more lateral part of MD produce labeled cells in the superior colliculus. Labeled cells are also found in the nucleus triangularis of the septum, possibly due to involvement of the medial habenular nucleus in the injections (e.g., Swanson and Cowan, J. Comp. Neurol. 186:621-656, 1979).

In contrast, the only projection to MD that was definitely labeled by injections of ^{3}H-GABA arises from the reticular thalamic nucleus. Injections that involve the habenular nuclei also produce some labeled cells in the nucleus triangularis of the septum, and in the endopeduncular nucleus and adjacent parts of the hypothalamus. Although a few, scattered cells are also labeled in the ventral pallidum in some cases, such cells are much more numerous following injections in which the habenular nuclei are heavily involved, and are not found after injections restricted to MD. Therefore, these cells appear to be related to the endopeduncular nucleus and to project to the habenular nuclei instead of MD.

A few cells in the posterior hypothalamus are also labeled following injections of either D-Asp and ^{3}H-GABA.

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225.11 HIGH AFFINITY NEUROTENSIN BINDING TO AFFERENT AND EFFERENT COMPONENTS OF THE VAGAL COMPLEX. J.P. Kessler*, P. Kitabgi* and A. Beaudet. (SPON: G. Karpati). Neuroanatomy Laboratory, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec, H3A 2B4, Canada and Centre de Biochimie du CNRS, Parc Valrose, 06034 Nice, France.

The cellular localization of specific neurotensin (NT) binding sites was investigated by radioautography in the rat nucleus tractus solitarius (NTS) and dorsal motor nucleus (DMN) of the vagus. Cryostat sections (5-20 µm-thick) from the medulla were incubated 1 h at 4°C with 0.1 nM ^{125}I-NT (2000 Ci/mmol) in the presence (non specific binding) or the absence (total binding) of 5×10^{-7} M non radioactive NT. After several buffer washes, the sections were either air-dried and exposed to LKB Ultrofilm or fixed with 4% glutaraldehyde and processed for radioautography using conventional dipping techniques. High labeling densities were measured by computer assisted densitometry throughout the rostro-caudal extent of the DMN. Moderate to high labeling densities were also present within the NTS. In the latter, the labeling was heterogeneous and heaviest rostral to the opening of the fourth ventricle, within the medial subdivision of the nucleus. To investigate the relationship of NT binding sites with afferent (terminals localized within the NTS) and efferent (preganglionic cell bodies of the DMN) components of the vagus, sectioning of the vagus nerve was performed immediately above the nodose ganglion (which contains the cell bodies of vagal afferents). Unilateral vagotomy resulted in a marked decrease (↓40%) in labeling density within the ipsilateral DMN without any significant modification in the contralateral DMN. DMN perikarya showed increased cytoplasmic basophilia, suggesting that the decrease in NT binding was due to retrograde changes within efferent DMN neurons and therefore that NT binding sites might be localized on the soma and/or dendrites of these cells. Unilateral vagotomy also resulted in a significant decrease (↓20%) in NT binding within the ipsilateral NTS. This decrease was uniform along the rostro-caudal extent of the nucleus. No significant modification of NT binding was found in the contralateral NTS when compared to sham lesioned animals. These results indicate that a small proportion of the labeled binding sites may be localized on vagal afferent terminals. Indeed, in sections of the nodose ganglion incubated with ^{125}I-NT, up to 15% of ganglion cells showed intra-perikaryal accumulations of specific NT binding sites suggesting that some vagal afferents are able to synthesize NT binding sites. In conclusion, the present results suggest that neurotensin receptor sites are present on both afferent and efferent components of the vagal complex. Supported by the MRC and a France-Quebec exchange program.

225.12 REGIONAL AND LAMINAR DISTRIBUTION OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN NINE REGIONS OF HUMAN NEOCORTEX. R.G. Struble, R.E. Powers*, L.C. Walker, P.J. Bruha*, R.L. Shockett* and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2182.

Quantitative neurochemical studies of somatostatin in human brain have disclosed regional variations in the amount of this neuropeptide in neocortex. Concentrations are highest in temporal and cingulate cortices; occipital cortex appears to have lower levels. To assess the distributions of somatostatin-like immunoreactivity in fibers and perikarya in neocortex, we examined, at autopsy, these parameters in the cortices of three humans (46-64 years of age) free of neurological disease. Fresh samples from multiple neocortical gyri were immersion fixed in 5% acrolein, cryoprotected, cut at 40 µm, and stained by the indirect antibody technique with polyclonal antibodies to somatostatin 14. Adjacent Nissl-stained sections were used to determine cortical laminae.

Among neocortical regions, there were marked differences in densities of fibers and perikarya. Cingulate cortex contained more than five times as many labeled perikarya as did occipital cortex. Cingulate and temporal cortices showed dense fiber plexes, while occipital cortex displayed few fibers. The distribution of fibers tended to vary among different laminae, with deeper neocortical laminae usually showing the highest concentration of fibers. The number of immunoreactive fibers in a particular neocortical region appeared to parallel the number of stained perikarya present in deeper laminae in that part of cortex. However, the number of stained perikarya in the subjacent white matter did not always correspond to the density of immunoreactive fibers in grey matter. For example, in motor cortex, most somatostatin-like immunoreactive perikarya were found in the grey matter, while, in occipital cortex, the majority of stained perikarya were located in white matter.

While generally agreeing with neurochemical studies of the distribution of somatostatin, the observation that immunoreactive perikarya occur in white matter emphasizes that care must be taken in dissections of human cortex for assays of somatostatin and other neurotransmitters.

225.13 VAGAL AFFERENTS IN THE SOLITARY TRACT NUCLEUS CONTAIN ANGIOTENSIN II RECEPTOR BINDING SITES. S.L. Stone*, T.T. Dinh*, S. Ritter and R.C. Speth (SPON: R. Abhold). Dept. of VCAPP, Washington State Univ., Pullman, Wash. 99164-6520.

Receptor binding sites for angiotensin II (AII) are present in the nucleus of the solitary tract (NTS) in the brain of the rat. These binding sites may be present on dendrites and nerve cell bodies, axon terminals from other brain nuclei or axon terminals of afferent cranial nerves which innervate this nucleus. To determine whether afferent terminals of the vagus nerve in the NTS of the rat contain AII receptors, the left nodose ganglion was surgically removed. After a 6 week survival time, the rats were anesthetized with sodium pentobarbital, perfused intracardially with ice-cold 0.9% saline and the brainstems were removed, frozen, sectioned on a cryostat at a thickness of 20 microns in the coronal plane, and thaw mounted onto microscope slides. The sections were incubated with 0.25 nanomolar 125-I-Sarcosine-1, Isoleucine-8-angiotensin II in the presence and absence of 1 micromolar AII to radiolabel AII receptor binding sites. The sections were apposed to LKB ultrofilm for 4-7 days to autoradiographically visualize the distribution of AII receptor binding sites which were defined as film areas in which the exposure exceeded that seen in film areas from the corresponding area of an adjacent section incubated with one micromolar AII. A group of unoperated control rats were also evaluated for brainstem AII receptor binding in parallel with the unilaterally nodose lesioned rats. Autoradiograms of sections from the unilaterally nodosectomized rats revealed a dramatic reduction in AII receptor binding in the NTS ipsilateral to the lesion. The density of AII receptor binding sites in the ipsilateral NTS was approximately 50% of that seen in the contralateral NTS. The reduction in AII receptor binding sites occurred primarily in the medial third of the NTS, just rostral to the obex. AII receptor binding in the contralateral NTS was not significantly different from that seen in the NTS of intact rats indicating that the fibers containing AII receptors predominantly innervate the ipsilateral NTS. These results indicate that AII receptors in the NTS are presynaptically localized on vagal afferents and suggest that AII may function as a modulator of vagal input to the brain.

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225.14 CAPSAICIN ATTENUATES ANGIOTENSIN BINDING IN THE BRAINSTEM AND SPINAL CORD OF ADULT RATS.
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Capsaicin is a potent neurotoxin which damages fine diameter afferent neurons. Recently we demonstrated that systemic capsaicin injection in adult rats produces extensive degeneration of nerve terminals and incoming fibers in areas associated with known projections of small diameter afferent neurons, as described previously in the neonatal rat. Receptor autoradiographic techniques have demonstrated angiotensin II (AII) binding in regions innervated by capsaicin-sensitive afferents. These areas include the dorsal horn of the spinal cord, the spinal trigeminal nucleus, and the nucleus of the solitary tract (NST). The purpose of this experiment was to determine whether any of these AII binding sites are present on capsaicin-sensitive afferent neurons.

Adult male Sprague-Dawley rats weighing 325-350 g were injected intraperitoneally with a series of 3 capsaicin doses (25, 50 and 50 mg/kg, respectively) or the capsaicin vehicle (10% alcohol/10% Tween 80). Injections were given during a 36 hr period. Subsequent behavioral tests demonstrated functional deficits referrable to trigeminal and vagal afferent damage, respectively: capsaicin-injected rats failed to exhibit the eyewipe response to corneal irritation (1% NH^4OH applied to the corneal surface) and did not suppress their food intake in response to cholecystokinin (2 and 4 µg/kg, i.p.). Six wks after capsaicin injection, rats were sacrificed and perfused transcardially with ice-cold 0.9% saline. Brains and spinal cords were removed and frozen. Cryostat sections (20 µm thick) were cut and thaw-mounted onto microscope slides. Sections were then incubated with 0.25 nanomolar ^{125}I-sarcosine-1, isoleucine-8-angiotensin II in the presence and absence of 1 micromolar AII to radiolabel AII receptor binding sites. The sections were apposed to LKB Ultrofilm for 4-7 days to autoradiographically visualize the distribution of AII receptor binding sites which were defined as film areas in which the exposure exceeded that seen in film areas from the corresponding region of an adjacent section incubated with one micromolar AII. Autoradiograms of capsaicin-treated rats revealed significant attenuation of AII receptor binding sites in the NST and spinal cord. A small reduction was also apparent in the spinal trigeminal nucleus. These results suggest that a substantial proportion of the AII receptor binding sites in these regions are located presynaptically on small diameter afferent neurons.

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225.15 DEVELOPMENTAL CHANGE ON DISTRIBUTION OF SUBSTANCE P AND ITS RECEPTORS IN RAT EMBRYO AND UTERUS. M.L. Swenberg*, R. Liu[1], and L. Chang*[1]. NIH Hypertension and Endocrinology Branch, [1]USUHS Grant No. RO7058, Dept. of Anatomy, Bethesda, MD 20892

The distribution of substance P (SP) and its receptor binding sites were observed in the developing whole embryo and uterus at various days of gestation. Immunohistochemical methods were used for detection of SP distribution while the autoradiographic technique was used for localization of the SP receptors.

Immunoreactive substance P (ir-SP) was detected in both central and peripheral nervous systems in developing embryoes as early as day 5 of gestation. Receptor binding sites of SP are first detected by day 17 in the following areas: midline structures of the brainstem and spinal cord, muscles, tongue, salivary glands, lungs, intestines, blood vessels, and the urinary bladder.

Ir-SP and receptor binding sites are comparable in distribution and density in the endometrium of both control and pregnant uteri. In the pregnant uterus, the receptors are condensed in the luminal side of the endometrium as a ring-like structure. During the course of pregnancy, the number of binding sites per gram weight of tissue decreased in proportion to the growth of the uterus.

The following conclusions are drawn based on these studies: The presence of ir-SP in tissues in addition to the nervous system throughout the course of embryonic development suggests that SP may function as more than as a neurotransmitter. The fact that the SP binding site first appeared at day 17 of gestation suggests that the sensory system may be fully differentiated at that time. The decreased density of binding sites in the pregnant uterus may contribute to the lessening of pain perception as the pregnancy progresses and the uterine cavity is distended.

225.16 AUTORADIOGRAPHIC LOCALIZATION OF CCK RECEPTORS USING ^{125}I-BOLTON-HUNTER CCK_8. D.L. Niehoff and J.K. Zwemer-Collins*. Dept. Neuroscience, Abbott Laboratories, Abbott Park, IL 60064

The presence of the gut peptide cholecystokinin (CCK) has been demonstrated in the CNS of several mammalian species, where it exists principally as CCK_8. Autoradiographic techniques have utilized ^{125}I-CCK_{33} (Zarbin et al. J. Neurosci. 3:877, 1983) or ^{3}H-CCK_8 (Van Dijk et al. J. Neurosci 4:1021, 1984) to localize receptors for CCK in brain. In order to examine CCK receptor distribution using the octapeptide fragment most prevalent in brain, while retaining the advantages of an iodinated ligand for autoradiography, we have utilized ^{125}I-Bolton-Hunter-CCK_8 (^{125}I-BH-CCK_8; Lin and Miller J. Pharmacol. Exp. Ther. 232:775, 1985) to localize CCK receptors in rat and guinea pig brain by autoradiography.

Sections of rat or guinea pig brain (8-10 microns) were labeled with 80 pM ^{125}I-BH-CCK_8 using a modification of the procedure employed by Moran, et al. (Brain Res 362:175, 1986). Blanks were generated by the simultaneous addition of 1uM CCK_8 to alternate sections. Labeled sections were exposed to tritium-sensitive film to produce autoradiograms.

In preliminary biochemical studies in guinea pig forebrain sections, ^{125}I-BH-CCK_8 reached equilibrium by 2.5 hr. and dissociated slowly (K_{-1}=0.0040 min^{-1}). The binding was saturable, of high affinity, and the rank order of potency for CCK-derived peptides was consistent with results obtained in membrane studies, biochemical studies in sections, or by autoradiography. BSA reduced specific binding by 65%; and was therefore excluded during incubation with the ligand without detrimental effect. HPLC analysis revealed that no significant degradation of ^{125}I-BH-CCK_8 occurred. Autoradiographic studies demonstrated a high density of binding sites for ^{125}I-BH-CCK_8 in cerebral cortex, olfactory areas (e.g. olfactory bulb and prepiriform cortex), limbic system, and terminal areas of mid-brain dopamine neurons (e.g. nucleus accumbens) in both species. We also observed moderate to high levels of binding in several regions which were not appreciably labeled using ^{125}I-CCK_{33} or ^{3}H-CCK_8, such as substantia nigra and lateral septum.

The observed distribution of ^{125}I-BH-CCK_8 binding supports putative roles for CCK in olfactory related behaviors, modulation of dopaminergic activity and cortical function. Differences in the localization pattern with ^{125}I-BH-CCK_8 and that found in previous studies could reflect overlapping subpopulations of receptors recognized by these ligands.

225.17 AUTORADIOGRAPHIC LOCALIZATION OF [MONO(^{125}I)IODO-Tyr^{10}, $MetO^{17}$]-VASOACTIVE INTESTINAL PEPTIDE (M-^{125}I-VIP) BINDING SITES IN THE RAT BRAIN. J.L. Martin*[1], P. Hof*[1], M. Dietl*[2], J.M. Palacios[2] and P.J. Magistretti[1] (SPON : M. Schorderet). [1]Dept. of Pharmacology, Centre Médical Universitaire, Geneva 1211 and [2]Preclinical Research, Sandoz Ltd., Basel 4002, Switzerland.

M-^{125}I-VIP prepared and purified by HPLC as recently described (Martin *et al., J. Biol. Chem.* 261, 5320, 1986), has been used in biochemical and autoradiographic studies of VIP receptors in slide-mounted sections of rat brain. This form of VIP which is monoiodinated on Tyr^{10} and oxydized on Met^{17} has a high specific activity (2050 Ci/mmol) and maintains the biological activity of native VIP. A series of kinetic and pharmacological experiments were first carried out to define conditions for incubation and to characterize the properties of VIP receptors. Slide-mounted sections were incubated for 90 min (except for kinetic experiments) at 22° in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$, 2 mM EGTA, 0.2 % BSA, 0.1 mM bacitracin and 60 pM M-^{125}I-VIP. Non-specific binding was determined in the presence of 1 µM unlabeled VIP and corresponded to approximately 50 % of total binding. Total binding was generally between 8000-9000 cpm/20 µm thick sections. After two 4 min washes in ice-cold buffer, sections were either wiped off the slide and counted in a gamma counter (biochemical experiments), or dried, exposed to ^{3}H-Ultrofilm (LKB) for 3 days and autoradiograms developed as previously described (Palacios *et al., Neurosci. Lett.* 25, 101, 1981). Association of M-^{125}I-VIP specific binding was time-dependent with maximal binding reached within 90 to 120 min. Half-time for dissociation, assessed by adding after 90 min of incubation 1 µM unlabeled VIP, was approximately 4 min. Unlabeled VIP as well as PHI and secretin, two peptides structurally-related to VIP, competed in a concentration-dependent manner for specific binding sites labelled by M-^{125}I-VIP with the following rank-order of potencies : VIP>>PHI>secretin. Autoradiographic studies (on 10 µm thick sections) revealed the highest density of VIP receptors in the following areas : olfactory bulb (except the int. granular layer), ant. olfactory n., dentate gyrus, the amygdaloid complex (particularly the basolateral n.), the n. accumbens, the lat. septal nn., the bed n. of stria terminalis, the med. and lat. habenula and various thalamic nn., including the vent. post. med. n., lat. dors. n., paraventricular n., parafascicularis n., lat. and med. geniculate. An extremely high density was observed in the superior colliculus. VIP receptors were seen throughout the entire neocortex, with a laminar distribution (densest in layer IV). Regionally, the highest density was observed in primary olfactory, ant. cingulate and entorhinal cortices. In the hypothalamus, the suprachiasmatic, paraventricular, interpenduncular and arcuate nn. are rich in VIP receptors. In the brain stem, the central grey, dorsal raphe, parabrachial n., vestibular n., cochlear n. and superior olive contain a high density of VIP receptors. VIP receptors were not visualized in the cerebellum. Thus it appears that VIP receptors are particularly dense in the limbic system and in areas of the brain involved in the processing of sensory inputs. Furthermore, a considerable matching exists between the distribution of VIP receptors and the presence of VIP profiles as revealed by indirect immunohistochemistry (Lorèn *et al., Neurosci.* 4, 1953, 1979).

225.18 AUTORADIOGRAPHIC LOCALIZATIION OF SEROTONERGIC, ADRENERGIC, CHOLINERGIC, HISTAMINERGIC AND SUBSTANCE P RECEPTORS IN THE CAT NUCLEUS SOLITARIUS. B.E. Maley and V.S. Seybold.(SPON:G.E. GOODE), Dept. of Anatomy, Univ. Kentucky Med. Ctr., Lexington, Ky 40536 and Dept. of Cell Biology and Neuroanatomy, Univ. Minnesota Med. Sch Minneapolis, Mn. 55455

The cat nucleus solitarius is located in the dorsal brainstem and is involved in the regulation of the cardiovascular and respiratory systems. Using receptor autoradiography the distribution of $alpha_1$-adrenergic ([^{3}H] prazocin), H_1-histaminergic ([^{3}H] pyrilamine), serotonergic ([^{3}H]lysergic acid diethylamide(LSD)), muscarinic cholinergic ([^{3}H] quinuclidinyl benzilate (QNB)) and substance P (I^{125} substance P) receptors in the cat nucleus solitarius was examined.

Adult cats were anesthetized with sodium pentobarbital (38mg/kg) and perfused transcardially with 0.1% paraformaldehyde. The brain was rapidly removed and frozen. Sections were cut on a cryostat and pre-incubated in the appropiate buffer prior to introduction of the radiolabeled ligand. Each of the ligands was used at or below its calculated K_D for the autoradiograms. Adjacent sections were incubated for total and nonspecific binding in order to determine specific binding. Following incubation in ligands the sections were rinsed, dried, and placed adjacent to emulsion coated coverslips.

Results of the study indicate that there was a differential distribution of ligand binding in the nucleus solitarius. The most striking pattern was seen with substance P. Its distribution was similar to that reported for the immunohistochemical localization the of peptide. Prazocin binding was more uniform in its distribution throughout the nucleus, while LSD binding was located in the medial and lateral portions of the nucleus solitarius. Binding for QNB was most prominent in lateral and parvocellular areas. Pyrilamine localization was less than the others; however some binding was present in the parvocellular region.

Results of this study indicate distinct patterns for the localization of receptor ligands in the nucleus solitarius. The presence of the binding sites indicate that the endogoenous neurotransmitters for these ligands are acting at the level of the nucleus solitarius.

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225.19 REGIONAL BIOCHEMICAL SPECIFICITY IN HUMAN AND ANIMAL QUANTITATIVE AND QUALITATIVE EEG Kenneth A. Bonnet, New York University School of Med.,New York, NY 10016

Several groups of investigators have reported the patterns of drugs affecting specific classes of features in electrophysiological(EEG) records in human, and animal subjects. Many studies have focused upon classification of drug types, or upon selective effects in a small electrode array or evoked potential. Recent studies in our laboratory have addressed the question of the biochemical specificity of individual neurotransmitter systems contributing to several parameters of EEG activities in 28 electrodes simultaneously in human control subjects under conditions of standardized EEG record taking, and with standardized evoked potentials recorded in the auditory and visual modes. Selective challenges that increase the activity of the dopaminergic, serotonergic, GABAergic and cholinergic systems of central nervous system. Computer-based analysis of all records(and topographic mapping)permits the regional discrimination of neurotransmitter-specific effects that are individual and that are discriminable from local cerebrovascular effects.

Selective neurotransmitter challenges to animals has shown good homology with human in many, but not all regions and EEG-specific paramteric effects. Selective stimulation of individual neurotransmitter systems have been systematically studied in drug-free schizophrenic, Gilles de la Tourette, depressed and clinically depressed individuals. Relative changes within individuals in these diagnostic groups that are induced by neurotransmitter challenge (i.e.,by increasing the activity of the individual neurotransmitter system) appear to have some utility in predicting drug response in therapeutic regimen. Pharmacological treatment has been followed in several of these individuals with repeat series. The parmacological specificity of different classes of antipsychotics, nootropics and antidepressives will be presented as studied in relationship to neurotransmitter systems in controls, and in patient studies.

Supported by the Courtney Block Fund for Brain Chemistry Research

MUSCARINIC RECEPTOR CHARACTERIZATION AND PURIFICATION

226.1 PURIFICATION AND MOLECULAR CHARACTERIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS: COMPARISON WITH ADRENERGIC RECEPTORS. A.R. Kerlavage*, S.P. Fracek, J.Y.-W. Lai*, C.M. Fraser*, S.M. Shreeve* and J.C. Venter*. Section of Receptor Biochemistry, Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bethesda, MD 20892.

Two molecular forms of the muscarinic cholinergic receptor have been consistently observed in pig heart as well as in brain and heart tissue from trout, frog, turtle, chicken and rat. In these tissues, two proteins having M_rs of ~94,000 and ~80,000 on SDS-PAGE were specifically labeled with [^{3}H]propylbenzilylcholine mustard ([^{3}H]PrBCM). We have developed a purification procedure which yields each of these proteins in a homogeneous form suitable for structural analysis. The four step procedure involves affinity chromatography on 3-(2'-aminobenzhydryloxy)tropane-Sepharose, concentration on hydroxylapatite, preparative SDS-PAGE and extraction of individual bands from the gel.

We have also developed a methodology for the unambiguous identification of neurotransmitter receptor proteins and comparison of their structural features. The method involves comparative peptide mapping of total proteolytic digests of purified receptor proteins by microbore reverse-phase high-performance liquid chromatography (RP-HPLC) in conjunction with covalent modification by specific receptor ligands or [^{125}I]-labeling. Chemical modification and digestion procedures can be carried out on one picomole of protein and several femtomoles of digested protein can be detected on microbore RP-HPLC. Cyanogen bromide digestion of partially purified [^{3}H]PrBCM-labeled muscarinic receptor from rat brain and subsequent analysis by HPLC revealed a single major [^{3}H]PrBCM-labeled CNBr fragment. The purified 94,000 Da and 80,000 Da [^{3}H]PrBCM-labeled proteins from rat brain were labeled with ^{125}I, digested with trypsin and also analyzed by this method. The two proteins exhibited very similar but clearly distinct tryptic maps. Most peaks which eluted in the hydrophobic region of the maps overlap for the two proteins while the 94,000 Da protein contained several additional peaks of low apparent hydrophobicity, raising the possibility that the 80,000 Da protein is a fragment of the 94,000 Da protein. Purified α_2-adrenergic receptors from human platelets and purified β_2-adrenergic receptors from guinea pig lung were similarly mapped. All of the receptors examined had unique tryptic digest maps although all were similar in the very hydrophobic region of the maps. Thus, this technique can be used to positively identify and characterize receptor proteins using very small amounts of material.

226.2 SIZE AND STRUCTURE OF PURIFIED MUSCARINIC CHOLINERGIC AND BETA ADRENERGIC RECEPTOR PROTEINS DETERMINED BY ELECTRON MICROSCOPY. C.M. Fraser*, A.P. Mariani*, A.R. Kerlavage*, J.Y.-W. Lai*, D. Robinson* and J.C. Venter* (SPON: T. Smith). Section of Receptor Biochemistry, Laboratory of Neurophysiology, NINCDS, National Institutes of Health, Bethesda, MD 20892.

Purified rat brain muscarinic cholinergic receptors and guinea pig lung beta$_2$-adrenergic receptors were negatively stained with uranyl acetate or ammonium molybdate and examined by electron microscopy in order to determine size, shape and quaternary structure. Muscarinic receptors were preparatively purified to apparent homogeneity by sequential affinity chromatography on 3-(2'-aminobenzhydryloxy)tropane-Sepharose, hydroxylapatite chromatography and size exclusion HPLC. When purification was performed using buffers containing digitonin the majority of the muscarinic receptor proteins appeared to be partially embedded in digitonin micelles with one to two receptor molecules per micelle. Approximately 50 percent of the receptor mass protruded from detergent micelles in agreement with known receptor topography (J.C. Venter, J.Biol.Chem. 258:4842, 1983). The majority of muscarinic receptor molecules appeared to be monomeric, cylindrical proteins with dimensions of 4.2 x 14.9 nm although occasional dimers were seen. The monomeric nature of the receptors agrees with target size analysis of membrane bound receptors (J.Biol.Chem. 258:4842, 1983). The size of the receptor determined by electron microscopy is in agreement with hydrodynamic data indicating a Stokes radius of 6.8 nm and an axial ratio of 1.95 (T. Haga, FEBS Letts. 113:68, 1980).

Guinea pig lung beta$_2$-receptors were purified to apparent homogeneity by sequential affinity chromatography on alprenolol-Sepharose, ion exchange chromatography and size exclusion HPLC. Beta-adrenergic receptors were shown to be proteins with dimensions of 3.4 x 13.0 nm and the majority were present in dimeric structures. These data are in agreement with target size data indicating a dimeric form for the membrane bound beta-receptor (C.M. Fraser and J.C. Venter, BBRC 109:21, 1982), and with hydrodynamic data indicating a Stokes radius of 5.8 nm and an axial ratio of 2.0. The slightly smaller size of the beta$_2$-adrenergic receptor relative to the muscarinic receptor is in agreement with the respective SDS-PAGE molecular sizes of 68,000 daltons and 80,000 daltons.

Receptor monomer	Stokes radius	EM radius	SDS-PAGE
Muscarinic	6.8 nm	7.4 nm	80,000 Da
Beta$_2$-Adrenergic	5.8 nm	6.5 nm	68,000 Da
Ratio Adrenergic/Muscarinic	0.85	0.88	0.85

226.3 RECONSTITUTION OF ATRIAL MUSCARINIC RECEPTOR. J.S. Aguilar*. (SPON: W.L. Klein) Instituto de Biologia Celular, Facultad de Medicina (U.B.A.), Bs As. Argentina.

The reconstitution of the cholinergic muscarinic binding sites (muscarinic receptors) from bovine atrial membranes into asolectin vesicles have been carried out by polyethyleneglycol precipitation (1). This procedure causes the formation of vesicles containing the muscarinic receptor (2). The kinetic and pharmacological properties of [^{3}H]-N-methyl-scopolamine ([^{3}H]NMS) binding to this reconstituted muscarinic receptor were studied. This binding is linear with the amount of protein, saturable and reversible. The apparent dissociation (K_d')constant determined by saturation experiments at equilibrium is 0.46 nM. By kinetic experiments an association rate constant (k_{on}) of 0.58 $min^{-1}xnM^{-1}$ and a dissociation rate constant (k_{off}) of 0.17 min^{-1} were obtained, whose ratio k_{off}/k_{on}=0.30 nM is in good agreement with k_d'. The potency of a range of muscarinic ligands in displacing [H]NMS is atropine> methylatropine> scopolamine> pirenzepine> exotremorine> gallamine> pilocarpine> carbamylcholine> bethanechol. The Hill coefficients for the antagonists atropine, methyl-atropine, scopolamine and pirenzepine are near to 1 indicating a single class of non-interacting binding sites for these ligands. In contrast, the Hill coefficients for the agonists and the cardioselective antagonist gallamine are ca 0.5 suggesting a more complex binding mechanism. The binding of the carbamylcholine but not of the [^{3}H]NMS is modulated by guanine nucleotides. These results indicate that asolectin fulfills the lipid requirements of the receptor related with its binding properties. Thus asolectin can be used as a starting lipid mixture to determine a more specific lipid requirement for the muscarinic function.

(1) J.S. Aguilar and E.L.M. Ochoa (1986) Neurochem. Int. 8:47-52
(2) A. Pellegrino de Iraldi, J.S. Aguilar and E.L.M. Ochoa (1986) Neurochem. Res. (in press).

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226.4 **DISCRETE POPULATIONS OF AFFINITY-PURIFIED MUSCARINIC RECEPTOR BINDING ACTIVITY IDENTIFIED USING KINETIC STUDIES.** G.R. Luthin and B.B. Wolfe. Dept. of Pharmacology, Univ. of Pa. Sch. Med., Philadelphia, Pa., 19104.

Muscarinic receptor binding activity was purified >1000-fold from rat forebrain using chromatography over 3-(2'-aminobenzhydryloxy)-tropane Sepharose and BioGel HTP (HTP fraction). The binding activity in the HTP fraction was further enriched following chromatography over DEAE-Sephacel (DEAE fraction). The association kinetics of (^{3}H)-QNB binding were followed at 25°C using the HTP and DEAE fractions. At all ligand concentrations tested (0.1-30 nM) association kinetics were biphasic, and were modelled to an initial fast-binding component (0-40 minutes) and a subsequent slow-binding component (40-120 minutes). The pseudo-first order rate constant of association for the fast-binding component was plotted as a function of QNB concentration. The data thus obtained were non-linear, suggesting that the fast-binding component may involve a 2-step binding mechanism (Model 1). Kinetic constants for the first (k-1/k1) and second (k-2/k2) steps were 3 nM and <0.008, respectively. The association kinetics for the slow-binding component, on the other hand, were best described using a first-order model, as the binding constant for the slow component was independent of ligand and receptor concentration. Dissociation kinetics were extremely slow for both components and could not be resolved into 2 phases. Atropine, pirenzepine and oxotremorine inhibited both fast- and slow-binding components with equal potencies. The hypothesis that the fast-binding component was derived from isomerization of the slow-binding component was tested by incubating HTP fraction with unlabelled QNB (1 μM) for 20 minutes, then removing unbound QNB by de-salting over Sephadex G50 and examining the binding properties using (^{3}H)-QNB. The fast-binding component did not spontaneously appear, but a slow increase in binding activity was observed only in the presence of ligand. From the above studies it appeared that although a single model cannot explain the results, a combination of Models 1 and 2 is consistent with all the data. The experiments suggest that there are 2 forms of receptor. One form can rapidly bind ligand and then isomerize to a highly stable binding state (Model 1). The other form can slowly isomerize into a state capable of binding ligand (Model 2). Most importantly more than half of the detectable binding activity is in the slow-binding form, and this complicates accurate assessment of total binding activity.

Model 1: $L + R \leftrightarrows LR \leftrightarrows LR'$

Model 2: $R \leftrightarrows R' + L \leftrightarrows LR'$

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226.5 EFFECTS OF TEMPERATURE ON SUBPOPULATIONS OF MUSCARINIC RECEPTORS DEFINED BY QUATERNARY ANTAGONISTS. John Ellis and Robert H. Lenox Neuroscience Research Unit, Dept. of Psychiatry, Univ. of Vermont College of Medicine, Burlington, VT 05405.

It has been reported by several laboratories that quaternary muscarinic ligands label fewer sites with high affinity than do their tertiary analogs. Thus, [3H]N-methylscopolamine (NMS) does not label as many sites as [3H]QNB does in rat brain, while unlabeled NMS, N-methylatropine, and gallamine compete for the binding of [3H]QNB in a biphasic manner. There is some evidence to suggest that the sites that have lower affinities for quaternary ligands represent desensitized receptors. We have begun to investigate the subcellular distribution of these sites and have found that freezing or heating various membrane preparations can alter the proportions of the sites.

Neural membranes are prepared by homogenizing rat forebrains in 30 vols of 40 mM NaK phosphate buffer, 1 mM EDTA, pH 7.4. The homogenate is centrifuged at 3000xg for 10 min ("pellet 1", 40 mg protein per g wet wt) and the supernatant from that centrifugation is sedimented at 50,000xg for 20 min ("pellet 2", 10 mg/g). Both pellets are resuspended in 40 mM NaK phosphate buffer without EDTA. The overall receptor density, measured as the binding of [3H]QNB, does not differ dramatically between the two fractions. When freshly prepared, pellet 1 has very few receptors with low affinity for quaternary ligands (ca 5%), while 20-30% of the sites in pellet 2 have low affinity for quaternary ligands. Freezing pellet 2 does not appreciably alter the binding properties with regard to quaternary ligands. On the other hand, freezing and thawing pellet 1 produces an increase in the proportion of sites with low affinity toward quaternary ligands (to ca 20%). That is, after freezing, pellet 1 more closely resembles pellet 2. We have not yet determined whether the appearance of these low-affinity sites is due to conversion of high-affinity sites or to unmasking of sites that were previously undetectable.

We have previously shown that the subpopulations of muscarinic receptors defined by gallamine and other quaternary ligands are stable and do not interconvert under our normal assay conditions over periods of hours. However, when pellet 2 membranes are preincubated to 50°C for 2 min, the sites that have low affinity for quaternary ligands disappear, while the binding of [3H]QNB decreases only slightly. Under the same conditions, the binding of [3H]NMS is increased by the heating preincubation. Thus, it appears that at elevated temperatures the sites with low affinity for quaternary ligands are converted into sites with high affinity for quaternary ligands.

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226.6 Thermodynamic Characterization of Muscarinic Agonist and Antagonist Interactions In The Bovine Hippocampus. J. A. Schoenheimer*, L. Antonian* and A. S. Lippa*. (SPON: C. A. Klepner). Matrix Research Laboratories Inc., New York, N.Y. 10031.

Studies concerning the multiplicity of agonist binding sites/states of the muscarinic acetylcholine receptor (mAchR) suggest that class A muscarinic agonists (e.g. carbamylcholine) inhibit antagonist binding in a complex manner revealing at least two sites/states and class B agonists (e.g. oxotremorine) recognize only a single site/state. Although the distinction between class A and class B agonist interactions with the mAchR has been demonstrated using a variety of biochemical and pharmacological techniques, the thermodynamic parameters of the mAchR have not been reported. We therefore chose to further characterize the multiple sites/states of the bovine hippocampal mAchR by defining thermodynamic parameters using mAchR antagonists as well as class A and class B agonists.

In this study the binding of the potent muscarinic antagonist 3H-QNB and the class B agonist oxotremorine were examined at 4 °C, 15 °C, 25 °C, and 37 °C. Scatchard analyses at each temperature revealed a ten fold increase in the kD value when the incubation temperature was lowered from 37 °C to 4°C; indicating a large decrease in the affinity of the receptor for 3H-QNB. The inhibition constant (Ki) for oxotremorine increased by a similar magnitude over the temperature range, suggesting a decreased affinity at lower temperatures. The thermodynamic factors ΔH, ΔG and ΔS were calculated for oxotremorine and 3H-QNB binding: the change in enthalpy (ΔH Kcal/mol) was determined by Van't Hoff analysis. Similar results were obtained for both the class B agonist oxotremorine and the antagonist 3H-QNB. In both cases the ΔG increased at lower temperatures, representing thermodynamically, the same decrease in affinity seen in the Ki and kD. The calculations also indicated that the interactions of oxotremorine and of 3H-QNB with the mAchR were primarily entropy driven(ΔS values were approximately 30 cal/mol deg and 45 cal/mol deg respectively), with negligible change in enthalpy ($\Delta H \approx 0$ Kcal/mol). The thermodynamic parameters of class A agonist interactions with the mAchR are presently under investigation.

226.7 AMITRIPTYLINE DISPLAYS SELECTIVITY AS AN M_2 MUSCARINIC RECEPTOR ANTAGONIST IN BRAIN. S. Nomura*, S.H. Zorn and S.J. Enna (SPON: G.A. Robison). Departments of Pharmacology and Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77025.

Cholinergic muscarinic receptors have been subdivided on the basis of their affinities for pirenzepine, an anticholinergic drug. Those receptors having a higher affinity for this agent are classified as M_1, whereas those having a lower affinity are designated M_2. Amitriptyline, and other tricyclic antidepressants, are known to possess anticholinergic properties. While receptor binding assays have suggested they may be nonselective with regard to cholinergic receptors, the present study was undertaken to examine this issue using functional measures of cholinergic receptor responsiveness in brain. Carbachol-stimulated inositol phosphate accumulation in rat brain cerebral cortical slices was used as an assay for measuring M_1 activity, whereas carbachol-induced inhibition of GTP-stimulated adenylate cyclase in rat brain striatal membranes was taken as a measure of M_2 receptor activity. Whereas amitriptyline was somewhat more potent than pirenzepine in competing for ^{3}H-quinuclidinyl benzilate binding sites in rat brain membranes, pirenzepine was found to be 10-fold more potent than the antidepressant as an inhibitor of carbachol-stimulated inositol phosphate accumulation. Conversely, amitriptyline was 15 times more potent than pirenzepine in blocking the inhibitory effect of carbachol on adenylate cyclase activity in brain membranes. Atropine, a nonselective muscarinic receptor antagonist, was substantially more potent than pirenzepine in both assays. Given the current classification of muscarinic receptors, these findings suggest that amitriptyline differs from pirenzepine and atropine with regard to its selectivity as a cholinergic antagonist. While it would appear that amitriptyline has some affinity for both M_1 and M_2 sites, the antagonist profile displayed with these two biochemical assays suggests a much greater affinity for the M_2 receptor subtype. Accordingly, amitriptyline, and perhaps other tricyclic antidepressants, may be useful for distinguishing and defining the pharmacological and biochemical properties of muscarinic receptors in brain. (Supported in part by USPHS grants MH-36945 and MH-00501).

226.8 THE STRUCTURE—ACTIVITY PROFILE OF COMPOUNDS RELATED TO NIPECOTIC ACID ETHYL ESTER, A CHOLINERGIC AGONIST THAT APPEARS SELECTIVE FOR A SUBCLASS OF MUSCARINIC SITES. S.H. Zorn, P. Krogsgaard-Larsen*, H. Hjeds*, and S.J. Enna. Departments of Pharmacology, Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77025 and Department of Chemistry, Royal Danish School of Pharmacy, Copenhagen, Denmark.

Nipecotic acid ethyl ester (NAEE) displays cholinergic agonist properties in a variety of behavioral, biochemical, and physiological tests (Zorn, S., et al., Fed. Proc. 45:922, 1986). The responses to NAEE suggest that it may be more selective for a subgroup of muscarinic receptors in brain, possibly the M_2 site. The present study was undertaken to compare the cholinergic agonist activity of NAEE analogs and related compounds in an attempt to identify those structural properties that are responsible for the selectivity of this agent. For the study, cholinergic M_1 receptor activity was assessed by measuring inositol phosphate (IP) accumulation in rat brain cerebral cortical slices, while M_2 receptor function was examined by studying the inhibition of GTP-stimulated adenylate cyclase in rat brain striatal membranes. Whereas NAEE and other esters of nipecotic acid were incapable of stimulating the production of IP at concentrations up to 1 mM, both guvacine ethyl ester and arecoline were active in this regard. In contrast, NAEE and its ester analogs were found to be potent inhibitors of GTP-stimulated adenylate cyclase, as were guvacine ethyl ester and arecoline. These findings indicate that whereas guvacine ethyl ester and arecoline are agonists for the muscarinic receptors mediating IP accumulation and inhibition of adenylate cyclase, NAEE and its ester analogs appear to be selective for those associated with the cyclase system. Thus, while it has been found that the ester moity is essential for imparting cholinergic agonist activity to nipecotic acid, it would appear that the esteratic portion of the molecule is not responsible for the selectivity of this compound. On the other hand, guvacine ethyl ester differs from NAEE only in that the former possesses a double bound in the ring structure, similar to arecoline. It would appear, therefore, that the absence of this double bond may be a critical factor with regard to the selective action of NAEE on a subclass of muscarinic sites. (Supported in part by a grant from the U.S. Air Force).

226.9 MUSCARINIC RECEPTOR BINDING IN SUBCELLULAR FRACTIONS OF RAT FOREBRAIN. G. Dohanich, S. Halpain, and B.S. McEwen. Dept. of Psychology, Tulane University, New Orleans, LA 70118 and The Rockefeller University, New York, New York, 10021

Muscarinic receptors exhibit heterogeneity in the binding of both agonists and antagonists. We examined these properties of heterogeneity in various subcellular fractions from rat forebrain. Subcellular fractionation was carried out by procedures modified from previously published reports (Cotman and Taylor, 1972, J. Cell Biol. 55: 696). A "P2" fraction was prepared and layered onto a discontinuous sucrose density gradient to separate mitochondrial, synaptic plasma membrane (SPM), light SPM, and myelin fractions. A synaptic junction (SJ) fraction was prepared from SPM by treatment with 0.3% Triton followed by centrifugation through 1 M sucrose. Binding of [^{3}H]N-methylscopolamine (NMS) and [^{3}H]quinuclidinyl benzilate (QNB) was assayed by incubating the tissue samples for 90 min at room temperature with saturating concentrations of radioligand, followed by rapid filtration over glass fiber filters.

Binding of both [^{3}H]NMS and [^{3}H]QNB was enriched in SPM, light SPM, and microsomal ("P3") fractions as compared to the crude homogenate. The P1 pellet, myelin, and mitochondrial fractions showed substantially less muscarinic binding relative to the homogenate. In contrast to other neurotransmitter receptors (like those for excitatory amino acids) muscarinic binding was not enriched in SJ fractions, indicating that muscarinic synapses probably are not associated with the asymmetric synapses ("Gray Type I") which primarily are isolated in SJ preparations by the methods employed here. These results agree with a morphological study which concluded that muscarinic synapses were mostly symmetrical, although some asymmetrical synapses could also be found (Wainer et al, 1984, Brain Res. 308: 69).

In agreement with previous reports, [^{3}H]QNB bound to 40-60% more sites than [^{3}H]NMS. Furthermore, nearly every fraction showed a similar binding ratio of [^{3}H]QNB:[^{3}H]NMS. Experiments in which a single concentration of either carbachol (25 uM) or pirenzipine (1 uM) was included in the incubation with [^{3}H]NMS showed that all fractions exhibited similar degrees of competition by these two compounds. A notable exception was the SJ fraction, where carbachol was significantly more effective and pirenzipine less effective in competing for [^{3}H]NMS binding. Detailed competition curves for both carbachol and pirenzipine on SJ and SPM fractions confirmed a substantial difference in the nature of muscarinic receptor binding to these two types of membrane preparations.

226.10 TWO PHARMACOLOGICALLY DISTINCT CLASSES OF CHOLINERGIC RECEPTORS IN NEMATODES. M.A.Segerberg* and A.O.W.Stretton. (SPON: Frank L. Siegel). Neurosciences Training Prog., Univ. Wisc., Madison, WI 53706.

There is evidence that excitatory neuromuscular transmission in the large parasitic nematode Ascaris is blocked by cholinergic antagonists and potentiated by anti-cholinesterases (Del Castillo, J., et al., ARCH.INT.PHYSIOL.BIOCHIM. 71:741-757, 1963). Furthermore, excitatory motorneurons contain choline acetyltransferase, the synthetic enzyme for acetylcholine (Johnson, C.D. and A.O.W.Stretton, J.NEUROSCI. 5:1984-1992, 1985).

Although earlier pharmacological experiments suggested that cholinergic mechanisms were nicotinic, we now present biochemical and electrophysiological evidence that there are also muscarinic receptors present in nematodes. The muscarinic antagonist N-methyl-scopolamine (NMS) binds with high affinity to homogenates of C.elegans, the small, free-living soil nematode. The equilibrium dissociation constant for this binding is $4.6 \pm 0.6 \times 10^{-10}$M. The forward rate constant is 6×10^6 L/mol-sec, and the reverse rate constant is 2.3×10^{-3}/sec. The maximum number of receptors is 15.8 ± 1.3 pmole/gm protein. These results are in agreement with previously reported figures (Culotti, J.G. and W.L.Klein, J.NEUROSCI. 3:359-368, 1983).

We find that larval Ascaris (L1-L2 stage) also has NMS-binding activity, approximately 5 pmole/gm protein, suggesting that muscarinic receptors are also present in Ascaris. Because of the small size of C.elegans, it is not yet possible to use electrophysiological techniques to investigate the function of NMS-binding sites in this organism. However, Ascaris has nerve and muscle cells which are large enough for intracellular recording. There are three classes of dorsal excitatory motorneurons in adult Ascaris: DE1, DE2, and DE3. Two of these classes, DE1 and DE3, project anteriorly in the dorsal nerve cord, where they form synapses with muscle cells. The third class, DE2, projects posteriorly. The direction of projection is relevant to the proposed model for anterior- and posterior-propagating locomotory waves (see, e.g., Chalfie, M., et al., J.NEUROSCI. 5:956-964, 1985).

Intracellular recordings made from these cells have revealed that synaptic outputs to dorsal muscle of all three classes are blocked by d-tubocurarine. NMS, however, blocks only the anterior-projecting motorneurons. We are currently investigating whether muscarinic receptors are also present at specific subsets of neuron-neuron synapses.

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226.11 MEDIATION OF NEURALLY-EVOKED SECRETION BY MUSCARINIC RECEPTORS IN GUINEA PIG DISTAL COLON. A. Kuwahara and H.J. Cooke. Department of Physiology, The Ohio State University, Columbus, Ohio 43210.

The colonic epithelium is supplied by enteric neurons whose cell bodies project to the mucosa primarily from the submucosal ganglia. Stimulation of submucosal neurons in the guinea pig distal colon evokes a tetrodotoxin-sensitive increase in resting short-circuit current (Isc) that is due primarily to chloride secretion. The aim of the present study was to investigate the contribution of cholinergic submucosal neurons to colonic secretion. Muscle-stripped preparations consisting of submucosal ganglia and mucosa were set up in Ussing flux chambers. Semi-circular aluminium foil electrodes were placed on the submucosal surface and rectangular, bipolar stimulus pulses were passed repetitively for 2 minutes in the plane of the tissue (0.5ms; 8Hz; 25V). Electrical field stimulation evoked an increase in resting Isc of 222±22 uA.cm^{-2};(n=8). Atropine (10^{-8}-10^{-5}M) evoked a dose-dependent decrease in the neural response to a minimal of 20-30% of control. Muscarinic receptor subtypes were investigated in the presence of the antagonists pirenzepine (M_1) and 4-diphenyl-acetoxy-N-methyl-piperidine (4-DAMP;M_2) and compared to atropine (M_1,M_2). At < 1x10^{-7}M, 4-DAMP and atropine decreased the neural response to 40% of controls, whereas pirenzepine was less potent (83% of control). Carbachol ($2.5x10^{-5}$M) increased resting Isc to a magnitude similar to the neurally-evoked response. The increase in resting Isc evoked by carbachol was reduced by 10^{-4}M tetrodotoxin, 10^{-4}M hexamethonium and 10^{-6}M atropine. Bethanechol ($2.5x10^{-7}$-$7.5x10^{-4}$M) evoked a dose-dependent increase in resting Isc, but it was less potent and less effective than carbachol. In the presence of tetrodotoxin, bethanechol ($5x10^{-4}$M) evoked a 35±5 uA.cm^{-2} maximal increase in resting Isc due to direct activation of the enterocytes. Addition of the M_1 agonist, McNeil-A343, ($5x10^{-5}$M) evoked a 37.2±21.2 uA.cm-2 increase in resting Isc. These results suggest that electrical stimulation of submucosal neurons evokes colonic chloride secretion that is mediated, in part, by acetylcholine's interaction with M_2 muscarinic receptors on enterocytes. The magnitude of the secretory response evoked by activation of muscarinic receptors on enterocytes can be further enhanced by interaction with M_2 muscarinic receptors located on neurochemically unidentified submucosal neurons that innervate the guinea pig mucosa. (Supported by NIH Grants KO4-AM01642;RO1-AM37240).

226.12 DIFFERENTIAL PRESYNAPTIC AND POSTSYNAPTIC EFFECTS OF ACETYL-AF64A, AN IRREVERSIBLE MUSCARINIC LIGAND WITH AGONIST PROPERTIES. S.P. Baker, P. Posner*, and E.M. Meyer. Depts. of Pharmacology and Physiology, Univ. Florida, Gainesville, FL, 32610.

Recent evidence suggests that the transduction of muscarinic receptor-activation into physiological responses may involve a variety of second messenger systems depending on the synaptic location and tissue. We therefore investigated the presynaptic and postsynaptic effects of a muscarinic agonist, acetylethylcholine mustard (acetyl-AF64A), which binds irreversibly to muscarinic receptors. Acetyl-AF64A induced a dose dependent (0.1-10 uM) loss of cardiac and brain muscarinic receptor binding to several ligands that was time-dependent, blocked by atropine, and attenuated by guanine nucleotides. Presynaptically, this agonist produced a protracted (up to 60 min) modulation (inhibition) of ACh release from rat cortical synaptosomes via muscarinic autoreceptors with a potency similar to that of oxotremorine. Oxotremorine inhibited ACh release for a shorter duration, suggesting that agonist-induced receptor-desensitization may involve previously activated, unbound receptors. The ACh release-inhibition induced by 50 uM acetyl-AF64A was blocked only temporarily by 1 uM atropine, was observed after washout of the agonist, and was not due to inhibition of choline-uptake or acetylation. These presynaptic effects on ACh release were compatible with the observed time-dependent IC50 (9 uM after 5 min at 37°) and irreversible binding characteristics of the drug in synaptosomal membranes.

In contrast to its presynaptic effects on ACh release, acetyl-AF64A (10-100 uM) produced only a transient decrease in the atrial beating rate as compared to carbachol or ACh. After pretreatment of atria with 100 uM acetyl-AF64A, there was a 20-fold increase in the carbachol EC50 value for inhibition of the atrial beating rate. In addition, there was a 50% loss of atrial muscarinic receptor binding to labelled quinuclidinyl benzilate or oxotremorine-M. The data suggest that acetyl-AF64A acts presynaptically as an irreversible agonist and postsynaptically as an agonist followed by an antagonist.

226.13 COMPETITION STUDIES OF MUSCARINIC RECEPTORS ON DENDRITES AND SOMATA OF ISOLATED CORTICAL PYRAMIDAL NEURONS. B.A. Vogt, D.L. Burns* and E. Townes-Anderson. Boston Univ. Sch. Med., Boston, MA 02118 and Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

Light microscopic autoradiographic techniques for slide mounted sections are now a powerful adjunct to receptor subtype analysis, both in terms of localization and binding properties. We have recently extended this technique for use with dissociated cells. In addition to having specific muscarinic acetylcholine receptor(AChR) binding, these cells were used in competition studies to calculate IC_{50} values. This approach provides the first direct evidence for specific AChR binding to identified neurons and differences in binding properties at different parts of the cortical neuron.

Neurons were dissociated from cingulate cortex (area 29c) of hooded, Long-Evans rats using enzymatic and mechanical procedures. The cells were plated onto concanavalin A-coated slides and either processed for electron microscopy or according to a radioligand binding assay. In the latter instance, cells were incubated in ^{3}H-propylbenzilylcholine mustard (PrBCM, $2.4X10^{-9}$ M) with or without atropine (10^{-6} M). Other cells were coincubated in cold pirenzepine (PZ, 10^{-4}-10^{-9} M) and ^{3}H-PrBCM. Autoradiographed, small pyramidal neurons were drawn and grains within 3 µm of each process plotted on the drawings. The mean number of grains per 10 µm of dendritic length or somal perimeter were calculated for total and atropine-blocked PrBCM binding and these values subtracted to determine specific binding.

Electron microscopy of dissociated neurons demonstrated that they were free of adhering presynaptic elements and that cytoplasmic organelles and postsynaptic densities were intact. No dendritic spines were preserved in these dissociations.

Specific PrBCM binding was observed to all somata and dendritic processes. Analysis of variance indicated that there were no significant differences in binding by order of dendrite (i.e. 1°, 2°, 3°). Nonspecific binding was an average of 33% of total binding. Both somatic and dendritic PrBCM binding was antagonized by PZ, however, PZ appeared to be more effective at dendritic sites. Thus, the IC_{50} value for somata was $1.5X10^{-6}$ M while that for apical and basal dendrites was between $1.2X10^{-7}$ and $1.9X10^{-7}$ M.

Pirenzepine sensitivity is characteristic of binding to the high affinity or M_1 muscarinic receptor and is present on both somata and dendrites of pyramidal neurons. However, choline acetyltransferase (ChAT) histochemistry suggests that no cholinergic synapses are formed between $ChAT^+$ terminals and somata of pyramidal neurons, but rather, that they are associated with smooth dendrites. It is possible, therefore, that lower affinity, somatic receptor binding is associated with extrajunctional receptors.

Supported by NIH grants NS18745 and EY06135.

226.14 HIGH AFFINITY AGONIST BINDING TO BRAINSTEM MUSCARINIC RECEPTORS IS ELIMINATED BY LOW PH. B.L. Anthony* and R.S. Aronstam (SPON: B.B. Gallagher). Department of Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 20912.

The effects of hydrogen ion concentration on high affinity agonist binding to muscarinic receptors were determined in membranes isolated from rat brainstem using [^{3}H]oxotremorine-M as a probe. [^{3}H]Oxotremorine-M bound with high affinity to a subpopulation of brainstem muscarinic receptors (<10% of the receptors identified with [^{3}H]methylscopolamine were labelled with 3 nM [^{3}H]oxotremorine-M). [^{3}H]Oxotremorine-M binding was constant between pH 7.0 and 9.0; the number of high affinity sites decreased below pH 7.0 and at pH 5.0 no binding was detected. This decrease was irreversible; when brainstem membranes were incubated for 1 hour at low pH and then returned to pH 8.0, [^{3}H]oxotremorine-M binding was not restored. In contrast, the total number of receptors (i.e., the number of [^{3}H]antagonist binding sites) was not affected by prolonged incubation at low pH. Agonist affinity for the surviving [^{3}H]oxotremorine binding sites and the sensitivity of agonist binding to guanine nucleotide were not altered in media of low pH (pH 5.5). These findings indicate that [^{3}H]oxotremorine-M binds only to receptors which are functionally coupled to guanine nucleotide-dependent regulatory proteins, and that this coupling is irreversibly inactivated in media of low pH.

Table 1. INFLUENCE OF PH ON THE CONCENTRATION OF [^{3}H]OXOTREMORINE BINDING SITES IN RAT BRAINSTEM MEMBRANES

pH	[^{3}H]Oxotremorine-M Binding Bmax[a]	%High Affinity[b]
8.0	34 + 0.7	5.5%
5.5	18 ± 2.7	3.0%
5.5 → 8.0[c]	25 ± 2.7	4.0%

[a] Density of [^{3}H]oxotremorine-M binding sites in fmoles/mg protein; means ± S.D., n = 4.

[b] [^{3}H]Oxotremorine-M (3 nM) binding sites expressed as percent of total [^{3}H]antagonist binding sites at pH 8.0 (0.62 ±0.08 pmol/mg protein).

[c] Membranes were incubated at pH 5.5 for 1 hour before binding was measured at pH 8.0.

227.1

WITHDRAWN

227.2 BLOCK OF DEPOLARIZATION-INDUCED Ca^{2+} UPTAKE INTO SYNAPTOSOMES BY ANTIBIOTICS. W.D. Atchison and C.M. Beaman* (SPON: T.M. Brody). Dept. of Pharmacology/Toxicology, and Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824.

Antibiotics of the aminoglycoside, lincosamide, tetracycline and polymyxin classes block neuromuscular transmission by a combination of pre- and postjunctional effects. The prejunctional effects in many cases are thought to involve competitive block of Ca^{2+} entry into the nerve ending. This has never been tested directly, however. The goal of the present study was to determine if several antibiotics known to block neuromuscular transmission would impair depolarization-dependent uptake of calcium into isolated nerve terminals (synaptosomes). Forebrain synaptosomes were prepared from male Sprague-Dawley rats by conventional methods. Antibiotics tested for potential block of Ca^{2+} uptake included the aminoglycosides streptomycin and neomycin, the lincosamide clindamycin, and polymyxin B. Drugs were applied in concentrations which ranged from 1-1000 µM. Uptake of ^{45}Ca was determined during depolarization induced by an elevated K^+ concentration (77.5 mM). Influx of ^{45}Ca during 1 and 10 sec of depolarization was used to assess Ca^{2+} uptake via a "fast, inactivating path", and total uptake, respectively. Clindamycin (1-100 µM) had no effect on either component of Ca^{2+} uptake following 1 or 10 sec of depolarization (n=3). Within the concentration range of 1-100 µM streptomycin (n=10) also produced no appreciable reduction in either total uptake or "fast" uptake. However, at 500 and 1000 µM, streptomycin caused a 17% and 19% reduction, respectively, in total ^{45}Ca uptake. Block of "fast" uptake was more pronounced; at 1000 µM streptomycin caused a 30% reduction in fast uptake. Neomycin which is believed to be a more potent neuromuscular blocker, did indeed cause a more pronounced reduction in the "fast" phase of uptake; at concentrations of 500 and 1000 µM, neomycin caused a 34% and 46% reduction, respectively (n=7). However, neomycin had no effect on total uptake of Ca^{2+}. Polymyxin B (5 µM) caused an 85% increase in uptake during the "fast" phase. Polymyxin B at 500 and 1000 µM caused a 25% and 26% reduction in total uptake, respectively (n=8). These results indicate that several antibiotics which cause neuromuscular block can alter depolarization-induced calcium uptake into presynaptic nerve terminals. (Supported by BRSG funds from the College of Veterinary Medicine and by NIH grant NS20683.)

227.3 EXOGENOUS CALCIUM BUFFER REDUCES SYNAPTIC TRANSMITTER RELEASE AND FACILITATION. Milton P. Charlton, George J. Iwanchyshyn* Dept. of Physiology, Univ. of Toronto, Ontario, Canada M5S 1A8

The diffusion of calcium ions inside nerve terminals controls the initiation and termination of transmitter release and should depend on the concentration and affinity of intracellular calcium buffer. To examine the role played by calcium buffers in transmitter secretion, we added exogenous calcium buffer to nerve terminals and determined changes in synaptic performance.

The crayfish opener muscle preparation was perfused with a 50 uM solution of the acetoxymethyl tetraester of bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM), a membrane-permeable calcium buffer. The amplitude of the sixth excitatory post synaptic potential (EPSP) in a six stimulus 100 Hz train was reduced by 83%+-8% (n=12) at 25C and by 57%+-9% (n=6) at 15C. The first EPSP in these trains was reduced by 63%+-22% and 18%+-13% respectively. Thus, EPSP's and facilitation were both reduced.

When 50 uM BAPTA-tetrapotassium salt, a membrane impermeant form of the calcium buffer, was applied there was no effect. This argues against nonspecific effects of BAPTA-AM. The effect of BAPTA-AM does not depend on reduction of muscle input resistance because reductions in excitatory postsynaptic currents (EPSC's) similar to those for EPSP's were seen when muscles were voltage clamped at rest potential. To check whether products of cleavage of the AM ester caused the effects of BAPTA-AM, we applied 2',7'-bis-(2 carboxyethyl)- 5,6 - carboxyfluorescein, tetra-acetoxymethyl ester (BCECF-AM). This pH indicator had, on the average, no effect on EPSP's. BAPTA-AM caused no significant changes in the amplitude or duration of intracellularly recorded presynaptic action potentials (experiments by Don Dixon). When BAPTA-tetrapotassium salt was injected into the presynaptic axon near the recording site, there was a dramatic decrease in EPSP amplitude. Because the BAPTA salt injected presynaptically exerts an effect similar to that of BAPTA-AM applied extracellularly, it is unlikely that the action of BAPTA-AM is solely postsynaptic. There was little recovery of transmitter release when BAPTA-AM was removed or when saline with twice normal calcium concentration was substituted. However, with prolonged high frequency stimulation, EPSP's facilitated to an amplitude greater than the level before treatment with BAPTA-AM.

These data indicate that BAPTA probably works presynaptically to affect transmitter release by increasing buffering of intracellular calcium. Calcium buffering sufficient to reduce transmitter release can be overwhelmed during repetitive stimulation. The site of action of calcium must be some distance from the calcium channel. (Supported by MRC of Canada).

227.4 REGULATION OF VOLTAGE-DEPENDENT CALCIUM CHANNELS IN PC12 CELLS BY CHRONIC CHANGES IN MEMBRANE POTENTIAL. E.M. DeLorme* and R. McGee (SPON:P.E.Gallant). Dept. of Pharmacology, Georgetown Univ.School of Medicine, Washington, D.C. 20007

Voltage-dependent Ca^{2+} channels (VDCC) play a critical role in the functioning of both neuronal and muscle cells. Upon depolarization these channels are responsible for the influx of Ca^{2+} which ultimately leads to neurotransmitter release and muscle contraction. However, little is known concerning the long-term regulation of VDCC in response to changes in cellular activity. The effects of chronic changes in membrane potential on the VDCC of a model neuronal cell were examined using pheochromocytoma cells, clone PC12. VDCC number in PC12 cells was measured using ^3H-nitrendipine binding to PC12 cell membranes. Exposure of the cells to growth medium containing 50 mM K^+ (Na^+ reduced to maintain osmolarity) depolarized the cells from the resting membrane potential of -65.4 mV to -40.1 mV as determined by the distribution of ^{14}C-triphenylphosphonium (^{14}C-TPP). Depolarization was maintained up to 4 days without toxicity. Graded elevations of K^+ caused graded decreases in ^3H-nitrendipine binding with a 45% loss after 3 to 4 days of depolarization. Scatchard analysis revealed a decrease in B_{max} with no change in the K_d for nitrendipine. Repolarization of the cells produced a return to control binding values within 1-2 days. In order to provide a functional correlate to the demonstrated changes in ^3H-nitrendipine binding, depolarization-dependent uptake of $^{45}Ca^{2+}$ was measured. Decreases in $^{45}Ca^{2+}$ uptake of 40% were observed in chronically depolarized cells which had been allowed to repolarize for 1 hr prior to activation of VDCC by exposure of the cells to 50 mM K^+. Both the time course of decreases in $^{45}Ca^{2+}$ uptake and the concentration-dependence of this effect corresponded with changes in ^3H-nitrendipine binding. As with binding studies, repolarization of the cells for 1-2 days caused a return to control values for $^{45}Ca^{2+}$ uptake. These results suggest that changes in neuronal cellular activity leading to changes in the average membrane potential may cause changes in the number of VDCC expressed by the cell. Supported by NSF Grant # BNF 82-16306.

227.5 INOSITOL-1,4,5-TRISPHOSPHATE INDUCED MOBILIZATION OF Ca^{++} FROM RAT BRAIN SYNAPTOSOMES. C.R.Gandhi* and D.H. Ross (Spon. D. Jones), Division of Molecular Pharmacology, University of Texas Health Science Center, San Antonio, Texas 78284.

Receptor stimulation by a variety of agonists has been shown to produce a rapid phosphodiesterase-dependent hydrolysis of phosphatidylinositol-4,5-bisphosphate to IP_3 and 1,2 diacylglycerol (1,2-DG). This is subsequently and rapidly followed by release of Ca^{++} from intracellular endoplasmic reticulum like storage sites. Recent studies have shown that IP_3 mobilized Ca^{++} from a variety of cell types is receptor-dependent providing a link between receptor mediated PIP_2 breakdown and Ca^{++} release. Since nervous tissue contains high concentrations of PIP_2 the present study has characterized the IP_3 release of Ca^{++} from saponin treated synaptosomes from rat brain.

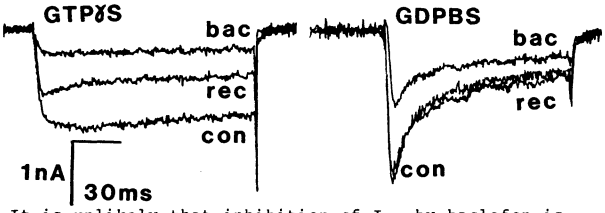
Inositol 1,4,5-trisphosphate (IP_3) was found to release Ca^{++} from presynaptic nerve endings made permeable with saponin. The K_m for IP_3 release of Ca^{++} was 0.4 µM with V_{max} 3-4 µM. Ca^{++} taken up into endoplasmic reticulum like storage sites was ATP-dependent. Addition of IP_3 produced a rapid release of Ca^{++} following equilibrium of uptake, which was complete within 60 sec. Ca^{++} reaccumulation occured to original levels in 5-7 min. No Ca^{++} release was detectable in absence of ATP. Ca^{++} release by IP_3 was not seen in absence of mitochondrial inhibitors oligomycin and sodium azide. Reaccumulation of Ca^{++} following IP_3 addition was prevented by vanadate a potent inhibitor of ATP-dependent Ca^{++} uptake. Cholinergic receptor activation with muscarine produced release of Ca^{++} which could be repeated 5-7 min later by addition of IP_3.

These studies demonstrate a viable IP_3-dependent release of Ca^{++} from endoplasmic reticulum-like stores in saponin treated synaptosomes. Ca^{++} release was nonmitochondrial and was dependent on the integrity of the storage sites, A23187 made the sites irreversibly permeable to Ca^{++}. Receptor stimulation and release of Ca^{++} is consistent with agonist activity in other cell preparations. This study provides a simple model with which to examine the effects of agonists on IP_3 mobilization of Ca^{++} in synaptosomes.

Supported by Neuroscience Program F33615-83-C-0624.

227.6 GTP ANALOGUES MODULATE CALCIUM CURRENTS AND THEIR INHIBITION BY BACLOFEN IN CULTURED RAT SENSORY NEURONES A.C. Dolphin* and R.H. Scott*, Dept. of Pharmacology, St George's Hospital Medical School, London SW17 ORE, UK. (SPON European Neuroscience Association).

Activation of $GABA_B$ receptors reduces calcium currents (I_{Ca}) in cultured rat dorsal root ganglion (DRG) neurones. To investigate the role of guanine nucleotide binding (N) proteins in the mediation of this response, the effect of intracellular nonhydrolysable guanine nucleotide analogues has been studied on I_{Ca} and its inhibition by baclofen. The whole cell recording technique was used with patch pipettes containing Cs acetate. ATP (2mM) was present, either with or without the GDP analogue guanosine 5'-0-3-thiodiphosphate (GDP-ß-S) or the GTP analogue guanosine 5'-0-2-thiotriphosphate (GTP-ɣ-S) (500µM). In the absence of either analogue the maximum I_{Ca} evoked at between -20 and +10mV from a holding potential of -80mV consisted of two components one of which was rapidly inactivating, and the other slowly or non-inactivating. In the presence of GTP-ɣ-S, I_{Ca} consisted largely of the non-inactivating component and the IC_{50} for its inhibition by (-)-baclofen was 2µM (n=8). In the presence of GDP-ß-S, the fast inactivating component (T_{inact} = 8.5±1.2ms, n=10) was more pronounced, and the potency of baclofen was reduced with the IC_{50} for baclofen being about 50µM (n=8). The figure shows the effect of 50µM baclofen on the maximum I_{Ca} in both cases.



It is unlikely that inhibition of I_{Ca} by baclofen is due to inhibition of adenylate cyclase since the effect was not diminished by 1µM forskolin or inclusion of 50µM cyclic AMP in the patch pipette. These data suggest that GTP binding to an N protein is involved in mediating inhibition of I_{Ca} by baclofen.

227.7 SPONTANEOUS, CALCIUM-INDEPENDENT QUANTAL TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. K. A. Alkadhi, Department of Pharmacology, University of Houston, Houston, TX 77004.

Infrequent, giant miniature endplate potentials (gMEPPs) can be recorded at untreated endplates of both mammalian and amphibian muscle. The frequency of these abnormally large gMEPPs has been reported to increase in the presence of certain drugs and toxins (e.g. Kim et al., J. Physiol., 356: 587, 1984). In the present investigation, the nature and characteristics of gMEPPs in the normal untreated and emetine-treated cutaneous pectoris muscle of the frog. After a latency of 20 min, emetine (10^{-5}M), an antiamebic drug, increased the frequency of gMEPPs by 10-fold without significantly affecting the frequency of the normal MEPPs. High Mg^{2+} (10 mM) or tetrodotoxin (1 µM) had no effect on the frequency of gMEPPs in absence or presence of emetine. The frequency of gMEPPs in untreated preparations is 0.016±0.002 Hz and has a slower rise time (0.693±0.38 ms) than the normal MEPPs (0.372±0.04, ms). No correlation was found between the frequency of gMEPPs and the frequency of normal MEPPs. Various procedures and drugs that increase or decrease intracellular Ca^{2+} concentration at the nerve terminals were tested in the presence and absence of emetine. The presence of ethanol (0.5 M), ouabain (0.2 mM), 10 mM Ca^{2+}, 10 mM Mn^{2+} or 7.5 mM K^+ although markedly increased the frequency of normal MEPPs had no significant effect on gMEPPs frequency, but when emetine was introduced in the presence of these agents it consistently increased the gMEPP frequency. Absence of Ca^{2+} in the bathing solution (0 Ca^{2+} + EGTA) decreased the frequency of normal MEPPs without affecting gMEPPs frequency. Emetine increased gMEPPs frequency even in Ca^{2+}-free solution. Hypotonic solution which markedly decrease normal MEPPs frequency, without affecting gMEPPs frequency prevented the emetine-induced increase in gMEPPs frequency. These results indicate that gMEPPs are different from normal MEPPs in many characteristics but significantly in being Ca^{2+}-independent.

227.8 INTERACTIONS BETWEEN MEPPS PRODUCED IN A REGION OF THE FROG NEUROMUSCULAR JUNCTION. J.P. Tremblay, R. Robitaille and G. Grenon*, Laval University, Quebec, Canada, G1K 7P4.

Experiments were made on cutaneous pectoris muscle of small Rana pipiens in order to study possible interactions between MEPPs. Five thousand MEPPs were recorded in low calcium solution with two intracellular electrodes located at each distal end of the neuromuscular junction. The electrodes were positioned under Nomarski optics. After the experiment, the preparations were fixed and neuromuscular junctions stained by NBT/ChE technique (Latinsky and Decino, J. Neurocytol. 9, 303, 1980).

We have calculated the probability of occurrence of a MEPP per second per µm of nerve terminal. The average probability was calculated for the whole junction and for the whole duration of the experiment. Regional probabilities were also calculated for different 35-µm-regions of the junction. These average regional probabilities are much lower in distal regions of neuromuscular junction than in the proximal regions (i.e. near the last myelinated segment). In order to study the possible interactions between MEPPs, we have also calculated the probability of MEPP occurrence during different intervals after a MEPP. For short intervals (0-20ms) this probability is lower than the average probability. For the individual regions, the probabilities at short intervals are also lower than their respective average regional probability. Therefore, it seems that the occurrence of a MEPP inhibits for a short period of time the occurrence of another MEPP. The degree of inhibition varies in the different regions. At longer intervals after a MEPP (i.e. 20 to 50 ms) the release probability increases toward normal value and a small facilitation occurs occasionally. The short term inhibition clearly indicates that there are interactions between MEPPs. Since regional inhibitions are also observed, this suggests that such interactions also occur within individual regions.

227.9 INTERVALS BETWEEN MEPPS PRODUCED IN A REGION OF THE FROG NEUROMUSCULAR JUNCTION ARE NOT RANDOMLY DISTRIBUTED. R. Robitaille, J.P. Tremblay and G. Grenon*, Laval University, Quebec, Canada, G1K 7P4.

The experiments were performed on cutaneous pectoris muscle of small frogs. Five thousand MEPPs were recorded in low calcium solution (1mM Ca^{++}, 10mM Mg^{++}), with two intracellular electrodes positioned at the distal ends of the junction under Nomarski optics. At the end of the experiment, muscles were fixed, the nerve terminals were stained with NBT (Letinsky and Decino, J. Neurocytol. 9, 303, 1980) and the postsynaptic AChE was revealed by the method of Karnovsky (J. Cell Biol. 23, 217, 1964).

The distribution of MEPP intervals is significantly different from a fitted exponential distribution (chi-squared test, $p<.001$). This confirms the result of Cohen et al. (J. Physiol. 236, 327-361, 1974). This result indicates that MEPPs do not occur randomly and suggests that there are interactions between them.

Simultaneous intracellular recording with two electrodes permits to evaluate the position of the release site producing each MEPP (Tremblay et al. Neurosc. Let., 51, 247, 1984). The activity of these release sites is not uniform along the frog neuromuscular junction. This confirms our previous results. The NMJ was divided in 10 equal regions of about 35µm. It was then possible to measure the time interval between MEPPs produced in one of these regions. The distribution of these time intervals is also significantly different from a fitted exponential distribution ($p<.005$). This confirms the results of Cohen et al. (Brain Res. 221, 382, 1981) obtained with extracellular recording of neuromuscular junction segment. In our experimental distributions of MEPP intervals for the whole neuromuscular junction as well as in individual regions, there are less short intervals(<20 ms) than predicted by the theoretical exponential distribution. However there are more 20-50 ms intervals than predicted by the exponential distribution. These results suggest that the occurrence of a MEPP in a region of the neuromuscular junction reduces the probability of occurrence of another MEPP in the same region for a short period and increases it slightly at longer intervals.

227.10 QUANTAL NEUROTRANSMITTER RELEASE FROM THE LARVAL HOUSEFLY (Musca domestica): THE EFFECTS OF NERVE TERMINAL DEPOLARISATION AND CALCIUM. G.R. Seabrook*, I.R. Duce* and S.N. Irving* (SPON: M.B.A. Djamgoz). Dept. of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

The disruption of neurotransmitter release processes in larval housefly muscles has been suggested to be involved in pyrethroid insecticide toxicity (Salgado V.L., Irving S.N. and Miller T.A., Pestic. Biochem. and Physiol. 20: 169, 1983). The mechanisms underlying neurotransmitter release in this and other insect neuromuscular synapses are as yet not fully understood.

After removal of the ventral ganglion, spontaneous and evoked extracellular post synaptic currents (EPSCs) were recorded from ventrolateral body wall muscles 6a and 7a of prepupal larvae using a single extracellular microelectrode and patch clamp amplifier. The extracellular calcium concentration, $[Ca^{2+}]_o$, was manipulated by changing the bathing saline calcium content between 0.1µM and 10mM. Evoked EPSCs were inhibited in saline containing <10µM $[Ca^{2+}]_o$; above this concentration the quantal content of the response was positively correlated with $[Ca^{2+}]_o$. Increasing $[Ca^{2+}]_o$ was also correlated with an increase in the rate of decay of these currents; this was attributed to block of the glutamate receptor channel by calcium ions. The frequency of spontaneous miniature EPSCs in normal (1mM Ca^{2+}) saline (ca. 0.4Hz) was not significantly affected when saline containing 0.1µM or 10mM Ca^{2+} was perfused. Nerve terminal stimulation (10 mins at 1Hz) suppressed the frequency of spontaneous release, but immediately following an EPSC (10-20ms after the stimulus) the probability of spontaneous release was increased 100 fold (ca. 43Hz). This "delayed release" increased with elevated $[Ca^{2+}]_o$ (four fold increase from 0.1 to 10mM Ca^{2+}) suggesting that it resulted from a transient increase in intraterminal calcium concentration. Electrotonic hyperpolarisation (200ms duration) of nerve terminals inhibited spontaneous release during the pulse. A depolarising current sufficient to elicit an EPSC produced a profile of spontaneous release similar to delayed release. Increasing the depolarising pulse potentiated miniature EPSC frequency. The frequency reached 80Hz before declining with larger depolarizations. This may be due to either suppression of calcium conductance, or disruption of some other process which modulates transmitter release.

These data demonstrate that recording individual synaptic currents can provide useful insight into neurotransmitter release mechanisms in insects, and they have further elucidated the role of Ca^{2+} in these processes.

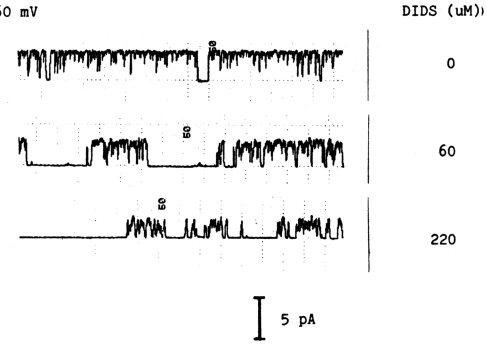
GRS is an SERC/Wellcome Pesticides CASE student.

227.11 EVIDENCE FOR ANION CHANNELS IN SECRETORY VESICLES. E.F. Stanley[1], G. Ehrenstein*[1], and J.T. Russell[2]. [1]Lab. of Biophysics, NINCDS and [2]Lab. of Dev. Neurobiol, NICHHD, Bethesda Md 20892.

We have recently proposed an "ion channel" model for exocytosis, wherein calcium triggers the influx of ions from the cytosol through channels into the secretory vesicles, leading to an increase in vesicle osmolarity, water entry, vesicle expansion and finally fusion (Stanley and Ehrenstein, Life Sci. 37:1985-1995). In this model, the opening of a calcium-activated potassium channel allows potassium ions to enter the vesicle, and an anion channel allows anions to enter. We now present evidence for the presence of an anion channel in secretory vesicles isolated from bovine neurohypophysis.

The vesicles were incorporated into lipid bilayers, which were voltage clamped. We observed single channels that were identified as chloride-permeable on the basis of reversal potentials in asymetric Cl^-solutions. These channels had a maximum conductance of 85 pS in a 1:0.02 M TrisCl gradient, were slightly voltage sensitive, and were blocked by DIDS in a dose dependent manner.

These findings indicate that the anion permeability of secretory vesicles can be accounted for by an anion channel. In addition they lend support to the "ion channel" model of vesicle exocytosis.



227.12 THE EFFECT OF CHLORIDE SUBSTITUTION ON AGONIST INDUCED AUTORECEPTOR ACTIVATION IN SUPERFUSED STRIATAL SLICES. L.X. Cubeddu and P.A. Diliberto*. (SPON: R.L. Glasser) Div. of Clinical Pharmacology, Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

The mechanism of autoreceptor-mediated inhibition of neurotransmitter release is not fully understood. Autoreceptor activation by agonists has been associated with a decrease in the electrical excitability of monoamine terminals thought to be due to hyperpolarization of the presynaptic membrane (Tepper, J.M., Trends Pharmacol. Sci. 6:251, 1985). The opening of autoreceptor-linked chloride channels has been proposed as a possible mechanism for receptor-mediated hyperpolarization (Vodyanoy, V., Neurosci. Lett. 62:103, 1985). Present experiments were designed to evaluate the Cl^--dependency of agonist induced inhibition of stimulation-evoked (SE) release of dopamine (DA) and acetylcholine (ACh) from superfused rabbit striatal slices. Slices were labeled with ^{3}H-DA and ^{14}C-ACh and superfused at a rate of 1 ml/min in Krebs-Ringer bicarbonate solution containing either 125.4 mM Cl^-(KRB) or 7.4 mM Cl^-(Low Cl^-). In Low Cl^- medium, 118 mM NaCl was substituted with 118 mM Na·isethionate. Cl^- substitution under these conditions results in an increase in spontaneous ^{3}H-DA efflux which decreases with time (Diliberto, P.A., Neurosci. Abstract, this meeting). Therefore, agonist action was assessed after 95 min of superfusion in Low Cl^- when total ^{3}H efflux had returned to control levels. Electrically stimulated (1 Hz, 120 pulses) release was measured in the absence and presence of agonists. Apomorphine (30 nM), a DA receptor agonist, inhibited the release of ^{3}H-DA and ^{14}C-ACh in KRB by 67% and 38%, respectively. In Low Cl^- release was inhibited by 40% (^{3}H-DA) and 25% (^{14}C-ACh). Carbachol (10 µM), a muscarinic receptor agonist, inhibited ^{14}C-ACh release by 50% and 63% in KRB and Low Cl^-, respectively. Carbachol enhanced the release of ^{3}H-DA by 65% in both media. SE release of both DA and ACh in the absence of agonists is lower in Low Cl^- than in KRB. This inhibition of release is reversed by sulpiride, a D_2-receptor DA antagonist, suggesting that synaptic DA levels in Low Cl^- are elevated and that DA autoreceptor and heteroreceptor-mediated inhibition is already activated. This may explain the apparent decrease in apomorphine effects in Low Cl^-. Thus, we believe that autoreceptor-mediated inhibition of DA and ACh release is achieved by a Cl^--independent mechanism(s).

Supported by NIH Grant #NS21645-01A1 to L.X.C.

227.13 THE EFFECTS OF CHLORIDE SUBSTITUTION ON SPONTANEOUS DOPAMINE EFFLUX AND ELECTRICALLY-EVOKED RELEASE OF DOPAMINE AND ACETYLCHOLINE IN SUPERFUSED STRIATAL SLICES. P.A. Diliberto* and L.X. Cubeddu. (SPON: A.K. Salm) Div. of Clinical Pharmacology, Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

Removal of chloride (Cl^-) from the superfusion medium results in increased spontaneous efflux of ^{3}H-dopamine (^{3}H-DA) from rabbit striatal slices preloaded with ^{3}H-DA and ^{14}C-acetylcholine (^{14}C-ACh). Reducing Cl^- from 125.4 mM (KRB medium) to 7.4 mM (Low Cl^-) or nominally zero Cl^- (0 Cl^-) by isethionate substitution resulted in large increases in ^{3}H efflux that peaked at 5-15 min and returned to near control levels 60 min after the medium change. ^{14}C-ACh release was not affected. At peak efflux, Low Cl^- and 0 Cl^- induced increases of 4 and 6 fold, respectively, in total ^{3}H overflow. This efflux was not Ca^{2+} dependent. Analysis of the composition of the ^{3}H efflux in terms of DA and metabolites revealed a concentration dependent increase in ^{3}H-DA in the superfusate. At peak efflux, ^{3}H-DA was increased by 18 and 41 fold in Low Cl^- and 0 Cl^-, respectively. ^{3}H-dihydroxyphenylacetic acid (^{3}H-DOPAC) efflux increased by only 3.5 and 5 fold in Low Cl^- and 0 Cl^-, respectively. At 50-60 min, ^{3}H-DA levels in Low Cl^- and 0 Cl^- were 4 and 13 times KRB values, respectively. ^{3}H-DA efflux was also examined in slices depleted of endogenous vesicular DA by reserpine pretreatment and superfused in the presence of pargyline and U-0521, inhibitors of MAO and COMT, respectively. Under these (RPU) conditions, Low Cl^- increased ^{3}H-DA efflux from RPU slices. Cl^- substitution also affected the stimulation-evoked (SE) release of ^{3}H-DA and ^{14}C-ACh. Slices were electrically stimulated (1 Hz, 120 pulses) 95 min and 145 min after superfusion was begun in either KRB, Low Cl^- or 0 Cl^- medium. Low Cl^- reduced SE release of ^{3}H-DA and ^{14}C-ACh by 21 and 52%, respectively; while 0 Cl^- inhibited 60 and 80%. Inhibition was reversed by sulpiride, a D_2-receptor DA antagonist. Similar inhibition (50%) of ^{14}C-ACh release in Low Cl^- occurred with RPU slices. Thus, Cl^- removal stimulates DA efflux. Data from reserpinized slices suggest that efflux can occur from extravesicular sources. Elevated DA levels may then be maintained by inhibition of a Cl^--dependent DA uptake process. The resulting increase in synaptic DA explains the sulpiride sensitive inhibition of the SE release of DA and ACh.

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227.14 ELECTROPHYSIOLOGICAL CORRELATES OF PRESYNAPTIC ALPHA-2 RECEPTOR-MEDIATED INHIBITION OF NOREPINEPHRINE RELEASE AT LOCUS COERULEUS SYNAPSES IN THE DENTATE GYRUS. H.C. Moises and R. Carlson.* Department of Physiology, University of Michigan, Ann Arbor, MI 48109.

Despite an abundance of evidence that presynaptic alpha$_2$ adrenergic receptors mediate inhibitory control of the release of norepinephrine (NE) from the terminals of locus coeruleus (LC) neurons, few studies have demonstrated the physiological significance of this 'autoreceptor'-mediated inhibition on NE-mediated synaptic activity within the mammalian brain. This question was addressed in the present study by examining the effects of systemic administration of alpha$_2$ receptor agonists and antagonists on the ability of LC stimulation to augment the population spike response evoked in the dentate gyrus by perforant path (PP) stimulation.

Single or two barrel-staggered glass microelectrodes (1-2 M resistance) were used to record the extracellular field potentials evoked by single pulse (0.2 ms duration) PP stimulation in the cell body and dendritic layers of the dentate gyrus in halothane-anesthetized rats. The PP was stimulated once every 15 s and the evoked responses digitized and stored on diskette for off-line analysis by computer. In each experiment, the intensity of PP stimulation was adjusted to elicit a population spike of half-maximal amplitude to ensure a clear enhancement of the response during pairing with LC stimulation. Stimulations of the LC consisted of 5 current pulses of 0.2 ms duration at 300 Hz, and were paired with two of every four PP test stimuli delivered.

LC stimulation delivered 35 ms prior to PP activation consistently produced a short-term enhancement of the dentate population spike (62.4 $\pm$ 7.2%, N=12), but did not significantly alter potentials measured in the dendritic layer. In 5 of 7 animals tested, administration of clonidine (3.75 ng/kg - 3.75 ug/kg, i.v.) reversibly abolished the enhancement induced by LC stimulation. The effect of clonidine was dose-dependent (est. EC_{50} of 0.125 ug/kg) and was blocked by i.v. administration of the alpha$_2$ antagonist idazoxan (0.125 - 0.875 ug/kg), which restored the potentiative effects of LC conditioning. Neither of these drugs in the dose ranges administered had a significant effect on the potentials evoked in the dendritic or cell layer by control presentations of PP stimuli.

To our knowledge, these results provide one of the first demonstrations of a functional blockade of LC-mediated physiological action on postysnaptic target cells as a result of presynaptic alpha$_2$-autoreceptor activation. (Supported by NIDA Grant DA-03365 and The Chicago Community Trust/Searle Scholars Program).

227.15 THE EFFECT OF QUINOLINIC ACID INJECTED INTO THE NUCLEUS BASALIS MAGNOCELLULARIS ON CORTICAL HIGH AFFINITY CHOLINE UPTAKE. R.H. Metcalf*, R.J. Boegman, R.J. Riopelle, and S.K. Ludwin. Departments of Pharmacology and Toxicology, Medicine, and Pathology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The mechanism by which central cholinergic neurons regulate their ability to synthesize acetylcholine has been the focus of intense investigation. It has been suggested that the supply of the precursor, choline, is a possible site in regulating the synthesis of acetylcholine. Stimulation of cholinergic neurons results in an increased uptake of choline by the sodium-dependent high affinity uptake system, as indicated by changes in the Vmax of uptake (Simon and Kuhar, J. Neurochem. 27: 93, 1975). We now report that changes in uptake capacity of basal forebrain cholinergic neurons projecting to the frontal cortex of the rat follow a specific time course after stimulation with the endogenous excitotoxin, quinolinic acid. Male Sprague-Dawley rats (250-275 g) were anesthetized with halothane and placed in a Narashige small animal stereotaxic apparatus. Using the appropriate coordinates, an injection cannula was placed into the right nucleus basalis magnocellularis and 120 nM of quinolinic acid in 1 µl saline was slowly infused. At specific times following injection, animals were sacrificed and synaptosomes from the frontal cortex were prepared. Synaptosomal uptake of ^{3}H-choline chloride by the ipsilateral frontal cortex was determined and compared to uptake in the contralateral hemisphere, sham-injected animals (1 µl, 0.9% saline), and normal animals. When high affinity uptake was performed in normal animals, no difference was seen between left and right corticies. However, in sham-injected animals there was approximately a 4% (n=5) decrease in uptake in the ipsilateral cortex. Three hours post-injection, a maximal increase of 45% in high affinity choline uptake was seen in the ipsilateral cortex. The quinolinic acid-induced increase in high affinity uptake decayed with time such that by 9 hours post-injection, uptake in the ipsilateral cortex returned to basal levels. By 1 day post-injection, uptake in the ipsilateral cortex had decreased by 25%, indicating that the toxic effects of qinolinate had already become evident. This loss of uptake activity progressed, and by 7 days post-injection a maximal decrease of 45% (n=5) in uptake activity was observed. Storage of synaptosomes on ice resulted in a loss of induced high affinity choline uptake such that by 6 hours synaptosomal uptake from ipsilateral and contralateral hemispheres were identical. Thus, stimulation of cholinergic neurons in the basal forebrain results in a time-dependent change in high affinity choline uptake in the frontal cortex. (Supported by the Medical Research Council of Canada, #MA9118)

228.1 **PRESYNAPTIC MECHANISM OF KINASE C-ACTIVATORS AT THE SQUID GIANT SYNAPSE.** G.J. Augustine, L.R. Osses*, S.R. Barry and M.P. Charlton. Dept. Biological Sciences, Univ. Southern California, Los Angeles, CA 90089, Dept. Physical Medicine, Univ. Michigan, Ann Arbor, MI 48109, Dept. Physiology, Univ. Toronto, Ontario M5S 1A8 and MBL, Woods Hole.

It has been proposed that the calcium- and phospholipid-sensitive protein kinase, kinase C, may play a role in mediating or modulating synaptic transmission. Consistent with this proposal, phorbol esters and other agents which activate kinase C profoundly increase transmission at the squid giant synapse (Osses et al., Biophys. J. 49:179a, 1986) and kinase C is found in squid synaptosomes (Unver et al., Soc. Neurosci. Abstr. 12, 1986). We have further explored the possible role of kinase C at the squid giant synapse by evaluating the physiological mechanisms which underlie the increases in transmission caused by the phorbol ester 12-deoxyphorbol 13-isobutyrate 20-acetate (DPBA). Enhancement of transmission by DPBA invariably is associated with a slight broadening of the presynaptic action potential. For example, 2 uM DPBA increases action potential duration by 15% while enhancing postsynaptic responses by approximately 400%. This small change in presynaptic action potential duration apparently is necessary for DPBA to increase transmission, because DPBA does not enhance transmission if presynaptic terminals are voltage clamped and stimulated with depolarizations of constant duration. We have also investigated the basis for DPBA-induced increases in action potential duration. Because presynaptic Na channels inactivate rapidly, broadening is likely to be due to an increase in presynaptic Ca current and/or a decrease in presynaptic K current. Ca and K currents were individually examined under pharmacological conditions which eliminate currents flowing through other presynaptic channels. DPBA (2 uM) produced no detectable change in Ca current magnitude in 6 experiments. However, in 2 experiments the same concentration of DPBA produced a 15% decrease in K currents flowing through delayed rectifier channels. In summary, our results suggest that DPBA enhances transmission by decreasing a presynaptic K current, which prolongs the presynaptic action potential and leads to increases in presynaptic Ca influx and resultant transmitter release. This mechanism is consistent with kinase C acting in a pathway which modulates, rather than mediates, transmitter release at the squid synapse. Supported by NIH grant NS21624 to GJA, Grass and Fogarty Fellowships to LRO, an NSF grant to SRB and an MRC (Canada) grant to MPC.

228.2 **KINASE C (Ca^{++}-PHOSPHOLIPID-DEPENDENT PROTEIN KINASE) IN SQUID OPTIC LOBE SYNAPTOSOMES.** E. Unver*, G.J. Augustine, S. Barry+, G.P. Miljanich* (SPON: W.O. McClure). Neurobiology Section, University of Southern California, Los Angeles, CA 90089 and +Department of Physical Medicine, University of Michigan, Ann Arbor, MI 48109.

Protein kinase C (PKC) may play a role in transmission at chemical synapses. At the squid giant synapse, phorbol esters and other PKC activators produce a dramatic enhancement of synaptic transmission (Osses et al., Biophys. J. 49:179a, 1986). This effect may be due to a reduction in a presynaptic K^+ current and subsequent spike broadening (Augustine et al., Soc. Neurosci. Abstr. 12, 1986). In this study we have asked whether PKC is present in squid presynaptic terminals, using squid optic lobe synaptosomes as a closely related and convenient system for biochemical identification and characterization of PKC.

Diacylglycerol (DAG) caused a several-fold stimulation of transfer of radioactive phosphate from ATP to the exogenous substrate, histone, in optic lobe homogenate and in a crude optic lobe synaptosome preparation. The specific activity of DAG-dependent phosphorylation was 0.75 nmolP/mg-protein/min in homogenates and 3.1 nmolP/mg-protein/min in synaptosomes. This 4-fold increase in activity suggests that PKC is enriched in nerve terminals. These levels of activity are comparable to those observed in mammalian brain preparations.

Polyacrylamide gel electrophoresis and autoradiography of the cytosolic subfraction of disrupted synaptosomes revealed nine endogenous polypeptides of apparent molecular weights of approximately 20, 40, 45, 50, 52, 55, 65, 160, and 220 kdaltons whose phosphorylation was increased two to seven-fold by DAG. The phosphorylation of one endogenous substrate, with an apparent molecular weight of 100 kdaltons, was decreased 3-fold by DAG. A similar analysis of the particulate synaptosomal subfraction was precluded by high levels of DAG-independent phosphorylation.

These results demonstrate the presence of PKC in squid neuronal tissue and suggest its enrichment in presynaptic nerve terminals, consistent with a potential role for this kinase in regulating synaptic transmission. The relatively high specific activity of PKC in squid optic lobes should permit purification of this enzyme and its substrates for biochemical analysis and microinjection experiments.

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228.3 STIMULATION-DEPENDENT PHOSPHORYLATION OF CHROMAFFIN CELL PROTEINS: IDENTIFICATION AND IN SITU PHOSPHORYLATION OF SUBSTRATE PROTEINS. J.W. Haycock, M.D. Browning and P. Greengard. Laboratory of Molecular & Cellular Neuroscience, The Rockefeller University, NY, NY 10021.

Treatment of chromaffin cells with secretagogues has previously been shown to increase the phosphorylation of two proteins (M_r≈60,000 and M_r≈95,000) which are evident in SDS-PAGE of total cell extracts (1). In the present studies we obtained evidence that acetylcholine (ACh) increased the phosphorylation of five proteins in intact chromaffin cells. Two of these proteins (M_r≈60,000 and M_r≈100,000) are evident in SDS-PAGE of total cell extracts and are presumably identical to those previously described (1). Whereas the M_r≈60,000 protein has been previously identified as tyrosine hydroxylase (TH, 2), the M_r≈100,000 protein is shown to be identical to 100K (a M_r=100,000 substrate for Ca++/calmodulin-dependent protein kinase III (3)). The three other proteins phosphorylated by ACh are protein IIIa and protein IIIb (two synaptic vesicle-associated phosphoproteins of M_r=74,000 and 55,000 respectively (4)) and 87K (an acidic, 87,000 M_r substrate for protein kinase C (5)).

In cells prelabeled with $^{32}PO_4$, addition of ACh or elevated K^+ rapidly increased the phosphorylation of all five substrates in a calcium-dependent manner. In the continued presence of ACh, addition of EGTA rapidly diminished the phosphorylation of 100K, proteins IIIa and IIIb, and 87K, but not TH, in concert with a diminution of catecholamine release. Addition of forskolin increased the phosphorylation of TH, 100K, and proteins IIIa and IIIb, but not 87K, whereas addition of phorbol 12,13-dibutyrate increased the phosphorylation of TH, proteins IIIa and IIIb, and 87K, but not 100K.

The data suggest that several different protein kinase systems are activated by ACh stimulation. The data also raise the possibility that the phosphorylation of 100K, proteins IIIa and IIIb, and 87K may be involved in mediating some of the physiological effects of calcium on secretion.

[(1) Amy & Kirshner (1981) J. Neurochem. 36, 847-854; (2) Haycock et al. (1982) J. Biol. Chem. 257, 12632-12640; (3) Nairn et al. (1985) PNAS 82, 7939-7943; (4) Huang et al. (1982) J. Biol. Chem. 257, 6524-6528; (5) Wu et al. (1982) PNAS 79, 5249-5253]

228.4 PHOSPHORYLATION OF A 96 kDa SYNAPTOSOMAL PROTEIN BY PROTEIN KINASE C: INHIBITION BY BAY K 8644. P.J. Robinson* and W. Lovenberg* (SPON: S.H. Buck). Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

Protein phosphorylation is an important mechanism by which various hormones and neurotransmitters achieve their physiological responses. Protein kinase C (PKC) is one of four major protein kinase (PK) systems in brain and is regulated by calcium and phospholipids. There are several specific neuronal substrates for PKC. Most are localized in the cytosol, while two membrane substrates, termed B-50 and the "87K" protein, have been characterized. In this study we characterized the phosphorylation by PKC of several synaptosomal proteins and demonstrated an inhibition of phosphorylation by the calcium channel agonist BAY K 8644.

A crude synaptosomal fraction (P2 pellet) was prepared from rat striatum lysed by resuspension in hypotonic medium (containing 5 mM Tris pH 7.4, 5 µg/ml leupeptin, pepstatin and chymostatin, and 5 M PMSF) and centrifuged at 25000xg for 10 min to separate cytoplasmic and membrane fractions. Samples were incubated with 30 mM Tris pH 7.4, 1 mM Mg^{2+}, 1 mM EGTA, and 40 µM [γ-^{32}P]ATP, with or without 11 mM Ca^{2+} and 20 µg/ml phosphatidylserine (PS), and the solubilized proteins separated on polyacrylamide gels. Many proteins were phosphorylated in the presence of Ca^{2+}/PS in both membrane and cytoplasmic fractions. A phosphoprotein of 96,000 MW, termed P96, was localized to the cytoplasm, and was entirely dependent on both Ca^{2+} and PS for labeling. P96 was not phosphorylated in the presence of Ca^{2+} alone, Ca^{2+} and calmodulin, cAMP or cGMP, it thus appeared to be a specific substrate of PKC. P96 was maximally phosphorylated after 60 sec incubation and slowly dephosphorylated over the next 5 min.

A range of drugs that affect calcium channels were examined for effects on the labeling of P96. At 10 µM, verapamil and nifedipine had no effect on P96 or the labeling of any other proteins. However, BAY K 8644 caused a significant inhibition of P96 labeling at 10 µM, with 50% inhibition at 19 µM. BAY K 8644 also inhibited Ca/PS-dependent phosphorylation of other proteins (including B-50 and "87K"), but had no effect on calmodulin- or cAMP-dependent PK (except at > 100 µM). BAY K 8644 did not displace binding of tritiated phorbol esters in lysed P2 fractions. However, inhibition of PKC was increased as PS concentrations were reduced, suggesting that the mechanism of action of BAY K 8644 is competitive inhibition of PS interaction with PKC rather than an effect on the phorbol ester binding site. A possible relationship between PKC inhibition and activation of calcium channels by BAY K 8644 appears unlikely, but cannot be discounted.

228.5 PHORBOL ESTERS POTENTIATE RAPID DOPAMINE RELEASE FROM MEDIAN EMINENCE AND CAUDATE NUCLEUS SYNAPTOSOMES. C. Shu* and M. Selmanoff. Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

Recent evidence indicates that phorbol ester activation of protein kinase C in the synaptic terminal modulates subsequent neurotransmitter release. In the present study we investigated the ability of phorbol esters to potentiate Ca^{++}-dependent, depolarization-induced release of preaccumulated ^{3}H-dopamine (DA) from median eminence (ME) and caudate nucleus (CN) synaptosomes. Synaptosomes were prepared from ME and CN tissue dissected from adult male rats and were loaded by incubation in 0.1μM ^{3}H-DA for 30 minutes at 30°C. Fractional release was subsequently measured in a 5 second time interval using a rapid filtration technique.

Phorbol esters were dissolved and stored (2×10^{-2}M) in 100% dimethyl sulfoxide (DMSO). The final DMSO concentration used was 0.0005% which was not found to alter basal (5mM K^{+}_{o}) or evoked release. Phorbol esters potentiated DA release in a concentration-dependent manner with the potency series (determined at 10^{-7}M) being phorbol 12-myristate 13-acetate (PMA,TPA)=phorbol 12,13-dibutyrate (PDBu)>>phorbol 12,13-diacetate (PDA)=12-deoxyphorbol 13-isobutyrate (DPB)=12-deoxyphorbol 13-phenylacetate (DPP)=phorbol (PHR) being similar to that observed in other systems.

The ability of the potent phorbol ester PMA to augment K^{+}-evoked DA release was characterized at a concentration of 10^{-7}M. Preincubation of ME or CN synaptosomes for 10 minutes with PMA resulted in an increase in the K^{+}-stimulated, Ca^{++}-dependent release of ^{3}H-DA. The potentiation averaged 39% with a range of 24-70% between experiments. The potentiation seen with K^{+}(75mM)-evoked release increased as the external Ca^{++} was increased from 0.01 to 3.0mM. When release was studied at 0.2mM Ca^{++}_{o}, the phorbol potentiation was found to be depolarization-dependent in that a consistent phorbol effect was not seen at low (5-20mM) but was seen at higher (30-75mM) K^{+} concentrations.

These data support the hypothesis that protein kinase C activation potentiates the intrasynaptosomal stimulus-secretion coupling mechanism(s) and that both nigrostriatal and tuberoinfundibular dopaminergic nerve terminals are affected by phorbol esters in a similar manner. (Supported by NIH grant HD-21351 and RCDA NS-00731 awarded to MS).

228.6 DOPAMINE RELEASE FROM RABBIT PREFRONTAL CORTEX IS MODULATED BY D2 DOPAMINE AUTORECEPTORS. R.K. Talmaciu*, I.S. Hoffmann* and L.X. Cubeddu. Dept. of Pharmacology, School of Pharmacy, Central Univ. of Venezuela and Div. of Clin. Pharmacology, School of Medicine, Univ. of North Carolina, Chapel Hill, N.C. 27514.

Experiments were designed to investigate whether dopamine (DA) release from prefrontal cortex DA terminals was modulated by presynaptic DA receptors. Slices of rabbit prefrontal cortex were incubated for 30 minutes with 3H-DA (0.2 uM; SA: 26 Ci/mmol) in the presence of 10 uM desipramine. The latter drug was used to prevent uptake of DA into norepinephrine terminals. Slices were superfused at 1 ml/min with Krebs-Ringer bicarbonate solution (desipramine-free) for 60 min before the sample collection. Depolarization was evoked by electrical stimulation. Two stimulations were applied on each experiment (S_1 and S_2). The ratio 3H overflow evoked by S_2/S_1 averaged 0.94 for stimulations at 0.1 Hz. Nearly 3.5% of tissue 3H was released by S_1 at 1 Hz, 120 pulses. The tissue content at the end of the experiment averaged 64 ± 4 nCi/slice. A calcium-dependent overflow of 3H was elicited by stimulations at 0.3, 1 or 10 Hz (120 pulses). Comparable overflows were obtained at 0.3, 1 and 10 Hz, thus flat frequency-release curves were observed. Apomorphine and LY-171555 inhibited in a concentration-dependent fashion the stimulation-evoked overflow of 3H. Apomorphine EC50 and Emax for inhibition of 3H-DA release averaged 64 nM and 85% inhibition, respectively. The effects of these agonists were antagonized by haloperidol (30 nM). Haloperidol (30 nM) produced a 40-fold shift to the right of the apomorphine dose-response curve for inhibition of stimulation-evoked 3H-DA overflow. Haloperidol per se enhanced the stimulation-evoked overflow of 3H. Greater facilitation was observed at 10 Hz (+70%) than at 1 Hz (+30%). Stimulation frequencies comparable to normal firing rates of mesocortical neurons (10 Hz) markedly reduced agonist-induced inhibition of 3H overflow. Apomorphine (100 nM) inhibited DA release by 34% at 10 Hz and by 64% at 1 Hz. These results indicate that mesocortical DA neurons projecting to the prefrontal cortex have release modulatory receptors of the D2 subtype. Part of the reported reduced "in vivo" efficacy of DA agonists in inhibiting DA turnover and firing rates may be due to the higher firing rates of the mesoprofrontal DA neurons.

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228.7 PRESSURE EFFECTS ON SYNAPTIC INTEGRATIVE FUNCTIONS. J. J. Kendig and Y. Grossman. Dept. of Anesthesia, Stanford Univ. School of Medicine, Stanford, CA 94305 and Dept. of Physiology, Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel.

There is as yet no convincing mechanism to account for the hyperexcitability associated with the high pressure nervous syndrome (HPNS). Pressure in the range of tens of atmospheres causes convulsions, yet depresses excitatory synaptic transmission. The present experiments were designed to examine whether the synaptic integrative properties of inhibition, facilitation, summation and tetanic potentiation respond to pressure in a manner which could explain an increase in neuronal excitability. The deep extensor muscles from lobster (Homarus americanus or Panulirus interruptus) were isolated and arranged to permit selective stimulation of single motor neurons. Muscle cells were impaled by a 3 M K acetate-filled microelectrode mounted on a remote-controlled micromanipulator. The preparation was constantly perfused by appropriate oxygenated artificial sea water solution, maintained at 19°C, and enclosed in a hyperbaric chamber. Inhibition was determined by stimulating the common inhibitor (CIn) motor neuron and the L_2 excitor (L_2Ex) separately and together. Facilitation was examined by a paired-pulse protocol of stimuli to a single excitor, and summation by stimulating each of two excitors over a range of interstimulus intervals. Potentiation was examined by repeated trains of stimuli to a single excitor. After control recordings were made, helium was admitted to the chamber to provide hyperbaric pressure up to 100 atmospheres (atm). The results showed that pressure depressed the inhibitory postjunctional potential and the inhibition produced by it to approximately the same extent as the excitatory postjunctional potential. Selective pressure sensitivity of GABA-mediated inhibition thus can be ruled out as a candidate mechanism for HPNS. Pressure at 100 atmospheres significantly enhanced facilitation at interpulse intervals shorter than 40 msec. There was no significant effect on summation. Tetanic potentiation was significantly enhanced at both 50 and 100 atmospheres. Pressure-induced enhancement of synaptic efficacy through an increase in facilitation and potentiation can counteract the depressant effect of pressure on the singly evoked synaptic response. Under conditions of suitable neuronal circuitry and impulse pattern, this mechanism could contribute to the increase in neuronal excitability of HPNS.

228.8 THE EFFECT OF DIFFERENTIATION ON GUANYLATE CYCLASE ACTIVITY AND cGMP LEVELS IN PC12 CELLS. Erica Eugster*, Kathryn Sandberg and Terry B. Rogers, Dept. Biological Chemistry, University of Maryland School of Medicine, Baltimore, MD., 21201.

We are interested in the possible role of guanylate cyclase and cGMP in the mechanism of neurotransmitter release. Guanylate cyclase activity was measured in PC12 cells (a cell line established from a rat adrenal pheochromocytoma) that were grown under a variety of differentiation conditions. PC12 cells offer a particularly interesting model for this study, in light of their highly differentiated transmitter synthesis and release systems.

PC12 cells were grown under the following conditions; sparse undifferentiated, which were grown to a density of about 1×10^{4} cells/cm², dense autodifferentiated, which were grown to 8×10^{5} cells/cm²; and cholinergic differentiated, which were sparse cells cultured with 50ng/ml nerve growth factor (NGF) for 7-10 days. After culturing, total cell homogenates were prepared for each cell type and separated into soluble and particulate fractions by differential centrifugation at 150,000xg for 1 hr. Guanylate cyclase activity was determined by quantitating the formation of [^{32}P]cGMP from [α-^{32}P]GTP. Under the assay conditions used, guanylate cyclase activity was linear with respect to tissue (up to 30μg protein) and time (up to 30 min). Considerable enzyme activity was observed in all the soluble and particulate fractions; however, differentiated cells showed a marked decrease of activity in the particulate fractions. The specific activities of guanylate cyclase (pmol cGMP formed /mg of protein /min at 37°C) in soluble and particulate fractions were respectively: sparse- 375 and 244; dense- 483 and 139; NGF- 414 and 143. Guanylate cyclase activities in differentiated PC12 cells were similar to rat brain synaptosomes in that the latter preparations showed much higher specific activity in the soluble fraction (253) when compared to the particulate (115).

These studies suggest that guanylate cyclase may play a role in some differentiated systems in the PC12 cells. Accordingly, basal levels of cGMP were measured in differentiated PC12 cells. The results (in pmol cGMP/mg of protein) were: sparse-15; dense-2; and NGF-7. Under conditions shown to evoke the release of neurotransmitter in these cells (Sandberg and Rogers, Soc. Neurosci. Abstr. 12, in press), increases in cGMP levels have been observed. In cells treated with 100uM veratridine at 37°C for 5 mins we found increases above control levels ranging from 67% in sparse to 46% in dense and 69% in NGF. Further experiments are needed to explore the possible correlation between stimulated cGMP levels and evoked neurotransmitter release in this system.(Supported in part by USARMDC contract DAMD-17-86-C6160.)

228.9 MECHANISMS OF PRESYNAPTIC FACILITATION AT CRAYFISH OPENER NEUROMUSCULAR JUNCTIONS. S.Sivaramakrishnan and G.D.Bittner. Dept. of Zoology, Univ. of Texas at Austin, Austin, TX 78712.

The probability of transmitter released by a single impulse in a presynaptic axon is greatly increased if the impulse is preceded by one or more conditioning pulses. This increased probability of release is known as facilitation. Facilitation is a form of synaptic plasticity that has been observed at many chemical synapses in both peripheral and central nervous systems. It is well known (B.Katz & R.Miledi, J. Physiol., 1967, 192, pp 407-436), that the amount of transmitter released by a single impulse in the presynaptic terminal of an axon depends on the amount of calcium that enters the terminal during the impulse. It has been suggested (B.Katz & R.Miledi, J. Physiol. 1968, 195, pp 481-492), that a constant, small fraction of the calcium that enters during an impulse remains behind in the terminal. This 'residual calcium' is thought to combine with the calcium brought in by the next impulse leading to an increased probability of release (R.S.Zucker & L.O.Lara-Estrella, J. Gen. Physiol., 1983, pp 355-372 ; and M.P.Charlton, S.J.Smith & R.S.Zucker, J. Physiol., 323, pp173-193).

However, alternate theories have been proposed (D.A.Baxter & G.D.Bittner, Brain Res., 1981, 223, pp422-428 ; J.Dudel et al, Pflugers Arch., 1983, 399, pp1-10) to explain facilitation at the crayfish opener neuromuscular junctions. It has been claimed that the level of presynaptic depolarization may directly affect facilitation.

Proponents of both theories of facilitation in the crayfish have been hampered by a major experimental difficulty, namely the inability to measure presynaptic depolarization accurately. We have been able to overcome this drawback in the crayfish opener muscle.

As a prelude to an examination of the residual calcium theory at opener neuromuscular junctions in the crayfish walking leg, depolarization-release characteristics of the excitor axon have been examined. Two microelectrodes have been inserted into a secondary branch of the excitor axon and one electrode into a muscle fibre. In the presence of tetrodotoxin in the bath, the excitor axon is depolarized to different extents by passing artificial current pulses of 1 msec duration through one intracellular electrode. The second intracellular electrode measures the amount of depolarization in the axon. Post-synaptic release can be measured with a third electrode placed intracellularly in a muscle fibre close to the site of current injection in the axon.

In order to test the residual calcium hypothesis, the excitor axon is artificially depolarized with twin pulses and transmitter release measured intracellularly in a muscle fibre. The amount of facilitation that occurs in the presence and absence of external calcium is being determined.

228.10 NON-LINEAR BEHAVIOR OF THE GABA-MEDIATED INHIBITORY POST-SYNAPTIC CONDUCTANCE IN RAT HIPPOCAMPAL NEURONS IN CULTURE. J.L.Barker, N.L.Harrison* and S.Vicini[1]* (SPON: H.G. Wagner). Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892 and [1]FIDIA Georgetown Institute for the Neurosciences, 3900 Reservoir Rd. N.W. Washington D.C. 20007.

Embryonic rat hippocampal neurons grown in dissociated culture form functional synaptic contacts, the inhibitory synaptic transmitter being γ-aminobutyric acid (GABA) (M.Segal and J.L.Barker, J.Neurophysiol. 52: 469, 1984). We have studied the inhibitory post-synaptic currents (IPSCs) in these neurons under 'physiological' conditions of low internal $[Cl^-]$. The whole-cell patch-clamp technique was used to record from rat hippocampal neurons of cell body diameter 10-20μm; patch pipettes were filled with 145mM K gluconate. The bathing medium contained 4mM Ca^{2+}/8mM Mg^{2+}. IPSCs were evoked by stimulation of a presynaptic neuron, either by intracellular current injection or by pulses of 20μM L-glutamate to the cell body. We have made the following observations: 1. IPSCs had a rapid growth phase (2-3ms.) and decayed as a single exponential, having a time constant of 10-40ms.(mean 24ms at -40mV). 2. The IPSC reversed in polarity between -55 and -75mV (mean -69mV); this was coincident with the reversal potential for currents evoked by exogenously applied GABA. 3. IPSC decay time constant was voltage-sensitive in some of the recordings, increasing with depolarization. 4. Measurements of peak IPSC amplitude revealed non-linear behavior of the synaptic conductance (g_{IPSC}). When g_{IPSC} at a given membrane potential was expressed relative to that at -100mV (=1.0), g_{IPSC} at -40mV was 1.7 ± 0.2 and g_{IPSC} at -20mV was 2.5 ± 0.5 (mean ± S.E.mean, n=6-10 cells). The net effect of this voltage-dependent behavior would be to increase the efficiency of GABA-mediated inhibition at depolarized membrane potentials.

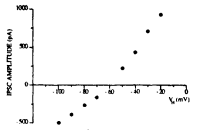

Fig.1 A plot of IPSC amplitude over a range of membrane potentials in one cell shows the marked non-linear behavior of the synaptic conductance.

228.11 DUAL ELECTRICAL RECORDINGS FROM SYNAPTICALLY COUPLED RAT HIPPOCAMPAL NEURONS IN TISSUE CULTURE G.D.Lange, V.Smallwood*,N.L.Harrison* and J.L.Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda MD 20892.

We have made long-term stable recordings from pairs of synaptically-coupled hippocampal neurons in dissociated tissue culture using the whole-cell recording technique. Patch pipettes were filled with (mM): 145 K gluconate or KCl, 1.1 EGTA/0.1 $CaCl_2$, 2 $MgCl_2$. In current-clamp or voltage-clamp recording from the postsynaptic element, postsynaptic potentials (PSPs) or currents (PSCs) were evoked by a single action potential in the presynaptic element. Inhibitory events were easily identified by virtue of their relatively long time-course and sensitivity to bicuculline and, in K gluconate recordings, by their reversal potential (-60mV). Both EPSCs and IPSCs could be evoked at low frequency (0.5Hz) for 30-40 minutes before appreciable 'run-down' of the synaptic signals became apparent. Synaptic transmission was depressed in low Ca^{2+}/high Mg^{2+} solutions, and failures of transmission were clearly apparent under these conditions (Fig.1), consistent with the 'quantal' hypothesis of synaptic transmission. The amplitude distributions of the synaptic signals under these conditions should allow us to estimate the quantal size for these two types of synapse in hippocampal culture. In addition, the ability to closely control presynaptic electrical activity will allow study of post-synaptic responses to diverse patterns of input in this simple model system, using analytical methods previously applied to invertebrate preparations (e.g. Krausz, Biol. Cybernetics 19: 217, 1975).



Fig.1 Failure of synaptic transmission at a GABAergic synapse. Two sequential records in 0.5mM Ca^{2+} are shown; in one the presynaptic action potential fails to evoke an IPSC. Such failures of transmission were not seen in high Ca^{2+}.

228.12 EFFECTS OF THE CALCIUM CHANNEL AGONIST BAY K 8644 ON SPONTANEOUS AND NERVE-EVOKED RELEASE OF TRANSMITTER AT THE NEUROMUSCULAR JUNCTION. S.M. O'Leary* and W.D. Atchison. Dept. of Pharmacology and Toxicology and Neurosciences Program, Michigan State University, East Lansing, MI 48824.

Whereas the effects of the dihydropyridine calcium channel agonist Bay K 8644 have been studied extensively on cardiac and smooth muscle, its effects on transmitter release from somatic motor nerve terminals remain to be elucidated. The goal of the present study was to characterize the potential effects of Bay K 8644 on transmitter release at the mammalian neuromuscular junction. Conventional intracellular microelectrode recording techniques were used to measure the effects of Bay K 8644 on miniature end-plate potential (MEPP) amplitude and frequency, and end-plate potential (EPP) amplitude from myofibers of the isolated hemidiaphragm of the rat. Muscle contractions due to electrical stimulation of the phrenic nerve were blocked using a modified "cut fiber" preparation. At a concentration of 750 nM, Bay K 8644 caused an increase in EPP amplitude within minutes of application into the bath. The increase in EPP amplitude was due to prejunctional effects since mean quantal content, as determined by the mean value method, was increased by Bay K 8644. Neither mean amplitude of MEPPs nor the amplitude distribution histograms were altered by Bay K 8644. Conversely, frequency of occurrence of MEPPs was increased following Bay K 8644 (650-750 mM). At these concentrations of the drug, MEPP frequency was elevated from 4-10 times pre-Bay K 8644 control values. Increased MEPP frequency typically occurred after 15-25 minutes of continuous exposure to Bay K 8644. Surprisingly, an increase in MEPP frequency in response to Bay K 8644 was also observed in 3 of 4 preparations treated with solutions to which no calcium was added. Results of the present study suggest that the calcium channel agonist Bay K 8644 is effective at increasing nerve-evoked and spontaneous quantal release of acetylcholine from the mammalian motor nerve terminal at concentrations less than 1 μM. Bay K 8644 was generously provided by Miles Institute for Preclinical Pharmacology. (Supported by NIH grants NS20683 and ES03299.)

228.13 THE EFFECT OF BayK 8644 ON SYNAPTIC TRANSMISSION BETWEEN MAMMALIAN SPINAL NEURONS IN CELL CULTURE. C. Yu* and P. G. Nelson. Lab. of Dev. Neurobiology, NICHD, NIH, Bethesda, MD 20892.

There is evidence from several systems that the agent BayK 8644 acts as a calcium channel agonist. It activates elements in the cardiovascular system and other tissues and enhances the probability of opening of one type of calcium channel (the L channel) in chick sensory ganglion neurons (Nowycky et al. Nature 316:440-443,1985). In view of this specificity for one species of calcium channel (BayK 8644 does not affect N or T calcium channel activation), we have evaluated the effect of BayK 8644 on synaptic transmission.

Mixed spinal cord (SC) and dorsal root ganglion (DRG) neuronal cell cultures were prepared from cells dissociated from 12-14 d. fetal mice. The cultures were about 4 weeks old when used in physiological experiments. Pairs of SC neurons were impaled and intracellular stimulation of one cell used to evoke monosynaptic responses in the other. We evaluated the effects on such responses of miniperfusion (from 3-5 μ pipettes) of BayK 8644 (1-5 μM) or nitrendipine (1 μM) vehicle control (DMSO corresponding to concentration present in the BayK 8644 solutions; this concentration of DMSO was also included in the bathing solution), high calcium solution (10-20 mM) and cobalt-containing solutions (10 mM). The bath solution contained 3 mM of calcium and magnesium. In other voltage-clamp experiments we confirmed that BayK 8644 did increase calcium currents in DRG neurons.

With most synaptically coupled SC-SC cell pairs (63%) BayK 8644 decreased the amplitude of the PSP or had no effect (17% of cell pairs tested). In particular, with inhibitory connection, 88% (15 out of 17) showed diminution, 1 of 17 no effect, and only 1 of 17 showed some increase. Nitrendipine, by contrast, which has little demonstrable effect on neuronal calcium channels, increased synaptic potentials in 43% of cases and in some of these the augmentaton was over 300%. On average in 28 cell pairs, NTP augments synaptic response less than 15%, a statistically non-significant amount. As expected, high calcium increases transmission substantially (69% augmentation by perfusing 10 or 20 mM calcium solution) and output was essentially blocked by cobalt.

Synaptic connections between different cell pairs were affected in a heterogeneous manner by BayK 8644 and NTP (although consistent effects were produced by calcium and cobalt). We hypothesize that different cell types may have different types of calcium channels involved in the transmitter release process.

228.14 TETANUS TOXIN INHIBITS THE EVOKED RELEASE OF ACETYLCHOLINE IN PC12 CELLS DIFFERENTIATED BY NERVE GROWTH FACTOR. Kathryn Sandberg and Terry B. Rogers, Dept. Biological Chemistry, University of Maryland School of Medicine, Baltimore, MD., 21201.

Tetanus toxin (T.T.) is a potent neurotoxin which binds selectively to neuronal tissue and inhibits release of neurotransmitters *in vivo*. In order to investigate the poorly understood mechanism of neurotransmitter release, T.T.'s effects were examined in a rat pheochromocytoma cell line (PC12). Previously, we have shown that T.T. binds to and is internalized by PC12 cells (Soc. Neurosci. Abstr., 11:1184, 1985). In order to examine T.T.'s effects on neurotransmitter release from PC12 cells, we have developed an acetylcholine (ACh) release assay that is a modification of a previously reported method (Green, L.A. and G. Rein. Nature 268: 349, 1977). PC12 cells, grown in 35 mm tissue culture dishes, were incubated overnight in choline free DMEM media containing [^{3}H]Choline (50 μCi/ml/dish). The following morning, these cells were preincubated with T.T., washed extensively and incubated under various conditions for 5 min. The supernatant was gently removed and microfuged for 5 min at 12000 x g. An aliquot of this supernatant was then incubated for 20 min in the presence of ATP (10 mM), $MgCl_2$ (10 mM) and choline kinase (0.01 units/ml) in order to convert all of the spontaneously released choline to phosphorylcholine. After the choline kinase reaction, the ACh was separated from the phosphorylcholine by an extraction with tetraphenylboron in 3-heptanone (10 mg/ml). The heptanone layer was reextracted with 0.4 N HCl to bring the ACh into the aqueous layer. This aqueous layer was then counted by liquid scintillation spectrometry. Typically, 1200 dpm of ACh were released per dish under depolarizing conditions (100 μM veratridine or 60 mM KCl) whereas only 400 dpm were spontaneously released in control dishes (Low Ca, High Mg).

We have found that T.T. (50 nM) inhibits evoked release by 90% but has little effect on spontaneous ACh release in PC12 cells which have been differentiated with nerve growth factor (NGF). At T.T. concentrations near the Kd (5.6 nM), this inhibition of ACh release occurred within the time frame of toxin binding to PC12 cells (3 hrs). Moreover, T.T. inhibited evoked release in 10 day old but not 5 day old NGF treated cells. Thus, it appears that the ability of T.T. to inhibit release depends upon the cell's state of differentiation. Horse anti-T.T. neutralized the inhibitory effects of T.T. which suggests T.T. inhibited ACh release in a manner pharmacologically analogous to the effects seen in vivo. Thus the PC12 cells offer an excellent system for studying the details of T.T.'s mechanism of action. Hopefully, these studies will provide insights into mechanisms of neurotransmission in general.

Support by USARMDC contract DAMD17-86-C6160.

228.15 PRESYNAPTIC ACTION OF HISTAMINE AT NICOTINIC CHOLINERGIC SYNAPSES IN MYENTERIC GANGLIA OF GUINEA-PIG SMALL INTESTINE. K. Tamura*, J.M. Palmer*, M. Schemann* and J.D. Wood (SPON: A. Humbertson). Dept. of Physiol., College of Medicine, Ohio State University, Columbus, Ohio 43210

Histamine is a putative neurotransmitter or modulator in the nervous systems of a variety of vertebrate and invertebrate species. There is evidence that histamine functions as a neurotransmitter in the cerebral ganglia of Aplysia (Mc Caman and Weinreich. 1982. J. Physiol. 328: 485-506); however, less is known about histaminergic actions in autonomic ganglia of vertebrates. The postsynaptic actions of histamine on myenteric neurons of the guinea-pig small intestine mimic slow synaptic excitation (Nemeth, Ort and Wood. 1984. J. Physiol. 355: 411-425). Presynaptic inhibitory actions of histamine were reported recently for an invertebrate species (Kretz et al. 1986. J. Neurophysiol. 55: 131-146). We now report on the presynaptic actions of histamine at nicotinic cholinergic synapses in Auerbach's plexus of the guinea-pig small intestine. Intracellular methods were used to record electrical behavior of myenteric ganglion cells in vitro. Fast excitatory post-synaptic potentials (EPSPs) were evoked by electrical stimulation of presynaptic fibers in the interganglionic connectives or by focal electrical stimulation applied at the ganglionic surface. Acetylcholine (Ach) or histamine were applied from fine pipettes by microejection with nitrogen pressure pulses of controlled amplitude and duration. Electrical stimulation evoked fast EPSPs in S/Type 1 and AH/Type 2 neurons. These were identified as nicotinic, cholinergic EPSPs by their susceptability to blockade by hexamethonium or curare. Application of histamine by pressure microejection or in the superfusion solution reversibly reduced or abolished the EPSPs in both types of neurons. Micropulses of Ach applied during histamine blockade of neurotransmission were identical to Ach responses in the absence of histamine. This suggested that the mechanism of synaptic blockade was presynaptic inhibition of the release of Ach. The presynaptic histaminergic receptors were unaffected by the H_1 antagonist, pyrilamine, and the H_2 antagonist, cimetidine. Dimaprit, which is a selective H_2 agonist, and 2-methylhistamine, which is a H_1 agonist, did not affect the EPSPs. This suggests that the presynaptic histaminergic receptors are neither the H_1 nor H_2 subtype. They appear to be of another subtype which remains to be identified for the enteric nervous system. (Supported by Tokai University Overseas Research Program stipend to K.T., NRSA AM07308 to J.M.P., DRG award Sche-267/1-1 to M.S., and NIH grant R01-AM26742 to J.D.W.)

228.16 THE SENSITIVITY OF AFTER HYPERPOLARIZATION IN RABBIT VAGUS NERVE TO THE TEMPORAL ORGANIZATION OF NERVE IMPULSES. D. Jeandupeux* and R.I. Birks. Physiology Department, McGill University, Montreal, Canada H3G 1Y6.

Synthesis and release of acetylcholine (ACh) in the cat superior cervical ganglion are markedly potentiated when tonic stimulation (2.5 Hz) is switched to a burst patterned (BP) form (40 Hz for 0.5 sec every 8 sec) (Birks, R.I., J. Physiol., Paris, 78, 417-419, 1982). ACh synthesis is also augmented when Na pump rate is increased (Birks, R.I., J. Physiol. 367, 401-417, 1985), and release is augmented when internal Na is increased (Birks, R.I., in preparation). If Na at the unmyelinated nerve terminals were to rise to a higher level during BP than during tonic stimulation this could account for the increase in ACh synthesis and release.

The question was approached using sucrose-gap recording from unmyelinated fibers of rabbit vagus nerve. In isethionate Locke, nerve activity is followed by a large after hyperpolarization (AHP) which consists of a slow electrogenic Na pump component and a fast gK component (Rang, H.P., & Ritchie, J.M., J. Physiol. 196, 183-221, 1968; Siegel, R.M., McGill University PhD Thesis, 1984). Since the amplitude of the pump component of the AHP is considered to be approximately proportional to internal Na (Thomas, R.C., Physiol. Rev. 52, 563-594, 1972), we compared the AHP following 3 min tonic and BP stimulation in nerves bathed in physiological chloride Locke solution at 37°C. In 12 experiments, in which 25 comparisons were made, the amplitude of the pump component, measured 20 sec after the stimulation, was 1.4 ± 0.06 (mean ± sem) times larger after BP than following tonic stimulation. The pump component following both types of stimulation was abolished by 1 mM ouabain.

In a second group of experiments the method developed by Rang and Ritchie (1968) to selectively activate the Na pump component of the AHP, while avoiding interference by the gK or accumulated K, was used. The nerves were stimulated as above, but in K-free Locke. One or five min after the end of the stimulation the solution was changed to normal Locke solution containing 5.6 mM K, which rapidly reactivated the Na pump to produce a transient hyperpolarization as the accumulated Na was extruded. In 7 experiments in which 20 comparisons of the K-activated response were made the amplitude of the response following BP was 1.3 ± 0.09 times that following tonic stimulation. Ouabain reversibly blocked the K-activated response. This evidence suggests that increased internal Na contributes to the potentiation by BP of ACh synthesis and release in ganglia.

228.17 ELECTRICALLY-INDUCED RELEASE OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVE MATERIAL FROM SLICES OF RAT CEREBRAL CORTEX. T.W. Vickroy and E.D. Cadman*. Abbott Laboratories, Neuroscience Research Division, Abbott Park, IL 60064

Cholecystokinin (CCK) is a family of bioactive "gut-brain" peptides which are present in unusually high concentrations within several forebrain structures. Previous studies with CNS tissues have demonstrated that a calcium-dependent efflux of CCK-like immunoreactivity (CCK-IR) occurs in response to chemically-induced depolarization. In the studies described here, a superfusion method has been developed in order to evaluate mechanisms which regulate electrically-evoked CCK-IR release from rat cortical slices.

Cerebral cortices from adult male albino Sprague Dawley rats were removed and slices were prepared and mounted between two copper electrodes in disposable polypropylene chambers. Superfusion of the slices with an oxygenated phosphate/bicarbonate-buffered salt solution containing peptidase inhibitors was carried out at 37°C and superfusate fractions (4 min.) were collected. Following a 30 min washout period, electrical field stimulations were applied to the slices by means of square wave pulses (i=2mAmp; duration=2msec; variable frequency) at t=6 min (S1) and approximately 30 min later (S2). Thereafter, slices were removed from the chambers and CCK-IR was extracted in 90% methanol as previously described (J. Neurochem. 42:1515, 1984). Quantitation of CCK-IR in superfusate fractions and tissue extracts was accomplished by radioimmunoassay and values are expressed as fractional release rates.

Initial studies with this technique demonstrate several similarities between the release of CCK-IR and that reported for other centrally active substances. The basal rate of CCK-IR release from rat cortical slices is stable and relatively low (fractional release rate = 0.05-0.1% / 3 min). In response to electrical field stimulation, the CCK-IR concentration in superfusate fractions increases in a frequency-dependent manner (7-12 fold elevation above baseline at 10 Hz) and release rates return to basal values following the stimulation period. CCK-IR release of similar magnitude is elicited during the S_2 period. Release (above baseline) does not occur during electrical field stimulation when the superfusion medium is replaced by a calcium-free medium containing EGTA.

In summary, this technique provides a reliable method for monitoring electrically-induced CCK-IR release from superfused slices of rat cerebral cortex. Preliminary studies indicate that the predominant form of CCK-IR which is released during depolarization is the sulfated octapeptide CCK-8. This procedure should be useful in delineating the mechanisms which regulate the neuronal release of this neuromodulatory peptide.

228.18 ARACHIDONIC ACID METABOLISM IN ISOLATED CEREBELLAR GLOMERULI. M.A. Schwartz* and R.V. Dorman* (SPON: D.M. Terrian). Dept. Biol. Sci., Kent State Univ., Kent, OH 44242.

Cerebellar glomeruli, including mossy fiber terminals, were isolated from rat cerebella, in order to determine the effects of membrane depolarization and calcium influx on the release of arachidonic acid from synaptic membrane phospholipids. The cerebellafrom 10 male Sprague-Dawley rats were pooled prior to isolation of the cerebellar glomeruli by differential centrifugation. The purified glomeruli fraction was labeled in vitro with [^{3}H]arachidonate prior to exposure to 45 mM K^+, in the presence and absence of external calcium. The prelabeled glomeruli were also exposed to 5 μM A23187 in the presence and absence of calcium. Total lipids were extracted following 15 min incubations at 37°C and the free fatty acids were isolated by thin-layer chromatography.

There was an 88% increase in free [^{3}H]arachidonate in preparations incubated for 15 min in Krebs-Ringer buffer containing 5 mM KCl. The labeling of the free fatty acid pool increased to 190% over 0 time controls when the K^+ concentration was increased to 45 mM. This depolarization-evoked accumulation of arachidonate was completely prevented when Ca^{2+} was omitted from the incubation medium. The involvement of calcium in arachidonate release was further evidenced by the inclusion of the Ca^{2+} ionophore A23187 to incubations that contained 5 mM K^+, since there was an increase in free arachidonate that was dependent on the concentration of external calcium. We suggest there is a correlation between Ca^{2+} influx, accumulation of unesterified arachidonate and neurotransmitter release mechanisms in the mossy fiber terminals isolated from cerebellar cortex.

This work was supported by AFOSR grant #86-0045.

228.19 AMMONIA DECREASES EXCITATORY SYNAPTIC TRANSMISSION IN CAT SPINAL CORD. W. Raabe. Depts. Neurology, VA Med. Ctr. and Univ. of Minnesota, Minneapolis, MN 55417.

Previously, it was reported that ammonia intoxication depresses the spinal monosynaptic reflex (Neurosci. Abstr. 11:855, 1985). The current study investigates the mechanisms by which ammonia affects excitatory synaptic transmission in the spinal monosynaptic reflex. Cats, anesthetized with pentobarbital and paralyzed with gallamine triethiodide were used. Extracellular recordings were obtained from the L7 or S1 dorsal roots, the cut L7 or S1 ventral roots and from motoneuron nuclei. Intracellular recordings were obtained from motoneurons. The nerves to the PBST, MG and LG muscles as well as the L7 or S1 ventral roots were stimulated at 0.5 to 1 Hz. Ammonium acetate (NH_4Ac), 20% solution in H_2O, adjusted to pH 7.4, was given i.v.

NH_4Ac i.v. had no significant effect on the resting membrane potential, the rheobase and the antidromic action potential of motoneurons. NH_4Ac decreased the intracellularly recorded EPSP. The EPSP decreased after a shift of equilibrium potential of the IPSP to the resting membrane potential, i.e. after the inactivation of Cl^--extrusion by NH_4^+. NH_4Ac i.v. also decreased the motoneuron pool EPSP recorded from the ventral root and the EPSP part of the focal synaptic potential recorded extracellularly from a motoneuron nucleus. When NH_4Ac decreased the motoneuron pool EPSP recorded from the ventral root, the effects of glutamate iontophoresis on the extracellularly recorded antidromic motoneuron spike were unchanged. When NH_4Ac decreased the EPSP in the focal synaptic potential, the spike generated by the invasion of presynaptic terminals of Ia afferents was unchanged or slighly increased.

These observations suggest that NH_4^+ decreases excitatory synaptic transmission by an action at a presynaptic site, either by inhibition of the release of transmitter or by depletion of transmitter. NH_4^+ inhibits glutaminase, the key enzyme for the utilization of glutamine as a precursor for (transmitter-) glutamate. NH_4^+ also inhibits the Na^+-dependent uptake of glutamate into synaptosomes (Exp. Neurol. 89:259, 1985). By either one or both of these mechanism, NH_4^+ may deplete Ia afferents of their presumed transmitter glutamate and decrease excitatory synaptic transmission from Ia afferents to motoneurons.

The observation that NH_4Ac i.v. affects the equilibrium potential of the IPSP before affecting the EPSP indicates that NH_4^+ tissue levels significantly higher than 1 mM are required to decrease the EPSP (cf. Brain Res. 303:67, 1984).

229.1 HETEROGENEITY OF OPIOID RECEPTORS IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS. S.M.I. Kazmi*, L.K. Srivastava* and R.K. Mishra, Neuropharmacology Laboratory, Depts. of Psychiatry & Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Receptor binding studies with relatively selective and highly potent opioid agonists, coupled with their differential pharmacological action, have established the multiplicity of opioid receptors (μ,δ,κ) in brain and a single class of enkephalin (δ) receptor in mouse neuroblastoma and mouse neuroblastoma x rat glioma hybrid (NG108) cells. Recent studies with human neuroblastoma cell lines derived from metastases of peripheral primary neuronal tumors have indicated the existence of functional opioid receptor sites, which may not be essentially homogeneous (Sonnenfeld et al., Anal. N.Y. Acad. Sci., 435, 208, 1984; Yu et al., J. Biol. Chem. 261, 1065, 1986). In the current study, we have characterized the opioid binding sites in human neuroblastoma SH-SY5Y cells using various ligands showing selectivity for separate types of opioid receptors.

Saturation binding experiments with (^{3}H)-(2-D-Penicillamine, 5-D-Penicillamine)-enkephalin (D-Pen^2-D-Pen^5, δ-selective) and (^{3}H)-dihydromorphine (μ-selective) showed high affinity saturable binding to SH-SY5Y cell membranes, which could be readily displaced by unlabelled DTLET (δ) and FK-33824 (μ), respectively. Scatchard analysis of the binding data revealed the equilibrium dissociation constant (K_D) and receptor density (B_{max}) as 1.4 $\pm$ 0.4 nM and 85 $\pm$ 12 fmoles/mg protein for (^{3}H)-D-Pen^2-D-Pen^5, and 1.7 $\pm$ 0.3 nM and 118 $\pm$ 16 fmoles/mg protein for (^{3}H)-dihydromorphine. The proportion of different types of opioid binding sites was estimated utilizing the selective inhibition of (^{3}H)-diprenorphine binding by D-Pen^2-D-Pen^5, D-Pro^4-morphiceptin and U-50488H. Various combinations of the three specific ligands yielded 55%:45% population of μ and δ sites, U-50488H (κ-selective) was relatively ineffective. FK-33824 and naloxone appear to have higher affinity than DTLET and D-Pen^2-D-Pen^5 in displacing (^{3}H)-diprenorphine (0.5 nM) binding to SH-SY5Y cell membranes. Guanine nucleotides and sodium chloride significantly reduced the potency of μ and δ-selective agonists in displacing (^{3}H)-diprenorphine binding. The observed heterogeneity of opioid receptors with the characteristic regulation by guanine nucleotides and Na^+ should offer a suitable model system to study the cellular and molecular mechanisms of the action of opioids.

Supported by N.C.I. Canada.

229.2 ANGIOTENSIN II RECEPTORS IN THE LUMBAR SPINAL CORD OF THE RAT. J.D. Penner*, S.R. White and R.C. Speth (SPON: R.J. Bull). Dept. of Vet. Comp. Anat., Pharmacol., Physiol., Washington State Univ., Pullman, WA 99164.

Angiotensin II-like immunoreactivity has been found in fibers in the substantia gelatinosa region of the rat spinal cord by several investigators (Ganten et al., Frontiers in Neuroendocrinology, 1978; Brownfield et al., Neuroscience, 1982; Lind et al., Neuroendocrinology, 1985) and in terminals outlining large somata in the human ventral spinal cord (de Lanerolle & Coen, Soc. Neurosci. Abstr., 1984). In isolated amphibian spinal cords, bath application of angiotensin II (AII) caused weak depolarization of both dorsal and ventral roots (Phillis & Kirkpatrick, Can. J. Physiol. Pharmacol., 1979). In the present study, we examined the lumbar spinal cord of the rat for the location of AII receptor binding sites detected by autoradiography and for the location of cells responsive to iontophoretically applied AII.

Twenty µm thick horizontal sections of rat lumbar spinal cord were mounted on microscope slides and incubated with [^{125}I]-Sar^1,Ile^8-AII for 60 min, dried and apposed to LKB Ultrofilm for a 3-9 day exposure period. Control sections were incubated in media containing an excess of unlabeled AII. Specific binding of [^{125}I]-AII was intense in the most dorsal region of the dorsal horn (primarily laminae I and II). Autoradiographic grain densities in the remaining portions of the lumbar spinal cord gray matter were low but detectably higher than in the surrounding white matter or in adjacent sections incubated with excess unlabeled AII.

In laminae I-III of the spinal cord, iontophoretically applied AII (45-90 nA, 30-90 sec) facilitated the excitability of all cells tested (25). More ventrally in the dorsal horn (laminae IV and V) AII facilitated excitability of 70% (14/20) of the cells examined. (Several cells in the dorsal horn were excluded from the analysis because current application to the pH 4 control solution also enhanced the excitability of the cells). In the ventral horn, AII (30-90 nA, 45-90 sec) enhanced glutamate-evoked firing of nearly every motoneuron tested (13/14). These results indicate that although AII receptor binding detectable by autoradiographic techniques is concentrated within the dorsal part of the dorsal horn of the spinal cord, cells that respond to local application of AII are found in abundance in both the dorsal and the ventral horn. (Supported by the WSU Research and Arts Committee.)

229.3 SOMATOSTATIN (SRIF) INHIBITION OF BASAL AND BIOGENIC AMINES, VASOINTESTINAL POLYPEPTIDE (VIP) AND FORSKOLIN STIMULATED ADENYLATE CYCLASE ACTIVITY IN DIFFERENT BRAIN AREAS. T.Florio*,O.Meucci*, E.Landolfi*,M.Grimaldi*,G.Magri* and G.Schettini. (SPON: M.Cronin) Department of Pharmacology,II School of Medicine, University of Naples, via Pansini 5, 80131 Naples, ITALY.

SRIF receptors have been characterized in different brain areas. Their coupling with adenylate cyclase (AC) activity has been shown in primary culture of neuronal and glial cells, and anterior pituitary cells, while less described is their action on AC activity in the brain. Given the growing bulk of evidence of SRIF involvement in regulating brain functions, we have undertaken the present study to characterize the SRIF effect on AC activity in frontal cortex (FC), hippocampus (HIPP), striatum (ST) and hypothalamus (HYPO), brain areas deputated to regulate different cerebral functions.

SRIF inhibited basal AC activity in all brain regions studied showing the highest degree of inhibition at 100 uM concentration (-42% FC, -35% ST, -28% HYPO, -24% HIPP). In basal conditions was possible to show a dose depedent (100 nM to 100 uM) inhibition of AC activity only in ST tissues. Both norepinephrine (NE) (10 uM) and dopamine (DA) (10 uM) stimulated AC activity in the brain regions studied. While in ST, HIPP and HYPO tissues, SRIF (1 uM to 100 uM) showed a slight dose dependent inhibition of both NE or DA stimulated AC activity, in FC tissue, the slope of the dose response curve of SRIF inhibition was more stip and pronounced. SRIF (100 uM) also inhibited VIP (1 uM) stimulation of AC activity in all brain areas studied, being less effective at the lower concentrations (1 and 10 uM) tested. The gratest degree of SRIF inhibition of AC activity was observed under forskolin stimulated conditions. SRIF (100 uM) reduced about of 50% forskolin (10 nM to 10 uM) stimulation of FC and HYPO AC activity, and of 20% HIPP AC activity, showing a non competitive pattern of inhibition.

These results show that brain SRIF receptors are coupled with AC activity, which may represent the post-receptor mechanism regulating SRIF-controlled brain functions.

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229.4 A COMPARISON OF THE STIMULATION OF INOSITOL PHOSPHOLIPID HYDROLYSIS AND OF CYCLIC GMP FORMATION BY NEUROTENSIN AND ITS ANALOGS IN NEUROBLASTOMA CLONE N1E-115. K. S. Kanba* and E. Richelson. The Departments of Psychiatry and Pharmacology, Mayo Clinic and Foundation, Rochester, MN 55905.

Neurotensin, a tridecapeptide originally isolated from bovine hypothalamus (Carraway and Leeman, J. Biol. Chem. 248:6854, 1973), is a putative neurotransmitter and has various biological activities in the central nervous system. Neurotensin stimulates not only the formation of intracellular cyclic GMP (Gilbert and Richelson, Eur. J. Pharmac. 99:245, 1984) but also inositol phospholipid metabolism (Snider et al., manuscript submitted for publication) in neuroblastoma clone N1E-115 cells. Although it has been reported that many neurotransmitters which stimulate cyclic GMP formation also stimulate inositol phospholipid hydrolysis, the relationship between these two biochemical events is unclear. We determined the EC_{50}'s for neurotensin and several related peptides at stimulating inositol phospholipid hydrolysis and at stimulating intracellular cyclic GMP formation in neuroblastoma clone N1E-115 cells under very similar experimental conditions.

Neurotensin(8-13) and acetylneurotensin(8-13) had the highest potencies for the stimulation of the hydrolysis of inositol phosphates, which were about 3 times as potent as neurotensin (EC_{50}=0.9 nM). On the other hand, fragments of the amino-terminal portion of neurotensin, such as neurotensin(1-6), neurotensin(1-8) and neurotensin(1-11) had no ability to stimulate this hydrolysis. These results support our previous study (Gilbert et al., Biochem. Pharmac. 35:391, 1986) in which several neurotensin analogs were compared for their ability to inhibit [^{3}H]neurotensin binding and stimulate intracellular cyclic GMP formation in intact N1E-115 cells and other structure-activity studies of neurotensin in various species in terms of receptor binding, pharmacology, electrophysiology and behavior; that is, the carboxy-terminal portion of neurotensin played an important role in its biological activities.

A strong correlation (r^2=0.97) was obtained between the EC_{50}'s for neurotensin and several analogs in the stimulation of the release of inositol phosphates and the EC_{50}'s for the peptides in the stimulation of cyclic GMP formation in neuroblastoma clone N1E-115 cells. These two different biochemical effects of neurotensin and its analogs appear to be mediated by the same receptor site. (Supported by Mayo Foundation and U.S.P.H.S. grant MH27692.)

229.5 NEUROBLASTOMA MODEL SYSTEM FOR STUDYING VASOPRESSIN RECEPTOR FUNCTION. P. Davies*,[1] R. E. Brinton,[2] and B. S. McEwen.[2] (SPON: C. S. RAINE). Departments of Pathology and Neuroscience,[1] Albert Einstein College of Medicine, Bronx, NY, 10461, and Laboratory of Neuro-endocrinology,[2] Rockefeller University, New York, NY, 10021.

We are interested in developing neuronal model systems to investigate the effector mechanisms of vasopressin receptors in brain. Towards that end, we explored the feasibility of using human neuroblastoma cell lines. In a clone of the cholinergic cell line, SK-N-MC-IXC, we found a direct stimulation of adenylate cyclase by arginine vasopressin (AVP). This stimulation was both concentration and time dependent. At a concentration of 500 nM AVP cyclic AMP accumulation was 50% above baseline levels while 1μM AVP produced a greater than 100% increase. Stimulation of adenylate cyclase increased sharply within 5 min and reached asymptote at 15 min with cyclic AMP levels declining following 30 min of incubation. The vasopressin analogs Phe^2Orn^8VT and VDAVP increased cyclic AMP levels with approximate epipotency while dVDAVP had no effect.

CONDITION	pmole cyclic AMP/mg protein/ml MEAN ± S.D.
Baseline	115 ± 7
10 nM AVP	113 ± 7
100 nM AVP	75 ± 5
250 nM AVP	69 ± 5
500 nM AVP	169 ± 30
1 μM AVP	232 ± 13
50 nM Phe^2Orn^8VT	164 ± 11
100 nM Phe^2Orn^8VT	163 ± 31
100 nM VDAVP	144 ± 34
500 nM VDAVP	163 ± 31
250 nM dVDAVP	112 ± 0

Initially we explored whether the AVP receptor modulated norepinephrine stimulated cyclase. These experiments showed that both NE and AVP induced accumulation of cyclic AMP and that when present in combination the accumulation of cyclic AMP was additive.

The ability of vasopressin to induce accumulation of cyclic AMP is strong evidence in support of a V_2 type receptor and is consistent with the findings of Abe, et al. (J. Physiol.; 337:665-685, 1983) for the existence of a vasopressin receptor in brain which is directly coupled to adenylate cyclase. We are currently exploring the existence of V_1 type AVP receptors in other human cell lines.

We thank M. Manning for generous gift of AVP and AVP analogs, and J. L. Beidler for providing the cell line for this study. Research supported in part by NIH-AG#02478 to P.D., and by a NRSA (MH#09224) to R.E.B.

229.6 ONTOGENIC CHANGES IN VASOPRESSIN BINDING SITE CHARACTERISTICS IN RAT BRAIN: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. F. Petracca*, D. Baskin, J. Diaz, and D. Dorsa (SPON: W. Stahl). GRECC, VA Medical Center, and Depts. of Pharmacology, Psychology, Biological Structure and Medicine, Univ. of Washington, Seattle, WA 98195

We have previously reported that the distribution of ^{3}H-arginine^8-vasopressin (^{3}H-AVP) binding sites in the Long-Evans rat brain changes markedly during postnatal development. Several regions of the neonatal rat brain, including the cingulate gyrus, exhibit intense AVP binding which does not persist into adulthood. This transient AVP binding is saturable and demonstrates specificity for neurohypophyseal peptides. The aim of the present study was to measure the affinity, number, and specificity of AVP binding sites in the developing rat brain.

For saturation studies, day 7 Long-Evans rat pups were sacrificed, and 10-micron sections of brain were cut at the level of the cingulate gyrus and dorsal hippocampus. The slide-mounted sections were incubated in Tris buffer solution containing concentrations of ^{3}H-AVP (40 Ci/mmol) ranging from .5 nM to 10 nM, in the presence or absence of 1 μM unlabeled AVP. For displacement studies, serial sections of day 7, day 8, and day 12 Long-Evans pup brains were incubated in buffer solution containing either 2.5 nM ^{3}H-AVP alone or in the presence of unlabeled AVP, oxytocin (OXY), arginine-vasotocin (AVT), luteinizing hormone releasing hormone (LHRH), or vasopressin pressor antagonist [1-(β-mercapto-β-, β-cyclopentamethylene proprionic acid), 2-(O-methyl) tyrosine]-arginine^8-vasopressin (M-AVP). Concentrations of unlabeled peptides tested ranged from 10^{-9} M to 10^{-5} M. Slides were apposed to LKB Ultrofilm for 30-90 days. Films were developed and analyzed by computer densitometry.

Saturation analysis in the cingulate gyrus resulted in a linear Scatchard plot, indicating a single class of sites with a K_D of 1.7 nM, and B_{max} of .1 fmol/mm^2 of tissue. Specificity studies in the cingulate gyrus resulted in the following rank order of potency in displacement of ^{3}H-AVP: OXY = AVT > M-AVP = AVP >> LHRH.

Data from saturation analyses were consistent with previously reported values for AVP binding in regions of the adult brain. However, the relatively high potency of OXY in displacing ^{3}H-AVP in the neonatal brain suggests that specificity of this developmental site is different from that reported in the adult brain. Thus, it may be that vasopressin receptor specificity, as well as anatomical distribution, changes over the course of development.

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229.7 LIGHT MICROSCOPIC AUTORADIOGRAPHIC LOCALIZATION OF VASOPRESSIN PRESSOR ANTAGONIST BINDING SITES IN THE RAT BRAIN, PITUITARY AND KIDNEY. F.W. van Leeuwen, E.M. v.d. Beek*, J.J. van Heerikhuize* and Y.P. Wan[1]. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands; [1] Dupont-NEN. Boston, MA, USA.

At present arginine vasopressin (AVP) meets nearly all criteria to be considered as a neurotransmitter. One of the last criteria to be fulfilled was the demonstration of specific receptors in the brain in close proximity to synaptic structures. Lately intense AVP labelling was found in the lateral septum, central amygdala, ventral hippocampus, nucleus of the solitary tract, olfactory nucleus and neural lobe (for references see 1). For several reasons including the fact that a potent pressor antagonist, $d(CH_2)_5Tyr(Me)AVP$, displayed a high affinity for central AVP binding sites, it was concluded that AVP binding sites in certain brain areas (e.g. the ventral hippocampus) resemble the V_1 vascular receptor type (1). [^{3}H]AVP will most probably label both AVP and oxytocin binding sites (2). Therefore, a more selective label is needed. The pressor antagonist $d(CH_2)_5Tyr(Me)AVP$ partly meets this need. Recently this antagonist was tritiated (S.A. 56.2 Ci/mmol) and light microscopic autoradiography was performed on a number of tissues known to contain V_1 or V_2 receptors.

The tritiated pressor antagonist selectively labelled a large number of brain areas. Dense labelling was obtained in the lateral septum, nucleus accumbens, anterior amygdala, rostral part of the central amygdala, dorsal hippocampus (gyrus dentatus) and nucleus of the solitary tract. Moderate labelling was obtained in the suprachiasmatic and dorsomedial hypothalamic nucleus and ventral hippocampus. Labelling was absent in the olfactory nucleus, ventromedial nucleus and neural lobe. In areas known or assumed to contain a different AVP receptor type no (supraoptic nucleus, adenohypophysis) or only minimal (kidney) labelling was seen.

The labelling of the dorsal hippocampus is intriguing since this area is hardly innervated by AVP fibres. This mismatch of fibres and binding sites can be explained in various ways (1). One of the explanations is that the immunocytochemical methods applied for the visualization of AVP fibres could still not be sensitive enough. Indeed the innervation areas of various AVP synthetizing nuclei (e.g. the locus coeruleus) have not yet been determined.

In conclusion, it seems possible to obtain a more selective labelling of AVP binding sites with the use of the tritiated pressor antagonist.

(1) Van Leeuwen, F.W., In: Vasopressin, principles and properties (Gash, D.M. and Boer, G.J., eds.), Plenum Press, in press.
(2) Audiger, S., and C. Barberis, EMBO Journal 4, 1407-1412, 1985.

229.8 SPECIFIC BINDING OF ^{125}I-desaminoTYR-PHE-nLEU-ARG-PHE-amide TO FMRFamide RECEPTORS IN THE BRAIN AND HEART OF HELIX ASPERSA. K. Payza* and M. J. Greenberg. (SPON: D. A. Price). Whitney Lab., Univ. of Florida, St. Augustine, FL 32086.

We have synthesized an analog of FMRFamide, ^{125}I-desaminoTyr-Phe-nLeu-Arg-Phe-NH_2 (^{125}I-daYFnLRFamide), that binds reversibly and specifically to receptors in total particulate suspensions of neural and cardiac tissues taken from the pulmonate snail, Helix aspersa. At equilibrium, specific binding is directly proportional to tissue concentration. Scatchard analyses show an apparent K_D of 10-20 nM in both heart and brain preparations, and a B_{max} of 100-200 fmoles per mg protein.

Several cardioexcitatory tetrapeptides displace bound radioligand: FMRFamide, FLRFamide, D-Phe-MRFamide, and acetyl-FnLRFamide have IC_{50}s close to 1 μM, whereas daYFnLRFamide and daYFLRFamide are more potent (IC_{50}s at about 20 nM).

The endogenous heptapeptides of pulmonate gastropod molluscs (pGlu-DPFLRFamide, GDPFLRFamide and SDPFLRFamide) are weaker displacers than the tetrapeptides, so they may be acting at a different receptor site. Neither Met-enkephalin, nor naloxone (100 μM), nor other peptides unrelated to FMRFamide, displace the radioligand.

We have developed an isolated, perfused preparation of the Helix heart which continues beating and remains pharmacologically responsive for more than 72 hours. We have used this preparation to correlate receptor binding with biological activity. For example, the tetrapeptide acetyl-FnLRFamide is a very efficacious excitatory agonist on the Helix heart. Its biological dose-response curve is parallel to its radioligand diplacement curve (Hill coefficient = 0.9), but is shifted one log-unit to the left. This shift implies the existence of spare receptors, or of an amplification step between receptor occupancy and the physiological response.

This demonstration of FMRFamide receptor-binding contributes to our understanding of the mechanisms underlying the pharmacological actions of the FMRFamide-related peptides. (Supported by NIH grant HL-28440.)

229.9 BRAIN OPIATE RECEPTOR BINDING IN DEVELOPING SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. M.D. Carrithers* and J.A. Weyhenmeyer, (SPON: A. Appelbaum) Neural and Behavioral Biology Program and College of Medicine, University of Illinois, Urbana, Il., 61801.

It has been suggested that opiates are involved in the central regulation of blood pressure; however, the exact role of the opioid peptides in this control system has not been elucidated. Their effects appear to be dependent on several factors, including the anatomical site and the type of ligand used. Understanding of these mechanisms has been complicated by the existence of multiple opiate receptor subtypes. The purpose of this study was to investigate the binding characteristics of opiate receptors in the developing spontaneously hypertensive (SH) rat and its normotensive control, the Wistar Kyoto (WKY) rat.

Male SH and WKY rats were sacrificed by decapitation at four, eight, and sixteen weeks of age while under CO_2 anesthesia. Whole brain minus the cerebellum was removed at 4°C. Tissues were homogenized in approximately ten volumes of 50 mM Tris buffer, pH 7.4, and centrifuged x 2 at 50,000 x g for 30 min. The final pellet was resuspended in Tris buffer. Homogenates were incubated in the presence of .08 to 10 nM of tritiated diprenorphine, a general opiate antagonist. Naloxone (10 µM) was used as a cold inhibitor to determine nonspecific binding. Membranes were incubated for 60 min at 25°C and then filtered under vacuum. Filters were washed twice with 5 ml of ice cold buffer, dissolved in a toluene-Triton based scintillation fluid, and counted with an efficiency of approximately 48%.

In all groups, linear Scatchard binding was observed in the concentration range of tritiated diprenorphine used. There was no significant difference between strains in the dissociation constant (K_d) at any age or in the binding capacity (B_{max}) at 4 and 8 weeks of age. However, an elevated binding capacity was observed in the 16-week old SH rat as compared to its normotensive age-matched control (WKY). These results suggest that central opiate regulatory systems may play a role in the maintenance of hypertension in the SH rat.

This work was supported by NIH HL27757 and Amer. Heart Assoc. (IL) grants to J.A.W. and a March of Dimes fellowship to M.D.C.

229.10 REDUCTION OF CCK-8 BINDING IN THE NUCLEUS OF THE SOLITARY TRACT OF CAPSAICIN PRETREATED RATS. E.E. Ladenheim, R.C. Speth and R.C. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843.

High affinity binding sites for cholecystokinin (CCK) have been detected in the nucleus of the solitary tract (NST), area postrema (AP) and spinal trigeminal complex (SP5) (Zarbin et al., 1983). These structures receive the terminals of small, unmyelinated, primary sensory neurons which are destroyed by the neurotoxin, capsaicin (Dinh and S. Ritter, 1985). To investigate the possibility that CCK binding is located on capsaicin sensitive sensory terminals, we have examined high affinity binding of ^{125}I Bolton-Hunter labeled sulfated CCK octapeptide (CCK-8) in the brains of capsaicin-treated and control rats. Adult male rats were anesthetized and treated intraperitoneally with natural or synthetic capsaicin or the injection vehicle, according to previously reported procedures (Ritter and Ladenheim, 1985; South and Ritter, 1985). At least 30 days after capsaicin treatment the animals were anesthetized with pentobarbital and perfused with ice cold 0.9% saline. Brains were removed and frozen, and 20 µm coronal sections were cut in a cryostat at -20°C. Brain sections were thaw mounted to slides and incubated with 44-88 picomolar ^{125}I CCK-8 in the presence or absence of 200 nanomolar unlabeled CCK-8. The sections were exposed to LKB Ultrofilm for 5 - 9 days after which the tissue sections were stained with thionin. Visual inspection and microdensitometric measurements of brains from vehicle-treated rats indicated that the distribution of CCK-8 binding was similar to patterns previously reported for normal animals using Bolton-Hunter ^{125}I CCK-33 (Zarbin et al., 1983). This binding included intense labeling of the AP, and moderate labeling of the NST and parts of SP5 (Moran, 1985). In capsaicin-treated rats, however, CCK-8 binding in the AP and medial NST was virtually eliminated, while binding in SP5 was minimally reduced. These results suggest that NST CCK-8 receptors may be located on capsaicin-sensitive primary sensory terminals. The CCK-8 receptors in SP5 presumably are located on terminals or cell bodies which are not damaged by capsaicin. Our data suggest the possibility that central, presynaptic CCK receptors may modulate the activity of some vagal afferent neurons. Supported in part by DHHS grants NS20561 and NS21805.

229.11 **CROSS-LINKING OF ATRIAL NATRIURETIC PEPTIDE TO BINDING SITES IN RAT OLFACTORY BULB MEMBRANES.** G.M. Wildey* and C.C. Glembotski. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Binding sites for [^{125}I]-atrial natriuretic peptide (ANP) in rat olfactory bulb membranes have been studied using pharmacological and biochemical methods. Various unlabeled ANP-related peptides were tested for the ability to inhibit the binding of the radioligand in membrane binding assays. ANP(92-126) and ANP(99-126) were the most potent inhibitors tested, both exhibiting an IC_{50} value of 0.40 nM. ANP(103-126) and ANP(103-123) were 3-times and 70-times less potent, respectively. ANP(111-126) was unable to inhibit the binding of the radioligand at a concentration of 1 µM. Several peptides unrelated to ANP were unable to inhibit the binding of the radioligand to rat olfactory bulb membranes. Membranes labeled with [^{125}I]ANP were incubated with cross-linking agents and subjected to SDS-PAGE followed by autoradiography. A band possessing an apparent molecular mass of 116 kDa was identified. The labeling of this band was progressively decreased by increasing concentrations of unlabeled ANP(99-126) (IC_{50} = 0.6 nM) and by several other ANP-related peptides at nanomolar concentrations. These results indicate that the ANP binding site in rat olfactory bulb membranes displays pharmacological and biochemical properties similar to peripheral ANP receptors. Supported by American Heart Grant-in-Aid 84 653.

229.12 INSULIN RECEPTORS IN THE PERIPHERAL NERVOUS SYSTEM: A FUNCTIONAL AND STRUCTURAL ANALYSIS. Robert J. Waldbillig and Derek LeRoith, Diabetes Branch, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, MD 20892

Unlike the vasculature of the CNS and peripheral nerve trunks, capillaries of peripheral nervous system ganglia are fenestrated. This raises the possibility that autonomic and sensory ganglia may be exposed to blood-borne insulin. Despite this, there has been no examination of this tissue for insulin receptors. The purpose of this study therefore, was to characterize insulin binding in bovine autonomic and sensory ganglia.

Insulin receptors from superior cervical and trigeminal ganglia were solubilized (1.0% Triton) and purified on wheat-germ agglutinin columns. The affinity and specificity of these receptors were determined by the competitive inhibition of A_{14} ^{125}I-porcine insulin binding by various concentrations of unlabeled pork insulin or human proinsulin. The tyrosine-specific kinase activity of these receptors was evaluated by assessing an insulin-stimulated incorporation of ^{32}P into exogenous substrate poly(Glu,Tyr)4:1. To determine the molecular weight of the receptor's binding α-subunit, disuccinimidyl suberate was used to crosslink ^{125}I-insulin to the subunit. Following solubilization with 1.0% SDS containing 5.0% mercaptoethanol, the sample was analyzed using SDS-gel electrophoresis and autoradiography.

The bovine superior cervical and trigeminal ganglia were found to contain specific, high affinity binding sites for ^{125}I-insulin (IC_{50}=0.5ng/ml). As is the case with other insulin receptors, pro-insulin was found to be 200 times less potent than insulin in competing with ^{125}I-insulin for binding. Also, as is the case with other insulin receptors, receptors from the peripheral nervous system were found to possess insulin stimulatable tyrosine kinase activity. The insulin-induced incorporation of ^{32}P into exogenous substrate was both time and dose-dependent. The apparent molecular weight of the receptor's binding subcomponent was found to be approximately 130,000 kDa. In this regard the peripheral nervous system insulin receptor is similar to the hepatic receptor but is larger than the brain insulin receptor (MW=120,000 kDa). In conclusion, the peripheral nervous system was found to contain highly specific receptors for pancreatic insulin. The characteristics of these receptors are virtually identical to insulin receptors found in classical target tissue.

229.13 BATCH VARIABILITY AS A SOURCE OF DPDPE ANAMALOUS BINDING. L. Taylor*, A. Mansour, S. J. Watson and H. Akil, (SPON. S.J. Watson), Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720, U.S.A.

The synthetic peptide [2-D-penicillamine, 5-D-penicillamine] enkephalin (DPDPE) is a delta selective agonist. The binding of [^{3}H]DPDPE to striatal and mid-brain sections was examined. Slide mounted 10 u tissue sections were incubated at 25°C with varying concentrations of [^{3}H]DPDPE in 50 mM Tris buffer, pH 7.5. After incubation, the slides were washed, dried and the binding quantified using liquid scintillation. Non-specific binding was evaluated in parallel experiments in the presence of 1 uM DSLET. The K_d and B_{max} values were determined with the LIGAND program (Munson and Rodbard, Anal. Biochem. 107, 220-229, 1980). The observed binding was consistent with a single receptor site having B_{max} = 172 pM and a K_d = 8.3 nM. However, one commercial preparation (Amersham, batch 8) suggested a two-receptor model (curvilinear Scatchard) with a B_{max} = 58 pM and a K_d = 3.5 nM as well as a B_{max2} = 116 pM and a K_{d2} = 181.9 nM. Analysis of this batch by HPLC indicated the presence of a second radiolabelled component. Removal of this material eliminated the curvilinear binding response. Investigators using DPDPE are cautioned to check the purity of their preparations by HPLC.

229.14 CHARACTERIZATION OF μ AND δ OPIATE RECEPTORS. S.McLean†*, J.Carcillo#* and B.Roth# (SPON: D. C. Hilt). †Laboratory of Neurophysiology, NIMH and #Naval Medical Research Institute, Bethesda, MD 20892.

Several laboratories have recently reported the purification and characterization of brain opiate receptors (Gioannini et al., J. Biol. Chem. 260: 15117-15121; Maneckjee et al. PNAS 82: 594-598; Simonds et al. ibid: 4974-4978). Following various purification schemes and SDS gel electrophoresis several protein bands have been described that migrate with M_r values ranging from 31,000 to 93,000. We report the crosslinking of human ^{125}I-beta-endorphin to crude brain membranes using the bifunctional reagents BSOCOES (Bis [2-(succinimidooxycarbonyloxy) ethyl] sulfone and DSS (Disuccinimidyl suberate).

Rats were sacrificed, their brains rapidly removed (minus the cerebellum) and homogenized by hand, in 50 mM K_2HPO_4, pH 7.4, 25°C with 1-10 μg/ml bacitracin. Tissue was incubated for 1 hr at 25°C with 2nM ^{125}I-beta-endorphin in the presence or absence of 1 μM bremazocine. Incubations were terminated by the addition of 1 ml ice cold buffer and centrifugation. The supernatant was discarded and the tissue resuspended in 0.2 ml of fresh buffer. BSOCOES or DSS freshly prepared in DMSO were added to a final concentration of 1mM and allowed to react for 15 min at 0°C. Crosslinking was terminated by the addition of an excess of 50 mM Tris and 1 ml K_2HPO_4, the tissue centrifuged, the supernatant discarded, the sample solubilized in SDS-PAGE buffer and then processed for gel electrophoresis using a 10% acrylamide separating gel and a 4.5% stacking gel. Radioactive gels were placed in x-ray cassettes with Kodak XAR-5 film and exposed for 7-14 days.

Similar results were obtained using either BSOCOES or DSS as the crosslinking agent. Major radioactive bands were found migrating to apparent M_rs of 67,000, 58,000, 45,000 and 33,000. A 110,000 band was occassionally seen. Labeling of all bands was reduced in the presence of 1 μM bremazocine. The 67,000 band is solubilized by low concentrations of detergents suggesting it is not an integral synaptic membrane protein. The 58,000 band overlies a major protein band. Two dimensional gel electrophoresis suggested an acidic pI for all major bands.

We have demonstrated that affinity cross linking of crude rat brain membranes produces 4 major bands of specifically labeled protein. Because beta-endorphin binds to both μ and δ sites it is not possible to determine whether the 4 bands represent subunits of an "interconverting" receptor, proteolytic degradative products or separate receptor components. Work is currently underway to resolve this issue.

229.15 AFFINITY CROSSLINKING OF ^{125}I-HUMAN BETA-ENDORPHIN TO CELL LINES POSSESSING EITHER MU OR DELTA TYPE OPIOID BINDING SITES. O.Keren, T.L. Gioannini*, J.M. Hiller* and E.J. Simon. Depts. of Psychiatry and Pharmacology, New York University Med. Ctr., New York, NY 10016.

We have used affinity crosslinking techniques to demonstrate that mu and delta opioid binding sites contain major peptide subunits that differ in molecular size (Howard, A.D. et al. J.Biol. Chem.260:10833, 1985; Howard, A.D. et al. Biochemistry 25:357,1986). Here we present additional evidence to support this finding. The NG108-15 cell line which contains exclusively delta sites and the human neuroblastoma cell line (SK-N-SH) which possesses predominantly mu sites (Yu, V.C. et al. J. Biol. Chem. 261:1065,1986) were bound and crosslinked with ^{125}I-human-beta-endorphin and subjected to sodium dodecyl-sulfate-polyacrylamide gel electrophoresis. Autoradiography of these gels revealed different labeling patterns for these two cell lines. NG108-15 cells showed bands consonant with peptides of M_r 53K and 25K. SK-N-SH cells demonstrated three bands of M_r 92K, 65K and 25K. All labeled bands were opiate receptor-related since they were eliminated when binding was carried out in the presence of excess opiates. Competition for binding revealed that the 53K and 25K bands from NG108-15 cells were selectively reduced or eliminated by the highly selective delta ligand, DPDPE, but not by the highly selective mu ligand, DAGO. In contrast, bands of M_r 92K and 65K from the mu site-bearing line, SK-N-SH, were reduced or eliminated by DAGO, but not by DPDPE.

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229.16 **PHOTO-INDUCED INACTIVATION OF NEUROPEPTIDE RECEPTORS WITH PEPTIDES CONTAINING p-BENZOYLPHENYLALANINE. W. F. Herblin, J. C. Kauer, and S. W. Tam*. E. I. du Pont de Nemours and Co., Central Research and Development Department and Biomedical Products Department, Experimental Station, Wilmington, DE 19898.**

The benzophenone chromophore has been incorporated into a synthetic amino acid (p-benzoyl-phenylalanine; Bpa) to produce a chemically stable photo-affinity probe for labeling receptors. The benzophenone group is especially advantageous since it is activated by long-wave UV which is less damaging to receptors. Two model peptides containing Bpa have been shown to be potential photo-affinity probes for the opioid and neurotensin receptors, respectively.

A morphiceptin analog was prepared in which the C-terminal proline was replaced by L-Bpa amide. The synthetic peptide showed substantial affinity for the mu opioid receptor (IC_{50}= 268 nM). IC_{50} values for delta and kappa receptors were 3670 nM and 8200 nM, respectively. Irradiation of rat brain membranes in the presence of the peptide (2.8 μm) induced apparent inactivation of an opioid receptor as indicated by a reduction in the subsequent binding of both ^{3}H-naloxone and ^{3}H-etorphine. The inhibition of ^{3}H-DADLE binding by naloxone in control and photoreacted membranes indicated selective inactivation of a mu receptor when the data were analyzed for multiple sites.

	Control		Photoreacted	
	Site 1	Site 2	Site 1	Site 2
Bmax (fmol/mg)	6.1	4.6	------	4.6
Kd (DADLE)	2.8 nM	1.2 nM	------	1.4 nM
Ki (Naloxone)	2.0 nM	53 nM	------	12 nM

An analog of a neurotensin fragment (NT 8-13) was prepared in which the tyrosine at position 11 was replaced with D-Bpa. This hexapeptide was as potent as an inhibitor of ^{3}H-neurotensin binding (Ki=4.4 nM) as was NT 8-13 itself (Ki=5.1 nM). Irradiation of the receptor-bound peptide induced a 75% reduction in the subsequent binding of ^{3}H-neurotensin as compared to control membranes which were irradiated in the absence of the peptide or incubated with the peptide but not irradiated.

230.1 INTERACTION OF ^{3}H-GLYCYLGLUTAMINE WITH CELL MEMBRANES PREPARED FROM CNS AND LYMPHOID CELL LINES.
R. J. Plishka and J. H. Neale, Department of Biology, Georgetown University, Washington, D. C. 20057.

Glycylglutamine is the terminal dipeptide of the POMC-gene product, β-endorphin-(1-31). Biochemical evidence suggests that this dipeptide is produced by proteolytic cleavage of B-endorphin-(1-31) in the intermediate pituitary and several brain regions (Parish et al., Nature 306:267,1983). We have reported immunohistochemical evidence of elevated levels of free glycylglutamine within cells of the intermediate pituitary (Brain Res. 360:403, 1985). Given the possibility that this dipeptide coexists with β-endorphin within neuronal and intermediate pituitary secretory vesicles, we have begun to examine the interaction between ^{3}H-glycylglutamine (31 Ci/mmol, provided by Dr. Y. P. Wan, New England Nuclear Research Products) and plasma membranes prepared from several different tissues and continuous cell lines.

Initial data from equilibrium binding assays of membranes prepared from spleen, thymus, liver, lung, kidney and brain indicate that radioligand interaction can be detected within these tissues and that this process is blocked by the presence of 500-fold excess unlabeled dipeptide. Focusing upon the ligand interaction with the nervous and immune systems, we find that the apparent radioligand binding to cerebellar membranes does not saturate in an assay range of 1-75 nM dipeptide, while the interaction with a transformed B-lymphocyte cell line has an estimated K_d of 10-15 nM. Binding of the ligand to nervous system versus lymphocyte membranes is further distinguished by the insensitivity of the former to excess unlabeled glycine or glutamine while the interaction of 10nM ligand with several lymphoid cell lines is affected by a 5 mM concentration of these amino acids in the incubation medium.

Data from the detailed characterization of the interactions between ^{3}H-glycylglutamine and cell surface membranes should provide a useful focus for subsequent analysis of this dipeptide's potential function within the nervous, immune and endocrine systems.

230.2 MONOCLONAL ANTIBODY TO SOMATOSTATIN EXHIBITS SIMILAR STRUCTURAL REQUIREMENTS FOR PEPTIDE BINDING AS PITUITARY SOMATOSTATIN RECEPTOR. S.B.Weissman* and L.L.Y.Chun* (SPON: V.T.Tran) Program in Neuroscience, Harvard Medical School, Department of Neurology, Massachusetts General Hospital, Boston, MA 02114

Somatostatin is widely distributed in peripheral organs and in the central nervous system where it exerts various physiological effects. In the pituitary it inhibits the release of growth hormone via specific receptors. Structure-function studies of somatostatin binding to rat anterior pituitary cultures using alanine-substituted somatostatin analogs indicate that amino acid residues 6-9 are important for the biologic activity of the peptide (Vale *et al.*, 1978). To obtain probes which can be used to examine this and related peptides, we have produced monoclonal antibodies (mAb) to somatostatin. The antigen recognition site of one of these antibodies exhibits a specificity similar to that of pituitary somatostatin receptors.

To prepare the mAbs (Balb/c x C57Bl/6)F_1 mice were immunized thrice with cyclic somatostatin coupled to bovine serum albumin. Spleen cells from the animal exhibiting the highest serum titer to the peptide were fused to the myeloma line, X63-Ag8.653, which does not itself produce immunoglobulin. Hybridoma supernatants were screened by single displacement radioimmunoassay with [^{125}I-Tyr^{1}] somatostatin as tracer.

Although we have obtained several lines which secrete mAbs that bind somatostatin with high affinity, only one antibody is discussed here. MAb 1E9 bound to the peptide with high affinity (ID_{50} for somatostatin is about 10 fmol by RIA). Immunologic cross-reactivity studies with several somatostatin analogs indicated that alterations in the N-terminal of the peptide did not affect antibody binding but modifications in positions 6 and 9 reduced it substantially. This is similar to the structural requirements for peptide binding to rat pituitary somatostatin receptors and suggests that that the binding specificity of this mAb may resemble that of the pituitary somatostatin receptor. No cross-reactivity was seen with other peptides tested (angiotensin, bradykinin, enkephalin-leu, growth hormone releasing hormone, neuropeptide Y, neurotensin, secretin, substance P, thryrotropin releasing hormone, vasopressin). In immunohistochemical studies this mAb was used to localize somatostatin in fibers of both rat and mouse median eminence and in both fibers and cell bodies in human cortex.

These studies indicate that the hybridoma approach is feasible for the generation of high affinity monoclonal antibodies which can be used for both structural and anatomical studies. In addition, the similarity of the antigen recognition site to that of the ligand binding site for a class of somatostatin receptors suggests that this reagent may also be useful for the generation of anti-idiotypic anti-receptor probes.

230.3 IMMUNOSTAINING OF MAGNOCELLULAR NEURONS OF SUPRAOPTIC AND PARAVENTRICULAR NUCLEI OF RAT HYPOTHALAMUS USING A VASOPRESSIN ANTI-IDIOTYPE ANTIBODY. D.J. Berlove*, D.T. Piekut and K.M. Knigge. Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

A primary anti-vasopressin (AVP) antibody was generated in a rabbit and its titer and characteristics determined by immunocytochemical methods. A second rabbit was immunized with purified IgG of the primary vasopressin antiserum and its titer was monitored with a precipitin test using partially purified AVP-binding protein from rat neural membranes. Brains of normal and Brattleboro animals were immunostained with anti-vasopressin and the vasopressin anti-idiotype antibody. Brains were fixed by intracardiac perfusion of 100ml of saline followed by 200ml of fixative containing 4% paraformaldehyde and 0.2% picric acid in 0.025M PBS buffer (sodium phosphate with 0.9% sodium chloride), pH 8.0. The PAP or ABC method of immunocytochemistry was performed on serial 40μm vibratome-cut sections of brains and pituitary glands.

The anti-idiotype antibody immunostained neurons in supraoptic and paraventricular nuclei of the hypothalamus of normal and homozygous Brattleboro rats. The distribution of immunostained perikarya in these hypothalamic nuclei together with the staining of median eminence and neural lobe was similar to that observed in normal rats using the primary vasopressin antiserum and suggests that vasopressinergic magnocellular neurons are stained. The appearance of the perikarya of neurons immunostained with these two antisera was distinctly different. Immunostaining was not observed in the suprachiasmatic nucleus using the anti-idiotype antibody. Although the Brattleboro rat does not synthesize vasopressin, receptors for this hormone are present and it appears that these neurons may be visualized by recognition of vasopressin receptors associated with vasopressin neurons by anti-idiotype antibodies. Absorption studies with vasopressin and a vasopressin-binding receptor protein further suggests that a receptor associated with vasopressinergic neurons is recognized by the anti-idiotype antibody. (Supported by grants NSF/BNS 83-10914, NIH/NS18626 and NIH/RCDA NS00869.)

230.4 CHOLECYSTOKININ RECEPTORS ARE LOCATED ON INTRINSIC STRIATAL NEURONES AND NOT ON STRIATAL DOPAMINERGIC AFFERENTS. C.R.Clark, D. J. S. Sirinathsinghji*+, N. Day*, I. J. M. Beresford*, J. K. Chambers*, J. Hughes*. Parke-Davis Research Unit, Addenbrooke's Hospital Site, Hills Road, Cambridge, CB2 2QB, U.K. +Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT, U.K.

Cholecystokinin (CCK) exerts a number of actions on the nigro-striatal dopamine (DA) system, including attenuation of 'DA-dependent' behaviour and modulation of striatal DAergic neurotransmission. How CCK exerts these effects is not known, although high concentrations of CCK and its receptors have been localized in the striatum. To further explore the nature of these CCK-DA interactions, studies were undertaken to identify the cellular location of CCK receptors within the striatum.

Rats were unilaterally infused with either 6-OH DA (8μg) into the nigro-striatal tract or ibotenic acid (5μg x 4 injection sites) into the striatum. Animals were sacrified 10 days post drug infusion and CCK receptors mapped by quantitative digital subtraction autoradiography. Striata were also dissected and CCK and DA content measured by radioreceptor assay and HPLC with electrochemical detection respectively.

Infusion of 6-OH DA into the nigro-striatal tract had no effect on either CCK content or CCK receptor number, although dopamine content was reduced by >98% on the lesioned side. All animals receiving 6-OH DA showed > 20 ipsilateral rotations per minute following injection of amphetamine (5mg/kg) confirming the effectiveness of the nigro-striatal lesion. Destruction of striatal interneurones with ibotenic acid in contrast resulted in a dramatic reduction (i.e. >50%) in CCK receptor number on the lesioned side with no detectable effect on dopamine content. Behavioural verification of this lesion was evidenced by ipsilateral bias and hyperactivity 1-2 days after ibotenic acid infusion.

These studies suggest that a large proportion of CCK receptors within the striatum are located on intrinsic neurones and not on dopaminergic presynaptic afferents. Studies are in progress to ascertain the identity of the CCK-receptor rich striatal interneurone.

230.5 MAPPING OF SUBSTANCE P RECEPTOR SITES IN THE HUMAN BRAIN: HIGH DENSITIES IN THE SUBSTANTIA INNOMINATA AND EFFECT OF SENILE DEMENTIA. M. Dietl[1]*, A. Probst[2]*, and J.M. Palacios[1] (SPON: B. Gähwiler). [1]Preclinical Research, SANDOZ LTD., Basle, [2]Dept. Neuropthatology, Inst. of Pathology, University of Basle, Basle, Switzerland.

Receptor autoradiographic techniques were used to study the distribution of putative substance P (SP) receptor sites in human postmortem materials. SP receptor sites were labeled in mounted tissue sections using [^{125}I]-Boulton-Hunter-SP. The pharmacological characteristics of SP binding to human brain tissue sections were similar to those seen in the rat brain. The regional distribution of SP receptor sites in the human brain was highly heterogeneous. The highest densities of SP sites were associated with brain nuclei known to contain the cell bodies of neurons utilizing acetylcholine, serotonin and noradrenaline as neurotransmitter, but not those using dopamine. Thus, the substantia innominata, including the nucleus basalis of Meynert, parts of the raphé linearis, but not the raphé dorsalis and the locus coeruleus, but not the substantia nigra were enriched in SP receptors. SP sites were also enriched in other brain areas including the nucleus caudatus, putamen and accumbens, neocortex, the hypothalamus, the hippocampus, the periaqueductal gray and nucleus interpeduncularis in the midbrain, the inferior olive and the dorsal and ventral horn of the spinal cord. The pattern of distribution of SP receptors in the control brains was comparable from case to case and apparently independent of postmortem delay and sex. Age, however, seems to influence SP receptor density as brains from patients dying in the first decade of life presented higher SP receptor densities than patients over 50 years of age.

The high densities of SP sites observed in control brains in the substantia innominata led us to examine these sites in the brains of patients dying from senile dementia (SD), a disease where cholinergic neurons in the substantia innominata are known to degenerate. The examination of several cases of SD revealed a loss of SP receptors only in cases where a clear cell loss was observed. In conclusion SP receptors have been visualized in human postmortem materials. The localization of these sites in nuclei where the cell bodies of neurons using well characterized neurotransmitters are located indicates that SP can play an important role in the regulation of the ascending pathways emanating from these neurons. Our results in the brains of SD patients further support the localization of SP receptors to cholinergic cell bodies in the substantia innominata.

230.6 AUTORADIOGRAPHIC LOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) BINDING SITES IN THE RAT BRAIN AND PITUITARY

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It has already been shown that LHRH can induce some behavioral effects in castrated male and female rats. These effects suggest that LHRH might have a neurotransmitter/neuromodulator role in the central nervous system. In order to identify the possible sites of action of LHRH in the rat brain, we proceeded to the localization of LHRH binding sites using slide-mounted frozen sections. The pituitary which contains large amounts of well characterized LHRH receptors was used as a control tissue. The radioligand used was an iodinated stable LHRH agonist [D-Ser(tBU)^6, des-Gly-NH^{10}] LHRH ethylamide ([D-Ser^6]LHRH-EA) (supplied by Dr. J. Sandow [2], Hoechst Co.). Incubation was carried out at room temperature for 1 hr in the presence of 10^5cpm iodinated [D-Ser^6] LHRH-EA. To determine non-specific binding, 10^7 M/l unlabeled [D-Ser^6] LHRH-EA was added to the incubation medium. Labeling was localized by exposing X-ray films to sections and also by dipping slides in liquid photographic emulsion (Kodak NTB-2). In the pituitary, an intense and diffuse labeling was observed throughout the anterior lobe. The intermediate and posterior lobes were free of any reaction. In the brain, the septal region, hippocampus, lateral and medial nuclei of the amygdala, and entorhinalis cortex showed a strong to moderate labeling. Only a weak reaction could be detected in the hypothalamus. In both pituitary and brain, the labeling was completely abolished by cold [D-Ser^6]LHRH-EA. Then the distribution of LHRH binding sites appears to be well correlated with the distribution of immunoreactive LHRH except in the hypothalamus which contains high concentration of LHRH and very small amount of LHRH binding sites. The present studies clearly indicate that LHRH has multiple sites of action in the rat brain and reinforce the hypothesis that this peptide could act as a neurotransmitter/neuromodulator in the central nervous system.

230.7 VIP RECEPTORS IN RAT BRAIN: CHARACTERIZATION BY BINDING AND COVALENT CROSSLINKING. J. Vasiloff*, M.S. Beattie, L.B. Campolito*, T.M. O'Dorisio*, J.C. Bresnahan, and M.S. O'Dorisio* (SPON: S. Tjioe). Depts. of Medicine, Surgery, Anatomy, Pediatrics, and Microbiol. and Immunol., and Neuroscience Program, Ohio State Univ. College of Med., Columbus, OH 43210.

The molecular components of vasoactive intestinal polypeptide (VIP) binding were investigated using both competitive binding and covalent crosslinking techniques. Frontal cortex was harvested from anesthetized, decapitated rats, dissected free of connective tissue, and homogenized in buffer A (20 mM Hepes, 2 mM $MgCl_2$, 5 mM EDTA, 1 mM 2-mercaptoethanol, 150 mM NaCl, 50 ug/ml phenylmethylsulfonyl flouride, pH 7.4) and the membrane containing fraction separated by differential centrifugation. Binding assays were performed by incubating 500 ug membrane protein in buffer A containing 100-150 pM ^{125}I-VIP in the absence or presence of unlabelled VIP or other competing peptides. ^{125}I-VIP bound to membrane protein was separated from free ^{125}I-VIP by filtration through Whatman GF/C filters presoaked in 0.3% polyethylenimine. Time course and temperature studies demonstrated steady-state binding from 15-90 min at 17°C. The percentages of total counts that were bound to membrane protein ranged from 9.4-14% with 40-62% of these counts representing specific binding. Computer-assisted fit of competitive binding experiments was used to calculate affinity constants and maximum number of binding sites. Binding affinity for VIP (Kd) was estimated to be 48 ± 21 nM with a Bmax of 12.4 ± 5.8 nM (bound/ gm protein).

^{125}I-VIP was covalently crosslinked to putative binding sites using the bifunctional reagent, disuccinimidyl suberate (DSS). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography demonstrated bands of Mr 50000 and 75000. The labelling of these bands was specific as evidenced by inhibition of labelling in the presence of 1 uM unlabelled VIP. The 75000 Mr band does not appear to represent a specific VIP receptor because it was observed when ^{125}I-VIP was treated with DSS in the absence of membrane protein; it more likely represents bovine serum albumin present in the commercial preparation of ^{125}I-VIP. These findings provide further evidence for the presence of VIP receptors in brain tissue, and demonstrate the presence of a 47K (50K minus VIP) protein comprising or associated with the putative VIP receptor in this tissue, as well as in human lymphoblasts (Wood,C.L. and O'Dorisio, S.M., J. Biol. Chem., 260:1243, 1985).

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230.8 NPY-BINDING IN RAT BRAIN WITH RELATION TO CATECHOLAMINERGIC SYSTEMS. M. Schultzberg, C. Köhler, T. Hökfelt, L. Terenius and M. Goldstein (SPON: C. Hildebrand). Astra Pharmaceuticals, S-151 85 Södertälje, Dept. of Histology, Karolinska Institutet, Stockholm, Sweden.

The occurrence of neuropeptide Y-like immunoreactivity (NPY-LI) in catecholamine (CA) neurones in certain nuclei in the medulla oblongata has recently been demonstrated. NPY is present in the majority of adrenaline cells of the C1-C3 group, in a smaller population of the noradrenergic locus coeruleus cells (A6), but not in the A2, A5 or A7 noradrenergic cell groups. NPY has not been found in dopaminergic neurones, but NPY neurones occur in e.g. the cerebral cortex, striatum, basal forebrain, the superficial laminae of the spinal cord and the spinal trigeminal nuclei (ntV), and these cells seem to lack CA:s. However, in several brain regions e.g. medulla oblongata, hypothalamus and striatum overlapping networks of NPY and catecholaminergic nerve terminals do exist. An autoradiographic technique was employed to study the spatial relationship between NPY receptors and noradrenaline/adrenaline receptors. The binding of tritiated NPY (or iodinated) and p-aminoclonidine (^{3}H-PAC) a selective α_2-receptor ligand, was studied in consecutive sections of the rat medulla oblongata, hippocampus and cerebral cortex. The binding of labelled NPY and PAC exhibited very similar distribution patterns in the medulla oblongata, with high concentrations mainly in the superficial laminae of the ntV and the dorsal vagal complex. Immunohistochemical studies of NPY and the CA synthesizing enzymes show overlapping distribution only in the latter area. In the ntV, NPY-immunoreactive fibres are more numerous. In the hippocampus, high grain density is found in strata oriens and radiatum, after incubation with labelled NPY, whereas ^{3}H-PAC-binding is low in these layers, but high in stratum moleculare. Fibres with NPY-LI are localized primarily in the molecular layer, and the noradrenergic terminals are diffusely scattered throughout the structure. In the cerebral cortex, α_2-receptors are present in a distinct band in lamina 1 and in lower number in lamina 4. Binding sites for NPY occurred in all layers, in particular laminae 1-3. NPY-immunoreactive and noradrenergic terminals, respectively, are seen in all layers, but most dense in laminae 1 and 4-6.

From these studies on a limited number of brain regions, it can be concluded that in some areas there was a good overlap between the distribution of nerve terminals containing endogenous messenger and its receptors, whereas other areas do not exhibit such an overlapping distribution. With regard to terminal fields of fibres with coexisting NPY and noradrenaline/adrenaline, there is a good correlation betwen the distribution of the two receptor types in the dorsal vagal complex.

230.9 DISTRIBUTION OF TRH RECEPTORS AND TRH IN THE CAT CENTRAL NERVOUS SYSTEM. R. M. Bogin*, M. S. Kreider, S. B. Caine*, S. Manaker, A. I. Pack* and A. Winokur. Univ. of Pennsylvania, Philadelphia, PA 19104.

In the cat, microinjection of thyrotropin releasing hormone (TRH) into certain areas of the central nervous system (CNS) changes ventilation and cardiovascular variables. To initiate a more systematic investigation of the effects, we undertook a study to determine the location of TRH and its receptors in the cat CNS. Using techniques previously described from our laboratory, quantitative autoradiograms for TRH receptors of the cat brain were produced; additional specimens were dissected, and radioimmunoassay for TRH was performed. Heterogenous distribution of receptors was observed in the cat brain. In the forebrain, large quantities of TRH receptors were found in the amygdala, hippocampus, claustrum, pyriform nucleus and tuberculum olfactorium. In the brainstem, high concentrations were localized to the dorsal motor nucleus of the vagus, the hypoglossal nucleus and the periaqueductal grey. The cerebellum contained few receptors. The largest quantities of the TRH tripeptide were noted in the hypothalamus and septum, with substantial amounts also obtained from olfactory bulb, corpus striatum and thalamus. Our results demonstrate that the distribution of TRH and TRH receptors in the cat brain is very similar to that previously described in the rat and human brain. They provide a basis for exploring the physiological and pharmacological effects of TRH in cats. (Supported by HL-08805 and NS-18332)

230.10 AUTORADIOGRAPHIC LOCALIZATION OF OPIATE BINDING IN RAT FOREBRAIN: EXAMINATION OF SPECIFICITY WITH A PHOTOAFFINITY-LABELLED ANALOGUE. C.B. Lacey*, W.G. Tatton, C.W.T. Yeung*, J.N. Nobrega, (SPON: J. Stevens). Playfair Neuroscience Unit, Toronto Western Hospital, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada, M5T 2S8.

The recognition of opioid peptides as putative neurotransmitters has prompted investigation of the regional and subcellular distributions of these peptides and their respective receptor sites. Localizations of ^{125}I-DAGO(Tyr-D-Ala-Gly-NMe-Phe-Gly-ol) and ^{125}I-DADLE[D-Ala^2,D-Leu^5]-Enkephalin were examined following in vitro incubation of serial coronal slices of frozen, unfixed rat forebrain. Recent evidence indicates the preferential labelling by these substances to mu and delta sites, respectively.

A photoaffinity-labelled enkephalin analogue, [D-Ala^2,p-N$_3$-Phe^4-Met^5]-Enkephalin (PAL-Enk), was utilized to produce an irreversible binding upon irradiation with UV light. The PAL-Enk was incubated with each of the two radioligands, with and without UV light activation, and its effects observed on each ligand's characteristic binding. Incubations with irradiated PAL-Enk demonstrated marked inhibition of ^{125}I-DADLE binding in all major cortical structures examined (e.g. the striatum, endopiriform nucleus, olfactory tubercle, and deep layers of the neocortex). In contrast, inhibition of ^{125}I-DAGO binding was not evident (e.g. in striasomal patches or in discrete portions of the thalamus). These results suggest a preferential affinity of PAL-Enk for delta binding sites.

The influence of different buffer mediums on the binding of each agent is reviewed for the purpose of selection of optimal binding conditions. The mechanisms of UV irradiation-induced competitive binding of PAL-Enk will be compared with the two other opioid radioligands, together with an evaluation of receptor specificity of these ligands.

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230.11 MU OPIOID BINDING SITES IN HUMAN BRAIN TISSUES. C.Pilapil, S.Welner*, J.Magnan, N.Zamir and R.Quirion. Douglas Hospital Research Centre, Verdun, Québec H4H 1R3 Canada, Departement de Pharmacologie, Faculté de Médecine, Université de Montréal, Québec, Canada and NHLBI, NIH, Bethesda, MD, USA.

The presence of various opioid receptor types in human brain has been reported by various investigators. However, their precise characterization and respective distribution have not been studied in detail. As a first series of experiments, we decided to investigate the distribution of μ opioid receptor binding sites in human brain using a highly selective ligand, [^{3}H]DAGO, in both membrane binding assay and in vitro receptor autoradiography. Membranes and whole human brain hemisphere sections were prepared as described before (Quirion et al, Life Sci. 33(1):227-230, 1983; Quirion, Eur. J. Pharmacol., 117:139-142, 1985) and then incubated for 60 min at 25°C in 50 mM Tris.HCl buffer in presence of various concentrations (saturation analysis) or 1.0 nM (autoradiography) [^{3}H]DAGO (44.7 Ci/mmol). The specific binding was calculated as the difference in radioactivity bound in the presence and absence of 1 μM cyclazocine. The results show that μ opioid receptor binding sites are very discretely distributed in normal human brain. High densities of sites are found in the posterior amygdala, caudate, putamen and certain thalamic and hypothalamic nuclei. Much lower densities are present in various brainstem nuclei including the pontine, hypoglossal and trigeminal nuclei. In the cortex, Brodmann areas #23, 21, 4, 6, 24, 20, 9, 22, 17, 30 and 7 (in decreasing order) are especially enriched in μ opioid binding sites. Other areas such as the globus pallidus, internal capsule and reticular formation contain only low densities of sites. These results reveal that μ opioid binding sites are discretely distributed in various regions of the human brain. We are currently investigating the possible alterations of these μ opioid binding sites in various neurological and psychiatric diseases to determine if opiates could be involved in certain aspects of the etiology of these diseases.

230.12 EFFECT OF CHRONIC HALOPERIDOL TREATMENT ON BRAIN CHOLECYSTOKININ RECEPTORS IN RAT. P. Gaudreau, J.N. Crawley and R. Quirion. Neuroendocrinology Laboratory, Notre-Dame Hospital Research Center, Montreal, Canada; Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, Maryland; and Douglas Hospital Research Center, Verdun, Canada.

The dopamine (DA) hypothesis in schizophrenia relies on the premise that hyperactivity of the mesolimbic DA system contributes to some of the symptoms associated with this disease. In the rat, immunocytochemical studies, combined with retrograde tracing, have revealed the co-existence of cholecystokinin (CCK)/DA neurons in the ventral tegmental area of the mesencephalon, which project into limbic structures (nucleus accumbens, central nucleus of amygdala, olfactory tubercles, and prefrontal cortex) (Hokfelt, T. et al., Neuroscience, 5, 2093-2114, 1980). Since there are indications that the therapeutic actions of antipsychotic drugs are related to a selective reduction of DA activity in mesolimbic and mesostriatal regions (White, F.J. and Wang, R., Science, 221, 1054-1057, 1983) and radioautographic data demonstrating the presence of high densities of CCK receptors in the terminal fields of these DA pathways (Gaudreau et al., Ann. N.Y. Acad. Sci., 448, 198-219, 1985; Van Dijk et al., J. Neurosci., 4, 1021-1033, 1984), it was of interest to study the effect of chronic haloperidol treatment on the amounts of CCK receptors in these forebrain regions. Haloperidol was administered s.c. (0.5 mg/kg/day), i.p. (2.0 mg/kg/day) or via osmotic mini pumps (equivalent rate to 0.1 mg/kg/day) to groups of rats for 24 hrs or 21 days. Control groups received 0.9% saline. All animals were sacrified 24 hrs after the last treatment. In vitro CCK receptor radioautography was performed with 34 pM Nα[^{125}I- desaminotyrosyl] CCK-26-33, as previously described by Gaudreau et al., (Ann. N.Y. Acad. Sci, 448, 198-219, 1985). Our data indicate that neither acute nor chronic (21 days) haloperidol treatment alters CCK receptor densities in rat cingulate cortex (Optical Density, control/treated: 0.850 ± 0.020/0.818 ± 0.019), fronto-medial cortex (0.672 ± 0.013/0.635 ± 0.021), olfactory tubercles (0.922 ± 0.026/0.880 ± 0.029), nucleus accubens (0.836 ± 0.055/0.810 ± 0.034) and caudate-putamen (0.880 ± 0.040/0.799 ± 0.023). These results are at variance with those reported by Chang et al., (Life Sci., 32, 871-878, 1983) in the guinea pig, where the maximal number of CCK receptors was increased in the olfactory tubercles/nucleus accumbens (29 ± 12%), fronto-medial cortex (20 ± 8%) and anterior cingulate cortex (21 ± 11%). Species differences could explain the observed discrepancies.

230.13 MODULATION OF ANGIOTENSIN II RECEPTOR EXPRESSION IN NEURONAL CULTURES BY PROTEIN KINASE C AGONISTS. C. Sumners, S.M. Fuentes*, F.T. Crews and M.K. Raizada*. Depts. of Physiology and of Pharmacology, College of Medicine, University of Florida, Gainesville, FL 32610.

In previous studies we have shown that stimulation of alpha$_1$-adrenergic receptors (α_1-R) by norepinephrine results in modulation of angiotensin II receptors (AngII-R), and also leads to increased inositol phospholipid hydrolysis. Since α_1-R stimulation results in activation of protein kinase C, these studies led us to investigate a possible role of this enzyme in the regulation of AngII-R expression. In the present study we have examined the effects of phorbol esters (PE) on AngII-R in neuronal cultures. These drugs are protein kinase C agonists and might be expected to mimic the effects of alpha$_1$-adrenergic stimulation on AngII-R. Primary neuronal cultures were prepared from the hypothalamus and brainstem of one day old Sprague-Dawley rats as described previously (Sumners et al., Am. J. Physiol. 250, C236, 1986). Cultures were grown for 10-14 days and consisted of 75-80% neurons, with the remainder being non-neuronal cells. Binding of AngII to neurons was assessed with the cells attached to the culture dish, using [^{125}I]-AngII as the radioactive ligand (Raizada et al., Brain Res. 297, 343, 1981). Treatment of neuronal cultures with PE (500 ng/ml) for 1-24 hours led to time dependent increases in [^{125}I]-AngII specific binding (60-80%), which reached a maximum at 4 hours. This increase was seen only with PE which stimulate protein kinase C, i.e. TPA (12-O-tetradecanoylphorbol-13-acetate) and 4-βPDD (phorbol 12,13 didecanoate) and not with inactive PE, e.g. 4-αPDD, and 4-αPE. Incubation of cultures with different concentrations of TPA or 4-βPDD (1-1000 ng/ml) revealed that their stimulatory action on [^{125}I]-AngII was dose dependent, with TPA being the more potent of the two. Maximal stimulation of [^{125}I]-AngII binding was found at 100 ng/ml (TPA) and at 500 ng/ml (4-βPDD). Saturation and Scatchard analyses with TPA and with 4-βPDD (both at 500 ng/ml, 4 hrs) revealed that both PE increase the number of AngII receptors with little effect on affinity (e.g. for TPA treatment: Controls, Bmax 164 fmol/mg protein, Kd 1.26 nM; TPA, Bmax, 343 fmol/mg protein, Kd, 2.06 nM).

In conclusion, protein kinase C agonists upregulate AngII receptors in neuronal cultures. These and other studies suggest that phosphorylation may be important in the regulation of AngII-R in neuronal cultures.

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230.14 INCREASED CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN RAT CEREBRAL CORTEX FOLLOWING CHRONIC ATROPINE TREATMENT. Errol B. De Souza and George Battaglia. Neuroscience Branch, ARC, National Institute on Drug Abuse, Baltimore, MD 21224.

Recent anatomical and behavioral studies have indicated interactions between CRF and acetylcholine in the rat cerebral cortex (Crawley et al. Peptides 6:891, 1985). Specifically, some brain stem cholinergic neurons projecting to the frontal cortex also contain CRF. In behavioral studies, CRF injected into the rat frontal cortex does not initiate, but significantly inhibits carbachol-induced "boxing" behavior. In addition, we have recently found that in Alzheimer's disease, decreases in CRF-like immunoreactivity and reciprocal increases in CRF receptor binding in affected cerebral cortical areas correlate significantly with decrements in choline acetyltransferase activity (De Souza et al. Nature 319:593, 1986). The present study was designed to investigate the functional consequence of cholinergic deficts on the regulation of brain CRF neuronal activity. Rats were chronically treated with atropine sulfate (14 days, 20 mg/Kg/day s.c.) and CRF receptors and CRF-stimulated adenylate cyclase activity were measured in discrete regions of brain. The effectiveness of the chronic atropine treatment was confirmed by demonstrating significant increases in the concentration (B_{max}) of muscarinic cholinergic receptors as measured by ^{3}H-NMS binding sites in the frontoparietal cortex (FPX) (30% increase) and hippocampus (20% increase) when compared to saline-treated control rats. Chronic atropine treatment did not alter the concentration of ^{125}I-Tyr^0-rat CRF binding sites in hippocampus, olfactory bulb, cerebellum, striatum and brain stem. In contrast, CRF receptors were significantly increased in the FPX of atropine-treated rats (B_{max}: 249 + 18 fmol/mg protein mean + SEM; n = 5) as compared with saline-injected controls (187 + 6). However, no significant changes in the V_{max} or EC_{50} of CRF-stimulated adenylate cyclase activity were observed following atropine treatment. In addition, atropine treatment had no effect on the guanine nucleotide regulatory protein (N_s) or the catalytic subunit activity as measured by GTP (100 uM)- and forskolin (100 uM)- stimulated enzyme activity, respectively. In summary, our data demonstrate that chronic atropine treatment results in selective increases in CRF receptors in the cerebral cortex without corresponding increases in CRF-stimulated cAMP production. These data suggest that the newly synthesized CRF receptors: 1) have not yet been functionally coupled to the guanine nucleotide regulatory protein (N_s) 2) may represent a subpopulation of receptors that are coupled to an alternative second messenger, and/or 3) may represent spare receptors. The data indicate that interactions between CRF and muscarinic receptors may be of importance in regulating cerebral cortical activity, however, the functional consequence of this interaction remains to be demonstrated.

230.15 CHARACTERIZATION OF AVP BINDING SITES IN THE VENTRAL SEPTAL AREA OF THE RAT BRAIN. P. Poulin* and Q.J. Pittman. Dept. of Medical Physiology, University of Calgary, Calgary, AB T2N 4N1, Canada.

Arginine Vasopressin (AVP) microinjected into the ventral septal area (VSA), but not other nearby regions of the rat brain evokes severe motor disturbances, including barrel rotations and myoclonic/myotonic movements. These AVP-induced motor disturbances appear to result from an interaction of AVP with specific receptors in the VSA and involve a "sensitization" process whereby a first exposure to AVP increases the likelihood of more severe motor disturbances upon subsequent AVP treatment. The mechanism by which this sensitization process occurs is yet unknown, but may be related to changes in receptor properties. To investigate this possibility, we have utilized radioligand binding studies to begin to localize and characterize putative AVP binding sites in the VSA. Subcellular homogenate fractions were prepared from the VSA, lateral septum (LS), as well as the previously studied hippocampus. Homogenate fractions were incubated in a medium containing Tris-HCl buffer (50 mM, pH 7.4), 3 mM MgCl2, bovine serum albumen (1 mg/ml) bacitracin (1 mg/ml) and various amounts of 3H-AVP, AVP and membranes (50-200 µg/tube). After 60 minutes incubation at 22°C, the reaction was terminated by the addition of 5 mls cold Tris-HCl buffer and immediately filtered through Whatman GF/B filters. Binding of 3H-AVP to homogenate fractions was saturable, temperature and time dependent, linearally related to protein concentration and reversible. Specific 3H-AVP binding sites in VSA were preferentially located in the synaptic plasma membrane fraction. Scatchard plot analysis indicates a homogeneous population of binding sites under these conditions with an approximate Kd of 3.5 nM. Comparable binding sites were characterized in the LS and hippocampus, with the LS showing the highest concentration of binding sites. 3H-AVP specific binding ranged between 30-65% of total binding.

These data provide the basis for further investigations examining possible changes in receptor properties in the VSA following pretreatment with AVP.

P. Poulin was an AHFMR Student. Supported by MRC.

230.16 INACTIVATION BY PHENOXYBENZAMINE OF TACHYKININ BINDING SITES IN GUINEA-PIG BRAIN. M. Tiberi* and J. Magnan* (SPON: P.-A. Lavoie). Dép. de Pharmacologie, Fac. de Médecine, Univ. de Montréal, Montréal, Canada, H3C 3J7.

The alpha-adrenergic antagonist Phenoxybenzamine (PBZ) is a relatively non-selective alkylating agent which has been successfully used to characterize different types of receptors (i.e. dopaminergic, opiate: Robson, L.E. and Kosterlitz, H.W., Proc. R. Soc. Lond. B., 205:425-432, 1979). We have investigated the possibility that PBZ might be a useful tool to help in the discrimination of the various postulated tachykinin binding sites.

^{3}H-Substance P (SP) and ^{3}H-Eledoisin (ELE) (New England Nuclear, Boston) both bind to high-affinity sites in crude membrane preparations from the guinea-pig brain. Scatchard analysis of saturation curves reveal a single class of sites for SP with an apparent K_D of 0.68 ± 0.07 nM and a single class of sites for ELE with a K_D of 5.0 ± 0.6 nM. PBZ (0.1 to 32 µM) was pre-incubated with membranes at 37°C for 20 min. After extensive washing of the membranes to remove excess unreacted PBZ, the specific binding of ^{3}H-SP (1 nM) or ^{3}H-ELE (3 nM) was measured at 25°C in presence of leupeptin (2 µg/ml), chymostatin (4 µg/ml), bacitracin (40 µg/ml), $MnCl_2$ (3 mM) and bovine serum albumin (0.1%).

Results indicate that PBZ produces a dose-dependant decrease in the specific binding of both ^{3}H-SP and ^{3}H-ELE. IC_{50} values for this inhibition were 2.61 ± 0.41 µM (n = 3) and 5.18 ± 1.14 µM (n = 4) respectively for the binding of ^{3}H-SP and ^{3}H-ELE. The inactivation produced by PBZ was not the result of a decrease in the affinity of the ligands for their respective binding sites but rather was due to a reduction in the number of available sites. Studies were then performed with unlabelled SP, ELE and other tachykinins as protecting agents against the inactivation produced by PBZ to try and determine if the binding sites for ^{3}H-SP and ^{3}H-ELE could be differentially protected from this alkylation.

The results of these experiments provide further evidence for the existence of separate, selective binding sites for ^{3}H-SP and ^{3}H-ELE in the guinea-pig brain. The caracteristics of these multiple sites will also be discussed.

[Supported by a grant from the Medical Research Council of Canada (to J.M.) and a F.R.S.Q. Studentship (to M.T.)].

230.17 RECEPTORS FOR THYROTROPIN-RELEASING HORMONE IN THE CNS: LACK OF A HIGH-AFFINITY BINDING SITE FOR THE ANALOG DN1417.
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Although TRH and its analog DN1417 both facilitate excitation of spinal lower motor neurons (LMNs) in cordotomized (T8-T9) rats, a more sustained response is produced by DN1417 (Beydoun et al. Ann Neurol. 18:150, 1985), despite the much lower (micromolar) affinity of the spinal TRH-receptor (assayed with [3H] MeTRH) for the analog (Hawkins and Engel, Life Sci. 36:601, 1985). Recently, Funatsu et al (J. Neurochem. 45:390, 1985) have claimed identification of a new TRH-receptor (TRH-R) in rat limbic forebrain (LF) that binds DN1417 and TRH with high-affinity but does not bind MeTRH. Since such a site could be responsible for the observed excitatory effects of DN1417 (and TRH), we have attempted to identify this DN1417-high-affinity receptor in rat LF, lumbosacral spinal cord (LSC) and pyriform cortex/amygdala (PC/A), using receptor assays with [3H] TRH and [3H] DN1417. Incubations with membrane suspensions of these tissues were performed for 2h at 0°C in the presence of protease inhibitors (0.5mM EGTA, 1mM iodoacetamide, 180μM bacitracin) to minimize the peptide catabolism found in their absence. Experiments were of two types, saturation and competition isotherms, and the data were analysed by computer programs (Lundon 1 and 2, Lundon Inc., Cleveland, OH). Saturation isotherms with [3H] TRH (0.2-40nM) on PC/A and LF identified only a single class of high-affinity receptor (PC/A:Kd=9±0.2nM, Bmax=93±6 fmol/mg ptn (n=2); LF:Kd=7±0.4nM, Bmax=27±3 fmol/mg ptn (n=2) (mean± SEM). When the experiments were repeated with [3H] DN1417 and extended to include all 3 CNS tissues, no saturable, high-affinity binding of the peptide was detected. Using higher [3H] DN1417 concentrations (up to 70 μM, a low affinity site was identified in PC/A (Kd=22±2 μM, n=2). Current studies are examining whether this represents DN1417 binding to the MeTRH receptor. Competition isotherms were performed with LF and LSC, utilizing [3H] TRH at 8nM and concentrations of unlabeled DN1417 from 5-6000 nM. The curves obtained with both tissues were monophasic (not biphasic for LF as found by Funatsu et al). Computed Ki values confirmed the low-affinity of the binding site for DN1417 at 1 and 2.6 μM respectively for LF and LSC, values similar to those obtained by us previously for DN1417 binding to the [3H] MeTRH-R in PC/A and whole spinal cord (Hawkins and Engel, 1985). Thus, using the methods and tissues described here, we have been unable to identify a DN1417-high-affinity receptor that could be responsible for the neurotransmitter-like excitatory action of DN1417 in the rat CNS. Our studies favor the view that the sustained spinal LMN excitatory response to DN1417 results from the low-affinity binding of the analog by a single type of TRH-R, which also binds TRH and MeTRH but with high affinity.
(Supported by ALSA and the MDA)

HORMONAL CONTROL OF BEHAVIOR I

231.1 A POSSIBLE ROLE FOR VITAMIN D IN CONTROL OF A SEXUALLY DIMORPHIC NEURONAL OSCILLATOR IN WEAKLY ELECTRIC FISH.
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The pacemaker nucleus of the weakly electric fish, Apteronotus, is a sensitive assay of neuromodulatory influences due to its uniquely precise regularity of discharge. In addition, the behavioral and functional significance of this structure is well understood. The medullary pacemaker nucleus controls the continuous, quasi-sinusoidal electric organ discharge (EOD) of these animals. EOD frequencies vary among individuals and are segregated within the species-specific range (700 to 1200 Hz) by sex and age. Among sexually mature apteronotids, males have frequencies at the high end of the spectrum and females at the low end. These fishes' EOD frequecies can be altered with implants of sex hormones; estrogen implants cause frequencies to decline significantly (Meyer,Leong & Keller,in press). That this is a physiologically relevant response is indicated by both the sexual appropriateness of the response and that similar treatments with testosterone cause frequency drops in species where the sexual dimorphism is reversed (i.e. in species where males are of lower frequency than females; Meyer,J. comp. Physiol.,1982).

Despite these suggestions of hormonal modulation of pacemaker frequency no sex hormone binding has been found in the pacemaker nucleus itself. This negative evidence has left open the possibility that EOD frequency is altered by a secondary agent which may be directly affected by estrogens. A chief candidate for this role is (1,25)dihydroxycholecalciferol, the active form of vitamin D. The rationale for suspecting this steroid hormone lies in its documented control by estrogens in mammals and by immunohistochemical studies done in Apteronotus which show high levels of vitamin D-dependent calcium binding protein in the pacemaker nucleus (Maler,Jande & Lawson, Br. Res.,1984).

We find that 100 ug implants of dihydroxy-vitamin D cause a significant decrease in EOD frequency comparable in degree and time course with that caused by estradiol. Lower dosages seem to elicit declines in frequency only noticeable in higher-frequency males. While the 100 ug dosages appear quite high, they are, in fact, smaller than those used in earlier sex hormone experiments which produce only moderate increases in serum levels of the hormone (Keller,Zakon & Sanchez,J. comp. Physiol.,1986). It remains for us to determine the serum effects of these vitamin D implants, both as regards effective levels of the hormone and possible secondary consequences. Nevertheless, this finding suggests a possibile indirect route for the control of pacemaker frequency by sex hormones and a neuromodulatory role for dihydroxycholecalciferol via vitamin D-dependent calcium binding protein.

231.2 MELANOTROPIN IS ALTERED BY ACUTE AND CHRONIC SOCIAL STRESS IN LIZARDS
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The lizard Anolis carolinensis typically has a green body color. However, when a male is "losing" in an episode of intraspecific aggression and when participating in a subsequently established social dominance hierarchy as a subordinate, it exhibits body color changes from green to brown. The dermal melanophores responsible for body color changes are known to be devoid of sympathetic innervation and are, therefore, controlled by circulating hormones. The activities associated with body color change are stressful in the sense of adrenal catecholamine and ACTH-corticosterone activation (Greenberg, Adv. Vert. Neuroethol., Plenum Press, 1983; Greenberg et al., Horm. and Behav., 1984).

While these hormones can affect body color, a principal hormonal influence on melanophores is the pituitary peptide melanotropin (MSH). To clarify the possible role of melanotropin in lizards we employed radioimmunoassay to determine its circulating levels immediately subsequent to the resolution of an aggressive interaction and several hours later, as social dominance relationships were being consolidated.

Blood sampled from lizards within five minutes of "winning" a fight had melanotropin levels significantly reduced from that of control animals while "losers" had only slightly elevated levels. Several hours later, however, melanotropin levels of social dominants (former winners) was similar to control values, while that of subordinates (former losers) was significantly elevated.

The behavioral concommitants of melanotropin elevation observed in other species include enhanced attention and are consistent with the behavior observed in winning/dominant and losing/subordinate lizards. Further, one could postulate an adaptive/survival value for a body color change in subordinate animals, known to select lower perch-sites than dominants.

231.3 SEX DIFFERENCES IN THE MONOAMINE CONTENT OF THE PREOPTIC-HYPOTHALAMIC AREA IN THE RING DOVE. S.R. Barclay and M.-F. Cheng. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

The preoptic-hypothalamic area, which controls sexual behavior and the pattern of gonadotropin secretion, is sexually differentiated by gonadal hormones during critical periods in development. Since monoamines are involved in processes mediating gonadotropin secretion and sexual behavior, we examined this brain region for sex differences in monoamine content. In addition, the effect of gonadectomy on monoamine content was determined.

The monoamine content of four brain regions was determined by high-performance liquid chromatography with electrochemical detection. The areas were the preoptic-hypothalamic area (HYP), the striatum (STR) containing the paraolfactory lobes and hyperstriatum, the neostriatum (NS) containing the archistriatum and neostriatum, and the optic lobes (OL). The minimal detectable level was 100 pg for each primary amine and its major metabolite: norepinephrine (NE), and normetanephrine (NME); dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA); serotonin (5HT), and 5-hydroxyindoleacetic acid (5HIAA).

Most of the significant sex differences were found in the HYP. There were significantly higher levels of NE, DA, and 5HT in the female's HYP compared to the male's HYP. No significant differences were found in the HYP content of metabolites. Also, higher levels of 5HT in the OL, and NE in the NS, and lower levels of DA in the STR, were found in the female compared to the male.

Another significant sex difference was the effect of gonadectomy on monoamine content. Ovariectomy results in an increased content of DA in the HYP, and a decreased content of DA in the OL. Castration results in an increased content of NE and 5HT in the HYP and OL. The castrated male's HYP monoamine content is similar to the monoamine content in the intact female's HYP, suggesting that circulating levels of androgen in the intact male ring dove contribute, in part, to the observed sex differences in this area.

It can be concluded from this study that there are sex differences in the monoamine content in the preoptic-hypothalamic area in the ring dove. That monoamine content in this brain area is responsive to changes in plasma levels of gonadal hormones is particularly interesting in view of our previous finding that male courtship behavior is modulated by the noradrenergic system. This suggests that the effect of androgens on monoaminergic activity in the brain may play an important role in the expression of male courtship behavior.

231.4 ACTIVATION OF MALE AND FEMALE SEXUAL BEHAVIOR WITH INTRACRANIAL SEX STEROID IMPLANTS IN THE JAPANESE QUAIL. James T. Watson, Frances Teunis*, Arnaldo Valedon*, and Elizabeth Adkins-Regan. Field of Neurobiology and Behavior and Department of Psychology, Cornell University, Ithaca NY 14853.

The Japanese Quail (*Coturnix japonica*) is an ideal model for studying the behavioral effects of sex steroid hormones. Treatment with testosterone propionate (TP) restores normal copulatory, courtship, and crowing behavior as well as cloacal foam gland development in castrated adult male quail. Estradiol benzoate (EB) treatment of male castrates activates male copulatory behavior as well as female receptivity, but does not activate courtship or crowing (Adkins & Pniewski, J. Comp. Physiol. Psychol., 92: 1169, 1977). Recent steroid autoradiographic studies by this lab have shown discrete populations of E-, T-, and dihydrotestosterone (DHT)-concentrating cells in the quail brain (Watson & Adkins-Regan, Soc. Neurosci. Abs., 11: 740, 1985). Intracranial implantation of minute amounts of sex steroids has been undertaken to examine the behavioral roles of the steroid-binding cell populations revealed by autoradiography, and to determine the neuroanatomical basis of the specificity of steroid effects on behavior.

Experimental subjects were adult, castrated, male quail, chosen for high sexual activity in pretests with hens. Subjects were implanted according to the method of Morgantaler and Crews (Horm. Behav., 11: 61, 1978). The subjects were anesthetized with Forane and placed in a Kopf stereotaxic modified for quail. 21 g steel cannulae were used to place pellets consisting of EB, TP, or cholesterol (Chol) mixed with charcoal and bone wax into sites in the brain. Following implantation, subjects were pair-tested for social behaviors on alternate days with intact male and female quail for two weeks, and tested twice for vocalizations in a recording cage. After the last behavior test, subjects were anesthetized heavily, perfused, and checked for testicular remnants. Their brains were removed, post-fixed, embedded in albumin-gelatin, sliced at 40 µm, stained with cresyl violet, and examined to determine location of implants.

EB implants in the preoptic area (POA) consistently activated male copulatory behavior, including head-grabs, mounts, and cloacal contact movements, without concomitant activation of female-typical receptivity. EB implants in posterior hypothalamic area (PHA) stimulated female-typical receptivity, without activating male copulatory behavior. EB implants in adjacent nuclei which contain E-concentrating cells, the periventricular nucleus (PVN) and the lateral septal nucleus (LS), had no effect on male or female copulatory behavior. Control implants of EB into a brain region which contains no E-concentrating cells, *lobus parolfactorius* (LPO), and Chol implants into POA did not activate copulatory behavior. In agreement with Barfield's findings in the fowl (Horm. Behav., 1: 37, 1976), TP implants in the preoptic area consistently activated male copulatory behavior without stimulating crowing, strutting, or foam gland development. TP implants in adjacent nuclei containing T-concentrating cells, PVN, LS, PHA, and lateral forebrain bundle area, did not activate male or female copulatory behavior.

These results are the first report of the neuroanatomical localization of sites responsible for estrogen activation of male and female copulatory behavior in a bird. The similarity of POA-EB and POA-TP activation of male sexual behavior is consistent with the hypothesis that POA neurons aromatize T to E and E directly stimulates cellular processes leading to behavioral activation. Further studies are planned using aromatase inhibitor implants into the POA, and EB, TP, and DHTP implants into other forebrain and midbrain regions. Supported by NSF BNS84-12083.

231.5 CYTOSOL AND NUCLEAR ESTROGEN RECEPTORS IN THE MALE ZEBRA FINCH BRAIN. Michael J. Walters and Cheryl F. Harding. Biopsychology Program, Hunter College, CUNY, New York, NY 10021.

There is a growing body of evidence which indicates that estrogens play an important role in the activation of many male zebra finch behaviors, including species-specific vocalizations which are used to court females. In the present study, we used a sensitive in vitro binding assay to detect the presence of estrogen receptors in various hypothalamic and vocal control nuclei.

Saturation assays were first performed on cytosol from the basal hypothalamus-preoptic area (BH-POA) of castrated males. Aliquots of cytosol were incubated with varying concentrations of ^{3}H-estradiol (0.01 - 2.7 nM) in the presence and absence of a 100-fold molar excess of unlabeled DES. Scatchard analysis indicated that estrogen receptor binding in BH-POA cytosol was saturable and that only a single, high affinity component was being measured (Kd = 0.31 + 0.06 nM, Bmax = 7.2 + 0.6 fmol/mg protein). Competition studies indicated that the assay was specific for estrogen receptors.

Individual nuclei from castrated finches were microdissected from 0.2 mm thick frozen sections. Cytosol fractions were then incubated with 1 nM ^{3}H-estradiol in the presence and absence of 100 nM unlabeled DES. Estrogen receptors were detected in all hypothalamic nuclei examined: the preoptic area (POA), the periventricular nucleus (PVM), and the infundibular region (INF). Concentrations of bound ^{3}H-estradiol ranged from 4.0 fmol/mg protein in INF to 11.5 fmol/mg protein in PVM. Low but significant receptor levels were also apparent in four of the five vocal control nuclei examined: the magnocellular nucleus of the neostriatum, (MAN), the caudal portion of the ventral hyperstriatum (HVc), the robust nucleus of the archistriatum (RA), and the dorsomedial region of the intercollicular nucleus (ICo). Bound ^{3}H-estradiol levels ranged from 0.9 fmol/mg protein in RA and HVc to 2.3 fmol/mg protein in MAN and ICo. No specific ^{3}H-estradiol binding was observed in Area X of the parolfactory lobe.

Since we have subsequently found evidence of significant levels of circulating estradiol in castrated males, nuclear exchange assays were performed on BH-POA extracts from castrates. Preliminary evidence indicates that saturable, high affinity binding is present in purified nuclear extracts obtained from castrated males (Kd = 0.43 nM, Bmax = 250.8 fmol/mg DNA).

These data suggest that castrated male zebra finches still possess substantial pools of endogenous estrogen which may account for the low levels of ^{3}H-estrogen binding observed in cytosol receptor assays and autoradiographic studies.

231.6 DOPAMINERGIC REGULATION OF MALE RAT COPULATORY BEHAVIOR: RELATIVE ROLES OF PRE- VS POSTSYNAPTIC DOPAMINE RECEPTORS IN THE MEDIAL PREOPTIC AREA. D. Bitran*, G. Holmes*, E. Hull, & K. Lookingland. Dept. Psychology, State Univ. New York at Buffalo, Amherst, NY 14226, and Dept. Pharmacol. Toxicol., Michigan State Univ., East Lansing, MI 48224.

Dopaminergic influence on male rat copulatory behavior is well documented. Disagreement exists, however, as to the proposed mechanism(s) by which dopamine (DA) agonists affect sexual performance. Improved sexual function has resulted from systemic administration of DA receptor agonists. Because the doses that facilitate copulatory behavior are lower than those that elicit stereotypy (a DA postsynaptic effect) some researchers have proposed that the facilitative effects of low doses of DA agonists result from preferential activation of presynaptic autoreceptors. By contrast, we have recently reported that microinfusions of apomorphine (APO) into the lateral ventricle (LV) or medial preoptic area (MPO) affected sexual behavior in a biphasic dose-dependent fashion: low doses inhibited, higher doses facilitated many of the same copulatory parameters (Hull et al., Brain Res., 370:73, 1986). We hypothesized that the lower doses impaired copulation by stimulating autoreceptors preferentially, while facilitation was mediated by postsynaptic receptor activation.

In order to clarify the role of presynaptic DA receptors in male copulatory behavior, we infused 6-OHDA into the MPO after pretreatment with desipramine (to spare noradrenergic terminals). Compared to sham controls, no differences were found in the copulatory behavior of 6-OHDA-treated males. Biochemical analysis (HPLC-ED) revealed a small (23%) but significant DA depletion in the MPO. DOPAC levels were unchanged suggesting that the system had compensated for the DA depletion by increasing turnover.

We hypothesized that further inhibition of DA synthesis in 6-OHDA-treated males would result in copulatory deficits. Indeed, a regimen of AMPT which did not affect copulatory behavior in normal males produced deficits in 6-OHDA-treated males.

The final experiments tested whether 6-OHDA had altered the biphasic dose-response curve of APO found in intact males. Removal of presynaptic receptors by 6-OHDA should attenuate the impairment produced by low doses of APO. Conversely, supersensitivity of postsynaptic receptors should enhance APO's facilitative effects. Indeed, these results were observed. In control males a very low dose of APO (100 ng) in the MPO significantly reduced ejaculation frequency; in 6-OHDA males no such inhibition occured. The attenuation of sexual behavior previously found with 200 ng APO in the LV of normals was not observed in 6-OHDA males. The facilitative effects of 500 ng APO in MPO or LV were not altered by 6-OHDA.

231.7 OPIATE REGULATION OF MALE SEX BEHAVIOR IN THE RAT: EFFECTS OF MORPHINE AND NALOXONE INFUSED INTO THE MEDIAL PREOPTIC AREA
L. Band*, R. Warner*, E. Pehek*, D. Bitran*, G. Holmes* and E. Hull (SPON: C. Smith) SUNY at Buffalo, Buffalo, NY 14260

Both clinical and experimental evidence indicates that opiates play an inhibitory role in male sex behavior. However, because studies conducted to date have employed systemic, intrathecal or intraventricular administration of agonists and antagonists, spicific sites of action have not been determined. The present studies investigated the role of an opiate system within the medial preoptic area (MPOA) in the regulation of sex behavior in male rats.

Male rats were implanted with indwelling cannulae positioned 1.0mm above the left MPOA and within the right lateral ventricle (LV). Each weekly behavioral test was preceded by an infusion of morphine sulfate (0, .2, .5 or 2ug) in .5ul saline vehicle into one of the two cannula sites. Five minutes following each infusion, males were placed with a receptive female and sex behavior was observed. Using an identical procedure, a second group of males were implanted with cannulae and received infusions of naloxone hydrochloride (0, .2, .5 or lug) in .5ul saline vehicle. Behavioral testing began fifteen minutes following each infusion.

The .5 and 2ug doses of morphine infused into the MPOA reduced ejaculation frequency while the 2ug dose increased the postejaculatory interval. These effects reflected a cessation of copulation. The lug dose of naloxone infused into the MPOA shortened ejaculation latency and the postejaculatory interval. The .5ug dose of morphine infused intraventricularly lengthened the intercopulatory interval and reduced copulatory efficiency. Both of these effects may have been related to increased mounting. Intraventricular infusions of naloxone produced no behavioral change.

The opposing effects of morphine and naloxone infused into the MPOA on measures of copulatory rate indicate that opiates endogenous to this structure play an inhibitory role in the regulation of male sex behavior. Further research is needed to clarify the sites of action for the effects of intraventricularly infused opiates.

231.8 ACTIVATION OF PENILE REFLEXES BY TESTOSTERONE IN RATS MAY NOT DEPEND ON PROTEIN SYNTHESIS.
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In two sets of studies we investigated whether some behavioral effects of testosterone (T) might occur through rapid neuronal mechanisms not dependent on protein synthesis. Such short-latency behavioral effects of progesterone (e.g., sexual receptivity in ovariectomized female rats) and other steroids have been described, but the latency of behavioral response to T has been slow. For example, in long-term castrated male rats, restoration of copulatory behavior is measured in days, and restoration of penile reflexes in hours. These latencies are consistent with the assumption that protein synthesis in neurons underlies these effects of T. However, electrophysiological studies of the brain suggest the potential for central neural effects of T measured in seconds or minutes, probably too fast to be accounted for by protein synthesis. We inquired whether more sensitive measures of penile reflex activity might reveal short-latency activation by T, and also investigated the effects of protein-synthesis inhibition on the activation of penile reflexes by T. Tests for penile reflexes occurred with the male restrained in supine position with the penile sheath retracted.

In one study we monitored the penile reflexes of long-term castrated rats visually and by EMG recordings from electrodes chronically implanted in the bulbocavernosus muscles (mBC). Tests started immediately after injection of oil vehicle or of 250 ug T im (into thigh) and 250 ug T propionate sc, and tests continued for 30 min after injection. Almost all visible penile responses were associated with mBC bursts, but many mBC bursts were accompanied only by twitching of the mBC, visible under the shaved scrotal skin. Mean latency from sheath retraction to first EMG burst was significantly shorter after T than after oil injection. Several males displayed EMG and penile responses within 6-8 min after T injection, a speed suggestive of a mechanism of steroid action other then protein synthesis.

In a separate set of studies, anisomycin was injected into castrated rats in doses and at intervals sufficient to inhibit protein synthesis by an estimated 80% for about 6-8 hr. Anisomycin did not delay the restoration of reflexes by injection of T, did not interfere with the maintenance of reflexes by sc-implanted Silastic capsules filled with T, and did not accelerate the decline in reflexes after removal of the capsules. Therefore, protein synthesis may not be critical to the role of T in activating penile reflexes in the rat.

(Supported by NIH grants HD-08933 to BDS and HD-21478 to RLM.)

231.9 OLFACTORY BULB REMOVAL DISRUPTS MASCULINE AND FEMININE REPRODUCTIVE BEHAVIOR IN MALE RATS.
A.R. Lumia*, F.E. Solomon*, M.Y. McGinnis*, B.D. Sachs. (SPON: M. Wilson). Psychology Department, Skidmore College, Saratoga Springs, NY 12866.

Bilateral olfactory bulbectomy has been shown to disrupt masculine sexual behavior and decrease androgen receptor binding in the amygdala and hypothalamus in males (Lumia, Zebrowski, and McGinnis (1984) Society for Neuroscience 10:826), In contrast, olfactory bulbectomy in females facilitates feminine sexual behavior and is correlated with increased amygdaloid estrogen receptor binding (McGinnis, Lumia, and McEwen (1985) Brain Res, 334, 19-25). The present experiment was undertaken to further investigate the role of the olfactory bulbs on gonadal steroid receptor binding and copulatory behavior.

In this study we examined the relationship among olfactory bulb removal, gonadal steroid receptor binding, and female and male behaviors. Sexually experienced male rats were either bulbectomized (BOB) or sham operated (Sham), castrated and implanted with either 10% or 100% (10mm) estrogen filled silastic capsules. Animals were then paired with a sexually vigorous male and given a series of four tests, one every third day, for female sexual behavior. Two tests were given with estrogen alone (E) or in combination with progesterone (P, 500ug) on alternating tests. Animals were then tested for male copulatory activity with a sexually receptive female. Males were tested with estrogen alone or in combination with dihydrotestosterone (DHT, two 100% 10mm silastic capsules).

Our findings demonstrate that bulbectomized males display significant decrements of all components of male copulatory behavior; only 3/12 BOB males achieved ejaculation. The magnitude of the copulatory deficits was unaffected by hormonal treatment. In contrast, Sham operates showed high levels of ejaculation through all tests. BOB males displayed less feminine sexual behavior than did Sham operates. For BOB males there was a facilitative effect of progesterone and repeated testing. BOB males given E + P displayed LQs similar to Sham operates. In sum, E alone or in combination with DHT is ineffective in reversing the BOB male copulatory defects. In contrast to BOB females, E did not facilitate lordosis in BOB males. However, it is interesting to note that BOB males were responsive to P.

Assay data are presently being compiled and evaluated.

(Supported by a grant from THE FREED FOUNDATION to A. R. Lumia).

231.10 HYPOTHALAMIC ANDROGEN RECEPTORS: EFFECTS OF CASTRATION AND HORMONE REPLACEMENT.
N.G. Simon, M.P. Perry*, and L.E. Hanninen*. Dept. of Psychology, Lehigh University, Bethlehem, PA 18105.

Changes in the concentration of androgen receptors (AR) have been noted in peripheral target tissues as a consequence of castration. Following a short-term increase 24-48 hr after gonadectomy (due to the depletion of endogenous steroids), receptor levels reportedly decline to undetectable amounts in androgen-dependent tissues (e.g., seminal vesicles, epididymis) and return to normal levels following androgen replacement therapy. Whether this same pattern of change occurs in neural target tissues, however, remains undetermined. This question is of interest since a comparable decrease in the brain might partially explain the relative refractoriness of long-term gonadectomized males to the behavior-activating effects of steroid stimulation.

CF-1 male mice were maintained in the laboratory in accordance with Federal guidelines for animal care. They were gonadectomized while anesthetized with nembutal between 55-65 days of age. Forty days later, half began receiving daily injections of 300 ug testosterone (Long-term + T) and half were given oil vehicle only (Long-term + Oil). Treatments were continued for 10 days and hypothalamic AR were assayed (hydroxylapatite method) using a 1-point procedure (nominal dose - 5nM) 48 hr after the last injection. Control males were castrated at approximately 115 days of age and AR were measured 48 hr later.

Specific AR binding in control males was 6.8±.29 fmol/mg protein (6 replications). In contrast, AR concentration in Long-term + Oil males was 4.0±.21 fmol/mg protein (5 replications), a 41% reduction in comparison to controls. Castrated males given 10 days of T exhibited 5.9±.36 fmol/mg protein of AR, a 13% reduction versus controls.

While the post-castration decrease in AR may have occurred during the treatment period, it appears more likely that long-term castration resulted in a decline in hypothalamic AR which can be overcome by T replacement therapy. This conclusion may provide a possible basis for the differences in behavioral sensitivity to androgens seen between long-term and short-term castrated males.

231.11 Alpha-adrenergic regulation of nuclear androgen receptor number in male rat brain. Robert J. Handa, Henry L. Stadelman, John A. Resko. (SPON. T. Condon). Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201. Div. of Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, OR 97006

Steroid hormones and catecholamines interact in neural tissues to regulate reproductive functions. In many cases, the effect of steroids appear to be catecholamine mediated. In the present study, we tested the hypothesis that adrenergic receptor antagonists alter androgen action on the brain by changing androgen receptor dynamics.

We treated groups of intact male Sprague/Dawley rats (250-300 g) for two hrs with saline (controls), prazosin (5 mg/kg BW), yohimbine (2 mg/kg BW) or propranolol (10 mg/kg BW). After sacrifice we collected trunk blood and assayed brain tissues for androgen binding of cytosolic and nuclear fractions. This assay utilized the binding of the synthetic androgen ^{3}H-R1881 and has been previously described in detail (Handa et al. Biol. Reprod. 24:293, 1986). Less androgen receptor ($p < 0.01$) was detected in nuclear fractions of preoptic area after treatment with the alpha-1-receptor antagonist, prazosin and the alpha-2-receptor antagonist, yohimbine. There was no effect of the beta-receptor antagonist, propranolol.

Treatment	N	Preoptic* Area	Basal Hypothalamus	Amygdala	Anterior Pituitary
Saline	6	125.3±12.7	146.7±28.2	81.3±14.0	159.9±17.0
Prazosin	6	83.8±12.6**	154.9±12.4	103.3±21.4	132.6± 8.0
Yohimbine	6	59.1± 8.6**	122.8±15.9	79.5± 9.7	144.9±15.8
Propranolol	6	140.4±23.4	180.5±36.8	85.9±11.9	148.5± 7.3

* Data presented as fmoles ^{3}H-R1881 bound/mg DNA.
** $p < 0.01$

Saturation studies revealed that the affinity of the androgen receptor for ^{3}H-R1881 was unaffected by any of the drugs tested ($K_d = 1.1 \times 10^{-9}$M in drug and control animals). The addition of excess amounts of radioinert prazosin, yohimbine and propranolol to the incubation tube revealed little competition of these drugs with R1881 for the androgen receptor.

These data show that the number of androgen receptors can be affected by alterations in the alpha-adrenergic system. This effect is confined to the preoptic area and suggests an additional role for adrenergic agents in the control of steroid mediated events. Supported by grants HDO6731 and HD18196.

231.12 REQUIREMENTS FOR DAILY TESTOSTERONE EXPOSURE AND PROTEIN SYNTHESIS TO ACTIVATE COPULATORY BEHAVIOR IN MALE RATS. M.Y. McGinnis*, M.C. Mirth*, A.F. Zebrowski* and R.M. Dreifuss* (SPON: J.E. Shriver). Dept. of Anatomy, Mt. Sinai School of Medicine, CUNY, New York, NY 10029.

The purpose of this experiment was to establish the number of hours of testosterone (T) exposure per day necessary to restore masculine sexual behavior and to determine whether protein synthesis is required for effective copulation. Long-Evans male rats weighing 200-250 g were screened for copulatory behavior. Animals completing two ejaculatory series were castrated and given a post-castration test 3-4 weeks later to confirm loss of copulatory behavior. On the day before treatment began an incision was made in the flank region and an open pouch formed for the insertion of two 10 mm T-filled Silastic capsules. This procedure permits insertion and removal of T capsules without anesthesia and with minimal disturbance to the rats. Capsules were inserted and removed every day to provide daily T exposures of 4,8,12,14,16,18 and 24 h (continuous). All animals were tested for copulatory behavior on days 2-3, 4-5, 7-8 and 9-10 of T treatment.

Rats in all groups showed mounting behavior. However only continuous (24 h) T exposure was effective in maintaining an ejaculatory response. The 14-18 h T exposures were equivalent and much weaker than 24 h T in activating complete copulatory behavior. Daily exposure to T for 12 h or less failed to activate copulatory behavior.

In a second experiment male rats were tested using the same paradigm except that only the 24 h T exposure was used. On the first day of capsule insertion daily sc injections of 2 or 20 mg Anisomycin (ANI) or saline were begun in order to block protein synthesis. Tests for copulatory behavior were conducted on days 3,7,10 and 14 of ANI treatment. Only 0-40% of ANI-treated rats ejaculated on any test day, regardless of dose. In contrast, all saline-treated rats completed two ejaculatory series by day 14.

The results suggest that 1) almost continuous daily exposure to T is necessary for effective copulation, 2) ongoing protein synthesis is required for the expression of male sexual behavior, and 3) copulatory behavior and LH secretion may be regulated by different androgenic mechanisms.

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231.13 FAILURE OF TESTOSTERONE TO REVERSE CONDITIONED AVOIDANCE OF COPULATION IN MALE RATS. G.J. Lawrence, S.W. Kiefer and C.W. Metzler*, Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506.

Following 1-6 trials in which male rats are made ill following copulatory encounters with estrous females that have been sprayed with a 2% almond solution, males display complete avoidance of copulatory behavior (Lawrence & Kiefer, 1984). Presently the effect of testosterone propionate (TP) was examined in male rats that had been trained to avoid copulatory behavior. Previous studies showed an increased rate of copulatory responses (mount, intromission, and ejaculation) when injections of TP were given to both copulating and non-copulating male rats (Beach & Fowler, 1959).

Two groups of sexually naive male rats were presented with an estrous female sprayed with a 2% almond solution; each training trial was terminated after an ejaculatory response or after 20 min if the male failed to ejaculate. Following each trial males were intubated; rats in the Experimental group (n=19) were given lithium chloride (LiCl) (2% body weight) to produce illness, and rats in the Control group (n=19) were given normal saline (NaCl). When an Experimental male failed to ejaculate on two consecutive trials, that male was yoked to a Control rat and both were assigned to one of four subgroups: (a) Exp/TP (Experimental, TP), (b) Exp/SL (Experimental, saline), (c) Con/TP (Control, TP), or (d) Con/SL (Control, saline). Assignment to subgroups was made according to the number of copulatory trials required to meet criterion. Daily 0.1 ml injections of TP 1 mg (TP subgroups) or NaCl (SL subgroups) were given for seven days, after which each male was tested with an almond solution-sprayed, estrous female (Test 1). The seven daily injections were repeated, and each male was again presented an odorous, estrous female (Test 2).

All experimental males showed persistent avoidance of copulation; 100% of these males failed to ejaculate in both Tests 1 and 2. Compared with the Exp/SL males, however, the Exp/TP males showed a significant increase in the display of mount responses in both test trials and a significant increase in intromission responses in Test 2. Significantly decreased latencies to mount, intromit, and ejaculate were noted in both test trials in the Con/TP males. Results indicated TP treatment for 14 days was ineffective in reversing conditioned avoidance of copulatory behavior in male rats. However, TP did produce sexual arousal, as evidenced by the significant increase in responses to initiate copulatory activity.

231.14 A COMPUTER-BASED CORRELATIVE APPROACH FOR IDENTIFYING THE LOCUS OF ACTION FOLLOWING INTRACRANIAL TREATMENT WITH LIPOPHILIC COMPOUNDS. J.A. Czaja and P.C. Butera*. Miami Univ., Oxford, OH 45056 and Niagara Univ., Niagara University, NY 14109.

The highly lipophilic nature of many psychoactive compounds allows them to diffuse readily through brain tissue. This characteristic complicates efforts to use direct intracranial treatment as a technique for identifying the loci at which such compounds act to produce particular behavioral and physiological effects. We have developed a computer-based correlative approach which takes advantage of the expected diffusion from intracranial implants of such substances in order to estimate potential sites of action. This approach is based on the premise that if the brain contains specific sites at which a lipophilic compound acts to modify a variable, then there should be a negative correlation between distances from that site to intracranial implants and the relative effects of those implants on that variable. Implants are filled, placed at various brain sites, and observations collected on changes in dependent variables. Implant placements are then confirmed by histology and exact locations quantified in three planes. Next, the entire brain is mathematically scanned at the chosen resolution (such as 0.1 mm). Individual correlation coefficients between the distance from each scanning site to the implant locations and the effects of the various implants are then calculated in order to identify potential sites of action.

To test this approach, 60 ovariectomized female guinea pigs were implanted with intracranial cannulae containing estradiol-17β and data collected on resulting changes in food intake. Treatment sites ranged through a brain volume of approximately 132 mm^3. Highly significant correlations ($p<.001$) were found between the effects of estradiol implants on food intake and their distance from an area in the vicinity of the paraventricular nuclei. Random reassignment of the feeding data among the implant sites produced equal or greater correlations in 9 out of 1000 reiterations of the total brain-scan procedure, operationally producing an estimated value of $p<.01$ for the observed results. This correlative approach therefore appears to provide an elegantly simple procedure by which one may utilize results from a relatively limited number of intracranial placements with a lipophilic compound to help localize the sites of action of that compound within the brain.

232.1 ESTROGEN RECEPTORS IN HYPOTHALAMUS-PREOPTIC AREA OF MALE AND FEMALE GRAY SHORT-TAILED OPOSSUMS (MONODELPHIS DOMESTICA). B.H. Fadem* and A.M. Etgen (SPON: A. Siegel). Dept. Psychiat., U.M.D.N.J.-N.J. Med. Sch., Newark, N.J. 07103 and Depts. Psychiat. and Neurosci., Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Marsupials provide an ideal model for studying the organizational role of gonadal hormones during early life on adult reproductive behavior since they are born in a developmentally immature "fetal" condition. The study presented here is the first of a series in which the presence and development of hormone receptor systems in the brains of gray opossums, small, South American marsupials, will be examined. Specifically, the present experiment was designed to determine whether the hypothalamus-preoptic area (HPOA) of adults of this species contains specific, high affinity, saturable estradiol binding sites.

Estradiol binding sites were characterized by incubating HPOA cytosols from gonadectomized male and female opossums with increasing concentrations of ^{3}H-estradiol (0.06-6.0 nM) in the presence or absence of excess unlabeled estradiol. Scatchard analysis revealed that the HPOA of both males and females contained high affinity (K_d=0.08-0.12 nM), saturable estradiol binding sites. There was a high concentration of binding sites (Bmax=20-30 fmole/mg protein), and there was no sex difference. Binding specificity was characterized by incubating HPOA cytosols from both sexes with 1 nM ^{3}H-estradiol plus 100-fold excess unlabeled estradiol, diethylstilbestrol (DES), 4-OH-tamoxifen (TX), dimethylstilbestrol (DMS), testosterone, dihydrotestosterone, progesterone, deoxycorticosterone, or corticosterone. The synthetic estrogen DES and the estrogen antagonist TX competed for ^{3}H-estradiol binding as effectively as estradiol itself. The weak estrogen angonist/antagonist DMS, the androgens and the adrenal corticoids were between 5- and 10-fold less effective at displacing radiolabeled estrogen than estradiol, DES and TX. Unexpectedly, 100 nM progesterone displaced approximately 40% of the specifically bound ^{3}H-estradiol. Thus the HPOA of both male and female gray opossums contain macromolecules with steroid binding properties characteristic of estrogen receptors.

Little is known about the role of gonadal hormones in the organization and activation of behavior in marsupials. Recent experiments demonstrated that gray opossums are behaviorally responsive to estrogen neonatally and in adulthood. The possible relationship between the presence of estradiol binding sites in the HPOA and the display of reproductive behavior in gray opossums will be discussed.

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232.2 ASYMMETRY OF NEURAL ESTROGEN ACTION. E. J. Roy and D. M. Lynn* Department of Psychology, University of Illinois, Champaign, IL 61820.

Asymmetries have been discovered in subcortical areas, including the hypothalamus. For example, Gerendai et al. (1978) reported that the right side of the hypothalamus has a higher LHRH concentration than the left side. Asymmetry of hypothalamic estrogen sensitivity was suggested by work of Nordeen and Yahr (1982), who found that unilateral implantation of estradiol into the brain of neonatal rats had different consequences depending on the side of application of the hormone. Exposure of the left hypothalamus to estrogen defeminized the females, whereas exposure of the right side masculinized them. We examined the effectiveness of unilateral implants of estradiol at inducing lordosis behavior in adult female rats. Ovariectomized (OVX) animals were implanted with bilateral stainless steel cannulas aimed at the rostral hypothalamus. One side of the hypothalamus was exposed to crystalline estradiol (diluted with cholesterol 1:10) in a 30 g cannula for 5 min. Six hrs later a second 5 min exposure was administered. Testing for sexual receptivity occurred 18 hrs later, 5 hrs after 1 mg progesterone s.c. One week later the animals received two pulses of estradiol in the opposite side of the hypothalamus. Order of treatment was counterbalanced. Application of estradiol to the right side was more effective than application of estradiol to the left side. The level of receptivity induced by this treatment varied, but within each animal the left was never more effective than the right. The mean lordosis quotient (LQ) was 80 when applied to the right side, vs. 49 when applied to the left ($p < 0.05$, n=8). In a between subjects experimental design, a single 5 min exposure to estradiol was also more effective when applied to the right (Rt LQ = 53 $\pm$ 13, Lt LQ = 8 $\pm$ 4, $p < 0.02$, n's = 10 and 6). Histological examination of the brains revealed no consistent difference in placement of the right and left cannulas. We have also examined the possibility of asymmetry in the distribution of cytosol estrogen receptors in the brain of OVX rats. In three separate experiments with n's of 4-5 each, the preoptic area (POA) consistently had more estrogen receptors on the right side (19-26% more). The hypothalamus did not show any consistent asymmetry across replications, and the amygdala showed no evidence of asymmetry in any experiment. Taken together, these results suggest that asymmetry in sensitivity to estradiol may occur in adult rats, possibly related to an asymmetry of estrogen receptors in the POA. Supported by NIMH grants MH33577 and MH00481.

232.3 SEX DIFFERENCES IN AROMATASE ACTIVITY IN MICRODISSECTED REGIONS OF THE MONKEY HYPOTHALAMUS AND LIMBIC SYSTEM. C.E. Roselli and J.A.Resko*, Dept. Physiology, Oregon Health Sciences Univ., Portland,OR 97201 and Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The conversion of androgens to active estrogenic metabolites by neural tissue is believed to be an essential component in the cellular mechanism of androgen-induced neuroendocrine responses. The enzyme complex which is responsible for the synthesis of estrogens from circulating androgens is called aromatase. We previously reported that testicular androgen, in addition to being the substrate for aromatase, also appears to be a major regulator of brain aromatase activity in adult rodents and primates. In the present experiment we wished to determine whether there are sex differences in the levels of aromatase activity in specific regions of the primate brain. To do this, we measured aromatase activity in ten nuclei and brain regions from intact adult female (n=6) and male (n=5) cynomolgus monkeys. Individual nuclei were microdissected from 600 um frozen brain sections. Aromatase activity was measured in tissue homogenates by a radiometric assay that uses the incorporation of tritium from [1β-^{3}H]androstenedione into $^{3}H_2O$ as an index of estrogen formation. The females were obtained during the early follicular phase of their menstrual cycles (i.e. serum estradiol = 20 $\pm$ 5(SE) pg/ml and progesterone = 0.2 $\pm$ 0.08 ng/ml). As expected serum testosterone was significantly greater in male than in female monkeys (15.7 $\pm$ 4.9 ng/ml vs. 0.7 $\pm$ 0.2 ng/ml; $p<.001$). The greatest levels of aromatase activity were measured in the medial amygdaloid nucleus (MA, 2.9 $\pm$ 0.2 $pmol \cdot h^{-1} \cdot mg\ protein^{-1}$); the ventromedial hypothalamus (VMH, 1.4 $\pm$ 0.3 $pmol \cdot h^{-1} \cdot mg^{-1}$); and the medial preoptic area - anterior hypothalamus (MPA/AH, 0.9 $\pm$ 0.1 $pmol \cdot h^{-1} \cdot mg^{-1}$) of the male. Significantly lower levels of aromatase ($p<.05$) were detected in these same areas in females (MA = 1.5 $\pm$ 0.3 $pmol \cdot h^{-1} \cdot mg^{-1}$; VMH = 0.4 $\pm$ 0.06 $pmol \cdot h^{-1} \cdot mg^{-1}$; and MPA/AH = 0.5 $\pm$ 0.07 $pmol \cdot h^{-1} \cdot mg^{-1}$). Despite substantial capacity to aromatize, no significant sex differences were found in any other hypothalamic or limbic nuclei. Thus, our results indicate that the areas containing the greatest levels of aromatase in the monkey brain appear also to contain a sex difference in the activity of this enzyme. These are the same areas that appear to be of fundamental importance to the expression of both the behavioral and neuroendocrine effects of androgens. Supported by NIH grant HD 18196.

232.4 MAINTENANCE OF PROGESTERONE-FACILITATED SEXUAL BEHAVIOR IN FEMALE RATS REQUIRES CONTINUED HYPOTHALAMIC PROTEIN SYNTHESIS AND NUCLEAR PROGESTIN RECEPTOR OCCUPATION. M.J. Moore* and J.D. Blaustein (SPON: P. Herron). Division of Neuroscience and Behavior, Dept. of Psychology, University of Massachusetts, Amherst, MA 01003

In ovariectomized (OVX) rats, the sequential administration of estradiol and progesterone results in sexual behavior, which begins within about 4 h and lasts until approximately 18 h after progesterone injection. However, the concentration of hypothalamic nuclear progestin receptors (NPRs) returns to baseline by 4 - 8 h after injection. This pattern differs from guinea pigs in which hypothalamic NPR levels remain elevated for the entire duration of the period of sexual receptivity. Experiments were performed to determine the role of ongoing progesterone-directed protein synthesis and NPR occupation in maintaining the full duration of sexual receptivity in rats.

OVX rats were injected with progesterone (500 µg) 44 h after injection of estradiol benzoate (2 µg). Injection of the protein synthesis inhibitor, anisomysin (100 mg/kg b.w.), 2 or 10 h later, caused the rapid inhibition of sexual behavior. This dose of anisomycin decreases protein synthesis in the mediobasal hypothalamus by 70% within 2 h after injection.

Because these results suggested that a protein synthesis inhibitor was capable of blocking sexual behavior long after NPRs were believed to have declined to baseline levels, the effects of a progesterone antagonist were studied. OVX rats were injected with progesterone (500 µg) 44 h after injection of estradiol benzoate. Injection of the antiprogestin, RU 486 (5 mg) 2, 6 or 10 h later resulted in inhibition of sexual behavior within 8 h. In fact, when RU 486 was injected 10 h after progesterone, sexual behavior was inhibited within 4 h.

Because the previous experiment suggested that sexual behavior could be inhibited by a progesterone antagonist at a time by which NPRs were thought to have decreased to baseline levels, we modified the NPR assay to increase its sensitivity. With this assay we documented that the concentration of NPRs was still elevated at 10 and 14, but not 18 h, after progesterone (500 µg) injection.

These results suggest that progesterone-facilitated sexual behavior in female rats requires an elevated level of hypothalamic NPRs throughout the period of sexual receptivity. In addition, sexual receptivity requires ongoing hypothalamic protein synthesis, at least as late as 12 h after progesterone injection. The hypothesis that ongoing protein synthesis and sexual behavior are dependent on continued NPR occupation is tenable, because we have now shown that the level of NPRs remains elevated throughout the period of sexual receptivity.

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232.5 CNS SITES FOR PROGESTERONE FACILITATION OF FEMALE SEXUAL BEHAVIOR. E.T. Pleim* and R.J. Barfield, Dept. of Biological Sciences, Rutgers Univ., New Brunswick, NJ

The intracranial hormone implant technique has been useful in investigations of CNS sites of hormone action on female sexual behavior but differences in techniques, dosages, priming and testing paradigms and sites of implant have made the interpretation of this body of literature difficult. For example, while several studies have found that unilateral implants of progesterone into several midbrain areas affect feminine sexual behavior in rodents, the most exhaustive studies have concluded that bilateral progesterone (P) stimulation of the CNS is most effective when applied to the ventromedial nucleus of the hypothalamus (VMN).

It was recently shown that in hamsters, a species less readily facilitated by intracranial progesterone, contralateral VMN-VTN (ventral tegmental nucleus) progesterone pellets were clearly more effective for induction of lordosis that bilateral pellets to either site alone. To follow up this finding, we decided to use contralateral stimulation of these sites in rats using the techniques of the workers who found the VMN to be the single most effective site of P facilitation in that species.

Ovariectomized rats were stereotaxically implanted with a unilateral guide tube directed to the VMN and another guide tube directed to the VTN of the opposite hemisphere. Subjects were primed with 8 µg EB in oil sc 2 days prior to behavioral testing. Following a pretest to check for responsiveness to EB priming alone, rats were stimulated with a 28 ga insert filled at the lumen with pure progesterone in each of the contralateral target sites. Subjects were tested for receptivity and proceptivity to a stud male 1 and 4 hours after cannula lowering. Preliminary evidence suggests that considerable receptive behavior (i.e. lordosis) may be elicited in rats with this staggered, contralateral implant technique.

These results, taken together with previous work employing similar methods at these implant sites, show that bilateral VMN stimulation with P is sufficient for the facilitation of female sexual behavior in rats, but not strictly necessary. In our hands, bilateral P at the midbrain (VTN) is not significantly facilitatory. However, stimulation of the VTN can synergize with subthreshold application of P to the contralateral VMN to effect female sexual behavior in rats, as well as in hamsters. Despite species differences in the form of lordosis and conditions sufficient for its expression, these results may indicate an anatomically diffuse progesterone sensitive system forming the neural substrate for facilitation of female sexual behavior in mammals.

232.6 REGIONAL SEX DIFFERENCES IN PROGESTIN RECEPTOR (PR) INDUCTION IN THE HYPOTHALAMUS OF THE RAT: EFFECTS OF DIFFERENT ESTROGEN TREATMENTS. J. D. Kranzler, T.J. Brown, A.S. Clark, N. J. MacLusky* and F. Naftolin*. Dept. of OB/GYN, Yale University Medical School, New Haven, CT. 06510.

In the rat, sex differences in behavioral response to estrogen and progesterone have been correlated with a sexual dimorphism in estrogen-induced PR synthesis in the hypothalamic ventromedial nucleus (VMN; Rainbow et al., Nature 300:648, 1982). Recently, it has been suggested that this dimorphism may depend on the estrogen dose used for PR induction, significantly higher hypothalamic PR levels being observed in females than in males after high dose (10µg/day, 3 days) but not after low dose (2 or 8 µg/200g body weight, 48h before sacrifice) estradiol benzoate (EB) injections (Etgen J. Neurosci. 5:2439, 1985). We have re-examined the induction of PR in microdissected hypothalamic and preoptic area nuclei from gonadectomized male and female rats, using three different estrogen treatment paradigms: [1] Forty-eight h following s.c. injection of 20 µg/kg. EB. [2] Three days after s.c. implantation of Silastic capsules containing 10% estradiol (E_2) in cholesterol (McGinnis et al., Neuroendo 33:158, 1981). [3] Three days after s.c. implantation of Silastic capsules containing 100% E_2.

Although no sex difference in PR induction in the entire hypothalamus was observed after EB injection, measurements in individual nuclei revealed a significantly higher PR induction in the VMN of the female than in the male following this treatment. No differences were observed in the arcuate-median eminence (ARC-ME) or medial preoptic area (MPO). With the 10% E_2 Silastic treatment, significant sex differences in PR induction were observed in both the ARC-ME (male:16.6 fmol/mg cytosol protein; female:27.1 fmol/mg protein) and VMN (male:6.5 fmol/mg protein; female:12.3 fmol/mg protein), but not in the MPO. In contrast, after the 100% E_2 Silastic treatment the sex difference in PR induction in the ARC-ME was abolished, while that in the VMN was attenuated below the limits of statistical significance ($p>0.05$; Students t test). These results confirm the existence of a sex difference in PR response to estrogen in the VMN and indicate that, after low dose E_2 Silastic priming, there may also be a sex difference in PR levels in the ARC-ME. These differences are not sufficiently large to be reflected in assays of the entire hypothalamic PR content. The results from the two Silastic E_2 treatments suggest that the sex difference in hypothalamic PR induction is less pronounced in the presence of sustained high circulating E_2 levels (Supported by NIH grants NS07724, NS07807 and HD 13587).

232.7 ESTRADIOL (E_2) AND PROGESTERONE (P) EFFECTS ON PROTEIN SYNTHESIS IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (VMN) AND PREOPTIC AREA (POA) OF THE FEMALE RAT. K.J. Jones, B.S. McEwen, and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

We recently provided evidence that short exposures to E_2 result in changes in protein synthesis in the VMN and POA, 2 brain regions with functionally opposed roles in lordosis behavior (Jones et al., Soc. Neurosci. Abs., 11, 738, 1985). These protein synthetic changes were correlated with ultrastructural alterations induced by E_2 in cell nuclei within the VMN (Jones et al., J. Comp. Neurol., 239, 255, 1985). In this study, we extended this analysis in order to examine the synergistic and independent effects of E_2 and P on protein synthesis in the VMN and POA.

Ovariectomized rats (OVX) were either 1) implanted with E_2 from time 0h to 6h, injected with P at 20h, and sacrificed at 24h, 2) implanted with E_2 from time 0h to 6h, injected with vehicle only at 20h, and sacrificed at 24h, 3) sham-implanted with blank capsules, injected with P at 20h, and sacrificed at 24h, or 4) sham-implanted and injected with vehicle only, and sacrificed at 24h. Punches of VMN and POA nuclei were collected and labeled for 4h in vitro with ^{35}S-methionine and -cysteine. Proteins were separated by 2-dimensional gel electrophoresis, fluorographed, and exposed to autoradiographic film. The fluorograms were quantified by computerized scanning densitometry and normalization procedures.

In the VMN, labeling of certain proteins was found to be either increased or decreased after administration of E_2 plus P. Those proteins altered by E_2 and P were different from those altered by E_2 alone. Also, either E_2 or P given alone resulted in changes in labeling of several proteins. In a subset of those proteins, the effects of E_2 on labeling were found to be counteracted by P, and vice versa. These same 4 points held true with regard to sex steroid effects on protein synthesis in the POA. However, the proteins affected in the POA by the hormones differed from the proteins affected in the VMN.

Comparison with our previous work suggests that the population of proteins affected at these temporal endpoints differ from the population of proteins affected during the very early phases of E_2 (i.e., 2h). The hormones appear to act with regional specificty, since the populations of proteins affected in the 2 brain regions do not overlap. Finally, the results are consistent with the hypothesis that, under hormonal conditions sufficient to induce lordosis behavior, protein synthetic changes exist in ventromedial hypothalamus and preoptic area. The specific functions of the altered proteins are not known and remain to be determined. Supported by NS 07080 (BMc), HD 05751 (DWP), and MH 15125 (KJJ).

232.8 INHIBITORY ACTION OF alpha-MELANOCYTE STIMULATING HORMONE ON LORDOSIS IN RATS: ROLE OF OVARIAN HORMONES. L.H. Raible*[1] and B.B. Gorzalka*[2] (SPON: R. Tees). [1]Dept. Psychology, Kalamazoo College, Kalamazoo, MI, 49007. [2]Dept. Psychology, The University of British Columbia, Vancouver, B.C., V6T 1Y7.

Previous research in our laboratory has indicated that centrally administered alpha-melanocyte stimulating hormone (MSH) exerts both a short (same day) and a long (1 wk later) term effect on sexual receptivity (lordosis) in rats. The short term effect appears to be mediated, in part, by MSH-induced alterations in serotonin activity. However, this factor seems less important in the production of the long term effect. The present series of experiments was designed to examine the role of estrogen and progesterone in the expression of the inhibitory actions of MSH. In Experiment 1, receptivity was induced by the administration of 10 µg estradiol benzoate (EB). Results indicated that MSH had no significant effects. This finding may have been due to either the large dose of EB administered or to the absence of progesterone (P). In addition, the possibility that an estrogen-progesterone interaction is required for the expression of the long term effect could not be eliminated. In Experiment 2, subjects received .8 or 2 µg EB in combination with either 200 or 500 µg P. The results of this study suggested that the inhibitory action of MSH was most noticeable when 2 µg EB was administered. Furthermore, it appeared that the long term action of MSH was attenuated by the higher dose of P (500 µg). Subjects in Experiment 3 received either 1, 2, 4, or 8 µg EB plus 200 µg P. Results indicated that the inhibitory action of MSH was most pronounced with the higher EB doses. When combined with the results of Experiment 1, this suggests that an estrogen-progesterone interaction may be involved in producing the inhibitory actions of MSH. Subjects in Experiment 4 received the standard 2 µg EB plus 200 µg P on the day of MSH administration. However, 1 wk later, one half of these subjects received 500 µg P rather than the standard 200 µg P. The results of this experiment indicated that 500 µg P significantly attenuated the long term action of MSH on receptivity. Thus, it is possible that the long term action of MSH is, in part, the result of an MSH-induced decrease in the sensitivity of the animal to progesterone. The mechanism by which EB may enhance the inhibitory action of MSH when progesterone is present is under investigation.

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232.9 OPIOID-SEROTONERGIC INTERACTIONS IN THE CONTROL OF FEMALE SEXUAL BEHAVIOR D.L. Allen, M. Frankfurt, and V.N. Luine. Laboratory of Neuroendocrinology, Rockefeller University, N.Y., N.Y. 10021 and Laboratory of Neuroanatomy, Montreal Neurological Institute, Montreal, Quebec, Canada H34 2B4.

Opiates and opioid peptides have been shown to alter lordosis responding in the female rat. Systemic or intraventricular morphine or beta-endorphin inhibit sexual behavior in estrogen and progesterone primed rats. Central or peripheral opiate antagonists facilitate lordosis after estrogen pretreatment. Interactions with monoaminergic systems may be important for the effects of opiates on sexual behavior. Pargyline, a monoamine oxidase inhibitor, blocks naltrexone-facilitated behavior. Naltrexone decreases serotonin turnover, as measured by the increase in serotonin levels after pargyline, in the preoptic area of estrogen-primed rats. The present experiments further investigate the effect of morphine on female sexual behavior and possible interactions between opiates and serotonin in the control of lordosis.

Ovariectomized rats were primed with estradiol benzoate (EB, 5 ug) and progesterone (500 ug, 48 hours later). After three hours, they were injected with saline or morphine, and sexual behavior was tested 10-20 minutes later. Morphine inhibited lordosis quotients (LQ) in estrogen-progesterone treated rats with an ED_{50} of 2 mg/kg.

In order to study interactions between opiates and serotonin in the control of lordosis, the ability of morphine to inhibit sexual behavior was tested in 5,7-dihydroxytryptamine (5,7-DHT) treated rats. Lesioning hypothalamic 5-HT terminals with 5,7-DHT facilitates lordosis responding in EB-treated rats. Rats were screened 48 hours after EB. Morphine (2 mg/kg) was injected and behavior was tested 10-20 minutes later. In 5,7-DHT lesioned rats, morphine had a slight, but nonsignificant effect on LQ (pretest: 98 ± 1, post-test: 81 ± 9). These data show that systemic administration of opiates inhibits sexual behavior in female rats, and suggest that the inhibition requires an intact hypothalamic serotonergic system.

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232.10 HYPOTHALAMIC 5HT LESIONS FACILITATE LORDOSIS WITHOUT AFFECTING STEROID RECEPTOR LEVELS V.N. Luine, J.E. Thornton, M. Frankfurt and N.J. MacLusky*. Rockefeller University, N.Y., N.Y.; Institute of Animal Behavior, Newark, N.J.; Neurological Institute, Montreal, Canada; Yale University, New Haven, Ct.

Serotonin endings in the ventromedial nucleus of the hypothalamus exert inhibitory regulation on female sexual behavior (lordosis). Depletion of serotonin by intrahypothalamic 5,7-Dihydroxytryptamine (5,7-DHT) alters the hormonal requirements for lordosis (Frankfurt et al, Brain Res. 340 127,1985). Following 5,7-DHT, estradiol alone (E_2) elicits maximal lordotic responding. We examined whether altered behavioral responsivity to E_2 might be related to serotonin-dependent modulation of steroid hormone receptor levels in hypothalamus.

Ovariectomized female rats received stereotaxic injections of 5 ug 5,7-DHT or vehicle into the hypothalamus. Lordosis quotients (LQ) were tested weekly after 5 ug of S.C. estradiol benzoate (EB). 5,7-DHT treated rats displayed L.Q.s > 88 while sham L.Q.s were < 6. At 30-45 days post 5,7-DHT, animals were sacrificed, 300 um brain sections made and the medial preoptic (mPOA), periventricular preoptic (PVPOA), dorsomedial (DMN) and lateral ventromedial (VMN) nuclei and arcuate-median eminence area (Ar-ME) were obtained by micropunch. For cytosolic progestin receptor (PR_c) measurements, rats received 2.5 ug EB for two days prior to sacrifice. Nuclei from 3 brains were pooled and PR_c were measured with ^{3}H-R5020 according to the methods of Parsons et al. (J. Neurosci. 2 1446, 1982). Nuclear estrogen-receptor complexes (ER_n) in nuclei from one rat were measured by exchange assay (Kranzler et al., J. Neurochem. 43 895,1984) 6 hrs. after injection of 10 ug of EB using 11-methoxy-16-(^{125}I) estradiol (specific activity 1043 Ci/mmol) as ligand (T. Brown et al., Neurosci. Abs. 1986).

No differences in PR_c levels were found between sham and 5,7-DHT groups in the nuclei examined. Levels of PR_c in the VMN and Ar-ME, 24 and 18 fmol/mg protein, respectively, were similar to previous studies utilizing higher E_2 doses. Levels in the POA and PVPOA, 20 and 12 fmol/mg protein, respectively, were approximately 50% lower than previous studies. This suggests that maximal induction of PR_c by E_2 occurs at lower doses in hypothalamic than preoptic sites. ER_n were also not different between sham and 5,7-DHT treated rats in the mPOA, Ar-ME or VMN. Levels ranged between 400 and 650 fmol/mg DNA.

These results suggest that serotonergic regulation of lordosis behavior does not involve modulation of estrogen nuclear receptor levels or E_2 induction of cytosolic progestin receptors. Other studies suggest serotonin modulates behavior through direct actions on neural circuits regulating lordosis.

232.11 MEASUREMENT OF CELL NUCLEAR ESTROGEN RECEPTORS IN MICRODISSECTED RAT BRAIN NUCLEI USING A [^{125}I] ESTROGEN LIGAND. T.J. Brown, N.J. MacLusky*, J. Zeilinski* and R.L. Hochberg* Dept. of OB/GYN, Yale Univ. Sch. of Med., New Haven, CT 06510.

Measurement of nuclear estrogen receptors in regions of the brain dissected using the Palkovits technique (Brain Res. 59:449, 1973) allows correlations to be made between the behavioral and neuroendocrine effects of estrogens and the occupation of estrogen receptors in specific brain structures. This approach has been limited by the necessity to pool tissue from several animals to obtain measurable levels of binding, using tritiated estrogen ligands. It has thus been impossible to relate the estrogen responsiveness of an individual animal to the regional distribution of cell nuclear estrogen receptors within its brain. In the present study, we have explored the possibility of using of an iodinated analog of estradiol, 11β-methoxy-16α-[^{125}I] iodoestradiol (MIE_2), for assay of estrogen receptors in microdissected areas of the rat brain. Because of the high specific activities possible with [^{125}I] labeled steroids (up to 2,200 Ci/mmol), it seemed likely that use of this compound might dramatically improve the sensitivity of the assay. MIE_2 binds to estrogen receptors specifically and with high affinity (K_d 10^{-9} M). A simple and rapid exchange assay has been developed using [^{125}I] MIE_2, which is sufficiently sensitive to allow measurement of cell nuclear estrogen receptors in microdissected brain nuclei from individual rats. Tissues are homogenized in 200 µl TEGD buffer (10 mM Tris-HCl, 1.5 mM Na_2EDTA, 10% glycerol and 1 mM dithiothreitol, pH 7.4) and centrifuged at 12,000 g for 1 min. The pellets are washed twice by resuspension in 200 µl TEGD and centrifugation at 12,000 g for 1 min. The receptors are then extracted with 0.4 M KCl in TEGD buffer containing 0.5 mM bacitracin. Following centrifugation at 12,000 g for 5 min, aliquots (75 µl) of the nuclear extracts are incubated with 5 nM [^{125}I] MIE_2 +/- 10^{-6}M diethylstilbestrol at 25°C for 4.5 h. Bound [^{125}I]MIE_2 is separated from free on Sephadex LH-20 minicolumns. To minimize nonspecific binding, the receptor complexes are precipitated from the column eluates with protamine sulfate in the presence of 'carrier' unlabeled brain cytosol protein (200 µg) and 1% Tween 80. The precipitates are washed with TEGD containing 1% Tween 80 and counted. Results using this method are indistinguishable from those obtained using [^{3}H] estradiol (E_2) and a more rigorous cell nuclear isolation procedure (Kranzler et al, J. Neurochem. 43:895, 1984). Moreover, the number of specific bound counts using MIE_2 with single animals is more than 4 fold higher than with [^{3}H]E_2 in tissue pooled from 5 rats (Supported by NIH grants CA37799 and NS07807).

232.12 ACTIVITY OF SINGLE NEURONS IN LIMBIC STRUCTURES DURING THE ESTROGEN-PROGESTERONE INDUCTION OF LORDOSIS RESPONDING IN FEMALE GOLDEN HAMSTERS. James D. Rose. Department of Psychology, University of Wyoming, Laramie, WY 82071.

In previous investigations, pronounced, rapidly-developing changes in the sensorimotor properties of midbrain neurons were identified following a lordosis-inducing progesterone (P) injection in estrogen-primed hamsters (Rose, J. D. Physiol. Behav., 1986 in press). The present study examined the activity of single neurons in limbic structures, particularly those known to have concentrations of estrogen and P receptors, during the hormonal induction of lordosis. Single unit activity was recorded through fine wire microelectrodes chronically-implanted in the lateral septal region, medial amygdala, bed nucleus of the stria terminalis, cingulate gyrus and hippocampus. The animals were given a priming injection of estradiol benzoate (10 ug) at the time of electrode implantation. Approximately 40 h later, single unit activity was recorded immediately preceding and following subcutaneous injection of P (500 ug). Recording was continued until after the animals showed lordosis to manual stimuli, approximately 3 h later. Recordings were also obtained following P injections given on the day after lordosis induction or in other animals which had not received estrogen priming. These injections served as controls for P effects not specific to lordosis induction. Movement correlates of unit activity were monitored with an accelerometer mounted on the recording cable and from video tapes. Behavior-related properties of these limbic neurons consisted of changes in firing associated with specific types of movement, such as head swings, shifts from one type of movement pattern to another (e.g. from walking to rearing), or increases or decreases in firing concomitant with changes in movement vigor. In comparison with midbrain neurons, these movement-correlated patterns of firing were less dynamic or specific to particular movement topographies. Lordosis inducing P injections produced changes, often a depression, in the overall activity levels, degree of movement-related firing and somatosensory responsiveness of limbic neurons in all of the regions examined. As in midbrain neurons, these P effects frequently began within a few minutes of hormone administration and were most pronounced at the time lordosis was elicitable. Unlike previous findings for midbrain neurons, the limbic neurons did not tend to develop firing patterns closely associated with the motoric elements of lordosis. These results are consistent with previous lesion and electrical stimulation studies of limbic structures in hamsters, which suggest a less central role of these brain regions in the control of lordosis than the mediobasal hypothalamus or midbrain. Supported by N.I.H. Grant NS13748.

232.13 THE INFLUENCE OF INTRASTRIATAL ESTROGEN ON BALANCE BEAM PERFORMANCE IN OVARIECTOMIZED FEMALE RATS. Peter J. Snyder, Sara A. Westgate*, Melissa M. Miller* Michael J. Jenuwine*, and Jill B. Becker. Dept. Psychology and Neuroscience Lab, The University of Michigan, Ann Arbor, MI 48104-1687.

There is now considerable evidence that estrogen can influence behaviors activated by dopaminergic drugs in the rat. These behavioral effects of estrogen have been correlated with changes in neurochemical indices of striatal dopamine (DA) activity, including the potentiation of amphetamine-stimulated striatal DA release. However, localization of the site of estrogen's action in this regard has been difficult, as either the hormone or the drug (or both) must usually be administered systemically. In an attempt to localize the effect of estrogen, we have examined the influence of intrastriatal hormone treatment on locomotor behavior across a balance beam. This behavior does not require drug treatment to be elicited and it shows the same pattern of estrous cycle dependent variation as amphetamine-stimulated striatal DA release.

Adult female Holtzman rats were ovariectomized (OVX) and guide cannulae aimed at the striatum were implanted bilaterally. Animals were then trained to walk across a suspended beam, 4 ft long and 0.5 inches wide. The bottoms of the rats' feet were painted with tempura paint prior to being placed on the beam and the footprints left on the beam were used to score footfaults. On 3 consecutive days baseline behavior was recorded. After baseline on the third day, crystalline hormones in 28 gauge cannulae were inserted bilaterally through the guide cannulae into the dorsal striatum. Animals received 1 of 4 treatments: 1) 100% cholesterol (CHOL); 2) 30% 17-beta-estradiol (B-E2; 70% CHOL); 3) 30% 17-alpha-estradiol (a-E2); or 4) 30% diethylstilbesterol (DES). Implants remained in the striatum for 6 hours. Behavior was tested 1 hr, 4 hr, 10 hr and 24 hr after hormone insertion and then daily for 6 days.

At 4 and 10 hr following implants of either B-E2 (the active estrogen in the rat) or the non-steroidal estrogen agonist DES there was a significant improvement in balance beam performance, relative to implants of CHOL or the inactive estrogen a-E2. At 4 and 10 hr post-implant of B-E2 or DES there was a significant decline in the total number of footfaults (footfaults made with front feet plus those made with back feet) relative to controls. These results indicate that estrogen acts directly in the striatum to influence balance beam performance and that this effect is both stereospecific and mimicked by an estrogen agonist.

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232.14 EXAMINATION OF SEX DIFFERENCES IN THE RESPONSE TO FRONTAL CORTICAL LESIONS. F.W. Schaerf*, T.H. Moran, J.R. Lipsey*, S. Ginsburg* and R.G. Robinson (SPON. G.McKhann). Department of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Previous studies have demonstrated that right frontal cortical suction lesions result in spontaneous hyperactivity in male but not in female rats (Lipsey, et al, Life Sci., 38, 2185, 1986). The present experiments were undertaken to examine some possible bases for this sex difference. In an initial experiment we examined the role of steroid hormones in mediating the response to cortical lesions. Female rats were ovariectomized and following acquisition of preoperative running wheel activity baselines, divided into four groups: sham lesion, right cortical suction lesion, sham lesion receiving a silastic testosterone implant, and lesion with testosterone implant. Postoperative activity was monitored for 30 days. Neither ovariectomied, $F(1,21) = 0.648$, $p > .1$, nor testosterone treated female rats, $F(1,21) = 0.167$, $p > .1$, demonstrated increased activity in response to cortical suction lesions, although the testosterone treatment itself greatly increased running wheel activity, $F(1,21) = 9.153$, $p < .01$. The results of this study indicated that estrous cycling itself was not preventing the response and simply providing the male steroid testosterone to ovariectomized female rats was not sufficient to induce the hyperactivity response to right frontal cortical suction lesions.

In a second experiment, we examined the ontogeny of responses to lesions in male rats. Twenty, 30, 50, 60 and postpubertal 90 day old male rats received either right frontal cortical suction lesions or sham surgeries. Activity was monitored for 30 days following lesions. Only 90 day old male rats demonstrated the characteristic hyperactivity in response to lesions, $F(1,14) = 8.635$, $p < .01$. These results suggested that some maturational process related to sexual maturity may be playing a permissive role in the expression of hyperactivity in response to cortical lesions. In order to examine this possibility, we examined the response to right hemispheric lesions in rats castrated prior to puberty. Rats were castrated on Day 55 and received either cortical or sham surgery on Day 90. Castration eliminated the response to cortical lesions, i.e. the postoperative activity of castrated animals receiving lesions or sham procedures did not differ, $F(1,9) = 0.031$, $p > .1$. Together these results suggest that some aspect of male sexual maturation is playing a role in mediating the hyperactivity response to right frontal cortical lesions.

232.15 PREGNANCY-INDUCED AGGRESSIVE BEHAVIOR AND PLASMA LEVELS OF PROGESTERONE IN THREE INBRED STRAINS OF MICE. S. Ogawa and S.C. Maxson. Lab. of Behavioral Genetics, Univ. of Connecticut, Storrs, CT 06268.

Among various kinds of female social behavior, the mechanism of pregnancy-induced aggressive behavior (PIA) still remains unknown. Previously, we found marked strain differences in the levels of PIA among five inbred strains of mice (Ogawa & Makino, *Behav. Neur. Biol.*, 40, 195, 1984). In addition, we have recently reported that the incidence and intensity of PIA in aggressive strains were associated with the days of gestation themselves and experiential effects such as repeated tests in early pregnancy were relatively small (Behavior Genetics Association meeting, 1986). Therefore, it is assumed that genetically-determined differences in pregnancy-associated factors might exist among the strains with different levels of PIA. In the present study, we assessed whether individual, strain, and/or gestational differences in PIA correlated with the variation in the plasma levels of progesterone in three different strains of mice.

Nulliparous females from AKR/J, BALB/cN, and DBA/2J (10-11 weeks of age) were mated with a CFW male during the night of estrus and placed in an individual cage the next morning if a vaginal plug was found (gestation day 0). Blood samples were taken from both behaviorally tested and untested mice (N=20/group/day/strain) during the early part of the dark phase of the light cycle on 1, 3, 5, 9, 13, or 17 days of gestation. Behavioral tests were conducted immediately before blood collection in the former group. Female aggressive behavior (defined as eight different behavioral acts) toward an unfamiliar olfactory bulbectomized, group-housed CFW male was recorded during a 10-minute observation period. Plasma levels of progesterone were determined by radioimmunoassay.

Plasma levels of progesterone tended to be highest in the most aggressive AKR mice on all days studied. The moderately aggressive DBA showed lower levels of progesterone than the AKR. Non-aggressive BALB mice showed the lowest levels of progesterone on all days. Gestational changes of PIA correlated well with plasma levels of progesterone in DBA but not in AKR.

These results suggest that strain differences in PIA might be due to the variation in the levels of plasma progesterone, and progesterone might have a facilitative effect on PIA. Whether these three strains are different in neural sensitivity to progesterone in addition to the peripheral circulating levels of progesterone will be further investigated in a supplemental progesterone treatment study to determine the mechanism of PIA.

This research was supported by a grant from The University of Connecticut Research Foundation.

232.16 PRENATAL STRESS AND MATERNAL BEHAVIOR IN VIRGIN RATS: RESPONSE LATENCIES ARE DECREASED IN MALES AND INCREASED IN FEMALES. Craig H. Kinsley and Robert S. Bridges, Department of Anatomy and Cellular Biology, Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, 45 Shattuck St., Boston, MA 02115.

Prenatal stress (PS) is a potent disruptor of the normal course of sexual differentiation. Examinations of PS have described the effects in males as comprising aspects of feminization and demasculinization, whereas in females, the effects are much less apparent and are more likely to affect such particular endpoints of reproduction as fertility and fecundity. The repeated findings that levels of various male-typical behaviors (e.g., copulatory behavior and intermale aggression) are reduced in PS males, coupled with the report that PS dampens the infanticidal tendencies of male mice (vom Saal, Physiol. Behav. 30: 675, 1983) prompted us to examine the possibility that prenatally-stressed male rats might display more female-like responses to young, viz., a reduced latency to respond maternally. Conversely, in PS females, where the effects are more subtle and where, to our knowledge, there has been no report of PS effects on maternal behavior in the nulliparous female, we wished to examine whether maternal responding would be disrupted by PS, as suggested by the results in the postpartum animal (e.g., Fride, et al., Life Sci. 36: 2103, 1985). Sprague-Dawley female rats were timed-mated (+sperm = Day 0). From days 14 through 21 of pregnancy one set of females was subjected to a thrice-daily regimen of heat and restraint stress (8:30 am, 12:30 and 4:30 pm). This treatment consisted of placing the rats into a plexiglas restraint tube over which was poised two 100 watt floodlights. Control females remained undisturbed throughout pregnancy. At parturition all offspring were fostered to untreated dams and weaned at 25 days of age. Testing for responsiveness to young occurred between 80-100 days of age. Following 24 hours of isolation, PS and Control males and females were exposed to three 1-7 day old rat pups. Tests for maternal behavior were conducted daily until a criterion of two consecutive days of full maternal behavior (FMB: Retrieval and grouping of, and crouching over, pups) was observed. PS males exhibited a significantly shorter latency (in days) to show FMB than Control males, $\bar{X} = 5.4$ vs. 7.7, respectively. PS females, on the other hand, took significantly longer to exhibit FMB than Control females, $\bar{X} = 7.0$ vs. 3.5, respectively. These data demonstrate that prenatal stress eliminates the sex difference normally observed in maternal behavior (i.e., females $\rangle$ males). Moreover, the data suggest that prenatal stress may actually feminize the male's responsiveness to young, while masculinizing the response of the female. (Supported by NIH Training Grant # 5T32-HD-07130, C.H.K trainee, and grants RSDA # K02-MH-00536 and NIH HD-19789 awarded to R.S.B.

232.17 RELATIONSHIP BETWEEN MATERNAL BEHAVIOR AND NUCLEAR ESTROGEN RECEPTOR BINDING IN PREOPTIC AREA AND HYPOTHALAMUS DURING PREGNANCY IN RATS. A.L. Giordano and J.S. Rosenblatt*. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

Female rats whose pregnancies are terminated (hysterectomized and ovariectomized) on Day 16 and are injected with estradiol benzoate (EB, 5ug/kg) at surgery will exhibit maternal behavior when tested 48 hr later. It has also been shown that lesions in the medial preoptic area (MPOA) will severely disrupt this behavior and that estradiol implants in the MPOA will stimulate maternal behavior in pregnancy-terminated animals. Lesions and implants in the ventromedial hypothalamus (VMH) of these animals have no effect on this behavior.

In the first experiment, we examined nuclear estrogen receptor binding in POA and hypothalamus (HYP) of Day 16 pregnancy-terminated females, 16 day hysterectomized and ovariectomized non-pregnant females, and diestrus I females hysterectomized and ovariectomized. All groups received either 5ug/kg EB or vehicle. Receptor levels were measured at 0 (no surgery or treatment), 6, 12, 24, and 48 hr after surgery and treatment. In animals that received EB, receptor concentrations in POA were significantly higher than in HYP at 0 hr in the pregnancy-terminated animals only. By 6 hr, no differences were found between these brain areas in any group. Within all groups in both POA and HYP, receptor levels increased significantly at 24 hr and decreased at 48 hr. No differences were found among the groups at any interval following treatment with vehicle (6 to 48 hr).

In the second experiment, we examined the profile of estrogen receptor concentration on days 8, 10, 13, 16, 19, and 22 (day of parturition) of pregnancy. Results showed that receptor levels in POA were significantly higher than in HYP beginning on D10 and these levels remained elevated through D22. Receptor concentration in HYP did not change from D8 through D19 but then significantly increased on D22.

In order to assess these changes in estrogen receptor binding with greater anatomical resolution, we measured nuclear estrogen receptors in microdissected brain regions during pregnancy. Nuclear estrogen receptor concentrations were determined in the medial preoptic nucleus, periventricular preoptic nucleus, ventromedial nucleus, periventricular anterior hypothalamus, paraventricular nucleus, and arcuate-median eminence on days 8, 16, and 22 of pregnancy. Differences between preoptic and hypothalamic nuclei concentrations of estrogen receptors are discussed in relation to initiation of maternal behavior and its development during pregnancy.

232.18 SERUM AND SALIVARY CORTISOL LEVELS IN THE DEXAMETHASONE SUPPRESSION TEST. D. M. Martin, D. C. Bernot*, D. R. Sweeney and M. Lehrer*. Psychiatric Diagnostic Laboratories of America, Princeton, NJ 08540 and Fair Oaks Hospital, Summit, NJ 07901.

Hypersecretion of cortisol and its resilience to suppression after the administration of dexamethasone has been strongly associated with major affective disorders in humans.[1] The Dexamethasone Suppression Test (DST) has been standardized requiring the sampling of serum cortisol levels minimally at 4 p.m. and 11 p.m. after one (1) mg of dexamethasone is taken at midnight the prior evening. Recent studies suggest that increasing the frequency of cortisol sampling increases the sensitivity and the diagnostic value of the test.[2] Obtaining DST blood samples for the minimum requirement of two samplings is difficult even under the best conditions for hospitalized patients and extremely inconvenient for outpatients. This restriction has severely limited the DSTs more widespread application in the diagnosis and treatment of affective disorders. The levels of most biologically active compounds, such as steroids including cortisol in saliva reflect the nonprotein bound or "free" levels found in the serum compartment.[3] Initial research on the DST measured total corticosteroids by a competative protein binding technique. Currently available commercial methods are more specific to cortisol but measure both protein bound and free cortisol. The measurement of salivary cortisol in the free or unbound form is not only a natural technical evolution of the DST but also yields a more convenient, patient oriented procedure. Salivary and serum cortisol levels were measured by our group in both normal controls and depressed hospitalized patients by a modification of a radioimmunoassay technique. The study demonstrated a strong correlation between serum and salivary cortisol determinations (r=0.99). These data will be presented suggesting that salivary cortisol analysis offers a nontraumatic and confidential approach to the DST especially well-suited for outpatients, adolescents, or inpatients with poor venous structures.

1) Carroll, B.J., British J. Psychiatry 140, 292, 1982
2) Sherman, B., et. al., Arch. Gen. Psychiatry 41, 271, 1984
3) Stall, F., Dorner, G., Endokrinologie 80, 158, 1982

232.19 CONDITIONED AVOIDANCE AND PLASMA CORTICOSTERONE IN MICE WITH EXPERIMENTAL DIABETES MELLITUS. W.P. Meehan* and L.J. Leedom* (SPON: D.F. Lindsley). Diabetes and Clinical Nutrition, LAC-USC Medical Center, Los Angeles, CA 90033.

It has long been recognized that experimental diabetes mellitus is associated with elevated plasma corticosterone as well as increased plasma glucose. Since disruptions in the pituitary adrenal axis are thought to effect avoidance learning, this study examined conditioned avoidance in mice with experimental diabetes mellitus. 32 male Swiss Webster mice (3 months old) were used in this study. 23 mice were given 180 mg/kg of streptozotocin for the induction of diabetes mellitus. 9 control mice received vehicle. 3 weeks following the streptozotocin injection, avoidance learning was assessed using a standard 2 chamber passive avoidance apparatus. Behavior of the mice was observed and recorded in chamber 1 as well as the latency to enter chamber 2. Following avoidance learning trials, mice were bled on day 5 via retro-orbital sinus puncture to obtain plasma for baseline glucose and corticosterone levels. Plasma glucose was determined using a Beckman glucose analyzer. Plasma corticosterone was determined using a competitive protein-binding radioassay. 16 of the 23 mice receiving streptozotocin became diabetic (plasma glucose > 250 mg/dl, m=694±18 std mg/dl). The other 7 mice were borderline diabetic with baseline plasma glucose < 250 mg/dl (m=163±21 mg/dl) (controls, m=151±18 mg/dl). Plasma corticosterone was greater in the diabetic mice than in the borderline and control mice (17.8±14.3 vs 4.37±2.5 vs 6.2±4.7 std ug/dl, $p<0.01$). On day 1 of the avoidance learning trial, latency to enter chamber 2 was similar in all groups, however 24, 48 and 72 hrs following the shock trial, diabetic mice latency to enter was significantly elevated above that of controls and borderline diabetic mice ($p<0.01$). On day 2, the diabetic mice were active while in chamber 1. Diabetic mice not fully reentering chamber 2 (14/16) reentered and withdrew from chamber 2 an average of 15±1 times. In conclusion, this study demonstrated that diabetic mice show greater passive avoidance than nondiabetic and borderline diabetic mice. Since in diabetes mellitus behavioral alterations are observed along with increased activity of the pituitary adrenal axis, it is suggested that the metabolic state of diabetes mellitus has observable effects on the central nervous system.

232.20 INCREASED PLASMA ALDOSTERONE DOES NOT ACCOUNT FOR THE ENHANCED SALT APPETITE OF THE RAT. R.R. Sakai*, B. Stamoutsos* and A.N. Epstein. (SPON: S.P. Frankmann), Univ. of Pennsylvania, Philadelphia, Pa. 19104-6018.

The salt appetite of rats is aroused by the combined actions of angiotensin II (ANG) and aldosterone (ALDO) on the brain (Sakai, NATO ARW, 1986) and can be suppressed by blockade of both hormones (Sakai and Epstein, AJP in press). In addition, salt appetite that is induced by sodium depletion is enhanced either by a prior depletion or by treatment of depletion-naive rats with ANG and ALDO one week prior to their 1st sodium depletion. The enhancement occurs abruptly after the second depletion or after the 1st depletion in the hormone pre-treated rat, and is expressed by a virtual disappearance of latency to drink and by increased volume of 3% NaCl intake (10-12 ml vs 6-8 ml). We now report that changes in plasma ALDO with successive sodium depletions does not account for the enhanced salt appetite.

Rats (male, S-D) were sodium depleted at weekly intervals by combining a natriuretic drug, furosemide (10 mg/rat, SC), with removal of ambient sodium (home cage washed, removal of 3% NaCl, and food replaced with a sodium deficient diet). At least 18 hours after the beginning of the depletion rats were either 1) sacrificed for collection of trunk blood or 2) were given access to 3% NaCl for 2 hours and allowed to express a salt appetite. Some of these rats were then given ad lib access to water, 3% NaCl, and Purina pellets until the following week when they were sodium depleted again and sacrificed. Blood was collected from the remaining rats after their 3rd or 4th depletions. Trunk blood was analyzed for plasma ALDO concentrations by radioimmunoassay (Diagnostic Products).

Plasma ALDO levels were: 0 dep = 139.3 pg/ml (n=19), 1st dep = 846.9 pg/ml (n=16), 2nd dep = 865 pg/ml (n=4), 3rd dep = 953.3 pg/ml (n=6) and 4th dep = 1726.6 pg/ml (n=6). Sodium depletion markedly elevated plasma ALDO but, except for the fourth depletion, successive depletions did not produce further elevations.

These data show that increases in ALDO levels do not account for the enhancement of salt appetite that is produced by a single prior depletion. And because the enhancement can be produced by treating the non-depleted rat with ANG and ALDO, we believe they mean that these hormones organize long-lasting changes in the brain that increase the animals avidity for salty substances in response to subsequent depletions.

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233.1 PINEAL AFFERENTS IN THE RAT DEMONSTRATED BY RETROGRADE TRACING METHODS Laura Stanley*, Kyoi Horikawa*, (SPON. D. Davies). Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, and Department of Neurosurgery, University of the Rukyus, Okinawa, Japan.

The influence of light and the rhythmic nature of pineal gland functions has brought about an assessment of neuroanatomical connections of the gland. Indirect evidence from evoked potential and histochemical studies support the idea that the pineal gland receives innervation from the superior cervical ganglion. Histochemical methods, demonstrating the absence or decrease in norepinephrine in the gland following superior cervical ganglionectomy, have been used to claim this direct connectivity. However, no direct anatmical methods appear to have been used to demonstrate the connection. Some workers have claimed that the only innervation of the pineal gland originates in the superior cervical ganglion. Therefore, this work was done to provide anatomical evidence to confirm the existence of such direct connections and to determine whether there are any others. Fast blue [1µl 3% (n=3)], rhodamine microspheres [1µl 100% (n=1)] and horseradish peroxidase [20% 1µl (n=4)] were used as retrograde tracers. One of the tracers was injected into the pineal gland of a rat using a microsyringe equipped with a small glass cannula (20-50 µ tip). After one to five days survival time, the brain and the superior cervial ganglion were removed, frozen and cut into 30 µ sections and mounted on slides for microscopic study using fluorescent and light microscopy. All three retrograde tracers consistently labeled neurons in the superior cervical gangilion. None were labeled in the thalamus or other structures of the brainstem, not even the habenula or the superior salivatory nucleus. Injection of the sphenopalatine ganglion did label neurons in the superior salivatory nucleus. Only faint labeling could be seen in the superior cervical ganglion subsequent to placing a fast blue soaked piece of gelfoam around the pineal gland as compared to an injection within the gland. Injection of fast blue (n=5), rhodamine microspheres (n=2), or horseradish peroxidase Sigma VI (n=5) into the subarachnoid space did not label neurons in the superior cervical ganglion or any brainstem nuclei. These results, for the first time, anatomically confirm the existence of a direct projection of the superior cervical ganglion into the pineal gland. The evidence does not substantiate the notion that innervation originates in the superior salivatory nucleus or habenular nuclei. Another brainstem nucleus was retrogradely labeled (accessory facial, n=2) but only by the fast blue tracer.

233.2 INNERVATION OF THE PINEAL GLAND OF THE RAT. J.W. Patrickson and T.E. Smith*. Dept. of Anatomy, Loma Linda University, Loma Linda, CA 92350.

The pineal gland is attached to the epithalamus by the pineal stalk. It has been shown that the pineal stalk contains pinealocytes and nerve fibers. These fibers were thought to be aberrant commissural fibers which formed "hairpin loops" within the stalk and proximal portion of the pineal gland (Kappers, J.A. Z. Zellforsch, 52:163-215, 1960). Recent morphological and electrophysiological studies have suggested that these fibers are not aberrant fibers as first thought; instead they represent a fiber tract that provides central innervation to the pineal gland.

The present study was designed to determine the origin of these central pineal fibers. The pineal gland of pentobarbital anesthetized Sprague Dawley rats (250-310g) was injected with horseradish peroxidase (HRP) and the conjugated form, wheat-germ agglutin-horseradish peroxidase (WGA-HRP). The pineal gland was approached by two methods: (a) stereotaxically and (b) directly, following surgical exposure of the gland. Discreet micropipet injections were made hydraulically with 0.5 µl of 30% HRP or 2% WGA-HRP. The animals were reanesthetized and perfused 24-48 hrs postinjection, and 30 µm frozen secions were made and processed using the chromogen tetramethyl benzidine as described by Mesulam (J. Histochem. Cytochem. 26:106-117, 1978). Retrograde labelled neurons were identified in the superior cervical ganglia. Anterograde labelled fibers were observed within the pineal stalk, lamina intercalaris and the medial habenular nucleus. Terminal fields were identified in lamina intercalaris and the medial habenular nucleus.

These results would suggest that the pineal gland of the rat is innervated solely by the sympathetic nervous system and that some of these fibers continue rostrally via the stalk to innervate components of the epithalamus. We further conclude that the remaining fibers of the pineal gland do not communicate synaptically with the pineal gland.

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233.3 EFFECTS OF PINEALECTOMY AND TIMED DAILY MELATONIN INFUSIONS ON WHEEL-RUNNING RHYTHMS IN DJUNGARIAN HAMSTERS. J.M. Darrow* and B.D. Goldman*. SPON: (R.J. O'Connell) Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Male Phodopus sungorus which were pinealectomized and castrated at 36 days of age showed a slower adjustment of the wheel running rhythm after transfer from long to short days than did pineal- intact castrated controls (long days=16L:8D, lights-off at 1800 hrs; short days= 10L:14D, lights-off at 1500 hrs). Whereas activity onset in the pineal-intact group had phase advanced from 18.17 ± .05 hrs to 15.50 ± .19 hrs within 15 days, the pinealectomized group showed a significantly later mean onset of wheel running (17.16 ± .74 hrs, $p<.05$) 15 days post-transfer. Also at this time, the mean duration of activity (α) was greater in the intact than in the pinealectomized group (11.68 ± .30 vs. 9.36 ± .64 hrs, $p<.01$). These results suggest that the pineal gland may modulate the rate of adjustment of wheel-running rhythms to changes in photoperiod.

To determine if the pineal hormone melatonin might influence locomotor activity in Djungarian hamsters, juvenile males were acclimated to running wheels, then pinealectomized and implanted with a subcutaneous catheter connected to an infusion pump. From the day of surgery on, hamsters were exposed to constant light (22 lux) and received 10 hr infusions of melatonin (100ng) or saline every 24.6 hrs. In the melatonin treated group, the period of the wheel-running rhythm (24.79 ± .06 hrs) was significantly greater than for the saline group (24.23 ± .07; $p<.001$), but not different from the infusion T-cycle. A tight correlation between daily onset of wheel-running and hormone infusions was observed (r values from .86 to .99), but correlation coefficients for the saline treated animals ranged widely (from .05 to .93). Interestingly, only 2 of 9 melatonin infused hamsters had regressed testes by 20 days of infusion. In contrast, wheel-running pinealectomized males receiving melatonin infusions on 16L:8D (10 hr duration, 100ng/day) had totally regressed testes after 15 days, whether melatonin, was administered during the day or at night. No difference in the phase of wheel-running activity was observed in day vs. night infused hamsters. Taken together, these results suggest a possible influence of melatonin on locomotor activity rhythms when administered under constant conditions, and may indicate an effect of constant light to slow the rate of melatonin-induced reproductive regression. Further studies are needed to determine if the effect of melatonin observed here is actually due to entrainment of the circadian rhythm of locomotor activity.

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233.4 TIMED MELATONIN INFUSIONS IN ADULT SIBERIAN HAMSTERS: EFFECTS ON BODY WEIGHT, BODY FAT, LIPID METABOLISM AND GONADAL ACTIVITY. T. J. Bartness* and B.D. Goldman* (SPON: F.R. Jackson) Worcester Foundation for Exptl. Biol., Shrewsbury, MA 01545

The change in the daylength is the environmental cue for many physiologically adaptive seasonal changes. The neuroendocrine transducer of the daylength cue is the pineal gland and its hormone melatonin (MEL). Previous studies by the authors have shown that: 1) in long day (LD)-housed adult Siberian hamsters, timed afternoon injections of MEL that extend the peak of circulating nocturnal MEL mimic the short day (SD)-induced gonadal regression, body weight and fat decreases, 2) pinealectomy (PINX) blocks all SD responses in hamsters moved from a LD to a SD and 3) timed s.c. MEL infusion simulating the SD duration of the nocturnal peak of circulating MEL in LD-reared PINX juvenile hamsters mimics SD-induced gonadal regression. Therefore, we wanted to further extend the observation that the duration of the circulating nocturnal MEL peak is the important parameter for the SD-induced regression of the gonads, and to also assess its importance for the SD-induced changes in body weight and lipid metabolism. In contrast to our previous work, the infusions were totally confined to the light portion of the 16:8 light:dark cycle and were performed in adults rather than juveniles. LD-housed male Siberian hamsters were PINX and given a 12 hr infusion of 100 ng of MEL or the saline vehicle each day for 5 wks. Hamsters were infused via a s.c. catheter. In addition, sham PINX hamsters were moved from LD to SD for 5 wks. Relative to saline-infused animals, the MEL-infused hamsters had fully regressed gonads (86.8±7.9 mg), decreased body weight reflected as decreased carcass lipid, decreased subcutaneous white adipose tissue (WAT) lipoprotein lipase activity (LPL) and increased sternal brown adipose tissue cytochrome oxidase activity. Although SD-housed hamsters had decreased body weight, carcass lipid and subcutaneous WAT LPL equal to that of the MEL-infused hamsters, the testes were not regressed after 5 wks of SD exposure. Thus, in adult Siberian hamsters 5 wks of a SD-type MEL duration mimics the effects of naturally-occurring changes in body weight (fat) and lipid metabolism, but accelerates the rate of gonadal regression. These results also suggest that the nocturnal phase of the circadian rhythm is not an overriding factor for the effects of MEL on numerous seasonal changes, but that the duration of MEL secretion seems most important.

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233.5 DEVELOPMENT OF CIRCADIAN CHANGES IN PINEAL AND RETINAL MELATONIN CONTENT IN THE EMBRYONIC CHICK. C.C.Chabot and M.Menaker, Institute of Neuroscience, Univ. of Oregon, Eugene, OR. 97403

In the adult chicken, circadian rhythms of pineal and retinal melatonin content persist in constant darkness (DD), with a period of approximately 24 hours, indicating regulation by a circadian clock. In the embryonic chick, significant day/night differences in melatonin content have been reported in the retina as early as embryonic day (E) 19 and NAT-activity differences in the pineal by E 16. We have examined the early development of melatonin rhythms in pineal and retina in order to determine when melatonin production comes under clock control in these tissues.

Eggs were maintained in an incubator in a 12:12 light/dark cycle, then subjected to DD starting at E 17 or E 20. The pineals and the retina/pigmented epithelium/vitreal (R/PE/V) complex of the eye were removed during mid-day or mid-subjective day or mid-night or mid-subjective night with the aid of an infra-red viewer. The samples were extracted and assayed for melatonin content by radioimmunoassay (Rollag and Niswender). The data were analyzed using ANOVA except for E 19 retina (T-test).

	E DAY	PINEAL MELATONIN LIGHT / DARK	R/PE/V MELATONIN LIGHT / DARK
LD			
	15	13±2(4) / 67±16*(6)	
	19	35±5(5) / 581±104*(6)	54±4(11) / 112±6*(12)
DD			
	20	349±59(5) / 609±46(6)	84±3(9) / 86±3(11)
	21	310±35(6) / 880±82*(6)	89±7(12) / 116±12(12)

Values= Mean±S.E.M.(n) *Significant Day/Night Difference(p>.05)

In a light cycle, significant day/night differences in melatonin content are found by E 15 in the pineal and by E 19 in the R/PE/V. In addition, the pineals of embryonic chicks in DD showed significant subjective day/night differences by E 21 but not by E 20 while the retinae showed no significant subjective day/night differences by E 21. Thus in the embryonic chick the photoreceptive input is coupled to melatonin production in both the retina and pineal gland before the circadian clock known to drive these rhythms is either coupled to melatonin production or is itself functional. In addition, both endogenously and exogenously driven day/night melatonin content differences develop in the pineal several days before they can be observed in the retina. (Supported by NIH HD13162 to MM and PHS GM07257 to CCC)

233.6 EFFECT OF AGE ON NOCTURNAL MELATONIN LEVELS AT REST AND FOLLOWING EXPOSURE TO BRIGHT LIGHT IN SHEEP. J. Seggie, G.M. Brown and M.D. Rollag. Departments of Neurosciences and Psychiatry, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada and Department of Anatomy, Uniformed Services University of Health Sciences, Bethesda, Maryland, U.S.A.

In all species studied, aged individuals evidence reduced nocturnal melatonin levels. Depressed patients evidence a 24 hour pattern of melatonin that is similar to that seen in elderly humans. It has been suggested that a common defect in the regulation of circadian rhythms may underlie both observations. Melatonin at various locations along the retinal-hypothalamic-pineal-axis has been implicated in the regulation of circadian rhythms that cue to light and dark. A further abnormality reported in depression is a supersensitivity to light as reflected by the ability of light to suppress nocturnal melatonin levels. Therefore, we questioned whether aged individuals, in whom melatonin levels parallel those seen in depression, might also exhibit melatonin supersensitivity to light.

The present study was undertaken in female sheep to determine the effects of age on serum melatonin levels and see if age modifies the ability of light to suppress nocturnal melatonin levels. In two studies sheep maintained on a 10 h light/14 h dark lighting regime evidenced a circadian rhythm of melatonin with low levels during the light and higher levels during the dark as measured using either the Rollag or CIDtech RIA. At two time points, 8 and 11 hours into darkness, old sheep, with a mean age of 8.6 years (range 6-11, N=6), evidenced significantly lower melatonin levels ($p < .05$) than young sheep, whose mean age was 1.1 years (range .9 - 1.2, N=5). Age and resting nocturnal melatonin levels showed a significant linear regression ($r= -0.75$, $p < .01$, N=11).

In the second half of the dark period, animals were exposed to bright light (1500 uW/cm^2) and blood samples were taken at 5, 10, 15 and 20 minutes after the onset of light. Young sheep responded to bright light with a precipitous decline in melatonin levels within 5 minutes of exposure (113 ± 18 to 39 ± 11, pg/ml, $p < .05$). Old sheep, in contrast, required 20 minutes of exposure to light before evidencing a significant decline in nocturnal melatonin levels in response to bright light (50 ± 9 to 13 ± 2 pg/ml, $p < .05$). These observations held up when the response was expressed in absolute terms as well as when it was expressed as a percentage of the nocturnal resting level for an individual. These data suggest that age related reductions in serum melatonin levels are not associated with supersensitivity of nocturnal melatonin to light. (Supported by The Ontario Mental Health Foundation, Medical Research Council and USUHS Protocol No. C07049.)

233.7 ^{3}H-MELATONIN RHYTHM IN CULTURED CHICK PINEAL CELLS. M. Zatz and J.R. Moskal. Lab. of Cell Biology, NIMH, Bethesda, MD 20892

Several laboratories have demonstrated the persistence of photosensitive rhythms related to melatonin secretion in cultured chick pineal glands (or dispersed cells). In order to investigate the regulation of such rhythms, we have developed a rapid and specific extraction assay for the ^{3}H-melatonin formed (from ^{3}H-tryptophan) and secreted by dispersed pineal cells in primary cell culture. Two hundred samples can be conveniently assayed in a day. Pineals from 75 one-day-old roosters are dispersed by trypsin, plated (in Medium 199, supplemented with 10% heat-inactivated fetal calf serum, penicillin-streptomycin, and glutamine) into fifty 16mm wells, and maintained (5% CO_2 in air, 37°C) under various lighting and drug regimens. After several days entrainment, media (containing 5μCi/ml [2,3-^{3}H]-tryptophan) are collected and replaced at fixed intervals.

[^{3}H]-Melatonin rhythms persisted in light-dark cycles (LD 12:12) for at least two weeks, though often with declining amplitude. Under constant red (R) light (Kodak #92 filter), the rhythm persisted for 3 cycles. Four hour pulses of white light (during the first such cycle) reduced ^{3}H-melatonin release acutely, and caused phase-dependent phase shifts compared to the rhythm in constant red light. Cycles of red and white light (LR 12:12 or LR 6:18, 2 phases) or red light and darkness (DR 6:18, 2 phases) were sufficient to drive the ^{3}H-melatonin rhythm for at least 5 cycles, with red light acting as subjective night or as subjective day, respectively. Cycles with 18h periods (LR 6:12, DR 6:12) were also followed, for at least 7 cycles. Elevated potassium (constant or pulsed) increased the amplitude in a light-dark cycle, and cycles of high and normal potassium could drive the rhythm (and determine the phase) in constant red light. Similarly, cyclic addition of norepinephrine (which acutely lowers ^{3}H-melatonin secretion) or of forskolin (which acutely raises ^{3}H-melatonin secretion) could drive the rhythm (and determine the phase) in constant red light.

With this approach, constant darkness cannot readily be used, and true free-running periods cannot be measured. Also, variability between preparations in amplitude and longevity remains a technical problem; the cells tend to aggregate into a ball in 6-7 days. In compensation, these primary, static cultures permit simultaneous comparisons of the effects of multiple, independent perturbations on virtually identical, cycling, photosensitive cells.

233.8 ENTRAINMENT BY PHOTOPERIODS OF ERG AND MOTOR ACTIVITY CIRCADIAN RHYTHMS IN CRAYFISH. B. Fuentes-Pardo and E. Moreno-Sáenz*. Depto. de Fisiología, Facultad de Medicina, UNAM. Apdo. Postal 70250, México 04510, D.F.

Long term simultaneous recording of electrical response to light (electroretinogram, ERG) of visual photoreceptors and motor activity in crayfish displays a circadian pattern for each record with a constant relationship between the circadian rhythm phases.

Circadian rhythms are susceptible to be entrained by periodical light stimulus changing their frequency just to be the same of the light stimulus. We have approached the problem of circadian rhythm entrainment by light, by analyzing the effects of the application of different photoperiods on ERG and motor activity rhythms in crayfish. Animals were kept in constant ambiental conditions required to simultaneously obtain the ERG at 3 min. intervals and the spontaneous pereiopods motor activity during 10 or more consecutive days, time necessary to maintain the crayfish 4 days at least in two of each following light regimes (L:D): 0:24, 24:0, 12:12, 20:4, 4:20. Results obtained were: both rhythms follow the Aschoff's rule to nocturnal species; i.e. in 0:24 or 4:20 photoperiods are more conspicuous; ERG circadian rhythm is more robust than motor circadian rhythm wich tend to become from bimodal to unimodal and even to damp in cosntant light; in 12:12 photoperiod both rhythms are more evident than in any other situation; when the circadian systems have been traslated from an initial photoperiod to any other, both rhythms maintain during several days, an attenuate but clear phase relationship associate with the previous "on" and "off" signals. These results suggest that in each rhythm operate, at least, two different oscillators with different light sensitivity, one associate with the "on" light and the other one with the "off" light.

233.9 PHASE SHIFT ON ELECTRORETINOGRAM CIRCADIAN RHYTHM INDUCED BY MONOCHROMATIC LIGHT STIMULUS IN CRAYFISH. V. Inclán-Rubio* and B. Fuentes-Pardo. (SPON: B. Ortega). Depto. de Fisiología, Facultad de Medicina, UNAM. Apdo. Postal 70250, México 04510, D.F.

Circadian rhythm is susceptible to be entrained by single or periodical external signals, changing phase or period, respectively. However, there is not knowledge about mechanisms involved in these process. We studied the effort generated by single monochromatic light stimulus on ERG circadian rhythms in crayfish, to analyze the mechanisms underlying the light phase shifting phenomenon. Adult crayfish, Procambarus bouvieri (Ortmann) were kept under constant environmental conditions, as required to obtain the ERG circadian rhythm during 10 or more consecutive days. Once the circadian rhythm reached its steady state, a 1000 lux, 50 minutes monochromatic (470, 530 or 630 nm) light stimulus was applied at different circadian times (CT). At 6th day, the phase shift (delay or advance) induced by monochromatic light stimulus was measured. Results obtained indicate that phase shifts depend on both, CT of light arrival and wavelenght of stimulus, in such a way that at 12 CT, the blue light generates the greatest advances (9.2h) while the red light applied at the same CT induces maximal delays (10h). Despite the fact that light intensity was the same for all the wavelenghts employed, changes in cycle amplitude, oscillation level and α:ρ ratio were also observed, consecutively to monochromatic light stimulus and depending on wavelenght value. Thus, the red light stimulus induces an increase in level oscillation detectable since the first day after be applied, and the green light has the opposite effect. These results suggest that the mechanisms involved in ERG circadian rhythm shifting phase by light are initiated with a visual photoreceptor's differential response, associated to the wavelenght impinging on them, which alters the oscillators output involved in ERG circadian rhythm in crayfish.

233.10 EFFECTS OF CALCIUM ON 2-DEOXYGLUCOSE UPTAKE IN THE SUPRACHIASMATIC NUCLEUS (SCN) IN VITRO. S.Shibata,G.C.Newman and R.Y.Moore,Depts. of Neurology and Neurobiology,SUNY-Stony Brook,NY11794.

The SCN is a circadian pacemaker in the mammalian brain. In vivo and in vitro experiments have shown a circadian rhythm in neural activity(Inouye and Kawamura,1979;Shibata et al,1982) and in metabolic activity(Schwartz et al,1981;Newman and Hospod,1986) in the SCN. Prior studies have also shown that replacement of Ca^{++} by Mg^{++} in medium abolishes the circadian rhythm of firing of SCN neurons (Shibata et al,1985) and intracellular inward Ca^{++} current of SCN neurons(Sugimori et al,1986).

To further elucidate the role of Ca^{++} in circadian rhythm generation in vitro,we measured 14C-2-deoxyglucose(2DG) uptake into SCN. Coronal hypothalamic slices,400u thick,that included the SCN and anterior hypothalamic area (AHA) were prepared from female Sprague-Dawley rats maintained in 12:12 light-dark cycle for 2 weeks.Slices obtained during the light portion of the cycle are referred to as subjective day whereas those obtained during the dark are subjective night.After 1 hr of pre-incubation at 37°C.,slices were incubated in Krebs solution containing 2DG(60uCi in 12ml) for 20 min (recirculating at 4.4 ml/min) and then rinsed for 30 min. The concentration of Ca^{++} was varied during the incubation and rinse. Slices were cryostat-sectioned at 20u,and the sections exposed against Kodak OM1 film for 2 weeks, and then stained with cresyl violet for histological analysis.

An estimate of local glucose utilization (LGU,ug glucose/100g tissue/min) was obtained using densitometry,14C standards(Amersham) and the in vivo kinetics constant obtained by Sokoloff et al. In normal Ca^{++} concentration medium (1.5mM) LGU is 39.3±2.1(Mean±S.D.)in SCN and 14.8±0.9 in AHA for subjective day slices,and 22±1.8 in SCN and 13.3±1.8 in AHA for subjective night slices.Slices obtained during subjective day and incubated in 0,0.375,0.75 and 1.5 mM Ca^{++} medium exhibit LGU of SCN of,25.4,31.3,33.1 and 39.3 respectively, while LGU of AHA is 14.4,14.1,11.2 and 14.8 respectively. In 0 mM Ca^{++} medium the LGU of SCN is 22.1±2.5 and 17.2±2.1 in subjective day and night,respectively.

The present results indicate that Ca^{++} is important for maintaining the rhythm in metabolic activity in the SCN. When Ca^{++} is replaced by Mg^{++},there is a reduction in SCN LGU that correlates with abolished neural firing activity in SCN. This can be interpreted as resulting from either a loss of Ca^{++} current within SCN neurons or the disappearance of synaptic activity. Since a rhythm is present in the fetus at embryonic day 19 (Reppert and Schwartz,1984) well before any rhythm in firing rate is observed (Shibata et al,1983), these alternatives potentially could be tested.

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233.11 **MECHANISMS UNDERLYING THE ACUTE AND CIRCADIAN RELEASE OF VASOPRESSIN FROM PERIFUSED RAT SUPRACHIASMATIC EXPLANTS *IN VITRO*.** D.J. Earnest and C.D. Sladek. Depts. of Neurobiology/Anatomy and Neurology, Univ. of Rochester Sch. of Med., Rochester, NY 14642

In mammals, the suprachiasmatic nucleus (SCN) of the anterior hypothalamus is involved in the central neural control of circadian rhythms. In accord with its regulatory role in the circadian organization of mammals, recent work suggests that measurable biological activities within the SCN oscillate with a circadian periodicity *in vitro*. Using a perifusion culture method, we have developed a system for studying circadian rhythms in vasopressin (VP) release from individual rat SCN explants for at least four cycles. The present experiments were conducted to determine whether the acute and the circadian release of VP by SCN organ cultures represent physiological mechanisms of release, rather than hormone leakage from degenerating neurons.

SCN explants were dissected from the hypothalami of male rats that had been maintained on LD 12:12. The explants included only the bilaterally paired suprachiasmatic nuclei, their rostral projections to the organum vasculosum of the lamina terminalis and the underlying optic chiasm. Individual explants were cultured under conditions of constant darkness and perifused with medium at a rate of 0.4ml/hr. Following a 6hr period of equilibration, serial samples of the perifusate were collected at 2hr intervals for 4 days, with the exception of the 1hr samples taken before, during and after exposure to a test stimulus. VP concentration in the medium was measured by radioimmunoassay.

In the first study, the effect of membrane depolarization on VP release was examined by exposing SCN explants to medium containing KCl (peak conc. = 41mM) for 1hr near the onset of the subjective night (i.e., when VP release is normally at a minimum) on days 2 and 4 in culture. Both of the KCl challenges induced a significant increase in VP release (1.3 - 2-fold) over that observed during the preceding intervals, suggesting that SCN neurons retained their capacity to release VP in response to membrane depolarization even on day 4 in culture. In addition to this acute effect on VP release, KCl exposure shifted the phase of the peak of the VP rhythm in the succeeding cycle relative to its temporal position prior to KCl stimulation and to its position in control explants.

In the second study, SCN explants were exposed to a low calcium - high magnesium media for 2hr near the onset of the subjective day (i.e., when VP release is normally at its peak) on days 2 and 4 in culture. Treatment with low calcium media had a significant effect in blocking the daily increase in VP release that normally occurs during this portion of the circadian cycle, suggesting that calcium-dependent synaptic transmission is an integral component of the mechanism underlying VP release by SCN explants. It is noteworthy that VP release was not completely blocked during treatment. In fact, hormone output during treatment was similar to levels normally observed during the subjective night. It was not clear whether exposure to the low calcium media had a phase-shifting effect on the VP rhythm. These results provide evidence that the acute release of VP by SCN explants is mediated by a calcium-dependent mechanism and that membrane depolarization alters VP release in an acute as well as a circadian fashion. Supported by MH-09129 (D.E.) and AM-19761 (C.S.).

233.12 **CIRCADIAN PROTEIN AND PHOSPHOPROTEIN CHANGES IN THE SUPRACHIASMATIC NUCLEI: THE DIFFERENCE BETWEEN NIGHT AND DAY.** M.U. Gillette, A.M. Reiman* and L.E. Lipeski*. Dept. of Physiol. and Biophys. & Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

The central role of the suprachiasmatic nuclei (SCN) of the hypothalamus in generating mammalian circadian rhythms is well established. Recent in vitro studies have demonstrated that the SCN are themselves intrinsic pacemakers. Circadian patterns of oscillation in both neuronal firing rate and vasopressin secretion are maintained by cultured SCN. Further, cAMP analogs induce phase-shifts of SCN rhythms in vitro indicating that the entrainment mechanism is also endogenous to this tissue (see Prosser and Gillette, this volume). However, little is known of the metabolic substrates which compose the 24-hr timekeeping mechanism. In order to gain insight into these processes, we have examined the day vs night protein and phosphoprotein composition of the SCN. Phosphorylation processes are major effectors of cellular change and might underlie diurnal changes seen in the oscillator, especially those modulated by cAMP.

Hypothalamic brain slices containing the SCN were prepared from six sibling Long-Evans rats (< 5 mo old) two hours before either the peak or trough of the circadian oscillation in neuronal firing rate. At the precise time that the slices had reached the extremes of this oscillatory cycle, they were removed from culture, frozen on dry ice in < 5 sec, and SCN were collected by micropunch (Palkovits). The pooled SCN from each time point were homogenized within 1 hour and back-phosphorylated by incubation at 37°C for 2 min with 10 μM γ-^{32}P-ATP (10 μCi, Amersham). Proteins were separated by two dimensional polyacrylamide electrophoresis (O'Farrell).

The silver stained gels exhibit numerous circadian differences in protein profiles. The differences include qualitative and well as quantitative changes in protein composition. Phosphorylatable proteins appear to correspond to proteins of which there are relatively few molecules per SCN. Densitometric scans of autoradiograms reveal that acidic proteins near 45 kD have more available phosphorylatable sites at night. Several basic proteins with molecular weights ranging from 28 kD to 110 kD are likewise more open to phosphorylation at the nighttime activity trough.

These results demonstrate that changes in proteins and phosphoproteins accompany the circadian oscillation in firing rate in the isolated SCN. Whether they are critical to pacemaker function or are part of output pathways remains to be determined. Supported by NSF-BNS 84-19342 and PHS-BRSG RR-7030.

233.13 LONG DAY LENGTHS INCREASE BRAIN WEIGHT AND DNA CONTENT IN THE MEADOW VOLE. J. Dark, K.A. Dark and I. Zucker, Dept. of Psychology, Univ. of California, Berkeley, CA 94720 and Brain Behav. Res. Ctr., Langley Porter Inst., Univ. of California--San Francisco, Eldridge, CA 95431

The weight of the brain in several rodents and insectivores is lower in specimens obtained in winter than in those collected in summer (Mezhzherin, Acta Theriol. 1964). Proximate factors that mediate such seasonal changes have not been identified.

The meadow vole, Microtus pennsylvanicus, undergoes several non-neural morphological seasonal changes which can be simulated in the laboratory by varying the duration of the daily photoperiod. We therefore evaluated the efficacy of day length in altering neural development in this species.

Male voles gestated and maintained for the first 70 days of life in a long day (LD) photoperiod (14 h light/day) had greater brain weights (5%; $p<0.05$) than corresponding males housed in a short day (SD) photoperiod (10 h light/day). Total brain DNA content was 7% greater in LD than in SD males ($p<0.05$). Similar changes were observed in a second experiment in which animals were placed in LD or SD at birth.

Neither brain mass nor DNA content of female voles were affected by photoperiod. Male voles had heavier brains with greater DNA content than their female littermates in long but not in short photoperiods. Increased androgen secretion in LD males may contribute to the seasonal sexual dimorphism in brain morphology.

The increased brain mass of male voles reared in long day lengths likely reflects an increase in neuron or glia number, although photoperiod also may affect cell configuration or chemical composition.

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233.14 PHOTOPERIODIC INFLUENCES ON BRAIN DEVELOPMENT OF MEADOW VOLES. I. Zucker and J. Dark. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

Male meadow voles maintained from birth in long photoperiods (14 h light/day:LD) have heavier brains with greater DNA content in adulthood than do their short day counterparts (10 h light/day:SD). Yaskin (Winter Ecology of Small Mammals, J.F. Merritt (ed.), 1984) in field studies of bank voles reported a 22% reduction of mass of the neocortex of autumn as compared to spring specimens. Experiments were done to determine whether photoperiod would similarly influence brain mass of several brain regions (neocortex, remaining forebrain and hindbrain) of meadow voles. A second experiment assessed whether photoperiod would influence brain mass in adult voles.

Male voles maintained from birth to 70 days of age in the long or short photoperiods had their brains quickly dissected and total brain weight was recorded. The neocortex was separated from the remaining portion of the brain which was subdivided into forebrain and hindbrain regions by a coronal cut just rostral to the superior colliculi; each brain region was weighed separately.

Total brain mass was significantly greater in LD as compared to SD voles maintained in the respective photoperiods from birth. Weights of individual brain regions were greater by 1.3-2.8% in LD than in SD voles but none of these differences were statistically significant. Thus, changes in neocortex were much smaller in the present study than in the related experiments on bank voles.

Male meadow voles placed in long or short photoperiods for 10 weeks in adulthood did not differ with respect to brain mass even though body mass was reduced by 20% in the SD animals. Photoperiod thus affects brain mass perinatally but not in adulthood.

Supported by Grant HD 02982

233.15 ALTERATION OF ADULT CIRCADIAN LOCOMOTOR RHYTHM EXPRESSION BY REARING CONDITIONS IN LIMAX MAXIMUS. I.G. Welsford and D.J.Prior(Spon: P.S. Dickinson). Physiol. Grp., School Biol. Sci., Univ. of KY., Lexington, KY. 40506

In our initial studies of the circadian locomotor rhythm in the terrestrial gastropod, Limax maximus, a significant difference in rhythm expression was observed between wild-caught and lab-raised animals. Eighty-three percent (20 of 24) of wild-caught Limax expressed a rhythm (measured by eye-fit and periodogram) while the percentage expression for lab-raised Limax was only 33% (12 of 36). During laboratory rearing animals were kept in the lab from egg to two months of age. After two months of age these animals were transferred to an environmental chamber where they were housed under L:D 14:10, thermophase:cryophase 18C.:12C. and 100% R.H. until they were tested. While in the laboratory these animals were exposed to a random L:D cycle, constant temperature (20 + 2.5 C.) and 100%R.H. We investigated the possibility that exposure to the laboratory conditions during development was responsible for the observed variation in adult rhythm expression between wild-caught and lab-raised animals.

Sixty-nine percent (20 of 29) of the Limax raised from egg to adult (five months old or reproductively mature) in the environmental chamber expressed a rhythm. In contrast, only 14% (5 of 36) of the Limax raised from egg to adult in the lab expressed a rhythm. Twenty-five percent (6 of 24) of the Limax raised from egg to 2 mos. of age in the lab then transferred to the environmental chamber expressed a rhythm. Two clutches of eggs were split, with half raised in the lab and half raised in the environmental chamber. The mean proportion of rhythm expression in the lab-raised animals was .25 + .08 ($\bar{x}$ + S.D.; 24 animals) while the proportion of expression in the environmental chamber-raised animals was .75 + .12 (23 animals). Seventy percent (14 of 20) of adult wild-caught Limax kept in the lab for up to 5 months expressed a rhythm.

There were no observed differences in the locomotor rhythms of the animals that expressed a rhythm regardless of rearing conditions. Furthermore, there were no observable differences between any groups in the general locomotor behavior parameters. These results suggest that exposure to random lighting and/or constant temperature during development alters the expression of the locomotor rhythm in adult Limax maximus without altering the mechanisms underlying the patterning of the rhythm.

234.1 SEROTONIN ONTOGENY IN THE SYMPATHETIC THORACOLUMBAR SPINAL CORD OF NEONATAL RAT: AN IMMUNOHISTOCHEMICAL STUDY. B.W. Newton, A.B. Burkhart* & R.W. Hamill. Department of Neurology, University of Rochester/Monroe Community Hospital, Rochester, NY 14603

5HT is a neurotransmitter implicated in control of autonomic functions, e.g. cardiovascular and sympathoadrenal function. The vast majority of spinal 5HT originates from brainstem projections which terminate upon sympathetic preganglionic neurons in the autonomic nuclei of lamina VII and X: intermediolateral n. pars funicularis and principalis (Ilf, Ilp), n. intercalatus (IC) dorsal commissural n. (DCN). Additionally, 5HT fibers are distributed throughout lamina X (X). 5HT fibers mimic this nuclear distribution. To study the ontogeny of 5HT fibers 0, 2, 4, 8, 12, 16, and 30 day (d) old rats were perfused transcardially with Zamboni's fixative and processed for the PAP technique. Spinal cords were serially sectioned horizontally at 40-70um with a vibratome. The 5HT antibody (Newton, et al., Brain Res. 376:155,'86) was diluted 1:5000 in phosphate buffered saline/3% normal sheep serum, 0.3% Triton and applied overnight at room temperature. Absorption controls demonstrated primary antiserum 5HT specificity.

A rostral-caudal developmental gradient was seen with a nearly adult pattern established by 30d. An initial pattern was seen in upper thoracic (UT) regions by 0d, middle thoracic (MT) by 2d, and lower thoracic-lumbar (LT) by 4d. In all cases the UT and LT patterns are more prominent than the MT pattern. Additionally, 5HT puncta and fine fibers develop before large 5HT varicose fibers are seen. As development occurs numbers of varicose 5HT fibers increase and their pattern within the autonomic nuclei become more complex. In UT regions Ilp, IC, DCN and X were established first (0d) with Ilf following (2d). In MT levels X (0d) was established first with Ilp (2d) and DCN (2d) second, then IC (4d) and Ilf (8d) following. At LT regions X (2d) was established first with Ilp, IC and DCN following (4d). Ilf (8d) developed last. As rats age the Ilp becomes more compact, delta-shaped and interconnected and Ilf increases in size in relation to Ilp. Additionally, DCN becomes broad as more IC's reach X. However, the number of IC's decrease and become more compact with age. X becomes filled with numerous large 5HT varicose fibers. In general, the 5HT pattern is established very early postnatally but its maturation process is protracted. IC and DCN mature most rapidly with Ilf achieving adult status last. 5HT neurons were seen at nearly all thoracolumbar levels and in all autonomic nuclei except Ilf. Adult rat spinal 5HT neurons have been described (Soc. Neurosci. Abst. 11:1018, '85). This study reveals that spinal sympathetic nuclei preferentially receive a dense 5HT innervation that differentially develops. Early spinal 5HT innervation suggests 5HT may be critical in autonomic spinal cord organization. Supported by U of R/MCH Research Fund & Neurobiol. of Aging Training Grant AGT32107 to BN.

234.2 IMMUNOHISTOCHEMICAL DISTRIBUTION OF SEROTONIN IN SYMPATHETIC NUCLEI OF RAT THORACOLUMBAR SPINAL CORD. M.A. Romagnano, B.W. Newton & R.W. Hamill. Department of Neurology, University of Rochester/Monroe Community Hospital, Rochester, NY 14603

5HT is a neurotransmitter implicated in control of autonomic functions, e.g. cardiovascular and sympathoadrenal function. The majority of spinal sympathetic 5HT originates from brainstem projections which synapse upon preganglionic neurons in autonomic nuclei of lamina VII and X: intermediolateral n. pars funicularis and pars principalis (Ilf, Ilp), n. intercalatus (IC) and dorsal commissural n. (DCN). To study the distribution of 5HT fibers in lamina VII and X, 21 adult Sprague-Dawley rats of either sex were examined with the PAP method. Rats were perfused with Zamboni's fixative and the spinal cords were sectioned horizontally at 40um with a vibratome. Sections were incubated for 48 hrs at 4°C in antiserum generated against 5HT coupled to bovine serum albumin (Newton, et al., Brain Res. 376:155,'86) diluted 1:5000. All antibodies were diluted in phosphate buffered saline/3% normal sheep serum with 0.3% Triton X-100. Absorption controls demonstrated primary antiserum 5HT specificity. Immunoreactive 5HT fibers were found in thoracolumbar spinal cord localized in nuclear regions containing preganglionic sympathetic neurons. Very dense numbers of 5HT fibers were seen extending from the dorsal lateral funiculus connecting with 5HT fibers in Ilp cell clusters. A moderate to dense band of 5HT fibers, adjacent to the white-gray border, connected adjacent Ilp's. Ilp periodicity slowly decreased from 460um in upper thoracic regions to 200um in lumbar regions. Large numbers of Ilf 5HT fibers extended from the pial surface to the Ilp and were oriented perpendicular to lateral funicular fibers. Often Ilf fibers followed blood vessels to Ilp. Immunoreactive 5HT fibers in moderate numbers were located in IC forming a series of transverse bands linking Ilp with DCN. Often multiple IC's projected from one Ilp in upper thoracic regions, while in the lumbar cord one Ilp gave rise to a single IC. IC periodicity slowly decreased from 420um in upper thoracic regions to 240um in lumbar regions. IC's merged dorsal to the central canal to join DCN. This dense band of DCN 5HT fibers was continuous throughout the length of the spinal cord. In addition, the remainder of lamina X contained moderate to scattered 5HT fibers adjacent to the central canal as well as a scattered plexus of fibers ventral to the canal. The distribution of 5HT fibers in these locations corresponds to previous reports. These data show that spinal autonomic nuclei preferentially receive a dense 5HT innervation indicating the importance of 5HT in autonomic control. Additionally, rat lamina X 5HT patterns are different as compared to dog and monkey (Kojima, et al., Cell Tiss. Res. 226:447,'82; 229:23, '83). Supported by U of R/MCH Research Fund & Neurobiology of Aging Training Grant AGT32107 to BWN.

234.3 IMMUNOHISTOCHEMICAL DEMONSTRATION OF 5-HYDROXYTRYPTAMINE AND L-GLUTAMIC ACID DECARBOXYLASE IN GLUCOCORTICOID-INDUCED SIF CELLS IN THE SYMPATHETIC GANGLION. L.Eränkö[1], H.Päivärinta[1], O.Häppölä[1], S.Soinila[1], H.Steinbusch[2] and J.-Y. Wu[3]. [1]Department of Anatomy, University of Helsinki. Siltavuorenpenger 20 A, 00170 Helsinki, Finland, [2]Department of Pharmacology, Free University, 1081 BT Amsterdam, The Netherlands and [3]Department of Physiology, Pennsylvania State University Milton S. Hershey Medical Center, Hershey, PA 170033, USA.

Formaldehyde-induced fluorescence method made possible the detection of two cell types in sympathetic ganglia: principal nerve cells (PN) and small intensely fluorescent (SIF) cells. It has been shown that PN cells contain noradrenaline, while depending on the ganglion, dopamine, noradrenaline or adrenaline has been detected in SIF cells. Recently also 5-hydroxytryptamine (5-HT) and L-glutamic acid decarboxylase (GAD) has been immunohistochemically demonstrated in SIF cells in the superior cervical ganglion of an adult rat. Treatment of newborn rats with glucocorticoids increases the number of SIF cells and induces phenylethanolamine-N-methyltransferase activity and appearance of adrenaline in them. On the other hand, similar glucorticoid treatment, causes only minute changes in the amount of dopamine and noradrenaline in the superior cervical ganglion of the rat. The aim of this study was to investigate the effect of glucocortricoid on the number of 5-HT- and GAD-immunoreactive cells.

In the superior cervical ganglia of early postnatal rats 5-HT immunoreactivity was localized in small cells (5-15 um in diameter) cells and large (15-30 um in diameter) cells, resembling in size SIF cells and PN cells, respectively. The relative number of 5-HT-immunoreactive PN cells decreased after the first postnatal week, and 5-HT immunoreactivity was localized only in SIF cells of adult rats. GAD-immunoreactive cells were not found in ganglia of newborn rats, but they were regularly observed in SIF cells of adult rat superior cervical ganglia, were they were found either solitary or in small clusters. Newborn rats were injected daily with hydrocortisone acetate for 5-7 days. Due to the glucocorticoid treatment, the number of 5-HT- and GAD-immunoreactive cells increased dramatically. The immunoreactive cells were quite evenly distributed in the ganglia, were mostly solitary but also formed small clusters. The immunoreactive cells were smaller than the nonreactive cells. On the basis of their high nucleus/cytoplasm ratio they were regarded to be SIF cells.

It has been suggested that glucocorticoids and the nerve growth factor have opposite effects on the developing primitive sympathetic precursor cells, the former favouring differentiation into SIF cell type and the latter into the PN cell type. The results of this investigation suggests that glucocorticoids also favour 5-HT- and GABA-ergic differentation.

234.4 LOCATION OF THE NEURONS PROJECTING TO THE AGANGLIONIC BOWEL OF ls/ls MICE. V.M. Tennyson, R.F. Payette*, T.D. Pham*, G. Mawe, T.P. Rothman and M.D. Gershon, Dept. of Anat. & Cell Biol. Columbia Univ. P&S, New York N.Y. 10032.

The enteric nervous system (ENS) is formed by the precursors of neurons and glia that migrate to the bowel from the neural crest. The ENS is abnormal in the lethal spotted (*ls/ls*) mutant mouse in that the terminal portion of the colon is aganglionic. We have previously reported that components of basal laminae (BL), including laminin and type IV collagen, are overproduced in the presumptive aganglionic bowel in *ls/ls* fetuses. In the adult *ls/ls* gut the muscularis mucosa of the aganglionic zone is thickened because the smooth muscle cells are hypertrophic and surrounded by reduplicated BL. We have postulated that the precursors of enteric neurons fail to colonize the affected region because the excessive BL favors neurite extension at the expense of precursor cell migration. We have now tested this hypothesis by examining the distribution of intrinsic enteric neurons of the submucosal and myenteric plexuses and the innervation of the gut by extrinsic axons. Our hypothesis predicts at least some penetration of the aganglionic bowel by the processes of intrinsic neurons and the growth of an abundant supply of extrinsic neurites into the aganglionic gut. Neurons and their processes were located by demonstrating acetylcholinesterase (AChE), classified as intrinsic or extrinsic by EM, and the location of perikarya was accomplished by retrograde tracing with True blue or WGA-HRP. Near the cecum the *ls/ls* colon contained both myenteric and submucosal ganglia, there were few extrinsic nerves, and the arrangement of the plexuses was similar to that of control mice. More distally (8-9 cm from the anus), a hypoganglionic zone was found in the mutant bowel, in which the numbers of submucosal ganglia gradually declined to reach 3% of control (4 cm from the anus). Below this point the myenteric plexus also became hypoganglionic while the submucosal plexus disappeared. The number of myenteric ganglia then declined distally, persisting only along the mesenteric border, until the aganglionic region was reached 1 cm from the anus. Both the aganglionic and hypoganglionic bowel contained large nerve trunks that were found by EM to have the structure of peripheral, not enteric, nerve. Peripheral ganglia, located just outside the intestinal musculature were attached to these nerves. Injections of tracers revealed that the longest intrinsic enteric neurites of ls/ls mice penetrated as far as 7 mm into the aganglionic colon, although they were slightly shorter (1.4 ± 1.3 mm) than the longest neurites of controls (14.7 ± 0.6 mm); however, most of the neurites in the aganglionic tissue were extrinsic axons. These axons were found to come from dorsal root and sympathetic ganglia. No retrograde labeling of the sacral parasympathetic nucleus was found in *ls/ls* mice, whereas it was observed in controls. Injection of the striated anal sphincter led to labeling of anterior horn cells in both *ls/ls* and control mice. These observations support the idea that the aganglionic bowel is a good substrate for axonal growth; intrinsic enteric neurites enter its orad boundery and extrinsic neurites enter it in a greater than normal abundance. On the other hand, the absence of target neurons is correlated with an evident failure of parasympathetic preganglionic fibers to innervate the aganglionic gut. Supported by grants NS 15547, HD 17736, BNS 09452, HD 21032, the MOD and the Dysautonomia Fdn.

234.5 TRACER INFLUX FROM THE SUBARACHNOID SPACE INTO "PARAVASCULAR" FLUID PATHWAYS IN THE MAMMALIAN BRAIN IS INFLUENCED BY STIMULATION OF SYMPATHETIC NERVES TO CEREBRAL BLOOD VESSELS. M.L. Rennels, O.R. Blaumanis* and P.A. Grady, Depts. of Neurology and Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201

After infusion into the subarachnoid space (SAS), horseradish peroxidase (HRP) enters the perivascular spaces (PVS) around penetrating arterioles and, within minutes, spreads longitudinally through the basal laminae enclosing their capillary branches throughout the CNS. Our recent hypothesis states that this unidirectional 'paravascular' HRP influx reflects a fluid/tracer circulation through these pathways that is facilitated by pulsations of the penetrating arterioles within their PVS (Brain Res., 326:47). This concept raises the possibility that paravascular fluid/tracer movements may be affected by altering the functional status of intraparenchymal arterioles. Of particular interest was a possible influence of the sympathetic innervation of penetrating arterioles, which, in the cat, is derived ipsilaterally from the superior cervical ganglia (SCG). Adult cats were anesthetized and the SCG exposed; a needle was then inserted into the cisterna magna. Continuous unilateral electrical stimulation (SS) of the SCG was administered at an intensity just adequate to evoke pupillodilation and HRP (4% in buffer) was introduced into the SAS by gravity flow through the cisternal needle. The total tracer volume infused (0.5 - 0.75 ml) was equal to the CSF outflow measured after cisternal puncture. Control animals received HRP without SS. After HRP circulation and SS of 4 or 10 min. duration, brains were fixed by intravascular perfusion of aldehydes and HRP was localized in Vibratome sections of the neuraxis using tetramethylbenzidine or diaminobenzidine as chromogens. In all animals, a thin layer of HRP reaction product (RP) appeared bilaterally over the pial and ependymal surfaces. In control sections, paravascular RP was uniformly distributed within the neuraxis. After 4 min. SS, however, a localized zone of RP appeared in the ipsilateral thalamus. Constriction and sludging of small vessels and diffuse tracer spread into the adjacent parenchyma was also observed. Continued SS for 10 min. was accompanied by a generalized increase in paravascular RP density bilaterally within the forebrain; the localized thalamic tracer pool and microvascular changes seen after 4 min. SS were no longer apparent. Although the mechanism(s) of these effects is unknown, our results are consistent with a neurovascular influence on tracer influx through the PVS. The sympathetic system may play a role in regulating paravascular fluid movements in the brain. Supported by Grant No. 2P50 NS16332-04A2, NINCDS, NIH.

234.6 **EFFECT OF TARGET REMOVAL ON MIGRATION OF SYMPATHETIC PREGANGLIONIC NEURONS IN CHICK EMBRYO.** Anita Prasad* and Margaret Hollyday (SPON: R.J. Dinerstein). Dept. Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago 60637.

The normal development of sympathetic preganglionic neurons ("Terni column" neurons) in the chick embryo was first studied in detail by Levi-Montalcini in 1950 (J. Morphol.86(2):253). We (Soc. Neurosci. Abstr. 11: 586, 1985) have recently confirmed and extended her observations. The Terni column is comprised of neurons within the avian spinal cord which project to the sympathetic ganglia and whose early development includes two migrations. Our primary focus has been on the secondary migration of these cells, from the ventral horn, to their adult position dorsomedial to the central canal. Experiments were done to determine what role, if any, the synaptic targets of the Terni column cells play in this secondary migration.

We have observed axons of Terni cells in sympathetic ganglia at St. 26. This coincides with the onset of the secondary migration. Subsequent progress of the secondary migration was therefore studied in a group of sympathectomized embryos. Using an electrocautery, neural crest (precursor of sympathetic ganglia and other cell types) was removed from the presumptive thoracic regions of the neural tube prior to the initiation of neural crest migration. These crest removals were done in St.13 and St.14 embryos. After survival times of 5-7 days following the neural crest removal, the embryos ranged in age from St.28 to St.35. At this time the embryos were removed from the egg, carefully eviscerated and laminectomized in order to expose the thoracic ventral roots. WGA-HRP was then injected into the ventral roots to retrogradely label motoneurons and any presumptive Terni column cells. Tissue was fixed, sectioned, and examined with the light microscope following a DAB reaction and cobalt-intensification step to visualize the HRP reaction product. Criteria for a successful crest removal included a total absence of dorsal root ganglia and sympathetic ganglia for at least three consecutive spinal segments.

Our results indicate that target removal has no apparent effect upon the development of the column of Terni. The time course of the secondary migration and the morphology of the migrating cells are very similar to what is observed in normal embryos. Therefore it would appear that the presence of the sympathetic ganglia is not essential for either the initiation or completion of the secondary migration of Terni column cells.

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234.7 THE ALLIANCE BETWEEN CHROMAFFIN TISSUE AND THE AUTONOMIC NERVOUS SYSTEM IN RABBITS: ANATOMICAL AND MORPHOLOGICAL DATA. J.A. Mascorro* (SPON: K.Kratz). Department of Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

It is well known that a catecholaminergic chromaffin system exists throughout the retroperitoneum in many species. The cells comprising this voluminous network are laden with dense granules and occupy intraadrenal (the adrenal medulla), paraaortic, paraganglion and intraganglion sites. The adrenal medulla is known for its endocrine character, however, the function of most extraadrenal chromaffin tissue is not known. This anatomical and morphological study examines extra-medullary chromaffin tissue and its association with the autonomic nervous system in order to elucidate the functional character of this unique tissue. Abdominal chromaffin tissue was located by utilizing a solution of potassium dichromate and glutaraldehyde. The interaction between dichromate and tissue catecholamines produced a gross chromaffin reaction which clearly "mapped" the extra-medullary chromaffin organs, while glutaraldehyde preserved their structure for concurrent morphological study.

Dichromate tracing revealed a continuity between the intraadrenal and paraaortic components of the chromaffin system. The paraaortic organs were elongated and bifurcated towards their caudal extent where they closely bordered the inferior mesenteric ganglion. Smaller paraganglion bodies as well as single or clustered intraganglion chromaffin cells also were seen.

Histological evaluation showed that the paraganglion and ganglion shared, and were separated by, a capsule rich in vascular and connective tissue elements. Distinct chromaffin cells were seen within the ganglion proper, but a continuity between paraganglion and intraganglion chromaffin cells or accompanying blood vessels was not evident. All paraaortic, paraganglion and intraganglion chromaffin cells contained numerous dense-cored vesicles and otherwise displayed fine structural features usually associated with chromaffin cells in general. Three chromaffin cells were observed in the metaphase stage of cell division and several more in varying degrees of necrosis. Synapses were not observed upon paraaortic or paraganglion chromaffin cells; cholinergic-like endings did occur, sparsely, on the intraganglion chromaffin cells. The ganglion chromaffin cells gave rise to granule-rich processes which neared, but did not actually contact, the principal neurons. Considering these data, it may be suggested that chromaffin cells juxtapositioned to sympathetic ganglia, or residing within the ganglion, perhaps release amine products into the ganglion proper. Conversely, highly vascularized and adrenal medullary-like paraaortic chromaffin organs may subserve as an extra-medullary source of catecholamines.

234.8 EVALUATION OF THE PARTICIPATION OF THE SYMPATHETIC NERVOUS SYSTEM IN THE PRESSOR EFFECT OF INTRACEREBROVENTRICULARLY INJECTED ANGIOTENSIN II. L.C.Lopes*, H.Milani*, W.A.Saad, L.A.A.Camargo*, J.V.Menani*, A.Renzi*, L.A. de Luca Jr.* and W. Abrao-Saad*. Departamento de Fisiologia e Patologia, Faculdade de Odontologia, UNESP, Araraquara, SP. e Departamento de Cirurgia, Faculdade de Medicina, USP, São Paulo, SP.

Central injection of angiotensin II (AII) causes an increase in mean arterial pressure (MAP) and in heart rate (HR). The objective of the present investigation was to study the effective extent of participation of the sympathetic nervous system (peripheral adrenergic endings and adrenal glands) in the changes in MAP and HR produced by intracerebroventricular (icv) injection of AII. Holtzman rats weighing 280 g and implanted with delay cannulae in the left lateral ventricle after treatment with guanethidine or adrenodemedullation were injected icv with 12 ng AII, and the changes in MAP and HR were recorded. Icv injection of 12 ng AII into normal rats caused increases in MAP of 14 $\pm$ 3 mmHg. In previously adrenodemedullated rats, this response was significantly reduced (an increase of only 5 $\pm$ 2 mmHg), but previous treatment with guanethidine did not change the increase in MAP due to icv injection of AII. The increase in HR observed after icv AII in normal rats (17 $\pm$ 6 bpm) was totally abolished by previous treatment with guanethidine (Δ HR = -13 $\pm$ 12 bpm) but was not changed by previous adrenodemedullation (Δ HR = 15 $\pm$ 8 bpm). The results suggest an important participation of the adrenal medulla in the increase in MAP, whereas the peripheral sympathetic endings may play a more important role in the change in HR caused by icv injection of AII.

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234.9 EFFECTS OF AXOTOMY ON THE FIRING PROPERTIES OF BULLFROG SYMPATHETIC GANGLION CELLS. P.A. Smith, M.E.M. Kelly* and T. Gordon
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Transection of the axons of vertebrate neurones results in a series of morphological, biochemical and physiological changes which may be related to the neurone's ability to regenerate an axon and reinnervate its target organ. We have shown that axotomy of B cells in bullfrog sympathetic ganglia results in various electrophysiological changes including an increase in spike width and a reduction in the amplitude and duration of the after-hyperpolarization (a.h.p.) which follows the action potential (a.p.) (Kelly et al., Neurosci. Letts. 1986, in press). These effects were attributed to a functional loss of calcium sensitive potassium conductance(s) (g_{KCa}). Since a.h.p. duration and the underlying g_{KCa} have been implicated in the regulation of the firing properties of these sympathetic neurones (Pennefather et al., Soc. Neurosci. Abs. 11, 148, 1985) the response of axotomized cells to depolarizing current pulses should resemble that seen in cells where g_{KCa} is blocked with Cd^{2+} or Mn^{2+}. We therefore used standard intracellular current clamp recording techniques to examine the response to depolarizing current in control, axotomized and Cd^{2+} or Mn^{2+} treated bullfrog sympathetic neurones. Cells in the IXth or Xth paravertebral sympathetic ganglia were axotomized by cutting the appropriate spinal nerve about 1 cm from the ganglia 14-47 days before electrophysiological investigation. Excitation of normal B cells by prolonged current pulses evoked one to five a.p.'s depending on the intensity of the stimulus. The cells subsequently remained silent throughout the duration of the pulse (adaptation). Superfusion of Ringer's solution containing 200 μM Cd^{2+} or 10 mM Mn^{2+}/0.18 mM Ca^{2+} had somewhat variable effects but at least in some neurones, firing was maintained throughout the entire duration of a depolarizing current pulse. In the light of these findings, we anticipated that axotomized cells would adapt less than normal and at least in some cells, firing would be maintained for as long as the neurones were depolarized. We found however that discharge patterns remained comparable between control and axotomized cells despite the attenuation of a.h.p.'s. This implies that other changes in ion channels must accompany the loss of g_{KCa} following axotomy and that these changes may serve to preserve neuronal activity within an apparently normal range. Such changes may be manifest in the marked increase in rheobase which we found to follow axotomy. This increase in rheobase may partly offset the loss of g_{KCa}. The maintenance of relatively normal discharge patterns may be condusive to regenerative processes.

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234.10 α-ADRENERGIC CONTROL OF CARDIOVASCULAR FUNCTION IN THE NEONATAL RAT: EFFECTS OF PHENOXYBENZAMINE ON RESPONSES TO HYPOXIA. F.J. Seidler*, K.K. Brown*, P.G. Smith* and T.A. Slotkin (SPON: A. Tadepalli). Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Stimulation of peripheral α-adrenergic receptors by circulating catecholamines derived from the adrenal medulla is essential for surviving acute neonatal hypoxia. We have evaluated the role of α-receptor mediated responses through assessment of the effects of phenoxybenzamine (PBZ) on cardiovascular performance during hypoxic challenge in urethane-anesthetized rats at two different developmental stages: 1 day old, where sympathetic tone is absent due to the immaturity of sympathetic efferents, and 8 days old, where sympathetic innervation of cardiovascular sites has become functional. In normoxic conditions, PBZ did not affect mean arterial pressure (MAP) or heart rate at 1 day of age. Reduction of inspired O_2 to 9% caused an immediate fall in MAP which was equivalent in controls and PBZ-treated rats. However, control 1 day olds maintained normal heart rates in 9% O_2 whereas PBZ-treated rats displayed falling heart rates and atrioventricular conduction abnormalities. The latter were characterized by a progressively worsening A-V block with eventual junctional escape, without abnormalities of ventricular conduction (normal QRS complex). Eventually, death ensued from heart failure. In 8 day old rats, PBZ administration caused a fall in MAP in normoxic conditions; heart rates were unaffected by PBZ. When inspired O_2 was lowered to 9%, both MAP and heart rates in control 8 day olds declined substantially; the latter reflected changes in sino-atrial rhythm unaccompanied by altered atrio-ventricular conduction. The adverse effect of PBZ on MAP was sustained throughout the hypoxic period, but no consistent exacerbation of the heart rate effect was noted. PBZ did not cause atrioventricular conduction defects in hearts of 8 day old rats exposed to hypoxia. These data support the view that the surge of catecholamines released by the adrenal medulla (1 and 8 day old rats) and by sympathetic nerves (8 day old rats) in response to hypoxia, promotes survival through cardiovascular adjustments mediated by α-adrenergic receptors. In the younger animals, effects on the myocardium predominate and heart rate can be maintained for a prolonged period; α-receptor blockade during hypoxia leads to a slowing of the sino-atrial pacemaker and appearance of conduction deficits, leading to cardiovascular collapse. In older animals, the contribution of α-adrenergic sites in sustaining myocardial function is lost, and instead the vascular beds become a primary contributor to catecholamine action. The spectrum of effects seen in the immediate postnatal period are commensurate with the unique requirements of the immature organism during the stress of birth and its associated hypoxia. (Supported by USPHS HD-09713 and NS-06233)

234.11 EFFECTS OF UNILATERAL MEDIAN NERVE CRUSH ON THE POSTGANGLIONIC SYMPATHETIC NEURONS IN THE STELLATE GANGLIA OF THE MACAQUE MONKEYS (MACACA NEMESTRINA). S.K. LEONG*, W.C. WONG.
Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511.

Two normal and five experimental adult monkeys were investigated. In two of the experimental animals the sympathetic postganglionic neurons whose axons are present in the median nerve were localized by the horseradish peroxidase method. In the remaining three experimental animals, the right median nerves were crushed at mid-arm level. They were then perfused 5, 15 and 30 days respectively after the operation. Perfusion of the three experimental and two normal control animals was preceded by a prewash with Ringer's solution and followed by a mixture of 3% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer. The right and left stellate ganglia were obtained and processed for electron microscopy, and their lower portions examined with a Philips 400T electron microscope. The histology of the normal stellate ganglion bears much resemblance to that of other species reported. The ganglion cells display a rich variety of size and shapes. Their nuclei are mostly eccentric in position. Binucleated neurons are present, but uncommon. The organelles and inclusion bodies present no special features. Each ganglion cell is surrounded by sheath cell cytoplasm which is arranged in one to several lamellae around the neuronal cell body or processes. Small cells containing large dense-cored granules are rare. Synapses and myelinated and nonmyelinated fibres are plentiful. Macrophages, mast cells and other connective tissue cells are present.

A notable feature in the normal ganglion is the presence of not a few darkened profiles. Many of them could be identified as dendrites by virtue of the presence within them of degenerating but recognizable mitochondria and diffuse ribosomes. The darkened profiles are either still attached to the cell bodies or completely engulfed by the sheath cell processes. Some of the neuronal cell bodies with attached darkened dendrites show typical chromatolysis. In addition to darkened profiles, neurons showing dense cytoplasm and degenerating mitochondria or dead cells whose morphology is beyond recognition are also present.

Five to thirty days after the median nerve crush the incidence of darkened profiles, chromatolytic neurons and dead cells is greatly increased in both the right and left stellate ganglia. It is surmized that the process of degeneration that occurs normally in the stellate ganglion is accelerated by damage to axons of the ganglion cells.

234.12 CAUDAL MESENTERIC GANGLION AND PLEXUS IN THE ALBINO RABBIT - TOPOGRAPHY AND COMMUNICATIONS. G. M. Constantinescu*, R. C. McClure and E.M.Brown* Laboratory of Anatomy, Dept. of Veterinary Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211

The literature concerning the caudal mesenteric ganglion and plexus in rabbit is meager. Since 1884, when Krause described them, researchers have focused their attention on sympathetic ganglia other than the caudal mesenteric. Brief descriptions were made by Craigie (1931, 1951). Barone et al (1973), neither illustrated nor described the caudal mesenteric ganglion in their "Atlas of Rabbit Anatomy".

According to Krause the ganglion is 7 mm long, 3 mm wide and 2 mm thick and angular, and located cranial to the origin of the caudal mesenteric A. According to Craigie in 1931, the same ganglion is narrow and curved. Craigie in 1951 described it as a slender and elongated body. In 1931 and 1951 Craigie located it cranial to the caudal mesenteric A. Only Krause mentioned an accessory caudal mesenteric ggl. All the authors agree unanimously with the presence of only one caudal mesenteric ggl. Craige made a simple mention about the presence of the caudal mesenteric plexus, from Craigie. The plexus was described in detail by Krause.

Based upon our research on 25 Albino Rabbits (20 female, 5 male, between 6-8 months old), we could first establish that the maximum length is 3-4 mm. The width and the thickness are difficult to establish, because it looks like a flat star with many communicating branches. It receives a direct branch from the 5th and sometimes the 6th sympathetic lumbar ganglion via the corresponding lumbar splanchnic Nn.V and VI, respectively, and indirectly from the other lumbar lympathetic ganglia via the intermesenteric Nn. (Kuntz, 1956) or plexus (Barone et al, 1973). The ganglion was located on the lateral side or caudal to the origin of the caudal mesenteric A., never cranial to the Artery. The accessory caudal mesenteric ggl. was inconstantly observed; when present it was located close to the origin of the left ovarian A.

The communicating branches are pre- and postganglionic. The preganglionic branches come from the lumbar sympathetic ganglia and the abdominal aortic (intermesenteric) plexus. The postganglionic branches along the right and left ovarian Aa. and the caudal mesenteric A., as well as the hypogastric N., which ends in the pelvic plexus.

The caudal mesenteric plexus was particularly prominent on the right side of the abdominal aorta.

234.13 ASSESSMENT OF TACHYCARDIA DENSITY ACROSS SLEEP STATES IN NORMAL INFANTS. V.L. Schechtman, K.A. Kluge*, and R.M. Harper. Neuroscience Program, Dept. of Anatomy, and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

The incidence of tachycardia in infants provides a useful index of certain pathological conditions. Normative values for occurrence of tachycardia during development, however, have yet to be described by sleep state. The definition of a tachycardia episode must reflect state, since baseline heart rate values vary considerably with state. Such state-dependent variation mandates that a single threshold value for tachycardia not be used. The present study examines the effect of sleep state on tachycardia density in normal infants.

Twelve-hour physiological recordings were made of 25 normal infants at 1 wk and at 1, 2, 3, 4, and 6 mo of age. Each minute of data was classified as waking (AW), quiet sleep (QS), rapid eye movement sleep (REM), or indeterminate state (IN) based on EEG, EOG, EMG, and motility criteria. Tachycardia density was assessed using 4 methods: 3 absolute thresholds (165, 180, and 200 beats per minute) and state- and age-dependent thresholds equal to the mean heart rate + 2 standard deviations (intersubject variance) + 2 times the interquartile range (intrasubject variance). In each case, an episode of tachycardia was defined as heart rate above the threshold for 10 or more consecutive beats. Mean tachycardia density in seconds per minute was determined for AW, QS, and REM for each recording. An ANOVA was performed to assess differences in tachycardia density across the 3 sleep states for each age.

At each age, all 3 absolute tachycardia thresholds showed greater tachycardia density in AW than in either QS or REM. In AW, heart rate was frequently above 165 bpm, thus use of the 165 bpm threshold showed mean waking tachycardia densities ranging from 10 to over 30 sec/min depending on age. The 180 and 200 bpm thresholds showed less tachycardia during AW and corresponding decreases in tachycardia in the other 2 states, resulting in significant state differences for each threshold at each age.

Use of the variable tachycardia thresholds resulted in mean tachycardia densities of 1 to 4 sec/min in each state and at all ages. At ages 2 mo and younger, tachycardia densities were comparable in the 3 states, but tachycardia density was greater in AW than in QS or REM in infants older than 2 mo.

These results suggest that any assessment of incidence of tachycardia must partition the effect of sleep state. Moreover, state-specific mean heart rate values, combined with measures of intersubject and intrasubject variation, should be used to determine tachycardia thresholds.

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234.14 SPONTANEOUS EFFERENT CERVICAL SYMPATHETIC ACTIVITY IN NEONATAL SWINE. H.L. Cohen, A.L. Sica, P.M. Gootman, P.G. Griswold*, M.R. Gandhi*, P.P. Rao*, Dept. Physiology, Downstate Med. Cent., SUNY, Bklyn, N.Y. 11203 and Dept. Ped. Cardiol. and Ped. Pulm. Div, Schneider Children's Hosp., New Hyde Pk., N.Y. 11042

An earlier paper reported that spontaneous efferent splanchnic activity in newborn swine (Gootman, et al., "Catecholamines Part A: Basic and Peripheral Mechanisms", Alan R. Liss, NY, 1984, p.369) was similar in many respects to efferent splanchnic activity in adult mammals (e.g., Gootman, et al., Brain Res., 1975, 87:395). Recordings of spontaneous cervical sympathetic activity indicate further similarities (Gootman & Cohen, M.I., Acta Physiol. Polonica 1973, 24:97). In the current series of experiments, spontaneous efferent cervical sympathetic discharge was monophasically recorded (bandpass 0.3-1000 Hz) in Saffan-anesthetized, paralyzed (C-10), artifically ventilated (100% O_2) piglets (5-26 days old) simultaneously with efferent phrenic discharge (bandpass 3-10 kHz) (monitor of the respiratory rhythm generator), EKG, aortic pressure and end-tidal CO_2. Body temperature was maintained at 38.5°C. Blood gases and pH were monitored before and after each experimental intervention using a Corning microsystem. Power spectral analyses were calculated using 1024 data point FFTs; cervical sympathetic activity was filtered between 1-128 Hz. Examination of power spectra revealed peak frequencies similar to those observed in neonatal splanchnic activity ca. 20 and 30 Hz. In addition, in some cervical sympathetics a peak was detected at ca. 10 Hz. Oscillations in phase with both the central respiratory cycle (maximum activity in inspiration, minimum activity in early expiration) and the cardiac cycle were also observed. Periodicities in cervical sympathetic discharge were exquisitely sensitive to bolus injection of epinephrine (0.5 μg/kg); power spectral peaks were markedly attenuated or eliminated. Hypoxia (15% inspired O_2 in N_2) resulted in an overall reduction in magnitude of cervical sympathetic peak frequencies. The present and earlier reports reveal that spontaneous sympathetic activity is present in newborn swine. Computer analyses of this sympathetic discharge indicate that at least some of the periodicities present in adult mammals can be observed at birth. (Supported by USPHS, NIH grant #HL-20864 to PMG & Parker B. Francis grant to ALS)

CNS NEURONS: VERTEBRATES AND INVERTEBRATES

235.1 MEMBRANE IMPEDANCE MEASUREMENTS USING SINUSOIDAL AND WHITE NOISE INPUT CURRENTS : EFFECTS OF ETHANOL. L. Takeuchi*, A. D' Aquanno*, B.L. Bardakjian*, and P.L. Carlen. (SPON:P.W. Kujtan). Playfair Neuroscience Unit, Institute of Biomedical Engineering, Departments of Electrical Engineering, Physiology, and Medicine, University of Toronto; Addiction Research Foundation; Ontario, Canada.

Intracellular recordings from dentate granule cells of hippocampal slices (Wistar rats) were made in normal and ethanol containing (50 mM) artificial CSF solution (perfusion rate 0.5-0.8 ml/min.). Membrane impedance (Z) was measured using either sinusoidal currents (5-500 Hz) or white noise (20-60 pA peak to peak) injected through the recording electrode. Sinusoidal input currents were used because each sinusoidal stimulus is composed of only one frequency, whereas each white noise stimulus is composed of a range of frequencies. All |Z| measurements were normalized with respect to the largest control value.

The averaged distributions of |Z| in the neurons under ethanol perfusion differed from those recorded in normal ACSF. |Z| measurements could be distributed in three frequency ranges: 1) in the range 5-10 Hz, |Z| was distributed between 0 and -3 dB for controls, while it increased from -2 to 0 dB in ethanol, 2) in the range 10-200 Hz, |Z| decreased from -3 to -12 dB for controls and decreased from 0 to -7 dB in ethanol, 3) beyond 300 Hz the |Z| was impossible to distinguish due to the inadequate capacity neutralization of the intracellular amplifier. The spectra obtained from the white noise stimuli were almost identical to those obtained from the sinusoidal stimulus, and can be appropriately (but not uniquely) modelled by a passive electrical network.

In summary: 1) sinusoidal and white noise input currents provide a useful approach for membrane impedance measurements, 2) ethanol perfused cells showed a smaller range of |Z| distribution for both sinusoidal and white noise inputs throughout the spectra. This reflects a difference in the signal processing characteristics (i.e. transfer function) of the neurons due to decreased membrane resistance and some basic change in the inductance of the membrane. These data will be incorporated into an algorithm to identify the mathematical transfer function which will enable the responses to be modelled.

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235.2 SQUID MEASUREMENTS OF MAGNETIC FIELDS GENERATED BY THE IN VITRO HIPPOCAMPAL SLICE. W.D. Knowles,[1] C.D. Tesche,* L. Krusin-Elbaum,* and R.D. Traub. IBM Thomas J. Watson Research Laboratory, Yorktown Heights, NY 10598.

Interpretation of SQUID (Superconducting QUantum Interference Device) measurements of the magnetic fields generated by brain tissue in vivo is impeded by poor theoretical understanding. This is due to the complex geometric and physiological configuration in vivo. We have developed a method of simultaneously recording the electrical and magnetic fields in the simplified in vitro hippocampal slice preparation. This preparation is much more amenable to theoretical analysis, and our computer model predicted magnetic fields similar to those observed experimentally; approximately 100 femtoTesla (100×10^{-15} T)

Guinea pig hippocampal slices were maintained at the surface of a static bath chamber at room temperature. The slices rested on a fixed array of stimulating and recording electrodes which penetrated the slice from below. Population field potentials were measured in CA1 near the pyramidal layer in response to stimulation of stratum radiatum near Schaffer collaterals. The bath contained 0.1 mM picrotoxin to maximize synchronization, and thus amplitude, of the neural signal. The chamber was then clamped on the bottom of a thin-walled tail of a liquid helium dewar which contained a thin film DC SQUID with a 1 inch diameter second order gradiometer pickup coil. The distance from the slice to the pickup coil was 17 mm. 500 to 3000 stimuli were averaged. The population electrical field potential was about 0.5 mV in amplitude and had a single negative peak. The vertical component of the magnetic field was approximately 100 femtoTesla in amplitude and consisted of two peaks. This approximately matched the results of a computer model of an array of 100 pyramidal neurons.

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235.3 **FINITE DIFFERENCE MODELLING OF NEURONAL POTENTIALS AND CURRENT DENSITIES.** A.S. Ferguson* and D. Durand. Applied Neural Control Laboratory, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

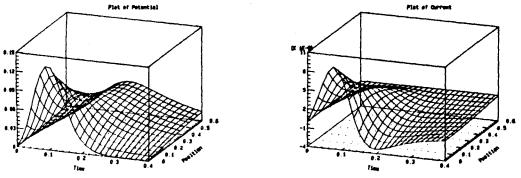
Finite difference equations provide a fast and accurate method for solving the cable equation and Poisson's equation for current flow in and around neurons. Solutions by other methods, notably empirical solutions, require greater computational time and may be impossible to solve when complex boundary conditions are imposed on the conducting region.

Intracellular potentials were computed from solutions to the parabolic cable equation using a fully implicit numerical scheme which has be shown to be stable under all conditions. Boundary conditions (either voltage or current) at both ends of a finite cable may be functions of time, allowing waveforms such as action potentials or synaptic input to drive passive dendritic cables. Speed of execution is much greater compared to solving with other methods such as Fourier transforms, convolution with impulse response, or using the method of images with the infinite cable response.

Extracellular potentials and current flow were computed from solutions to Poisson's equation in cylindrical coordinates. By using axially symmetric conditions, a three dimensional problem can be reduced to two dimensions. An ADI (Alternating Direction Implicit) scheme is used to solve Poisson's equation with boundary conditions established by neuronal trans-membrane current flow. The model allows the extracellular space to be anisotropic and to contain "foreign" bodies such as nerve cuff electrodes and extracellular electrodes. Neurons with restricted extracellular space or neurons surrounded by simultaneously conducting neurons have also been modelled with this method.

Finite difference methods allow the easy incorporation of anisotropies, boundary conditions and different geometries within a model. Coupled with a computational scheme such as ADI, the model can be described, modified, and solved faster than several methods (which may require summing infinite series of exponentials or Bessel functions) without any loss in accuracy.

Shown below are the intracellular potential (left) and current (calculated by a fully implicit scheme) for a sealed-end dendrite driven by an action potential. Dendrite length is 0.6 lambda, lambda is 0.1 cm, and tau is 16 msec.



Supported by NSF Young Investigator Presidential Award and by the Whitaker Foundation.

235.4 ELECTRICAL AND GEOMETRICAL NON-UNIFORMITIES IN MOTONEURONE CABLE PROPERTIES, J.D. Clements* and S.J. Redman. Experimental Neurology Unit, John Curtin School of Medical Research, Australian National Univ. Canberra, A.C.T. 2601

Two basic assumptions in the Rall model of the motoneurone are that the membrane resistivity (R_m) is uniform for soma and dendrites, and that the dendrites can be collapsed to an equivalent cable with a uniform diameter. The response of a motoneurone to a brief current pulse could not always be fitted using this cable model (Iansek & Redman, J.Physiol., 234:613, 1973) and it was then suggested that the soma may be a region of lower R_m. Histological studies have revealed some tapering of dendritic branches and unequal electrotonic distances to dendritic terminations (Barrett & Crill, J.Physiol., 239:301, 1974; Ulfake & Kellerth, Brain Res., 307:167, 1984) suggesting that the assumption of a uniform diameter dendritic cable is incorrect. Both assumptions were investigated in these experiments.

Lumbar motoneurones in cats anaesthetised with sodium pentobarbitone were penetrated with double-barrelled microelectrodes. One barrel was filled with 3KMCl, the other with 10% HRP in 3MKCl. The voltage response to a brief pulse of current and the current response to a voltage clamp step were recorded. The motoneurones were subsequently injected with HRP and their morphology was reconstructed from serial sections. A compartmental model of each neurone was created using the reconstruction. Specific membrane capacitance was assumed to be 1 $\mu f/cm^2$ and specific cytoplasmic resistance was fixed at 70 Ωcm. Identical current pulses and voltage clamp steps to those used in the experiment were applied to the model, while R_m and soma shunt resistance (r_{sh}) were adjusted to obtain model transients which best fitted the recorded transients.

Consistent results were obtained from 6/7 neurones. The values obtained for R_m ($K\Omega cm^2$)/r_{sh} ($M\Omega$) were 10/1.9, 20/1.5, 50/1.9, 17/2.4, 50/1.9, 40/1.0. If electrode leak is assumed negligible and all shunt resistance is attributed to a lower R_m for somatic membrane, the corresponding values of R_m(soma) are .20, .10, .16, .27, .16, .08 $K\Omega$ cm^2. The time constants for these neurones were 5.4, 4.1, 4.3, 6.4, 4.6, 4.0 ms. The equivalent dendritic cable had a constant diameter for the first 1000 μm; then an approximately linear taper to zero diameter occurred over the next 3000 μm. In 5 neurones for which secure HRP staining was achieved, there was little variability about this standard profile. When the diameter of the equivalent cable was calculated as a function of electrotonic distance from the soma, the dendritic diameter tapered at different rates for different neurones, reaching zero at 1-3λ from the soma. These results indicate that dendritic R_m will be underestimated from the measured time constant and that the cable parameters (L and ρ) calculated using a uniform diameter dendritic cable model will be incorrect.

235.5 THE ROLE OF DENDRITIC DIAMETERS IN MAXIMIZING THE EFFECTIVENESS OF SYNAPTIC INPUTS. W.R. Holmes. Mathematical Research Branch, NIDDK, NIH, Bethesda, MD 20892.

The effectiveness of an individual synaptic input in changing the potential at the soma depends strongly on the dendritic geometry and the position of the synapse within the dendritic tree. Given the dendritic branching pattern, the particular weighting given an individual input depends on the distance of that input from the soma and in particular on the diameters of the processes in the dendritic tree. The purpose of the present study was to show how dendritic diameters, along with the specific membrane and longitudinal resistances Rm and Ri and the magnitude of the synaptic conductance change, g, could affect the weighting of synaptic inputs by determining the loci of inputs operating at maximal effectiveness. For the present study a synaptic input is considered to be operating at maximal effectiveness when changes in dendritic diameters will produce a smaller potential change at the soma.

The special role of process diameters was examined analytically in a single cylinder and numerically in a model of a reconstructed cortical pyramidal cell. For current input into one end of a cylinder of length ℓ, the maximum potential at the other end was obtained when the cylinder diameter was chosen so that the electrotonic length, L, was 3 ($L=\ell/\lambda$, where λ is the space constant). For a constant conductance into the end of the cylinder, maximal effectiveness occurred for a value of L less than 3; how much less depended on the magnitude of g. In the reconstructed cortical pyramidal cell, the diameters of all processes were increased 20, 50, or 100% or decreased 20 or 50% from the values specified by the reconstruction. The peak transient potential change due to proximal, mid-dendritic, and distal synaptic inputs was determined in each case for three values of Rm and two values of g. The proximal input was most effective when process diameters were reduced 50%. The mid-dendritic and distal inputs were most effective with increases in dendritic diameters for g=20nS, but with decreases in diameters for g=2nS.

For each input there is a set (probably non-unique) of dendritic diameters which maximizes the effectiveness of that input. Paradoxically, a synaptic input at a given physical distance from the soma may produce a larger change in soma potential when it is at a larger electrotonic distance from the soma than a smaller one. The dendritic diameters determine which inputs are operating at maximal effectiveness. Changes in Rm or Ri that may occur with learning or in certain disease states may shift the loci of inputs operating at maximal effectiveness. This would change the weighting of synaptic inputs and possibly affect neuronal function.

(This work was supported in part by NSO7845 and AFOSR).

235.6 **STIMULATION OF ANODIC BREAK EXCITATION IN HIPPOCAMPAL GRANULE CELLS.** G.L.F. Yuen and D. Durand. Applied Neural Control Laboratory, Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Hippocampal granule neurons were observed to exhibit a variety of responses including anodal excitation (see for example Fricke, R.A. and Prince, D.A. J. Neurophy., 51: 195, 1984). To the best of our knowledge, the only successful quantitative description for this phenomena was Hodgkin and Huxley's model of the squid axon (Hodgkin, A.L. and Huxley, A.F. J. Physiol., 117: 500, 1952). In the squid model, the production of the anodic break was contingent upon proper timing relationships and conductance profiles of the various voltage-dependent conductance gates (m,h and n). Conversely, the ability of a granule cell model to produce anodal break would also be an important indication of the validity of its active parameters.

A 10-compartment model of a granule cell was implemented on a computer with one active somatic compartment and nine passive dendritic compartments. The passive parameters were adjusted to conform to published values of cell input resistance (65 Mohms), electrotonic length (0.9) and time constant (25 msec) (Durand, D. and Carlen, P. J. Neurophy., 54: 807, 1985).

Pilot simulations using a single compartment with active parameters from previous models of squid axon, hippocampal CA1 and CA3 neurons (see Traub, R.D. Neurosci., 7: 1233, 1982) or modified versions of these indicate that a low firing threshold (less than 10 mV) was necessary for generating an anodic break. Furthermore, when the passive dendritic compartments were attached to the various active compartments, the active compartments lost the ability to produce any anodal excitation. This is probably due to the higher threshold resulted from the attached dendritic tree which acts as a load.

Hippocampal granule cells were often observed to have a threshold of greater than 20 mV. However, results from our simulations so far predict that no anodic break is possible at this high threshold. Therefore, we suggest that granule cells which exhibit anodal excitation probably have low threshold of less than 10 mV.

The relationship between threshold and anodal excitation in granule cells will next be assessed intracellularly using the hippocampal slice preparation .

We express our gratitude to D.H. Perkel for providing the simulation software. Supported by NIH grant # IROI AA 06773.01.

235.7 DYE AND ELECTROTONIC COUPLING BETWEEN HIPPOCAMPAL NEURONS IN CULTURE, M. O'Beirne and B.A. MacVicar. Dept. of Med. Physiol., Univ. of Calgary, Calgary, Alberta, T2N 4N1, Canada.

Electrotonic and dye coupling have been demonstrated in both the hippocampal slice preparation and in the in vivo hippocampus. Some controversy still exists as to the extent of coupling and many characteristics have not yet been described. This is primarily due to the difficulty in obtaining simultaneous intracellular recordings in slice preparations and in vivo. Dissociated cultures provide a simple system in which coupling between CNS neurons may be studied. Dissociated neurons grow in a monolayer, in which individual cell bodies are visible and may be impaled without injuring any surrounding cells. Therefore, we have examined the incidence and properties of electrotonic and dye coupling between hippocampal neurons in dissociated cultures.

Hippocampi were asceptically removed from rat fetuses (18 days gestation). After mechanical dissociation, the cells were plated onto polylysine coated glass coverslips. Cultures were maintained in DMEM + 10% horse serum at 37^{0}C for up to 4 weeks. Some cultures were exposed to 0.2 µg/ml insulin for 3-4 days before recordings were made. Neurons were impaled with glass microelectrodes filled with either 4.5% Lucifer Yellow (LY) for staining, or with 0.15 M KCl. Live stained cells were visualized using epifluorescence optics in the recording chamber. Simultaneous intracellular impalements of pairs of neurons were obtained with KCl electrodes to directly examine electrotonic coupling between neurons.

Neurons stained with LY in culture had diverse morphologies particularly with respect to their neurites. Some cells had processes extending 400 µM whereas other had processes confined to the area immediately around the soma. Cell bodies were typically round although some were pyramidal in shape. Dye-coupling was observed in 5 out of 33 neurons (15%) injected. Up to 3 neurons were stained after injecting one cell. In some cases somas were apposed and in other cases neurites were apposed.

Electrotonic coupling was directly observed between some pairs of simultaneously impaled cells. The coupling ratio was approximately 0.1 and the junction was non-rectifying. Imposition of large voltage gradients between cells did not appear to alter the coupling between cells. High frequency signals were attenuated by the junction.

Therefore electrotonic and dye coupling are present between hippocampal neurons in dissociated tissue culture. This will be an excellent system in which to rigorously analyze the properties of electrotonic junctions between CNS neurons.

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235.8 LOCAL CIRCUIT SYNAPTIC INTERACTIONS IN THE CA3 SUBFIELD OF DEVELOPING HIPPOCAMPUS. J.W. Swann. Section for Dev. Neurophysiol., Wadsworth Ctr. for Labs & Research, NYS Dept. of Health, Albany NY, 12201.

Interest has developed in recent years in the role of local circuit interactions in brain dysfunction, particularly seizure generation. Our laboratory has been studying the physiological mechanisms responsible for the pronounced capacity of the CA3 subfield of hippocampus from 1 and 2 week old rats to generate prolonged seizure-like discharges. In experiments reported here we have employed dual intracellular recordings from pairs of neurons in the CA3 subfield to study synaptic interactions.

Hippocampal slices (500 µ thick) were taken from rats 8 to 11 days of age and bathed in normal artificial cerebrospinal fluid. Intracellular recordings were obtained from the soma of 2 neurons in stratum pyramidale. The tips of the electrodes were usually within 100 µm of each other. Intracellular current passage via active bridge circuits was used to generate trains of action potentials in one cell while the other cell was held at a fixed membrane potential. Resulting synaptic events were studied.

We have found that in 1 week old rat hippocampus very few pairs of cells were synaptically coupled. Both electrical and chemical synaptic interactions were seen but in less than 10% of the pairs. These results are similar to the frequency and types of synaptic interactions reported in CA3 subfield of mature slices. However, during the course of our recordings we have found that between 90 to 100% of the pairs of cells spontaneously received synchronous bursts of synaptic potentials. These were considered synaptic events since their polarity was reversed by membrane polarization while their frequency remained unaltered. These post synaptic potential (psp) bursts were structurally complex and appeared to consist of both inhibitory and excitatory synaptic potentials. In some pairs of cells the psp bursts were excitatory in one cell and triggered action potentials and inhibitory in the other resulting in cessation of repetitive firing. We have been unable to trigger these psp bursts by trains of action potentials in individual neurons. Each cell appears to receive synaptic input from a common source. The psp bursts are easily elicited by direct electrical stimulation of populations of pyramidal cells several hundred microns from the recording site. When elicited they had a discrete threshold and at these low stimulation strengths were not graded in amplitude but in fact occured in an all-or-none fashion. Our recordings differ dramatically from previous reports of synaptic interactions in the CA3 subfield of mature hippocampus in which synchronous synaptic events were recorded in a small minority of neuron pairs. Thus, the presence of these synaptic events may reflect a fundamental difference in local circuit function in immature and mature hippocampus. Supported by NIH Grant NS 18309.

235.9 IMMUNOCYTOCHEMICAL AND ELECTROPHYSIOLOGICAL CORRELATES OF DEVELOPING MAMMALIAN CEREBELLAR NEURONS *IN VITRO*. P.E. Hockberger, H. Y. Tseng*, and J. A. Connor. Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

Development of the rodent cerebellum is a protracted process that begins prenatally and requires almost one month of additional postnatal development to reach maturity. We are currently analyzing various aspects of cerebellar differentiation using explant cultures from embryonic and postnatal rats. The explant cultures contain several key components of the intact, developing rat cerebellum: radiating glial processes, proliferation of granule cell neuroblasts, and progressive differentiation of both neuronal and glial cell types. We have concentrated our efforts so far on the analysis of two neuron types, granule and Purkinje cells.

Using immunocytochemical and electrophysiological methods, we have followed the differentiation of granule cells from neuroblast to relative maturity. These cells developed electrical excitability and immunoreactivity (to neuron-specific enolase antibodies) *in vitro* over a time period which corresponds to their migration into the (internal) granular cell layer *in vivo* - i.e., between postnatal days 5-20. Electrical excitability included synaptic potentials, overshooting action potentials, and spontaneous firing patterns. Iontophoretic application of GABA or glutamate evoked membrane potential responses on immature cells, and the GABA response (i.e. hyperpolarization) was present throughout the differentiation period. Glutamate responses, on the other hand, were rare in more mature granule cells. Kainate application mimicked the depolarizing effect of glutamate, but adenosine, NMDA, and quisqualate were ineffective agonists at all stages examined. After one month in culture, spontaneous excitability involved only slow, subthreshold depolarizations and overshooting action potentials could no longer be evoked with current stimulation. Voltage clamp records indicated that although somatic currents were now absent, axonal currents were present. The loss of somatic spike generation and response to glutamate are consistent with observations in adult cerebellum *in vivo*.

We have recently begun to analyze the differentiation of Purkinje cells in culture under similar circumstances (also see Gruol, D., Franklin, C., *Soc. Neurosci. Abstr.*, 11:149, 1985). At present, we have identified a population of cells which develops large apical dendrites, with some secondary branching, and which reacts positively with antibodies that stain Purkinje cells *in vivo*. These putative Purkinje cells develop electrical excitability only if the cerebellar tissue was removed from the animal prior to postnatal day 2. Since climbing fibers begin synapse formation around postnatal day 3 *in vivo*, differentiation *in vitro* may be impeded following this event. Cultured Purkinje cells of embryonic origin were hyperpolarized by GABA and depolarized by glutamate (typically eliciting a transient bout of spikes), but we have not as yet analyzed the development of these responses.

In the adjoining abstract (Connor, Tseng and Hockberger, these abstracts) we report on the use of Ca imaging techniques for analyzing short-term and persistent intracellular Ca^{2+} changes in granule and Purkinje-like cells exposed to local or bath application of various neurotransmitters.

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235.10 TRANSMITTER AND DEPOLARIZATION INDUCED CHANGES IN [Ca^{++}] in GRANULE AND PURKINJE NEURONS FROM RAT CEREBELLUM MEASURED BY FURA-2 FLUORESCENCE IMAGING. J. A. Connor, H.-Y. Tseng* and P. E. Hockberger, Dept. of Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

Digital imaging in conjunction with the fluorescent indicator fura-2 have been employed to examine intracellular Ca^{++} changes in cerebellar granule and Purkinje neurons from rat, grown in explant cultures. The system allows spatially resolved measurements to be made on a time scale of 2 to 3 sec. Responses of both cell types to glutamate and K-induced depolarizations have been assessed. Electrical correlates of the responses to these treatments have been measured by whole-cell patch recording.

Granule cells: K^+ challenges of 15 and 25 mM (normal 4.7 mM) were given to cells at stages of development from 2 to 30 days *in vitro* (DIV). Changes in Ca^{++} were induced at all stages, but as cells matured and expressed higher densities of several types of membrane ion channels (Hockberger and Connor, Soc. Neurosci. Abst., 11:508,1985), [Ca^{++}] changes grew larger. Cells maintained over 10 DIV responded to 25 mM K^+ with a transient burst of action potential firing. During the first 30 s of such activity, [Ca^{++}] rose to levels exceeding 1 μM from resting levels of 60 to 80 nM. Peak responses were followed by rapid (60 to 90 s) decline to plateau levels ranging from 150 to 250 nM that were maintained for the duration of the high K exposure. Resting levels were restored within 2 or 3 min. in normal K. Blocking spike activity with TTX (300 nM) reduced the initial peak by 50% or more but had no effect on the plateau phase or on the steady depolarization (20 mV for 25 mM K, 10 mV for 15 mM). TTX had no effect on the intrinsically smaller response of the less mature cells. Where Ca entry was restricted to moderate levels, the response showed pronounced facilitation for repeated exposures to high K even though resting levels of Ca^{++} were rapidly reestablished between challenges. The facilitated response was maintained for intervals as long as 4 to 7 minutes making it of potential interest as a mechanism of short term plasticity. Glutamate caused a transient depolarization and [Ca^{++}] increase in the granule neurons during a 5-7 day window in their development, but they were unresponsive to focal applications at the soma after about 14 DIV.

Purkinje neurons: Cells were studied after 15-17 DIV when excitability was well established. Neurons were often spontaneously active and in such circumstances showed Ca^{++} levels in the 200-300 nM range. Application of current from an external patch electrode was sometimes capable of silencing a cell and reducing the levels. Brief (5-7 s), focal applications of glutamate (50-100 μM in pipette) caused an increase in Ca^{++} to levels often > 500 nM that developed over a time course of 30 to 60 sec. and persisted for periods as long as 5 to 8 minutes, far outlasting the electrical activity induced. Comparable Ca^{++} changes induced by transient exposure to high K in the same neurons recovered within 60 to 90 s (see Hockberger, *et al*, previous abstract). Therefore, fundamentally different mechanisms may be involved in generating the two responses.

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235.11 SYNCHRONOUS ACTIVITY IN NEOCORTICAL EXPLANT CULTURES. B. Wolfson M. J. Gutnick, and F. Baldino, Jr. Central Research and Development, E. I. du Pont de Nemours and Co., Experimental Station, Wilmington, DE 19898

We have previously shown (Wolfson et al., Neurosci, Abstracts *11*: 508, 1985) that the electrophysiological properties of neurons in neocortical explant cultures are essentially the same as those seen in other neocortical preparations. However, neurons in these cultures differ from those in neocortical brain slices in that they spike spontaneously and often display spontaneous epileptiform activity. In the present study, we used intracellular recording to examine the mechanisms responsible for the generation of synchronous activity in neocortical explant cultures.

Explant cultures of parietal cortex were prepared using standard techniques. Intracellular recordings were made with micropipettes filled with 4 M potassium acetate and placed visually with the aid of an inverted phase-contrast microscope. During recording, cultures were maintained at 36 +/- 0.5°C and superfused with a balanced salt solution (BSS) or BSS containing pharmacological agents. Focal electrical stimulation was achieved with a bipolar electrode placed 300-400 μm distant from the recording electrode.

Most explants showed some form of spontaneous synchronous activity. This could appear as bursts of spikes, as showers of psps, and in some cultures, as prolonged, ictal episodes. Spontaneous and evoked epileptiform depolarizing shifts (DSs) were augmented by addition of 25 μm bicuculline or removal of Mg^{++} (normally 1.5 mM) from the BSS. Low Mg^{++} also markedly increased spontaneous IPSP amplitude and frequency. Many neurons showed evidence of a transient low-threshold Ca^{++} current manifested as a TTX-resistant burst response following a hyperpolarizing pulse. In some cases this burst response appeared to be triggered by spontaneous IPSPS. The form, but not the frequency of bicuculline-induced DSs could be modified by intracellularly injected current, indicating a voltage-sensitive component. There was no evidence of voltage-dependence of DSs induced by Mg^{++} withdrawal. Both types of DSs were reduced by the NMDA receptor antagonist, 2-AVP (20 μm).

These results indicate that synchronous activity in neocortical explant cultures reflects both synaptic and intrinsic mechanisms. Furthermore, they demonstrate that NMDA receptors play an important role in epileptogenesis in the cultures. Finally, the data emphasize that although blockade of GABA-mediated inhibition is potently epileptogenic, seizure-like activity can also be sustained in a limited cortical circuit in which inhibitory mechanisms are intact.

235.12 **ELECTRICAL RECORDINGS FROM SINGLE, ISOLATED VERTEBRATE CNS NEURONS CULTURED IN A THREE-DIMENSIONAL EXTRACELLULAR MATRIX.** Richard D. Nathan and Penelope W. Coates. Depts. Physiol. & Cell Biol. & Anat., Texas Tech Univ. HSC, Sch. Med., Lubbock, TX 79430.

We have shown previously that single CNS neurons, which are isolated from embryonic chick cerebral hemispheres or spinal cords and are not in contact with or contacted by other cells, rapidly regenerate two morphologically distinct types of processes - axons and dendrites - when cultured in a three-dimensional (3-D) extracellular matrix (ECM) consisting of a hydrated collagen lattice (Cochran & Coates, SN Abs. 11: 762, 1985; Coates, Dev. Brain Res. 25:11-20, 1986). We now demonstrate the feasibility of studying the electrophysiological properties of these neurons. Cells were plated at low density (10^2/dish) to assure that the neurons remained isolated. The matrix was cast to a depth of 1-2 mm in plastic culture dishes and was hydrated with 1 ml of Medium 199 containing 10% heat-inactivated fetal calf serum but no antibiotics/antimycotics. A patch-clamp/whole-cell voltage-clamp system (Dagan, model 8900) was used to record a) single-channel currents from cell-attached patches, b) transmembrane potentials during the injection of rectangular currents, and c) whole-cell currents during voltage clamp. Microelectrodes were filled with 155 mM K-gluconate or CsCl, and resistances were 15-20 MΩ . The resistance of seals between the patch pipette and neuronal membrane ranged from 10 to 20 GΩ . After 2-4 days in 3-D culture, both cerebral and spinal neurons exhibited single-channel currents. After only 2 days, the latter preparation also displayed whole-cell currents similar to those observed in situ. For example, we recorded a transient outward current that increased at positive potentials and a transient inward current that was completely inactivated by holding potentials $\leq$ -40 mV. We have yet to characterize the ionic nature of these currents. Within 7 days, rectangular currents injected through the recording electrode evoked action potentials in single, isolated cerebral neurons. These results demonstrate that in 3-D ECM culture, single, isolated CNS neurons quickly recover some properties of excitability displayed by their counterparts in vivo. Moreover, neuron cell bodies, their axons and dendrites attach strongly to the matrix, simplifying microelectrode attachment and allowing stable electrical recordings to be made. Thus, this culture system provides a convenient method to investigate inherent electrical properties of individual neurons from different regions of the vertebrate CNS. Testing the effects of extrinsic factors including neurotransmitters, hormones, other cells and pharmacological agents on such neurons should also be facilitated. (Supported in part by TTUHSC BRSG.)

235.13 EFFECTS OF PHENCYCLIDINE AND OTHER K^+ CHANNEL BLOCKERS: ELECTROPHYSIOLOGICAL STUDY OF SPINAL CORD NEURONS IN CELL CULTURE. L.G. Aguayo* and E.X. Albuquerque (SPON: C.N. Allen). Dept. Pharmacol. Exp. Therap., Univ. Maryland Sch. Med., Baltimore, MD 21201.

The processes by which spinal cord and other central neurons regulate membrane excitability include the transient and delayed rectifier outward currents. The delayed outward current which is similar to that seen in the peripheral nervous system is activated during depolarization and does not inactivate when the membrane potential is held at positive potentials. Earlier studies from this and other laboratories have suggested that blockade of the delayed current could form the basis for the psychopathological behavior induced by phencyclidine (PCP) (Albuquerque et al., Proc. Natl. Acad. Sci. USA, **78**:7792, 1981). Recent evidence with a photolabile analog of PCP indicated that the putative PCP receptor may be a K^+ channel (Sorensen & Blaustein, Soc. Neurosci. Abs. 11:316, 1985). Since PCP receptors are known to exist in the spinal cord, we studied its effects on the fast transient and delayed outward currents recorded from spinal cord neurons grown (10-20 days) in cell culture. Sodium channels were blocked with tetrodotoxin (1 µM) and solutions containing low-calcium in the presence of Mg^{2+} or Co^{2+} (5mM) were used to block Ca^{2+} currents. PCP decreased the amplitude and prolonged the decay phase of the action potentials recorded at holding potential of -70 mV. PCP was more effective than tetraethylammonium (TEA) or 4-aminopyridine (4-AP) in reducing both transient and delayed currents. The time course of the onset and recovery of both currents in the presence of PCP (0.1 - 0.5 mM) disclosed that the transient current was more sensitive to PCP blockade and recovered more slowly when microperfused to the cell soma. The amplitude of the transient current during control experiments was always larger than that of the delayed current. In the presence of PCP the transient current measured at 12 msec had the same amplitude as that measured 60 msec after the peak onset. It appeared that 4-AP (5 mM) was more potent in blocking the fast transient current while TEA (10 mM) affected the delayed current more effectively. Both currents were also reduced by about 10% when the cell soma was perfused with Co^{2+}. This suggested that a small fraction of the total outward current is Ca^{2+}-activated K^+ current. The PCP blockade of K^+ currents in central neurons may provide the basis for explaining the pathophysiology of this hallucinogenic agent. Additionally, such effects coupled with the profound anticholinergic effects of the drug can be of fundamental importance to the understanding of the schizophrenic properties of this frequently abused drug. (Support: NIDA Grant DA02804).

235.14 VOLTAGE DEPENDENCE OF PATTERNED OUTPUT OF LATERAL HABENULA NEURONS. K. S. Wilcox, M. J. Gutnick, and G. R. Christoph. Central Research and Development Department, E. I. du Pont de Nemours and Company, Wilmington, DE 19898

We have previously shown that neurons of the lateral habenula (LHb) nucleus possess a transient low threshold calcium conductance which, in part, controls the firing pattern of these neurons (Wilcox et al., Neurosci. Abst., 11: 509, 1985). We now further examine the electroresponsive characteristics of LHb neurons and describe their rebound responses to transient hyperpolarization. Intracellular recordings were made in in vitro brain slices of guinea pig diencephalon. Anesthetized guinea pigs were decapitated and their brains were rapidly removed and immersed in cold Ringer solution. Coronal sections (400 μm thick) were cut with a vibratome and maintained in oxygenated Ringer at 36°C.

The rebound responses of LHb neurons to termination of injected hyperpolarizing current pulses consisted of one or more of the following characteristics. In some neurons, the time course of return to resting potential following the pulse was markedly slowed. This response, which persisted in TTX, was blocked by 4-aminopyridine suggesting that it reflects transient activation of a voltage-dependent K^+ conductance (A current). In many neurons, the hyperpolarizing pulse was followed by a dampening series of membrane potential oscillations (5-7 Hz) with superimposed bursts of Na^+-dependent action potentials. The overall duration of this oscillatory response increased as a function of amplitude and duration of the preceding hyperpolarizing pulse. This rhythmic oscillation persisted in TTX and apparently reflected an interplay between Ca^{++} and K^+ conductances. The most commonly encountered rebound response to hyperpolarizing pulses consisted of a single calcium spike followed by a prolonged depolarizing (10-15 mV) potential that often lasted longer than 1 second. This depolarization, which itself is TTX resistant, could serve as a generator potential for Na^+-dependent action potentials. During this prolonged depolarization, the apparent membrane resistance was increased. In neurons held at a slightly hyperpolarized level, these responses could be triggered by spontaneous PSPs.

Anatomical evidence suggests that the LHb serves as a relay for flow of information from limbic forebrain structures to midbrain. The present data indicate that LHb neurons generate a variety of complex output patterns which reflect conductance mechanisms intrinsic to their membranes. Our results lead us to predict that hyperpolarizing influences in these cells will significantly amplify transmission through this relay.

235.15 THE EFFECT OF MESENCEPHALIC RETICULAR STIMULATION ON THALAMIC RETICULARIS NEURONS. B. Hu, M. Steriade and M. Deschênes. Lab. Neurophysiol., School of Medicine, Univ. Laval, Quebec, Canada G1K 7P4.

On the basis of anterograde and retrograde transport studies it has been shown that the reticular thalamic complex, including its visual sector, the perigeniculate nucleus, receives a strong input from the mesencephalic reticular formation (MRF). This input originates from cholinergic and non-cholinergic neurons intermingled within the same mesencephalic region. The effect of MRF stimulation on reticularis thalami neurons was studied in cats under various types of anesthetic conditions. Cells were recorded intracellularly in the rostral pole of the reticular complex and in the perigeniculate nucleus. In all reticularis neurons MRF stimulation (2-5 shocks at 200 Hz) produced a transient blockage of spontaneous or evoked spindle oscillations. This was associated with a large and long-lasting (up to 2 seconds) hyperpolarization. Hyperpolarizations decreased in amplitude when the resting potential was hyperpolarized and they were accompanied by a 30% to 40% decrease of input resistance. This phenomenon was observed under barbiturate anesthesia and in nonanesthetized deafferented preparations. Pretreatment of animals with scopolamine (0.8 mg/kg) abolished this hyperpolarization. In all reticularis neurons the hyperpolarization was preceeded by a shorter-lasting depolarization appearing at latencies of 7-10 msec. This depolarization was less prominent under barbiturate anesthesia and remained insensitive to scopolamine. It is concluded that the MRF cholinergic input hyperpolarizes reticularis neurons. It remains to be demonstrated if the scopolamine-resistant early depolarization is also cholinergic or if it results from activation of a non-cholinergic but yet not chemically-defined input.

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235.16 THE EFFECT OF MESENCEPHALIC RETICULAR STIMULATION ON RELAY NEURONS OF THE DORSAL LATERAL GENICULATE NUCLEUS. M. Deschênes, B. Hu and M. Steriade. Lab. Neurophysiol., School of Medicine, Univ. Laval, Quebec, Canada G1K 7P4.

On the basis of retrograde transport studies, it has been shown that the lateral geniculate nucleus (LGN) receives a massive input from the mesencephalic reticular formation (MRF). This input originates from cholinergic and non-cholinergic neurons intermingled within the same mesencephalic region. The effect of MRF stimulation on LGN neurons was studied in deeply barbiturized cats and in unanesthetized encephale isolé cats after destruction of trigeminal input. In addition, bilateral enucleation and visual cortex ablation were performed. Under barbiturate anesthesia, MRF stimulation (2-10 shocks at 200 Hz) blocked rhythmic spindle oscillations in LGN neurons without any apparent effect on membrane potential. Long trains of stimuli lasting for 200 msec produced a slow depolarization and tonic spike discharges at a latency of about 2 seconds. In non-anesthetized preparations, MRF stimulation also blocked spindle oscillations but in addition it produced a slow depolarization with a latency of 8-10 msec that lasted for about 300 msec. This depolarization could trigger tonic spike discharges or generate spike bursts depending upon the cell resting potential. Yet, no clear change in membrane resistance has been detected during this response. Our results strongly suggest that previous failure to observe change in LGN membrane potential following MRF stimulation was due to the use of anesthetics. It remains to be demonstrated if these responses are cholinergic.

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235.17 GENERATION OF FOCAL SPINDLE WAVES BY FIELD EFFECT IN CAT ANTERIOR THALAMIC NUCLEI. L.Domich, D.Paré, G.Oakson*, M.Deschênes and M. Steriade. Lab. Neurophysiol., School of Medicine, Univ.Laval, Québec, Canada G1K 7P4.

At odds with most thalamic nuclei, anterior thalamic nuclei of the cat do not receive inputs from the thalamic reticular (RE) nucleus (J. Comp. Neurol. 1984, 229: 531-547). We confirm this observation: after injections of WGA-HRP into anteromedialis (AM) and anteroventralis (AV) nuclei, heavy retrograde labeling was obtained in mamillary nuclei whereas the RE nucleus was left virtually free of labeling. Recent evidence shows that the RE nucleus plays an essential role in the genesis of spindle rhythms. Spindle oscillations are abolished in thalamic nuclei disconnected from the RE nucleus (J. Neurophysiol. 1985, 54: 1473-1497) while they are preserved in the deafferented RE nucleus (This meeting: Steriade et al.). Since anterior nuclei are naturally deprived of RE inputs, it was expected that anterior nuclei would not display spindle rhythms.

Indeed, intracellularly recorded cortically-projecting neurons in AV-AM nuclei did not exhibit spontaneous oscillations of membrane potential within the frequency range of spindle waves, and these oscillations could not be evoked by cortical stimulation. The absence of spontaneous and evoked spindle rhythms was observed in anterior thalamic cells despite the fact that they displayed intrinsic membrane properties similar to those described in other thalamic nuclei. In unanesthetized cerveau isolé preparations, extracellular recordings of single neurons in AV-AM nuclei also showed lack of focal spindle oscillations and spindle-related rhythmic spike bursts, thus contrasting with rhythmic spindle-burst events in simultaneously recorded intralaminar thalamic neurons. With barbiturate administration, however, focal spindles appeared in AV-AM nuclei, but they were not associated with rhythmic bursting in anterior thalamic single cells.

Since spindle oscillations did not appear intracellularly, their presence in extracellular recordings at high levels of EEG synchronization after barbiturate administration was thought to be mediated by field effects. To test this hypothesis, lesions of anterior nuclei were performed by means of H_2O_2 microinjections. Such lesions did not interfere with the extracellular focal spindles induced by barbiturate administration whereas they suppressed the field potential evoked synaptically by stimulation of mamillary bodies. These results support the conclusion that spindle waves recorded from anterior thalamic nuclei are field potential effects generated actively in neighboring thalamic nuclei.

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235.18 EFFECTS OF MICROWAVES ON MOUSE EEG'S AND EVOKED POTENTIALS. B. Pulford* and M.W. Luttges.(SPON:R.Gerren).Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309

Enhanced microwave applications have led to increases in the type and amount of exposure experienced by biological systems. The full impact of such exposures awaits more quantitative knowledge of microwave dosimetry and related bioeffects. The U.S. microwave standard for human exposure recommends a limit of 10^{-3}W/cm^2 regardless of exposure time. Eastern European countries base microwave exposure limits on a reciprocal relation between time and intensity of exposure. Few studies have been done to explore the biological consequences of such major differences in dosimetry. This report summarizes the differences in brain electrical activity produced by a wide range of microwave time - intensity reciprocities.

Several sources of CW microwave radiation at a frequency of 2450 MHz were used for exposures. Eight orders of magnitude of exposure times (.003 to 30,000 secs) and eight orders of specific absorption rate (SAR) values (10^{-6} to 10 W/g) were encompassed. Pre-trained mice, with chronically implanted carbon-loaded Teflon electrodes, were restrained and placed within a waveguide system that oriented the head such that an H-field maximum was produced in the cranial region. Spontaneous electrical brain activity samples were recorded both before and immediately after exposure. A series of auditory evoked responses also were collected for the same time periods.

The spontaneous electrical recordings indicated considerable brain sensitivity to varied microwave durations in the absence of intensity changes. Brief duration exposures were correlated with low voltage, high frequency brain recordings. As the duration increased, the recordings exhibited higher voltages and lower frequencies. The late components of the evoked response have shown the most sensitivity to time-intensity changes. Voltages decreased as exposure time was increased, while the intensity was held constant.

This study suggests that, even at low levels of intensity, the length of time of microwave exposure needs to be taken into account when setting microwave exposure limits.

235.19 IN VIVO LABELING OF SEROTONIN CONTAINING NEURONS BY 5,7-DIHYDROXYTRYPTAMINE IN APLYSIA. B. Jahan-Parwar, K. S.-Rozsa*, J. Salánki*, M. L. Evans and D. O. Carpenter. Wadsworth Laboratories, New York State Department of Health, Albany, NY 12201.

5-Hydroxytryptamine (5-HT) has been implicated in the modulation of arousal state, locomotion, feeding, and several simple reflexes in Aplysia. With the aim of elucidating the mechanisms by which 5-HT modulates arousal and locomotor behavior, we have labeled the 5-HT containing system of Aplysia neurons using the 5-HT analogue, 5,7 dihydroxytryptamine (5,7-DHT). This substance and the related 5,6 DHT have been shown to specifically label 5-HT neurons in the leech (Lent and Dickinson, Brain Res. 300: 167, 1984) and Helix neurons (K.S.-Rozsa et al. Comp. Physiol. Biochem., in press) with only transient modification of neuronal excitability. Eight Aplysia californica (200-300g) received an intrahemocoelial injection of 5,7-DHT (10 mg/kg body weight) in sea water containing 0.5 mg/ml ascorbic acid. This treatment was repeated a week later. A control group received the vehicle solution only. Sixty days following the first 5,7-DHT injection, the animals were dissected and the nervous system processed for electrophysiology and whole mount viewing. All 5,7-DHT treated animals inked immediately after injection and displayed strong pedal waves for several hours following injection. The behavior returned to normal and no discernable sensory or motor deficits appeared subsequently. The cerebral, pedal, abdominal but not buccal and pleural ganglia of all treated animals showed clearly identifiable, darkly brown pigmented cells in a distribution pattern similar to that of serotonin neurons stained by immunohistochemical method (Ono and McCaman, Neurosci. 11: 549, 1984; Goldstein et al., Neurosci. 11: 535, 1984). Electrophysiological analysis of labeled neurons revealed normal values of resting and action potentials and synaptic input. The synaptic input from the serotonergic cerebral giant cells to their buccal followers were functional. Labeled abdominal RB cells were voltage clamped and ACh and 5-HT applied by rapid perfusion. Responses to both agonists were identical to those of untreated animals in terms of conductance change, voltage sensitivity and desensitization. These results suggest that 5,7-DHT labels serotonergic neurons in vivo without long term alteration of neuronal physiology and behavior. The ability to identify the 5-HT containing neurons will considerably facilitate studies of synaptic and functional organization of the serotonergic system and its role in modulation of behavior. (Supported by NSF grant BNS8313061 to B. J-P.)

235.20 IN THE SNAIL A-CELL, THE SPONTANEOUS ACTION POTENTIAL AND THE STEADY STATE CURRENT-VOLTAGE RELATIONSHIP ARE MODIFIED BY PROLONGED PHYSICAL INACTIVITY. Kathleen Shaver Madden* (SPON: W. G. Van der Kloot). Department of Pharmacology, New York Medical College, Valhalla, New York 10595

When faced with reduced food supplies, the land snail becomes quiescent and can remain immobile for months before resuming physical activity. These experiments were done to assess the effects of prolonged physical inactivity on the frequency and duration of the action potential spontaneously fired by the A-cell. Then, ionic currents were studied in the A-cell from active and inactive snails.

Snails, Helix aspersa, were housed in environmental chambers (ca. 20°C, 60-80% humidity). They were offered free access to water and either fed or unfed for at least two months before experiments. Physiological snail saline contained (in mM): 80 NaCl, 4 KCl, 7 $CaCl_2$, 5 $MgCl_2$, 5 TES (pH 7.8). Low resistance (≤4 MOhm), glass microelectrodes (3 M KCl) and conventional methods for recording intracellular voltages and the results of two electrode voltage clamp experiments were used.

Prolonged inactivity decreased the firing frequency and duration of the action potential in the A-cell and increased the after-hyperpolarization. It also increased the magnitude of the net ionic current determining the N-shaped configuration of the steady state (80 msec) current-voltage (I-V) relationship and associated with a calcium-activated potassium current in the A-cell. Exposing preparations to the mixed oxygenase inhibitor ETYA (5,8,11,14-eicosatetraynoate-K^+, 12 uM, ≥45 min) tended to reverse the effects of prolonged inactivity on the I-V relationship.

The increased potassium conductance can account for effects on the action potential and the after-hyperpolarization in the A-cell from inactive snails. Certain other neurons in snail ganglia may show similar activity-dependent increases or decreases in ionic currents which may help to explain some types of adaptive behavior.

Snail ganglia contain oxygenases mediating the synthesis of prostaglandins and other eicosanoids. The effects of ETYA on ionic currents in the A-cell implicate oxygenase activity as one process that may be affected by motor activity. Extracellular ensembles of endogenous eicosanoids may be important factors modulating calcium and potassium currents in the A-cell. (Supported by grant 20774 NINCDS)

235.21 INHIBITION OF THE LATERAL GIANT FIBER IN THE ISOLATED NERVE CORD OF *Lumbricus terrestris*. J.L. Johnson. Dept. of Physiology & Pharmacology, USD School of Medicine, Vermillion, SD 57069

The dorsal giant fibers within the earthworm ventral nerve cord (VNC) mediate two independent escape responses. Consequently, it is of interest to clarify how activity in the medial giant fiber and lateral giant fiber (LGF) is modulated. The purpose of this study was to record the effects of the longitudinally projecting small fiber tracts upon the LGF at the rostral end of the isolated VNC. At this location, the LGF is minimally responsive to segmental afferent input. The small fiber tracts (maximal conduction velocity = 0.71 ± 0.05 m/sec; M ± SEM; N = 11) first of all induced a depolarization in the LGF which lasted for 55-64 msec and had a maximal amplitude of 0.25-0.4 mV. These small fiber tracts also induced a separate long latency hyperpolarization of the LGF which was graded in nature. The hyperpolarization ranged from 1-1.8 mV in amplitude and lasted for 120-160 msec. Subthreshold electrical depolarizations of the LGF were capable of initiating conducted action potentials when applied during the time of the small fiber induced depolarization of the LGF, but failed to do so when applied during the time of the small fiber induced hyperpolarization. The small fiber induced hyperpolarization was not capable of blocking the conduction of LGF action potentials once initiated. On the other hand, the LGF depolarization induced by the small fiber tracts at the P2 stimulus was always blocked when applied during the time of hyperpolarization induced by a P1 conditioning stimulus. This may be an effective means of LGF deafferentation at the rostral end of the VNC. The massive activity induced within the segmental neuropile microstructure by the small fiber tracts of importance in generating these effects upon the LGF was readily obtained as well in each preparation. The maximal duration of such evoked activity ranged from 175-214 msec. Currently, electrical stimulation of the small fiber tracts is the only means of evoking LGF hyperpolarization, since activation of segmental afferent input alone never evoked such an effect (Smith, P.H. & Mittenthal, J.E., J. Comp. Physiol., 140: 351, 1980). Even though the small fiber induced depolarizations and hyperpolarizations were shown to significantly modify LGF activity, the very low amplitude for these responses suggests that the small LGF collaterals may be the primary sites for input and not the LGF per se. This requires further study. SUPPORTED BY GRANT 202-4680-005 FROM THE UNIVERSITY OF SOUTH DAKOTA TO THE AUTHOR.

235.PO SYNCHRONOUS CONDUCTION IN SMALL GROUPS OF CNS FIBERS. A.L. Towe, Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195

Many physiological observations imply that small groups of CNS fibers arising from neighboring neurons conduct at the same speed over long distances. Though how this occurs in unclear, arguments may be made that point to a possible general mechanism. Most CNS fiber spectra range from 0.5 to 3 um or more, with modes near 1.0 to 1.5 um, imposing a problem for synchrony of conduction. Recent EM studies show that the extracellular current-carrying medium in CNS pathways is highly limited; the interperiod line of myelin is present between the sheaths of adjacent fibers; each oligodendrocyte wraps many different axons; no basement membrane is present; nodes are long, align over small regions, and usually do not have cellular material intruding, as in PNS fibers; myelin sheaths are half the thickness required for maximum conduction speed and they show certain preferred values. Taken together, these observations suggest that axons arising in small clusters of cells are wrapped by the same set of oligodendrocytes, their nodes align, and their myelin sheaths join to closely that external current must flow in the interiors of neighboring fibers. By sharing their myelin, the "sheaths" thus formed are about the "right" thickness for optimum conduction speed. At the same time, only half as much myelin need be developed and maintained, in comparison with the amount needed for independent fiber conduction. Thus, by involving the nodes of adjacent fibers, action potentials may be closely synchronized to yield the optimum summation of input to their target sites, while at the same time using the smallest possible number of cells.

At rest, the energy involved in charge separation across nodal membrane is minimal, because the interiors of adjacent axons have the same potential, and the "outside" of one axon is the "inside" of neighboring axons. Because the capacitance across any adjacent nodes is low, the capacitative current required to bring any node to firing level is minimal, and may occur without any significant hyperpolarization of adjacent nodes. Independent fiber conduction in the PNS may represent an adjustment to both the mechanical and the thermal stresses to which such nerves are exposed. Asynchrony of timing has been minimized in the PNS by increasing fiber size, thereby decreasing conduction time. By contrast, CNS pathways are protected from these stresses by an encasing body armor, and have evolved a mode of conduction allowing precise timing with minimum metabolic cost. This solution may be present not only in the CNS, but also in places where the myelinated fibers are not exposed to significant mechanical stresses.

236.1 ORIGIN OF THE ELECTRORETINOGRAM AND DETERMINATION OF CONTRAST CODING IN THE COMPOUND EYE IN DIPTERA. L.J. McCook*[1] and S.B. Laughlin.*[2] (SPON: R. Croll). [1]Dept. of Biology, Dalhousie University, Halifax, N.S. Canada B3H 4J1; [2]Dept. of Zoology, Cambridge University, England.

The electroretinogram (ERG) provides a quick, simple, non-invasive means of recording visual responses, especially in insects. Unfortunately, these benefits are outweighed by uncertainty about the exact cellular origins of the ERG. We have recorded the ERG of the compound eye in the sheep blowfly Lucilia cuprina in response to 1 second rectangular pulses of light of various amplitudes, using both increments and decrements in light intensity, and using both light-adapted and dark-adapted animals. The waveform and stimulus-response characteristics of the ERG recordings were compared to those of intracellular recordings of the photoreceptors and the first order interneurons, the large monopolar cells.

Similarities in waveform and response dynamics support a previous suggestion (M. Heisenberg, 1971, J. Exp. Biol. 55: 85) that the sustained and transient components of the ERG originate in the responses of the photoreceptors and the large monopolar cells, respectively. The intensity range required for ERG response saturation and the time-course of the ERG transient components are significantly different from those of the monopolar cells. However, response frequency distributions indicate that these differences may arise because the ERG is a low-pass filtered version of the intracellular responses. This low-pass filtering may arise from the electrical properties of adjacent cell membranes, through which photocurrent leaving the receptor terminals must pass (S.R. Shaw, 1984, J. Exp. Biol. 112: 225). The idea that the ERG originates in a low-pass filtered, extracellular expression of the receptor and monopolar cell responses provides a detailed and testable explanation for the origins of the ERG. Such an explanation will give us an estimate of the usefulness of the ERG as an indicator of the responses of the visual neurons.

The response of large monopolar cells to contrast is matched to the environmental contrast distribution normally experienced, which maximizes the cells information carrying capacity (S.B. Laughlin, 1981, Z. Naturforsch. 36c: 910). We are investigating whether this matching is genetically hard-wired, or is modifiable by the visual environment experienced during development. Initial results for flies reared under high contrast conditions (stroboscopic illumination) suggest that there may be some environmental modulation of the monopolar cell contrast response. This result is a rare demonstration of plasticity in the neural development of an invertebrate, particularly unexpected in such a short lived species.

236.2 EFFECT OF DEVELOPMENT STAGE ON SPECTRAL SENSITIVITY IN CRAYFISH. M.L. Fanjul-Moles* and B. Fuentes-Pardo. (Spon: G. Meza-Ruiz). Depto. de Fisiología, División de Investigación, Fac. de Medicina, UNAM. Apdo. Postal 70250, México 04510, D.F.

Microspectrophotometric and electrophysiologic studies about spectral sensitivity in crayfish have been reported, however there are some differences between results attributables, perhaps, to technique employed, species, geographic distribution and functional state of visual system at moment of experiment; moreover, spectral sensitivity would change depending on the animal age. To detect putative changes in spectral sensitivity during development after hatching, the electrical response to light (electroretinogram ERG) of crayfish visual photoreceptors was recorded. Procambarus clarkii juvenile forms, from 1 to 12 weeks old, were allowed to dark adapt during 2 hours, before being stimulated with 50 lux, 10 ms monochromatic (300-400nm, 400-500nm, 500-600nm, 600-700nm) light flash. Immediatly after juevenile forms received a background adapting monochromatic light, while the ERG to a 100lux, 100ms, test light flash was recorded. Data obtained using both methods correspond to: 1) full dark adapted ERG amplitude; 2) time required to reach the light-adaptation state, i.e., the required time for ERG to dissapear as a consequence of the monochromatic light stimulus. The most important results are: 1) Highest responsiveness to ultraviolet (UV) light is present in the youngest animals (1 to 16 days old); 2) In these stages a high response to blue light, a low response to green light, and no response to red light are observed; 3) Older animals (4-12 weeks old), progressively show a reduction in their UV response; 4) In this group, a decrease in blue light response, up to 10 times lesser and a noticeably increase in green light response, up to 50 times than of those of younger animals, and the appearance, for the first time, of a red light response, were simultaneously observed. Results suggest that, a filtering action of screening pigments on incident light, as well as, functional photopigment changes are present during crayfish juvenile forms development.

236.3 CARBONIC ANHYDRASE LOCALIZATION IN MOLLUSCAN PRIMARY SENSORY NEURONS. R.L. Ridgway and S.B. Moffett, Dept. of Zoology, Washington State University, Pullman, WA 99164-4220.

Carbonic anhydrase (CAH), an enzyme catalyzing the reversible hydration of carbon dioxide, is typically found at high levels in ion-transporting and secretory cells. For many years this enzyme was thought to be restricted in neural tissues to glial cells. However, CAH activity has recently been detected in dorsal root ganglion neurons and peripheral sensory nerves of the rat (Wong et al., J. Histochem. Cytochem. 31:293, 1983; Riley et al., Neurosci. 13:189, 1984). These reports led us to test for the presence of CAH in invertebrates, where primary sensory neurons are often more amenable to experimental manipulation. We have used the histochemical method employed in the vertebrate studies to examine the distribution of CAH activity in cryostat sections of the eyes, tentacles, labia, and central nervous system (CNS) of the pulmonate snail Melampus bidentatus. Staining was absent from tissue sections exposed to 0.01 mM acetazolamide (a specific inhibitor of CAH), and from sections where the sodium bicarbonate substrate was omitted from the reaction medium.

In the eyes, CAH activity was localized to photoreceptor cells (the soma of which are interspersed between non-reactive pigment cells of the retina). Photoreceptor cell axons containing the dense CAH reaction product converge to form the optic nerve and terminate within the ipsilateral cerebral ganglion. CAH activity in the tentacles was restricted to a subset of primary sensory neurons having undetermined sensory function (the possibilities include chemo-, mechano-, or photoreception). Axons from these neurons converge to form a single large fascicle within the tentacle nerve and terminate in varicosities within the neuropil of the ipsilateral cerebral ganglion. Many primary sensory cells of the labia also exhibit CAH activity. Axons from these cells form fascicles within the labial nerves and enter the ipsilateral cerebral ganglion. Again, the specific function of these cells cannot be inferred from their morphology alone. CAH-positive axons are discernible in nearly all neural pathways. Many of these axons probably derive from sources (e.g. foot, heart, viscera, head skin) that have not yet been investigated. A small number of neuronal somata within the CNS also show CAH activity. The possibility exists that these cells are higher order sensory neurons. Interestingly, primary sensory cells of the statocyst organs did not show CAH activity.

While CAH activity appears in many primary sensory cells of Melampus, neither its presence nor absence can at this time be directly correlated to any specific structural or functional characteristic (roles in intracellular pH balance and/or transmitter function are possibilities). Supported in part by grants from Sigma Xi and Wash. St. Univ. to RLR.

236.4 LIGHT ADAPTATION, SEROTONIN, AND EFFERENT NEURONES ALL MAY AFFECT VISUAL RESPONSIVENESS IN A CIRCADIAN-INSENSITIVE INSECT RETINA. L.A. Donovan*, P.M. Thompson* and S.R. Shaw, Department of Psychology, Dalhousie University, Halifax, N.S., Canada B3H 4J1

Among the insects, the dipteran compound eye is the classical example of a "fast" retina, for its ability to respond to 200Hz flicker and to recover very rapidly (in seconds) from light adaptation. Nässel et al (1983, Brain Res. 280: 361) report that an efferent, serotonergic neurone TAN 3 has endings ramifying widely between the retina and first synaptic zone (lamina) of the blowfly Calliphora, suggestive of a cell type that in other arthropods mediates slow circadian changes in retinal sensitivity. To investigate the possible role of this efferent, we first examined the visual system of Calliphora for evidence of circadian rhythmicity, in long-term electroretinogram (ERG) recordings. The ERG is readily interpretable in Diptera because its prominent 'ON' and 'OFF' L-waves originate from the large second-order monopolar (L) cells of the lamina (Heisenberg 1971, J.Exp.Biol. 55: 85; L. McCook & S. Laughlin, this meeting). L-waves therefore provide an index of any sensitivity changes occurring either in the postsynaptic L neurons, or in the photoreceptors driving them.

No circadian changes in responsiveness were found in the retina or lamina tested in constant darkness, even with ERG measurements averaged over several days from stable preparations. Short-term fluctuations in sensitivity of about 20% remain unexplained, but were not circadian. Nor were conditional circadian changes observed, that needed a light trigger to be manifested. Behaviorally, the flies were normal in exhibiting a pronounced circadian eclosion rhythm, but no circadian cycle was evident in the distal visual responses.

Our attempts to trigger a circadian cycle with light pulses revealed an unanticipated aspect of visual sensitivity, that the pace of dark-adaptational recovery is controlled not by one but by at least three independent processes, distinguished by different time constants, T. The fastest ($T_1 \simeq 1$ min) originates within the photoreceptors, and in intracellular recordings is followed by a hitherto undescribed slower exponential process (T_2 = 10-30 min). A process believed to be T_2 appears also during postsynaptic L-wave recovery, and is followed there by another even slower, so far undescribed exponential process, T_3 = 1.5-3h. T_3 is absent from photoreceptors, and therefore must be a postsynaptic phenomenon, possibly added through efferent action. Both T_2 and T_3 alter in different light adapting regimes, in a way that may point to their origin in efferent control. Preliminary tests show that 5-HT injected into the retina does change visual sensitivity, producing both medium and long term effects. Supported by NSERC grant A9593 (S.R.S).

236.5 DIRECTION-SELECTIVE LISTENING IN THE CRICKET TELEOGRYLLUS OCEANICUS: POSSIBLE SYNAPTIC MECHANISM. G.S. Pollack. Dept. of Biology, McGill Univ., Montreal, Quebec, Canada H3A 1B1.

Pair formation in crickets is mediated by an acoustic signaling system, in which females are attracted to the calling song produced by conspecific males. An important cue for recognition of the signal is its species-typical temporal pattern. Females often must detect their species' pattern under acoustically unfavorable conditions, e.g., when the temporal structure of the song of an individual male is obscured by overlain songs of nearby singing males, both of the same and of different species. Previous work showed that the directionality of the auditory system may play an important role in permitting a cricket to discern stimulus temporal patterns under such conditions. An identified interneuron, the omega neuron, was shown to encode selectively the stimulus pattern played from the soma-ipsilateral side in preference to the pattern played from the contralateral side when both patterns were played simultaneously, even though the contralateral pattern was encoded when it was played alone. The present work concerns the mechanisms that may be responsible for side-selective encoding of the stimulus temporal pattern.

Intracellular recordings from omega neurons show that long-duration stimuli, including the species' calling song, produce in addition to EPSPs and spikes a hyperpolarizing potential, the strength and duration of which vary directly with the strength and duration of the stimulus. The hyperpolarization appears to act as an automatic gain control which damps the neuron's excitability according to the intensity of stimulation. Thus, although a contralateral stimulus of given intensity might be able to drive the neuron when it is presented alone, when a different pattern is presented simultaneously, and at the same intensity, from the ipsilateral side the "automatic gain control" results in a decrease in excitability so that now, because of the neuron's directional selectivity, only the ipsilateral stimulus is sufficiently intense to bring the neuron to threshhold. The intensity-dependence of the hyperpolarizing potential allows this mechanism to operate over a wide range of stimulus intensities.

Preliminary results indicate that the hyperpolarization is due to a conductance-increase IPSP. It shows frequency sensitivity, being induced by 5 kHz (cricket-like) sounds but not by ultrasound, despite the fact that the omega neuron is sensitive to ultrasound. Although the IPSP has a long latency (ca. 50-70 ms), lesion experiments show that the circuitry needed to generate it is entirely within the prothoracic ganglion. The time course of the IPSP is slow, with rise and decay times of up to several seconds. Investigations are continuing on its nature and source.

236.6 WIND-SENSITIVE HEAD HAIRS: A MECHANOSENSORY PATHWAY ACTIVATING FLIGHT IN THE HAWKMOTH *MANDUCA SEXTA*. Edmund A. Arbas and J.G. Hildebrand. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721

To initiate studies of the olfactory control of flight in *Manduca sexta*, we have begun to characterize neural pathways of various sensory modalities that influence flight motor activity in the CNS. Observations with restrained moths indicate that mechanical stimulation of the head with an air current is a powerful stimulus for activating flight motor activity. It is well known that similar stimuli activate flight in locusts via actions on the antenna and on a system of mechanosensory head hairs. Like locusts, *Manduca* possesses large antennae that are exposed to air currents during flight, but its head is covered with numerous large scales. Removing the head scales by gentle teasing revealed that the head also bears 100-200 curved hair sensilla, the largest of which measures 200-300 μm in length. Hairs are distributed with irregular density over the posterior part of the head capsule, while discrete hair patches are evident at the margins of the frons near the eyes.

Central projections of sensory neurons associated with the hairs were revealed by filling cut hairs with cobalt-lysine followed by standard histology. Hair-cell axons enter the brain by 2 bilaterally symmetrical paths: via the anterior tegumentary nerve (ATN) and via another, distinct tegumentary nerve (TN). The ATN joins the antennal nerve near the antennal lobe (AL), and the hair-cell axons form a thin tract that runs laterally around the AL and branch in the antennal mechanosensory and motor center (AMMC) of the deutocerebrum. Fibers running in the TN enter the tritocerebrum near the frontal connective and maxillary nerve and form branches that mingle with fibers of the ATN in the AMMC. Fibers originating from the 2 paths then project together through the subesophageal ganglion (SEG), sending branches toward the midline at several levels (possibly conforming to the mandibular, maxillary, and labial neuromeres of the fused SEG), and descend in the ventral nerve cord to the pro- and pterothoracic ganglia.

The responses of the hair cells to stimulation were examined by recording extracellularly from the TN. Phasic bursts of action potentials were recorded from TN axons in response to air puffs applied to the head or to stroking of hairs. Sensitivity is not diminished when scales are left on the head, and it appears that the scales may act as wind vanes which, when deflected, bend the head hairs interspersed among them.

The remarkable similarity in the structures of the head hairs of *Manduca* and those of locusts and in the patterns of projection of the hair-cell axons in the CNS of both insects suggests that cephalic mechanosensory hairs represent a pathway of sensory inputs that is highly conserved among pterygote insects. [Support: NIH NS-23405]

236.7 CENTRAL PROCESSING OF OLFACTORY INFORMATION IN THE BRAIN OF THE LARVAL TOBACCO HORNWORM *MANDUCA SEXTA*. H. Itagaki and J.G. Hildebrand. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

As part of our continuing studies of the processing of chemical-sensory information in the insect CNS, we have begun an investigation of central olfactory pathways in larval tobacco hornworms, *Manduca sexta* (Lepidoptera:Sphingidae). These larvae offer several advantages that make them favorable animals for the study of olfactory mechanisms. (1) Schoonhoven and Dethier (1966) showed that there are only 32 olfactory receptors located in the two antennae and several more in the mouthparts. This small number of olfactory primary afferents simplifies our investigations. (2) The central projections of the olfactory receptor axons are known from the previous work of Kent and Hildebrand [*Phil Trans Roy Soc (Lond)* in press (1986)]. (3) The sensory responses of the olfactory receptors to a variety of natural plant odors and pure odorants have been investigated by Dethier, Schoonhoven, Hanson, and others. This wealth of background information has aided our investigation.

We have approached the larval olfactory system using physiological and neuroanatomical techniques in order to correlate the physiological properties of odor-sensitive neurons in the larval brain with their morphology. Intracellular recordings were made from desheathed larval brains bathed in physiological saline solution, by means of beveled microelectrodes filled with a saline solution of Lucifer Yellow CH. Most of the recordings have been made from neuronal cell bodies, yielding attenuated action potentials, although several neuropil impalements have also been obtained. Chemical stimuli were delivered via an olfactometer that applied a constant stream of clean air past the antennae; the air was diverted through a glass cartridge containing odorant(s) in order to stimulate the antennal receptors. Cells that responded to either pure odorants (e.g. *trans*-2-hexenal), or to extracts or squashes of host-plant (tobacco or tomato) leaves, or to both pure and plant-derived odors were stained intracellularly with Lucifer Yellow by passage of hyperpolarizing current pulses through the dye-filled electrode. The brains were then fixed, dehydrated, and cleared before the cells were drawn with the aid of a camera lucida. Selected brains were further processed by embedding in paraffin and sectioning at 8 μm.

So far, we have found 3 types of responses to odors in the CNS neurons. (1) Normally quiescent CNS neurons that respond with a phasic burst of spikes when the antenna is stimulated with odor. (2) Tonically active neurons that increase their firing frequency in response to an odor stimulus. (3) Tonically active neurons that show a decrease in firing frequency or stop firing in response to an odor. [Supported by NIH grant AI-23253.]

236.8 SYNAPTIC RELATIONSHIPS BETWEEN LOCAL AND PROJECTION NEURONS IN THE ANTENNAL LOBE OF THE SPHINX MOTH *MANDUCA SEXTA*. T.A. Christensen and J.G. Hildebrand. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Extending our studies of the mechanisms underlying olfactory information processing in the brain of the sphinx moth *Manduca sexta*, we have recently focused our attention on the role(s) of synaptic inhibition in the antennal lobe (AL), the first-order olfactory center in the deutocerebrum. We are beginning to probe pairs of neurons in the AL with intracellular recording and staining techniques in order to test for synaptic interactions between olfactory information-processing CNS neurons. These AL neurons fall into two broad morphological categories: *local interneurons* (LNs), which are confined to the AL and have arborizations in most or all of the spheroidal glomeruli ("knots" of synaptic neuropil), and *projection neurons* (PNs), which arborize in single glomeruli and project axons to other parts of the brain.

Impaling two AL neurons simultaneously (one LN and one PN in most of the 30 cases to date) has permitted the following observations: (1) Spiking activity (either spontaneous or current-induced) in an LN only occasionally appears to elicit a postsynaptic response in a PN impaled simultaneously. Subthreshold depolarization of an LN has never been correlated with a physiological response in a PN. Thus a given LN seems not necessarily to interact synaptically with all PNs in the AL. Alternatively, spikes generated in one region of the LN (nearest the microelectrode impaling it) may not propagate to the region of synaptic contact with the PN impaled by the second electrode. This is consistent with the idea that LNs may have multiple sites of spike initiation in physiologically isolated cellular compartments [Matsumoto & Hildebrand *Proc Roy Soc* B213:249 (1981)]. (2) In cases in which a definite connection was established, LNs have been shown to inhibit PNs. This observation is consistent with previous evidence that a majority of LNs are GABAergic inhibitory interneurons [Hoskins et al. *Cell Tiss Res* in press (1986), and Christensen et al. *Soc Neurosci Abstr* 11:163 (1985)]. (3) No reciprocal interactions (from PN to LN) have been observed.

We have also begun to examine the responses of pairs of AL neurons to stimulation of the antenna with behaviorally important odors such as sex pheromones. The sex pheromone bombykal can hyperpolarize some LNs, and this in turn may be followed by a delayed depolarization in a postsynaptic PN, suggesting that the PN may be depolarized by disinhibition. As further evidence of this, hyperpolarization of the LN by current injection can lead to spiking activity in a previously quiescent PN. Most paired recordings, however, do not clearly reveal synaptic relationships. Thus it appears that the combined effects of several presynaptic cells (presumably LNs) are needed to change the firing of a given PN. [Supported by NIH grant AI-23253.]

236.9 IDENTIFIED LOCAL INTERNEURONS OF THE CRICKET CERCAL AFFERENT SYSTEM AND THEIR POSSIBLE ROLE IN SENSORY PROCESSING. Deane A. Bodnar, Gwen A. Jacobs, and John P. Miller. Graduate Group in Neurobiology, University of California, Berkeley, CA 94720.

The cercal afferent system of the house cricket, Acheta domesticus, serves as a model for understanding the functional organization of afferent inputs and the processing of sensory information. We have begun to study the integrative role of local interneurons in this system. In previous work Rod Murphey and his collaborators have characterized the directionally selective attributes of the wind sensitive afferents and the giant ascending sensory interneurons they innervate. Recently we have identified a pair of local interneurons which are also wind sensitive. These two cells, referred to as 9LD-1 and 9LD-2, have extensive dendritic arborizations which are confined to localized regions of the filiform neuropil and lack a projecting axon. Their spontaneous activity is characterized by psp oscillations at approximately 40 Hz. Both cells are directionally selective in their response to wind stimuli producing a sustained EPSP in their maximal wind orientation. These cells do not produce action potentials either during spontaneous activity or evoked wind responses. As would be predicted from their dendritic structure, 9LD-1 responds best to wind from the front of the animal, while 9LD-2's optimal stimulus orientation is from the rear. However each cell possesses a dendritic region which does not appear to receive significant synaptic input. It is likely that this branch serves as an output site of the cell.

One focus of our experiments is to determine what functional role these interneurons play in this sensory system. It is known from previous work that the giant ascending sensory interneurons receive polysynaptic inhibition which sharpens their directional sensitivity to wind. The anatomical and physiological characteristics of the local interneurons implicates them as possible inhibitory interneurons. One means of testing this hypothesis is to remove one of the local interneurons from the sensory circuit and observe the changes in the response to wind stimuli of an ascending interneuron. This was done by injecting 9LD-2 with a fluorescent dye and then obtaining an intracellular recording of one of the ascending interneurons,10-3. The directional sensitivity of interneuron 10-3 was measured before and after 9LD-2 was killed by photoinactivation. The results of this experiment suggest that 9LD-2 has an inhibitory influence on 10-3.

Research is currently in progress to investigate more directly the integrative characteristics and functions of these local interneurons.

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236.10 DIRECTIONAL PROPERTIES OF THORACIC INTERNEURONS IN COCKROACH GIANT INTERNEURON PATHWAYS J. Westin, A.J. Pollack, R.E. Ritzmann and R. Todd Wiblin. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

Two sets of thoracic interneurons have been identified which receive inputs from giant interneurons (GIs) and excite those leg motor neurons that are excited by the GI pathways during escape. These have been designated as the dorsal posterior group (DPG) interneurons, which receive inputs only from the ventral GIs, and the T cell interneurons, which receive inputs from the dorsal GIs. The pattern of connections that have been established to and from these interneurons suggest that they may play a critical role in the escape pathway. One hypothesis is that these interneurons decipher the information on wind stimulation that is contained in the various GIs and then utilize this information to control the escape turn away from the wind puff.

If this hypothesis is correct one would expect that the inputs from the GIs confer directional biases on at least some of the thoracic interneurons. In this study we have determined the directional sensitivity of several of these thoracic interneurons utilizing intracellular recording and dye injection. The majority of these cells show directional bias. For example the Reverse J cell receives stronger excitation from wind coming from the side contralateral to its soma. Since the motor output is always stronger on the contralateral side these biases should result in the appropriate turn away from a wind directed at the contralateral side.

In order to determine the basis of this bias we are comparing the inputs from the various individual GIs to each thoracic interneuron. Some of the interneurons are very asymmetric both in morphology and GI input. For example Cross cell appears to have its dendritic field restricted to the side ipsilateral to the cell's soma. This cell has shown positive inputs only on that side. Other DPG interneurons (including Reverse J cell) have more symmetrical dendritic fields and clearly receive inputs on both sides. In these cases we will determine whether the directional wind properties can be attributed to quantitative differences in the inputs from various different GIs. Alternatively they may result from morphological properties of the cells.

Supported by NIH grant NS-17411 to RER.

236.11 CHARACTERIZATION OF PROPRIOCEPTIVE INPUTS TO DPG INTERNEURONS IN THE COCKROACH. M. Murrain and R.E. Ritzmann. Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

When the cockroach turns directionally away from a wind stimulus, the position of the legs is probably important in determining the motor output. We have investigated the responses of a group of cells to stimulation of proprioceptors on the legs. These cells, which are called DPG or dorsal posterior group interneurons, are thought to play an important role in the escape behavior. They have been found to receive monosynaptic input from ventral giant interneurons (vGIs), which carry directional wind information from the cerci (Ritzmann and Pollack, 1986, in press). As well, these cells have directed motor output.

The proprioceptors that were stimulated were the trochanteral campaniform sensilla (trCS), the trochanteral hair plates (trHP), and the femoral chordotonal organ (feCO). They were stimulated using a piezoelectric crystal. The crystal was used to either bend the hairs of the HP, apply pressure to the trochanteral cuticle to excite the CS, or lift the strand of the feCO, to excite the cells of the CO. The DPG neurons were impaled intracellularly, and filled with Lucifer Yellow for identification.

All of the DPG's investigated so far receive some input from proprioceptors, although not all receive input from all types of proprioceptors on all legs. All DPG interneurons show some bias in the response to proprioceptors. There are three types of bias seen in the responses. The first is a sensory bias; cells tend to respond more strongly to one type of proprioceptor than the other two. There is a definite left/right bias seen in all DPG's. Also, there is a segment bias; the response to stimulation of proprioceptors in the cells segment tends to be greater than the response to proprioceptors in another segment.

A model will be described for how these biases accentuate already inherent biases in the proprioceptors, and how this can be used by the escape system to help in determining motor output.

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236.12 ANATOMY AND PHYSIOLOGY OF SOUND SENSITIVE DESCENDING AND T-SHAPED INTERNEURONS IN THE PROTHORACIC GANGLION OF THE CRICKET TELEOGRYLLUS OCEANICUS. G. Atkins* and G.S. Pollack (SPON: V. Pasztor). Dept. of Biology, McGill Univ., Montreal, Quebec, Canada H3A 1B1.

Behavioural experiments have shown that flying crickets steer towards conspecific calling songs having carrier frequencies of 5 KHz (positive phonotaxis) and away from ultrasonic stimuli (negative phonotaxis). Although much is known about the processing of auditory information within the prothoracic ganglion, where auditory receptors terminate, little is known about how auditory information is delivered to the motor circuitry that accomplishes steering, which is located primarily in the meso- and metathoracic ganglia. We have begun to search for sound sensitive neurons that project from the prothoracic ganglion to lower ganglia.

Using intracellular recording and staining techniques we have identified ten such interneurons in the cricket Teleogryllus oceanicus, all of which receive auditory input within the prothoracic ganglion. Only 3 of these have processes that overlap with the terminal processes of auditory sensory neurons, and therefore may receive direct input from receptor cells. The remaining 7 interneurons must receive their auditory input indirectly. All but one of the interneurons exhibit branches within the prothoracic ganglion which, based on anatomical evidence, are likely to be output terminals. Mesothoracic projections were stained in 8 of the neurons, and in 7 cases were extremely similar to the presumed output branches within the prothoracic ganglion.

Examination of the neurons' spectral sensitivities revealed that 5 of the interneurons respond best to tones having carrier frequencies near 5 KHz while the remainder respond best to ultrasound. Based both on their responses to various sound stimuli and on their morphology in the mesothoracic ganglion, all 5 of the ultrasound-tuned cells are candidates for providing information to motor circuitry involved in flight phonotaxis. The 5 neurons tuned to 5 KHz are less likely to provide direct phonotactic input to flight motor circuitry.

The potential roles of these interneurons in flight phonotaxis are being examined by mapping the connectivity between the interneurons and the motor systems for flight.

236.13 **THE CRAYFISH HORSESHOE NEUROPIL IS THE HOMOLOGUE OF THE INSECT VENTRAL ASSOCIATION CENTERS.** **E. M. Leise and B. Mulloney.** Dept. of Zool., Univ. of Calif., Davis, CA 95616.

In the ventral regions of insect ganglia are large areas of neuropil that contain terminal branches from primary sensory afferents. Within these neuropils, the ventral association centers (VACs), some of the afferent projections are known to be somatotopically arranged. A large ventral neuropil, the horseshoe neuropil (HN), also exists in crayfish. To find out if the HN is organized similarly to the insect VACs we made a series of axonal fills with cobalt chloride of branches of the nerve that carries sensory afferents to the ganglion. We then studied the central projections of these filled sensory afferents to determine where in the ganglionic neuropils they project.

We filled branches of one of the second pair of nerves (N2s) that exit from each of the first five abdominal ganglia. This pair of nerves carries the axons of the slow and fast abdominal extensor motor neurons, the muscle receptor organs (MROs) and axons from at least three fields of sensory hairs on the abdominal carapace. Field 1 is a patch of hairs on the ipsilateral ventral rib, in the segment of origin. Field 2 is a rectangular area on the ipsilateral pleural plate, also in the segment of origin. Field 3 is an arc of hairs on the adjacent caudal pleural plate.

We made two types of axonal fills. In one, a window was cut in the ventral carapace and the nerve to Field 1 was dissected out and filled in vivo. In the second type of fill, the anterior branch of one Nerve 2, which carries all of the sensory axons except those associated with the MROs, was filled in an isolated nerve cord. Short filling times (4 hrs in vitro and 14-26 hrs in vivo gave the best results.

The central projections of the filled axons were studied in whole mounts and sections. Axons from the sensory afferents enter the ganglion in Nerve 2 and bend anteriorly to branch in the ipsilateral arm of the HN. No axons were found that extended caudally, but many afferents send axons to anterior ganglia in the ventral intermediate tract. Several afferents also send terminals to the contralateral hemiganglion in the curve of the HN and in DC6 and DC7, two commissures in the second commissure layer. In summary, the HN receives a few dendrites from abdominal motor neurons, but is densely innervated by the sensory afferents from hairs on the dorsal and ventral carapaces. This information leads us to suggest that the HN is the homologue of the insect VACs.

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236.14 PROPERTIES OF THE INTEGRATED CIRCUIT MEDIATING DIRECTIONAL SELECTIVITY IN A MOVEMENT SENSITIVE NEURON. N. Franceschini[x], A. Riehle[x], A. Le Nestour[x] (SPON: J. Requin) Laboratoire de Neurobiologie (C.N.R.S.) 13402 Marseille (France).

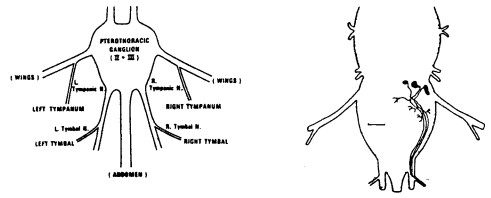
In an attempt to understand the basic process of sequence-discrimination underlying motion detection in a visual system we record the activity of an identified, spiking neuron called H1 (Hausen K., Z. Naturforsch. 31c, 629, 1976) present in the third optic ganglion (the lobula plate) of the fly. This unique neuron, which characteristically responds to horizontal motion presented from back to front to the ipsilateral eye, displays a huge receptive field and more or less directly integrates the responses of a multitude of Elementary Movement Detectors (EMD's) which are wired down with fine grain behind the receptor mosaic.

Our strategy is to make the response of a single EMD stand out from the H1 activity. The method consists in applying a temporal sequence of excitation to only 2 neighboring receptor cells (R1 and R6) that are selected in vivo among the 50.000 receptors of the retinal mosaic (Riehle, A., Franceschini, N.: Exp. Br. Res. 54, 390-394, 1984; Franceschini, N., Neurosci. Res. Suppl.2, 17-49, 1985). Optical microstimulation of R1 and R6 --which lie only 3.5µm apart-- induces a vigorous response (e.g. 100Hz) in H1, provided the sequence of two flashes simulates motion in the preferred direction (R1 then R6). A basic requirement for a response to occur is that the second flash be presented to R6 within a characteristic "temporal window" that starts at $t_1 \simeq 10$ms and ends at $t_2 \simeq 230$ms following the first flash. Whereas this window sets limits to the range of speed that can be sensed by the EMD under study (360°/sec down to 16°/sec), the existence of a minimum delay ($t_1 \simeq 10$ms) makes the circuit immune to synchronous flicker. A sequence of two steps (of light or darkness) induces a prominent response only if both steps have the same sign (e.g. an ON-step on R1 followed by an ON-step on R6). Moreover the response to a sequence of OFF-steps does not show the same time course as the response to a sequence of ON-steps. The former displays a conspicuous "rebound effect" that is not seen in the latter. We conjecture that the facilitatory control concealed in this highly non-linear microsystem is split into two independant pathways responsible for the ON- and OFF-response respectively.

236.15 SOUND PRODUCTION IN ARCTIID MOTHS: RHYTHMIC BEHAVIOUR ELICITED BY SIMPLIFIED AUDITORY INPUT. J.H. Fullard and J. Yack*. Department of Biology, Erindale College, University of Toronto, Mississauga, Ontario, Canada L5L 1C6.

Certain tiger moths (Lepidoptera: Arctiidae) respond to high-frequency (30 kilohertz) acoustic stimuli by emitting trains of clicks, presumably as a defense against the attacks of predators, including echolocating bats. The moths' sounds are produced by a pair of metathoracic tymbals which, in *Cycnia tenera*, appear to be activated by single large pterothoracic motoneurons with their soma located near the auditory input of the moth's two receptor cells. The moth produces its sounds by alternately buckling each tymbal on each side of its thorax thus the acoustic response constitutes a stereotyped, rhythmic behaviour of significant ethological importance. Anatomical and backfilling studies (CoS and Lucifer Yellow) indicate that the motoneurons innervate the tymbal muscle (basalar coxal branch) via the motor branch, IIIN2a. Extracellular recordings suggest a large unit neurogenically activating the tymbals. De-efferented preparations continue to exhibit cyclic motor output in isolated ganglia indicating the circuitry involved lies within the pterothoracic ganglion and that the behaviour is centrally-generated. Studies with monaural preparations suggest that, in *C. tenera*, the tymbal motoneuron may be activated by the second, less sensitive auditory receptor, the A_2 cell and may be monosynaptically connected. Asymmetry in the motor output combined with what is known of the moth's sensory input has resulted in a neural model of this central oscillator. The existence of a range of behavioural thresholds in other arctiid species from sensitive to non-acoustic suggests that this circuit may prove useful in neuroethological studies of the evolution of rhythmic behaviours and the cellular bases for auditory-based behavioural habituation.

This research was supported by the Natural Sciences and Engineering Research Council of Canada.



(left) Thoracic innervation of arctiid tympanum and tymbal, (right) three motoneurons from Lucifer Yellow backfill of tymbal nerve in *Halysidota tessellaris*. Scale bar 50 µm.

236.16 BAND-PASS FILTERING AND INTERVAL CODING AT A SENSORI-MOTOR SYNAPSE IN CRAYFISH BRAIN. R.M. Glantz and H.B. Nudelman, Dept. of Biology, Rice University, Houston, TX 77005

It is widely held that impulse frequency is a universal code for information transmission in nervous systems. There are however, numerous cases where sensory stimuli modify a neuron's firing pattern or interval distribution with only modest effects on the mean rate. The sustaining fibers (SF) of the crayfish optic nerve provide a particularly striking example of this phenomena (Glantz and Nudelman, 1976 J. Neurophysiol. 39:1257). As the intensity of constant, broad field illumination of the eye increases, the SF discharge exhibits an increasingly pronounced periodic component and a higher proportion of interspike intervals of ~100ms (range 60 to 180). Since the SFs monosynaptically excite oculomotor neurons (OMN) (Glantz, Nudelman and Waldrop, 1984) it is possible to assess the influence of interspike intervals on the efficacy of synaptic transmission.

Impulse trains of SFs and OMNs were recorded extracellularly for extended periods (30-90 minutes) in constant broad field illumination. The impulse trains were digitized and the synaptic efficacy was measured from the intensity of the cross-correlation function between SF and OMN impulses. The possible role of the SF interspike interval was assessed with two correlation procedures:

If one looks backward in time from the occurrence of an OMN spike there is a high probability of a SF spike in the period 2-5 ms just preceding the OMN spike. This is the expected result for a monosynaptic connection. In addition to this narrow, short latency peak, the OMN to SF correlations also reveal a large but broad peak of SF activity at 80 to 100 ms prior to the OMN spike. The result indicates that in the period preceding an OMN spike the effective SF pulse trains contain two spikes. One at the normal synaptic delay (2-5 ms) and a second one about 100 ms earlier.

An alternative approach is to examine the intensity of cross-correlation function (from SF to OMN) as the interspike interval of the SF is systematically varied. This analysis makes use of the standard correlation procedure, but the reference event is defined as the second of a pair of SF impulses separated by a range of intervals. The calculation of the correlation is then repeated for each interval class in the SF discharge. The results indicate that if a SF spike is preceded by another at an interval of about 100 ms, the spike is 50 to 150% more effective in eliciting an OMN spike than a randomly selected SF spike. The 100 ms intervals are 10 to 100x more effective than brief intervals (3-30 ms) and 50 to 200% more effective than long intervals (e.g. 200 ms). Thus the SF to OMN synapse is a band-pass filter with maximal transmission near 10 spikes/sec.

We conclude that SFs encode the intensity of broad field illumination by the relative preponderance of a single interval class and that the message is appropriately decoded at the SF to OMN synapse. Supported by NSF Grant No. BNS 83-1229.

236.17 BIFURCATIONS, PHASE-LOCKING AND INTERMITTENCY IN THE PERIODICALLY STIMULATED SLOWLY ADAPTING STRETCH RECEPTOR. R. Budelli*, O. Diez-Martínez*, H. Quijano* and R. Pérez-Pascual*. (SPON: M. R. Budelli). Departamento de Ciencias Fisiológicas, Universidad Autónoma de Puebla, Puebla, Pue., Departamento de Fisiología, Facultad de Medicina, UNAM; Instituto de Física, UNAM. México, D. F., 04510, MEXICO.

This paper deals with the application of dynamical systems techniques to study a periodically stimulated biological pacemaker. We have used one of the most regular neural oscillators, the slowly adapting stretch receptor (SAR) of the crayfish, as a model of a forced neuronal pacemaker. Although it is well known that neural oscillators phase-lock to periodic stimuli at some frequencies, the kind of bifurcations that occur when a small frequency variation causes phase-locking disappearance or change had never been explored systematically.

Results were represented as Poincare maps of the circle in itself. This allowed a simple and precise comparison between experimental results and mathematical models derived from dynamical systems theory. Experiments were carried out in isolated abdominal SAR's. The action potential discharge of the SAR neuron was recorded extracellularly. Trains of regular brief lengthenings were applied to the receptor muscle. Stimulus frequencies varied randomly between 1.6 and 1.0 times the spontaneous discharge rate. When the stimulus frequency was close to, either the spontaneous discharge rate, or its sub-multiples, phase-locking between stimuli and spikes occurred. Phase-locking disappeared through a tangency bifurcation with intermittency appearing when stimulus frequency either decreased or increased. For intermediate frequencies other kinds of phase locking were observed (e.g. 4:3, 4 spikes: 3 stimuli). Other types of bifurcation leading to chaos or bistability were not found. The Poincare maps helped to understand how phase-locking occurs, but also to show through which kind of bifurcation it disappears.The data obtained are consistent with the conclusions of Arnold's theorem, so for the stimulus intensities employed the Poincare map seems to be a diffeomorphism. Furthermore, our results indicate that a model with only one variable such as the leaky integrator is sufficient to explain the behavior of this periodically stimulated pacemaker.

INVERTEBRATE LEARNING AND BEHAVIOR III

237.1 IN VITRO CLASSICAL CONDITIONING OF THE APLYSIA GILL WITHDRAWAL REFLEX INVOLVES MULTIPLE MECHANISMS. Elaine Colebrook, Ken Lukowiak and George Mpitsos. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1 and Mark O. Hatfield Marine Science Centre, Oregon State University, Newport, Oregon 97365.

Both associative and non-associative conditioning of a defensive withdrawal response have been demonstrated in the intact Aplysia californica and in the motor neurons of its isolated nervous system. The increased response has been attributed to facilitation at the central sensory/motor neuron synapse (Hawkins, R.D. et al (1983) Science 219 p.400).

A semi-intact in vitro preparation of A. californica was used to simultaneously monitor the responses of the gill and a gill motor neuron (L7 or LDG1) during classical conditioning or sensitization. The conditioned stimulus (CS) consisted of a tap to the siphon and depolarization of the pedal nerves served as the unconditioned stimulus (UCS). The CS preceded the UCS by 500 msecs in the experimental group (classical conditioning) or by 3 seconds in the control group (sensitization). The response of both the gill and the motor neuron(s) were measured before, during and after 10 training trials.

Both cell and gill increases were observed after training in 46% of classically conditioned (CC) and 27% of sensitization control (sens) preparations. The cell, but not the gill, response increased in 32% of CC and 27% of sens preparations. Only one preparation in each group showed an increased gill response in the absence of an increased cell response. Neither an increased cell or gill response occurred in 18.2% of CC and 36.4% of sens preparations. CC preparations were more likely, therefore, to show a facilitated response after training than sens preparations. No correlation existed between the response of the gill motor neuron and that of the gill.

When motor neurons were depolarized before and after training, the elicited gill withdrawal to the same number of action potentials significantly decreased in the sens group. There was no significant difference in this gill response before and after classical conditioning.

It seems, therefore, that some habituation occurs at the neuromuscular synapse during training. A facilitatory mechanism, in addition to and distinct from presynaptic facilitation at the central sensory/motor synapse, opposes this habituation and is more likely to do so following associative conditioning than non-associative conditioning. This mechanism tends to occur later than presynaptic facilition and may involve a change in the peripheral nervous system or in central neurons having a direct influence in the periphery. (Supported by AHFMR).

237.2 LONG-TERM SENSITIZATION IN APLYSIA INCREASES THE TOTAL NUMBER OF VARICOSITIES OF SINGLE IDENTIFIED SENSORY NEURONS. C.H. Bailey and M. Chen. Ctr. for Neurobiol. & Behav., Depts. Anat., Neurol. and Psychiat., Columbia Univ., P&S, and NYS Psychiat. Instit., N.Y., N.Y. 10032.

An issue central to the study of learning and memory is the functional relationship between synaptic structure and the persistent changes in synaptic effectiveness that accompany long-term behavioral modifications. We have addressed this issue by examining the family of morphological events at identified sensory neuron synapses that may underlie simple forms of learning and memory in the gill-withdrawal reflex of Aplysia californica. In an earlier study (Bailey and Chen, 1983), we combined intracellular labeling techniques with serial reconstruction approaches to analyze the fine structure of identified sensory neuron presynaptic terminals (varicosities) in long-term sensitized and control (untrained) animals. Our results indicated that the number, size, and vesicle complement of sensory neuron active zones were larger in animals showing long-term sensitization than in control animals. To maximize the chances of finding labeled sensory neuron processes in thin sections, multiple cells in each animal were injected with horseradish peroxidase (HRP) and it was not feasible, therefore, to examine if long-term sensitization had any effect on the total number of presynaptic varicosities per sensory neuron.

In the present study, we have addressed this question directly by analyzing the total axonal arbor of single HRP-filled sensory neurons from control (N=8) and long-term sensitized animals (N=10; Pinsker et al., 1973). Within 48 hrs of retention testing, a single sensory neuron from each animal was intrasomatically pressure-injected with HRP, incubated for 2 hours, fixed, histochemically processed and embedded in Epon. Each cell was completely reconstructed using serial 20 μm slab-thick sections and the total number of HRP-labeled varicosities for each sensory neuron was counted through a blind procedure. We have found that long-term sensitized animals have approximately twice as many varicosities per sensory neuron (2697 ± 277 S.E.M.) compared to control animals (1320 ± 99 S.E.M., $t=4.25$, $p < .01$).

These findings, combined with our earlier ultrastructural observations, suggest that each mechanoreceptor sensory neuron in Aplysia possesses a profound presynaptic capacity for structural plasticity following long-term training. Using the slab-thick section approach, we now plan to examine which of these anatomical changes are necessary for the maintenance of long-term memory by exploring the time course over which they occur and, in particular, their duration relative to the persistence of the memory assessed behaviorally. In this fashion, we hope to be able to establish a more causal relationship between synaptic architecture and the learning process as well as increase our understanding of the morphological mechanisms that may contribute to the initiation and stabilization of a long-term memory trace.

237.3 HEMOLYMPH FROM SENSITIZED APLYSIA MODULATES BODY WALL TENSION AND HEART RATE. J.K. Krontiris-Litowitz, B.F. Cooper* and E.T. Walters. Dept. of Physiol. & Cell Biol., Univ. Texas Med. Sch., Houston, TX, 77225.

Defensive behavior in Aplysia has proven useful for examining general cellular mechanisms of behavior and learning. It is not yet known, however, whether endocrine and paracrine signals play an important part during defensive stress in Aplysia, as they do in many other organisms. As a first step in examining humoral contributions to defensive behavior in Aplysia we have withdrawn hemolymph from intact donors following sensitizing shock to the tail and applied it to 2 simple recipient preparations isolated from the CNS: the tip of the tail and the heart. The shock parameters were the same as previously used to produce site-specific sensitization and associative LTP in Aplysia.

In the isolated tail preparation perfused with artificial seawater (ASW), 0.5 ml of hemolymph collected from the body wall of the neck of unshocked donors (control hemolymph, CH) always caused a small, long-lasting contraction (up to 10 min). In 6 of 7 preparations hemolymph taken 5 min after strong sensitizing shock of the tail of the donor ("stimulated hemolymph", SH) produced a contraction (up to 23 min) that was larger than that produced by CH. SH withdrawn 10 or 20 min after the sensitizing shock had little effect.

In the isolated heart preparation perfused with ASW, CH usually had no effect. The effects of SH on heart rate (HR) depended upon the site of hemolymph collection and upon prior sensitizing experience of the donor. SH collected near the body wall in the neck of naive donors 5 min after strong tail shock did not increase HR (N=7). By contrast, SH collected from the anterior aorta 5 min after shocking naive donors, increased HR in 4 of 6 preparations. Interestingly, SH collected from aortae of the 2 "ineffective" donors in this group increased HR when stimulated a second time 1 or 2 days after the first stimulus sequence. The possible contribution of previous sensitizing experience to modulatory activity in the hemolymph received further support by the observation that HR was increased by SH from 4 of 4 donors previously parapodectomized and subjected to an aversive classical conditioning paradigm (see Erickson & Walters, this volume).

Hemolymph (SH) in recently sensitized Aplysia probably contains factors released from the body wall (Cooper et al., this volume). The present results suggest that such factors may have endocrine functions, perhaps contributing to the general "balling-up" response and to the increased HR (Dieringer et al., 1978) observed in Aplysia following noxious stimulation.

237.4 PROPERTIES OF HUMORAL FACTOR(S) RELEASED FROM APLYSIA BODY WALL BY SENSITIZING STIMULATION. B.F. Cooper*, J.K. Krontiris-Litowitz and E.T. Walters (SPON: A. Eskin). Dept. of Physiol. & Cell Biol., Univ. Texas Med. Sch., Houston, TX 77225.

Noxious mechanical or electrical stimulation causes persistent local contraction of the body wall of Aplysia. Under natural conditions this response probably functions to seal wounds, preventing loss of hemolymph. We are investigating the properties of humoral factors released from the body wall that contribute to wound sealing and perhaps to other effects of noxious, sensitizing stimulation (see Krontiris-Litowitz et al., this volume).

Isolated body wall was stimulated electrically or mechanically and the inner surface washed with a small volume of artificial seawater (yielding "stimulated body-wall wash", SBW). Control wash (CBW) was obtained from unstimulated body wall. 0.1 ml of SBW but not CBW always caused contraction (for up to 45 min) of body wall from the tail, midbody, rhinophore, and tentacle, and of isolated columellar muscle. In addition, SBW increased the heart rate in 6 of 13 isolated heart preparations.

Release of the factor(s) was blocked by perfusing the donor body wall with 30mM Co^{2+}. The possibility that SBW has a direct effect on muscle was indicated by its ability to cause (reduced) contractions in recipient body wall perfused with 30mM Co^{2+} (7 of 8 preparations). SCP_B sometimes caused similar, long-lasting contractions in ASW, but never in 30mM Co^{2+}. FMRFamide caused brief contractions that were blocked by Co^{2+}. 5-HT, dopamine, and octopamine failed to produce reliable contractions in ASW or Co^{2+}.

SBW could potentiate the effects of small amounts of 10^{-8}M SCP_B on body wall contraction; the combined effect exceeded the sum of the individual effects (n=5). This synergism was not observed, however, in 2 preparations in which the SCP_B alone caused a near maximal contraction (compared to 10^{-6}M SCP_B). Synergism was not observed between SBW and 5-HT.

Preliminary characterizations are consistent with the possibility that the factor(s) is a peptide. Activity is reduced by pepsin digestion. Activity is retained in 1000 MW cut-off dialysis tubing, but passes through 3500 MW tubing. It survives heating to 100°C for 30 min and storage for 5-7 days in ASW or hemolymph at 4°C. Activity after 100-fold purification (filtering, boiling and centrifuging) remains for at least 6 weeks when stored at -20°C.

In view of the common co-occurence of neuroendocrine substances in central and peripheral tissues in other animals, we were interested in examining possible effects of SBW on identifed central neurons. Perhaps surprisingly, we have not yet observed direct effects on sensory, motor, or interneurons.

237.5 STUDIES OF MOTOR CONTROL OF APPETITIVE FEEDING BEHAVIOR IN *APLYSIA*. G. Bablanian, K.R. Weiss and I. Kupfermann. Cntr. for Neurobiol. & Behav., Columbia Univ. and NYS Psychiatric Inst., New York, NY 10032.

We have been studying the motor control of the appetitive phase of feeding behavior, in the gastropod, *Aplysia californica*, utilizing a variety of preparations. The appetitive phase of feeding behavior consists of head lifting, head waving and orientation of the head to food. The muscles controlling this behavior, located in the anterior dorsal neck and lateral tegument of the animal, are innervated primarily by pleural nerve 1 (PL1) and pedal nerves 2, 3 and 5 (P2, P3, P5). Electrical stimulation of PL1 and P3 caused lateral and longitudinal contractions of the loosely overlapping neck musculature; while stimulation of the branches of P5 caused longitudinal contraction of the wide band of lateral tegumentary muscle. Lateral contraction of the entire head region occurred during stimulation of P2.

Recordings from these nerves, in free-moving animals, showed an increase in extracellular activity during head lifting, at the onset of appetitive feeding behavior. Directionally specific inhibition and excitation in neural activity occurred in PL1, P3 and P5 during leftward and rightward movements of the head (head waving). Cobalt and nickel backfills of these nerves revealed cell bodies in the cerebral, pedal and pleural ganglia. The neurons are therefore putative motor neurons for the neck and tegumentary muscles involved in appetitive behavior. This evidence suggests that appetitive control of feeding may involve the coordinated activity of several different ganglia.

237.6 NEURAL CORRELATES OF CONDITIONING IN LATERAL AND MEDIAL B-PHOTORECEPTORS OF HERMISSENDA. M.S. Bridge* and T. Crow (SPON: F. Robinson). Dept. of Physiol. and Ctr. for Neurosci., Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

A conditioning procedure consisting of pairing-specific stimulation of the visual system and gravity-detecting statocysts in the marine mollusk Hermissenda produces a long-term modification of normal positive phototactic behavior. The learning is expressed by a significant suppression of the initiation of locomotion in the presence of the CS (light). Associated with the conditioned modification of phototaxis is a reduction in the amplitude of the generator potential in the primary sensory neurons (type-B photoreceptors) of the pathway mediating in the CS (Crow, 1985). Since each eye of Hermissenda contains three type-B photoreceptors, it is possible that the B-photoreceptors are not functionally equivalent in the expression of the cellular changes produced by conditioning. In order to test this hypothesis we have examined the light responses of B-photoreceptors in the medial and lateral position in the eyes of conditioned animals and random controls.

Previous work with the intact nervous system revealed a significant overall reduction in the amplitude of the generator potential of conditioned animals (N=13) as compared to random controls (N=13)($p<.01$). An analysis of the time course of adaptation revealed that the significant difference in the generator potential between groups was first expressed 90 sec after light onset ($p<.05$). In order to examine if the change in the generator potential is intrinsic to the B-photoreceptors the experiments were conducted with preparations where the photoreceptors were surgically isolated by cutting the optic nerve. B-photoreceptors in the lateral (N=8) and medial (N=8) positions in the eyes of conditioned Hermissenda were compared to lateral (N=14) and medial (N=6) B-photoreceptors of random controls. The mean amplitude of the generator potential at the offset of a 2 min light step was not significantly different for lateral ($\bar{X}$=26.5 mV) and medial ($\bar{X}$=25.3 mV) B-photoreceptors from random controls. In contrast to the results from the random controls the generator potentials of medial B photoreceptors from conditioned Hermissenda were significantly smaller than the potentials from lateral photoreceptors ($\bar{X}$=14.4 mV vs $\bar{X}$=20.3 mV, $p<.01$). Light responses of lateral and medial B-photoreceptors were significantly smaller ($p<.05$; $p<.01$) than the responses of lateral and medial B-photoreceptors from the random controls. These results indicate that the plasticity intrinsic to B-photoreceptors of conditioned Hermissenda is quantitatively different for photoreceptors in different positions within the eyes. However, both medial and lateral B-photoreceptors exhibit diminished responses to light after conditioning.

237.7 PAIRING- AND STIMULUS-SPECIFIC FOOD AVERSIONS IN HERMISSENDA. R.Wu, L.Grover, & J.Farley. Prog. in Neurosci. & Behavior, Princeton University, Princeton, NJ 08544.

The reduction of Hermissenda's phototaxic behavior by light-rotation pairings has proven to be a useful preparation for understanding the cellular bases of some simple characteristics of associative learning. We are interested in extending this analysis to some of the more complex characteristics of associative learning, such as blocking, overshadowing, and higher-order conditioning. Such experiments presuppose a cue, other than light, which Hermissenda can learn about. Thus, we have developed a chemosensory aversion-learning paradigm using the same UCS (rotation) that has been used in conditioning of phototaxis. Bite strike latencies to two types of food (selected from clam, scallop, or oyster depending upon the experiment) were measured prior to conditioning. Subsequently, one food was presented in either a paired (CS_P) or unpaired (CS_{UP}) fashion (separate groups of animals) with rotation, while the second food was not presented (CS_{NP}). Pairings (n=15) were arranged by placing each individual animal in a beaker of seawater for 30 sec, flavored with CS_P extract, and simultaneously rotating the beaker on a turntable (30 sec, 2.2 g; ITI = 2.5 min). Animals spent the ITI in a beaker of unflavored seawater. Unpaired trials were identical to paired, except that chemosensory exposure and rotation were always separated by 30 - 90 sec. Bite strike latencies were again measured to CS_P (or CS_{UP}) and CS_{NP} at 1.5 and 24 hr retention intervals. Pairings resulted in significant increases in both CS_P and CS_{NP} bite-strike latencies at 1.5 and 24 hr (see Table 1); CS_P latencies were significantly longer than CS_{NP} at 1.5 hr retention interval, however. Unpaired presentations resulted in significant increases to CS_{UP} at 1.5 but not 24 hr; CS_{NP} latency increased significant at 24 hr. Comparisons of CS_P vs. CS_{UP} latencies revealed these to differ significantly at 24, but not at 1.5 hr. Thus, at 24 hr post-conditioning, suppression of bite-strike behavior is both stimulus- and pairing-specific.

Table 1
Bite-strike latencies (sec)

	Paired (n=26)						Unpaired (n=23)					
	pre		post (hr)				pre		post (hr)			
			1.5		24				1.5		24	
	CS_P	CS_{NP}	CS_P	CS_{NP}	CS_P	CS_{NP}	CS_{UP}	CS_{NP}	CS_{UP}	CS_{NP}	CS_{UP}	CS_{NP}
X	5.9	6.4	17.3*	9.8*	13.3*†	9.9*	6.5	6.1	13.3*	7.4	8.6	9.5*
S	5.8	6.2	10.9	8.0	9.6	9.6	4.8	6.1	11.2	5.3	8.9	8.5

* $p < .05$, pre vs. post; † $p < .05$, paired vs. unpaired

Supported by NSF BNS-8316707 to J. Farley.

237.8 AMINERGIC INPUTS IDENTIFIED IMMUNOCYTOCHEMICALLY MODULATE cAMP LEVELS IN AN OLFACTORY PROCESSING CIRCUIT. T. Yamane*, A. Gelperin & K. Delaney* (SPON: M. Lampert). Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

The procerebrum (PC) of the terrestrial slug, Limax maximus, contains on order 5 x 10^5 local interneurons. These interneurons are densely interconnected synaptically (Zs.-Nagy & Sakharov, 1970) and receive direct axonal projections from fibers in the olfactory nerve. Since behavioral experiments indicate that Limax can form both short term (minutes) and long term (weeks) odor memories, we are investigating the computational characteristics of the PC as a possible locus for odor memory formation and storage.

To determine the neurotransmitter content of intrinsic cells and extrinsic fibers in the PC, 30 µm sections of the PC were exposed to antisera raised against serotonin, dopamine and small cardioactive peptide B (SCP_B). FITC, Texas Red or HRP labelled secondary antibodies were used to reveal sites of serotonin-like, dopamine-like and SCP_B-like immunoreactivity (SLI, DLI, SCP_BLI, respectively). Fibers showing prominent SLI and DLI were evident throughout the neuropil and cell body layers of the PC. No intrinsic cells were found to show SLI or DLI. SCP_BLI, however, was localized in both scattered intrinsic cells (8-10 µm) and extrinsic fibers. SLI and SCP_BLI did not appear to be present in the olfactory nerve projection to the PC.

The first functional assessment of the effect of serotonin(5HT) and dopamine (DA) on the PC was made by measuring their affect on the level of cAMP in the lobe. Freshly isolated lobes were incubated in saline containing DA or 5HT (50-100µm) and cAMP determined by RIA on aliquots of PC homogenate. Protein determinations were also done so that results were compared as pmoles cAMP per µgm protein. 5HT reliably increased cAMP in the PC by up to 20 fold. The elevation was transient, with peak cAMP occurring 0.5-1.0 minutes after 5HT application. DA also elevated cAMP transiently, but only by a factor of 2-3. Incubation of the PC with amine and a phosphodiesterase inhibitor (IBMX, 1 mM) prolonged the peak of cAMP elevation.

These results will be extended by examining the effect of SCP_B on cAMP in the PC and used to optimize electrophysiological assessment of the effects of these three neurotransmitters on synaptic transmission and information processing in the PC (Gelperin, Tank & Chiel, 1986).

Gelperin, A., Tank, D. W., Chiel, H. J., 1986, Soc. Neurosci. Abstr. 12.

Zs.-Nagy, I. & Sakharov, D. A., 1970, Tissue and Cell, 2:399-411.

237.9 CELLULAR AND SYNAPTIC RESPONSES RECORDED FROM AN OLFACTORY PROCESSING CIRCUIT. A. Gelperin, D. W. Tank and H. J. Chiel (SPON:H. Feder). Molecular Biophysics Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974

The procerebrum (PC) of the terrestrial slug *Limax maximus* has a distinctive architecture. The half million cell bodies are all located in the distal pole of the PC, separated from the main body of the cerebral ganglion by a large neuropil in which extensive synaptic interconnections occur. The neuropil receives a direct projection from a tract of fibers in the olfactory nerve (ON), suggesting the involvement of the PC in olfactory information processing. We initiated these studies to determine the tractability of the PC for electrophysiological analysis.

A preparation of the cerebral ganglion with the PC completely desheathed and the ON dissected free from its origin at the digitate ganglion was mounted in a chamber on the stage of an inverted compound microscope. The distal end of the ON was held in a suction electrode and stimulated with pulses of 0.2-2.0 volts, 2.0 msec duration. Spontaneous or evoked extracellular currents were recorded with a virtual ground circuit using a 5 µm patch microelectrode positioned at various levels within the cell body or neuropil layers. The boundary between these layers as well as the outlines of cells on the exposed distal surface of the PC were clearly visible.

A single shock to the ON produced a sequence of extracellular currents, as shown in a typical recording from the neuropil (Fig. 1A). Such single stimuli elicited current fluctuations composed of an early phase which persisted in high Mg^{++} medium, and a late phase which was eliminated by high Mg^{++} (Fig. 1B). Loose-seal extracellular patch recordings from neuronal somata in the PC showed action potential-like waveforms, which on occasion were elicited by ON stimulation and occurred at a time coincident with the late phase of the evoked neuropil response.

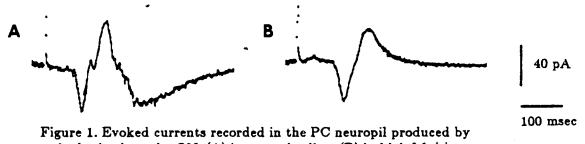


Figure 1. Evoked currents recorded in the PC neuropil produced by a single shock to the ON. (A) in normal saline, (B) in high Mg^{++}.

A second class of events was observed in neuropil recordings. Spontaneous slow oscillations (~ 0.5 Hz) of extracellular current flow were recorded in normal saline (Bullock, 1945). In high Mg^{++} medium, these slow oscillations disappeared and short trains (~4 sec duration) of rhythmic current flows (~ 5Hz) were observed. These trains of rhythmic currents could be triggered by ON stimulation.

A population of individual neurons was mechanically dissociated from the cell body layer of the PC. The isolated cells adhered to polylysine coated dishes and remained viable for 1-2 days in normal saline. Three size classes of neurons were observed. Spontaneous action potentials were recorded from isolated PC neurons with cell-attached patch microelectrodes.

These studies demonstrate the tractability of the PC for system and cellular analysis (see also Yamane, Gelperin & Delaney, 1986). They represent a first step in the analysis of olfactory processing in the *Limax* PC.

Bullock, T. H., 1945, *Yale J. Biol. Med.* 17:657-679.

Yamane, T., Gelperin, A., Delaney, K., 1986, Soc. Neurosci. Abstr. 12.

237.10 **PREY AVOIDANCE LEARNING IN Pleurobranchaea californica.** R.-C. Huang* and R. Gillette. (SPON: A.N. Spencer) Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

Neural mechanisms of associative learning in Pleurobranchaea have been studied using laboratory paradigms where food-avoidance behavior is learned through the association of food stimuli with electric shock. Although food-avoidance is of likely adaptive significance to foraging by this predator, no natural model has been known. We find that Pleurobranchaea quickly learns to avoid predation on the aeolid nudibranch Flabellinopsis iodinea, one of many aeolids which carry stinging nematocysts in their cerata. Upon initial presentation, all of 24 subjects vigorously bit the aeolids but quickly rejected them from the buccal cavity. A bout of cyclic egestion movements of the radula typically followed, lasting up to 1.5 minutes, itself followed by active avoidance locomotion. In 6 subsequent 2 min. trials separated by 10 min. intervals, animals showed increasing latencies in proboscis extension and biting; by trial 7, 79% of animals had failed to bite and show active avoidance at least once; 50% on the last trial . In a 2nd day of training 90% showed avoidance without biting. Avoidance learning was highly selective: latencies and thresholds of feeding responses to an homogenate of squid were unchanged. Retention was demonstrable 4 days after training.

A procession of 5 behavioral stages could be defined in animals learning full abstention from Flabellinopsis. In terms of proboscis extension (E), the bite (B), rejection (R), "tasting" with the lips of the proboscis (Ta), withdrawal of the head region (W), and active avoidance (AA), the stages are: 1) E --> B --> R --> AA; 2) E --> Ta --> B --> R --> AA; 3) E --> Ta <--> W--> AA; 4) E --> W --> AA; and 5) W --> AA.

Our observations are compatible with a neural model based on simultaneous sensory excitation of and mutual inhibition between motor networks for feeding and avoidance (Soc. Neurosci. abs. 11, 78.2, 1985). A neural mechanism of food avoidance learning in Pleurobranchaea (London & Gillette, PNAS in press) can be incorporated into that model, based on suppression of feeding by learning induced hyperexcitability in elements of the feeding oscillator which act to lock the oscillator in a retraction phase. Thus, in the trained animal learned suppression of feeding releases latent avoidance behavior. Predictions and tests of the model will be presented. Supported by NSF BNS 83-08551.

237.11 CHANGE IN DUM (OCTOPAMINERGIC) NEURON ACTIVITY ACCOMPANIES POSTURAL LEARNING IN THE LOCUST. S.Sombati*, S.A.Chesnut*, M.Woollacott, G.Hoyle+ and R.R.Forman. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The metathoracic ganglion contains a cluster of about 30 dorsal unpaired median (DUM) neurons, some of which are confined to the CNS while others supply neurosecretory-type endings on skeletal muscles. They release octopamine which modulates neuromuscular transmission and muscle tension. These neurons have bifurcating axons exiting the ganglion on both sides. Each neuron has 3-5 spike-initiating zones and one can be independent of the others. All have central branches. Their central functions are unknown but octopamine has been shown to have a central modulatory role, including arousal.

We examined the role of DUM neurons in leg position learning of the locusts Schistocerca americana and Schistocerca gregaria using modified Forman paradigm. The locusts were immobilized, except for one femoro-tibial joint of a hind leg, and dissected dorsally to expose the metathoracic ganglion to permit intracellular recordings from DUM neurons. An on-line computer was used to monitor the joint angle and train the animal by operant conditioning using both heat to the contralateral leg and low frequency sound as negative reinforcement. The trained metathoracic tibia occupied a position window, preset in either extension or flexion, away from rest position. Both flexion and extension learning can be achieved in the same animal in separate sessions. Furthermore, various degrees of flexion or extension learning can be carried out. Cutting both the anterior and the posterior connectives to the ganglion did not impair its ability to learn.

Activities of 12 different DUM neurons were examined during the training sessions. Before training, DUM neurons showed spontaneous activity of soma spikes with the frequency of about .1-.5 Hz. Most of them showed an increased activity during training and usually a few brief bursts (5-20 spikes) occurred about 1/2-1 second before the tibia occupied a desired window. The activity then ceased upon learning and the spikes reappeared 10-20 seconds later, but at lower frequency than that observed before training. In a few neurons the activity recovered to a higher frequency (up to 4 fold). The spikes which reappeared were of axonal type as characterized by attentuated amplitude without large undershoots. This was a more prominent feature when a shift in basal tonus was the learning strategy used by the animal to achieve extreme flexion or extension.

We conclude that changes in DUM neuron activity correlate with leg position learning of the locust. The role of these DUM neurons in learning will be discussed. Supported by NIH grant 1RO1NS21033 and a grant from the Whitehall Foundation.

237.12 NEURAL CORRELATES OF THE PROBOSCIS EXTENSION RESPONSE IN THE BLACK BLOWFLY, *PHORMIA REGINA*. R.S. Edgecomb and L.L. Murdock. Department of Entomology, Purdue University, W. Lafayette, IN 47907.

The black blowfly can taste with contact chemosensory hairs on its legs and on its labellum. Stimulation of these sensilla with a suitably sweet solution will elicit proboscis extension. Each sensillum contains five receptors. Four of these are taste receptors sensitive to different substances; their dendrites extend into the lumen of the hair. The fifth is a mechanoreceptor situated at the base of the hair. To determine where the axons from these taste hairs project in the central nervous system (CNS), selected hairs were cut and stained with cobaltous chloride. The hairs chosen were those most likely to contact a potential food source, namely, the most anterior (1), middle (6) and most posterior (11) of the 11 "largest" labellar hairs, and the D hairs from the 5th tarsomere of each leg.

The central projections of axons from labellar hairs 1, 6 and 11 are limited to the subesophageal ganglion (SOG). Axons from hairs 1 and 11 project medially and have mutiple contralateral branches. Axons from hair 6 remain in the ipsilateral hemiganglion and have one dorsal contralateral projection. The technique used for staining sensory neurons from labellar hairs often resulted in single axons staining in the CNS, thereby permitting the characterization of particular cell types for each hair. Each of the three labellar hairs gives rise to one axon which projected into a common dorsal region of the SOG having both a contralateral projection and an ipsilateral projection coursing dorsolaterally into a region between the posterior antennal center and the SOG.

Sensory neurons from taste hairs in the leg project primarily to the ipsilateral side of the corresponding neuromere in the fused thoraco-abdominal ganglia. From each leg nerve a bundle of axons projects anteromedially from their respective neuromeres, joining axons from all legs from one side, and then passing into the ipsilateral half of the SOG via the ventromedial region of the cervical connective. These fibers project to the same region as the dorsolateral branch of the common labellar axon. Staining of axons from single D hairs on the 5th tarsomere revealed that only one of the axons from each D hair projects into the SOG. All other axons remain in the ipsilateral half of the corresponding neuromere.

Extension of the proboscis results from the contraction of three muscles— the protractor of the fulcrum (ProFul), the adductor of the apodeme (AdA) and the extensor of the haustellum (ExHst). The motoneurons innervating these muscles were identified by filling appropriate cut nerves with cobaltous chloride using either a petrolatum well or a polyethylene suction micropipette. Four motoneurons with cell bodies in the ipsilateral side of the SOG innervate the AdA and ExHst via the maxillary nerve. Their cell body locations are variable, ranging from the root of the labellar-maxillary nerve to the dorsolateral region of the SOG. The central projections of these motoneurons are primarily in the ipsilateral hemiganglion in the dorsolateral, posterior and ventromedial neuropil. A few ventromedial branches project contralaterally. Filling the branch of the labro-frontal nerve, which innervates the ProFul, with cobalt revealed one motoneuron having a cell body near the root of the labrofrontal nerve. The central projections of this motoneuron follow a similar pattern to the other extension motoneurons in the maxillary nerve. This motoneuron differs from the other extension motoneurons in having more extensive contralateral ventromedial projections and an anteriorly projecting branch arising from the ventromedial branch along the midline.

237.13 REGULATION OF TARSAL AND LABELLAR THRESHOLDS IN THE BLACK BLOWFLY, PHORMIA REGINA. L.C.Sudlow *, R.S.Edgecomb, and L.L.Murdock. Department of Entomology, Purdue Univ., W.Lafayette, IN, 47907.

When the tarsal or labellar chemosensory hairs of the black blowfly touch an appropriate feeding stimulus, e.g., aqueous sucrose, the fly extends its proboscis to feed. Tarsal and labellar thresholds can be determined as the mean acceptance threshold (MAT), the logarithm of the minimum sucrose concentration that elicits proboscis extension from the average fly. Tarsal MAT's are highest after a meal and decline during starvation. Labellar thresholds have received little systematic study.

When 3-day-old starved flies were fed single 3.7 or 15 ul meals of 250 mM sucrose, tarsal and labellar MAT's both rose. Tarsal and labellar MAT's peaked 10-30 min after the meal, then declined at similar rates over the next 5.5 hr. For each meal volume, tarsal MAT's were higher than labellar MAT's at every time point.

In experiments designed to explore the relationships among crop weight and labellar and tarsal MAT's, flies were allowed to feed ad libitum on either 62.5 or 250 mM sucrose for 4-5 days, and were then removed from the food. Crop weights and labellar and tarsal MAT's were determined at times ranging from 1 hr to several days of deprivation. With flies fed either 62.5 or 250 mM sucrose, both tarsal and labellar MAT's were highest 1 hr after removal from food, and subsequently declined at similar rates. For each concentration of sucrose fed, crop weights were highest at the earliest times, and declined logarithmically thereafter.

Information from the foregut and abdominal stretch receptors, carried via the recurrent nerve (RN) and the median abdominal nerve (MAN) respectively, play an important role in meal size regulation. To determine whether these nerves have a role in labellar threshold regulation, either nerve was transected in flies previously fed 250 mM sucrose for 4-5 days. RN-transection caused an immediate and significant decrease in tarsal MAT but not in labellar MAT. MAN-transection had no significant effect on either labellar or tarsal MAT's. To confirm that RN affects tarsal but not labellar thresholds, RN-transected, starved flies were fed a single 15 ul meal of 250 mM sucrose. RN-transection did not prevent the post-meal elevation of labellar MAT's, but tarsal MAT's, although significantly higher than prefeeding MAT's, were significantly lower than MAT's in sham-operated flies.

These results suggest that tarsal and labellar taste thresholds in the blowfly are regulated in similar, but not identical ways. Crop weight appears to have an important relationship to both tarsal and labellar thresholds. However, the information from the foregut stretch receptors affects only the tarsal threshold pathway. Information from the abdominal stretch receptors has no effect at all either on tarsal or labellar thresholds. These observations suggest the existence of as yet unidentified mechanisms governing tarsal and labellar thresholds.

238.1 AUTORADIORGRAPHIC LOCALIZATION OF TRITIATED FUCOSE INCORPORATION MIRRORS LECTIN BINDING PATTERNS IN THE EARLY POSTNATAL MOUSE BRAIN. D.A. Steindler and N.G.F. Cooper. Dept. of Anatomy and Neurobiology, Univ. of Tennessee/Memphis, Memphis, TN 38163.

Glycosylated molecules have been presumed to play roles during central nervous system (CNS) morphogenesis. Previous biochemical studies (Dutton and Barondes, J.Neurochem.,17:913, '70) have also determined that systemic administrations of radiolabeled glycans, such as fucose, become incorporated in glycoproteins in developing mouse brain. Postnatal day 2 ICR mice were anesthetized and received intraperitoneal injections of 3H fucose (NEN), and survived 1hr-19 days at which time they were cardiac perfused with mixed aldehydes. Vibratome sections were incubated in peroxidase-labeled lectins such as lotus tetragonolobus (specific for alpha-L-fucose) or processed for light microscopic autoradiography. Following survival times of 1hr., 3H fucose autoradiographic labeling, seen with darkfield illumination, is primarily associated with CNS blood vessels and meninges, although there is a light, uniform labeling throughout the CNS. By 3 hr. post-injection, a characteristic image of subcortical boundaries already appears, and is maximal by 27 hr. where, for example, the molecular layers of the developing neocortex and cerebellum are intensely labeled. 3H fucose labeling reveals patterns unique to every CNS structure, and they relate to cytoarchitectonic boundaries and developing substructural arrangements (e.g. five rows of labeling in the trigeminal complex and thalamic barreloids related to mystacial vibrissae). Higher resolution brightfield microscopy reveals the label to be predominantly associated with undefined elements of the neuropil rather than being concentrated over neuronal somata. Between 5 and 8 days postinjection, the intensity of labeling is beginning to diminish, with the band of molecular layer labeling in the cerebellum still present. By 19 days, most patterns are no longer visible. The histochemical image of lectin binding patterns in all of these cases is identical to the maximal images of radiolabeled fucose incorporation. These findings support a notion that particular glycoconjugates, involved in recognition phenomena related to pattern formation, are degraded in the third postnatal week (also see Reeber et al., J.Neurochem.,35:1273,'80). The finding that astrocytes are a major site of 3H fucose incorporation in the adult brain (Reisert et al.,J.comp.Neurol. 176:453,'77), in addition to our previous findings on lectin labeling related to glia (Steindler and Cooper,J.comp.Neurol.249, '86; Cooper and Steindler, Brain Res.,'86), support a hypothesis that glia sequester the infused radiolabeled glycan observed here, and synthesize glycosylated molecules that convey pattern formation cues. Supported by grants NS-20856 and EY-02708.

238.2 GRADIENTS OF GLYCANS PRESENT IN THE DEVELOPING MOUSE STRIATE CORTEX. N.G.F. Cooper and D.A. Steindler. Dept. of Anatomy and Neurobiology, Univ. of Tennessee/Memphis, Memphis, TN 38163

Lectins and antibodies have been used to show correlations between the existence of patterns of intrinsic molecules and the development of cytoarchitecture within the somatosensory cortex (Cooper and Steindler, J.comp.Neurol.,'86; Brain Res.,'86). In this study, patterns of lectin binding and 3H fucose incorporation in the mouse striate cortex are compared at the light microscopic level, and lectin binding sites are also evaluated at the electron microscope level. The peroxidase conjugated lectins specific for either fucose, mannose, galactosamine or glucosamine were used to examine the disposition of glycans in aldehyde-fixed parasagittal sections of cortex from postnatal day 1 through adult mice. Autoradiographs were prepared following injections of 3H fucose in early postnatal mice allowing for a 1 day survival time. The disposition of glycoproteins seen in autoradiographs mirrored the patterns detected with all of the lectin-peroxidase conjugates and show that glycans in this region of the cortex are concentrated in 4 laminae which alternate with 4 glycan-poor laminae. This pattern differs from that seen in thin sections, where lectin binding sites are more uniformly dispersed throughout the cortex. Therefore, the lectin-positive laminae seen in thick sections are areas of high concentration of glycans and a gradient of glycans is present between them. Under the EM, cross sections of lectin-positive circular profiles, suggestive of growth cones, are present in regions of neuropil where most immature neurites reveal only plasma membrane-associated binding sites. The growth cone-like profiles contain lectin-positive vacuoles and we hypothesize that these profiles represent glial processes that are secreting lectin-positive material observed between axons, dendrites, and cell bodies. The lectin-positive material associated with all plasma membranes appears matrix-like. Glial-associated lectin binding can be detected in the adult CNS with WGA, but it is more discrete, being more closely associated with glial processes that surround synaptic terminals and not generally present throughout the neuropil or enriched in laminae. Also in the adult, a lectin-positive lattice can be seen covering the surface of neuronal cell bodies and dendrites with lectin-negative sites, representing sites of synaptic apposition. We hypothesize that the lectin binding sites represent a glycan 'bridge' between neurons and glia and we predict that endogenous receptors for glycans must exist on both glial and neuronal cell membranes. During development of the visual cortex, these glycans could play a role in sorting neurons into laminae or in mediating selective neuronal interactions between the cells of presumptive laminae. Supported by EY-02708 and NS-20856.

238.3 A MONOCLONAL ANTIBODY (WCC4) IDENTIFIES DEVELOPMENTALLY REGULATED GLYCOLIPIDS IN THE RAT CNS. S.F. Suchy*, M. Yamamoto, L. Barbero* and G.A. Schwarting* (SPON: L.M. Hemmendinger). Department of Biochemistry, E.K. Shriver Center, Waltham, MA 02254

A monoclonal antibody (WCC4), raised against a suspension of rat pheochromocytoma (PC12) cells, recognizes a ganglioside with a developmentally regulated pattern of expression in the CNS of the rat. Immunohistochemical and biochemical studies of gangliosides isolated from PC12 cells and rat brain reveal the WCC4-reactive ganglioside to be α-galactosyl, α-fucosyl GM1. Treatment of the ganglioside extracts with α-galactosidase results in a loss of WCC4 immunoreactivity, whereas no loss in immunoreactivity is observed after treatment with other glycosidases (endo-ß-galactosidase, ß-galactosidase, ß-hexosaminidase, α-fucosidase or neuraminidase). The expression of this ganglioside during development was examined by TLC immunostaining of lipid extracts from embryonic and postnatal rat brain (E13 to adult). The ganglioside, undetectable in embryonic brain, appears at PO and increases in concentration through the first several postnatal weeks until approximately P26, after which it decreases slightly to adult concentrations. WCC4 also reacts postnatally with at least two neutral glycolipids which are developmentally regulated and which have different temporal patterns of expression. In addition, Western blot analysis of whole brain homogenates reveals that the WCC4-reactive epitope is present on two prenatally expressed glycoproteins at E17, but that immunoreactive glycoproteins are absent at P6 and in the adult.

Immunocytochemical studies of the adult rat brain reveal that intense, diffuse immunoreactivity is present in the stratum lacunosum moleculare of the hippocampus, in the stratum moleculare of the dentate gyrus, in the molecular layer of the pyriform cortex and the entorhinal cortex, and in the olfactory tubercle. There is also a punctate staining of neurons in the entire brain. Immunohistochemical analysis of WCC4 reactivity during development reveals that the punctate staining of neurons is present from E17. However, the anatomically restricted, diffuse immunoreactivity which is present in the adult does not appear until P3, and it resembles that of the adult by P6. Studies to investigate whether WCC4 immunoreactivity is associated with specific presynaptic, postsynaptic or glial elements are in progress.

238.4 IMMUNOHISTOCHEMICAL LOCALIZATION OF UNIQUE C-TERMINAL PEPTIDES OF β-TUBULIN IN DEVELOPING RAT BRAIN. W. S. T. Griffin and I. Ginzburg. *Univ. of Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235 and Weizmann Inst., Rehovot, Israel 76100.*

The diverse functions of microtubules include chromosomal movement, cell division and migration, creation and maintenance of cytoarchitecture, intracellular transport, and secretion. This diversity suggests possible heterogeneity of the tubulin isoforms assembled into into microtubules and their distribution. Progressive changes in cell shape during neuronal morphogenesis depend on the redistribution and possible interconnections of microtubules with other cytoskeletal elements. This is further evidence of the necessity for regulation of the synthesis and/or assembly of heterogeneous tubulin isoforms necessary for cell differentiation and function. In organs composed of many different cell types, *e.g.*, brain, microtubule heterogeneity could be a reflection of cell type diversity. There is region-specific differential distribution of tubulin isoforms in the brain, but there is also expression of multiple tubulin isoforms in individual neural cells. Some of the isoforms are derived from the family of tubulin genes, but those in excess of gene number must be the result of posttranslational modification. We have sequenced 3 unique rat β-tubulin cDNAs and found an interspecies regional heterogeneity within the 15 amino acids in the C-terminal domain. We hypothesized that the divergence reflects different functional properties and, if so, that such sequences could be cell type specific and/or have unique intracellular locales. To test this hypothesis, we synthesized and raised antibodies against a number of peptides spanning the 15 amino acids in the C-terminal region of some of the β-tubulin isoforms. Two peptides designated 1 and 2, correspond to porcine β-tubulin positions 416-425 and 416-431, respectively. A third, designated peptide 4, corresponds to rat β-tubulin positions 426-445. The antibodies were characterized by immunoblotting, indirect radioimmunoassay and peroxidase-antiperoxidase staining. Radioimmunoassay and immunoblot analysis showed that these antibodies cross reacted to some extent with each other and β-tubulin but did not cross react with α-tubulin. Control serum was nonimmunoreactive. Immunohistochemical localization of the antibodies with their immunoreactive products showed that Peptide 1 preferentially stained axons, Peptide 2, neurons and axon terminals, and Peptide 4, glia. We will show developmental variations of this preferential staining. *Supported in part by NIH AI-14668 and AG-05587.*

238.5 DEVELOPMENTAL EXPRESSION OF CALCITONIN GENE RELATED PEPTIDE ON CEREBRAL ARTERIES. S.H. Tsai*, J.M. Tew, Jr.*, and M.T. Shipley (SPON: R. Cardell), Departments of Anatomy/Cell Biology and Neurosurgery, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0521

Calcitonin gene-related peptide (CGRP) is a peptide recently discovered by recombinant DNA technology. CGRP has been found in the central and peripheral nervous system and on vessels where it is a most potent vasodilator. We have recently demonstrated that CGRP fibers from the ipsilateral trigeminal ganglion (V_1) heavily innervate the arterial wall of anterior cerebral circulation in adult rats. The present study investigates the developmental expression of CGRP on cerebral arteries in newborn rats.

Immunocytochemical staining with CGRP antisera (generously supplied by W. Vale, Salk Institute, California) was performed on whole-mount cerebral arteries. The day of birth is called postnatal (PND) 1. On PND 1-2, CGRP is found only on the wall of anterior cerebral (ACA) and anterior communicating arteries (ACoA). The fibers are largely confined to one or two longitudinally oriented bundles, but in all cases a few fibers take a zigzag course around the vessel. On PND 3-4, the number of fibers increases on the wall of ACA and ACoA and first appear on the carotid (CA) and middle cerebral arteries (MCA). On PND 15-16, the innervation pattern becomes a meshwork of longitudinal and circumferential fibers on the wall of ACA and ACoA, the pattern typical of adult animals; the density of fibers increases on the wall of CA and MCA. At this time, a few CGRP fibers are found on the wall of basilar and vertebral arteries. By PND 64, the overall distribution of GCRP on the wall of cerebral arteries appears similar to the adult.

The expression of CGRP in fibers innervating cerebral arteries appears to begin at or near birth. The cerebral arteries in the anterior part of the circle of Willis (ACA and ACoA) receive the first fibers and throughout development have the densest innervation. ACA and ACoA supply the anterior hypothalamus, stratum, medial and ventral sides of cerebral hemisphere and olfactory bulbs. The early development of CGRP expression in these cerebral arteries may be related to regulation of blood flow to the hypothalamus, the basal forebrain and olfactory cortical structures and, therefore, may play a role in supporting homing and feeding behaviors in developing animals.

Supported by NS 23348, USADAMD 17-86-C-6005 and UC Neuroscience Training Program.

238.6 DIFFERENTIATION PATTERNS IN THE MEDIOBASAL HYPOTHALAMUS AND IN THE PITUITARY OF THE RAT FOR STAGE SPECIFIC EMBRYONIC ANTIGEN 1 AND ALKALINE PHOSPHATASE. E.A.J.F. LAKKE*, E. MARANI* and A.H. EPEMA* (SPON: T. van Wimersma Greidanus). Lab. of Anatomy and Embryology, NL 2333 AL Leiden, The Netherlands.

The development of the mediobasal hypothalamus (MBH) of the rat was studied with the use of a panel of monoclonal antibodies (MAB) and enzyme histochemical techniques (Marani, E., Progress Histo-Cytochem., 16-4:1-170, 1986). Of these results those obtained with the use of MAB 3B9 immunocytochemistry and alkaline phosphatase (AP) histochemistry will be presented. MAB 3B9 is specifically directed against fucosyl-N-acetyl-lactosamine, also known as stage specific embryonic antigen 1 (SSEA1).

At 12 hour intervals from the 10th day post-coitem (pc) onwards rat embryos were removed from the uterus under deep aether anaesthesia. Cryostate serial sections with a thickness of 16 µM were made. All sections were intercepted on chrome-alumn subbed glass slides. MAB 3B9 was employed using the peroxidase-antiperoxidase technique. AP histochemistry was done according to Marani (Progress Histo- Cytochem., 16-4:1-170, 1986).

At day 16 pc labeling of SSEA1 was found in the lateral pre-, supra- and retrochiasmatic areas. In the rostral infundibular area diaminobenzidine reaction product was localized immediately periventricular, while in the caudal infundibular area the label was seen in the medial part of the MBH. In the ventral midline the ventricular matrix and the mantle layer were free of SSEA1 in all areas mentioned.

From the 18th day pc onwards SSEA1 was found in the periventricular matrix of the prechiasmatic area, continuous with labeling of the suprachiasmatic nucleus (SCN). Dorsal from the SCN the peri- and paraventricular nuclei were positive. In the retrochiasmatic area labeling was again found periventriculary, though not continuous with the labeling of the SCN.

While AP was complementary to SSEA1 in the lateral and medial MBH and in the arcuate nucleus, it was co-localized with it in the SCN and in the peri- and paraventricular nuclei.

In the pars intermedia of the pituitary a zonal pattern was observed for both SSEA1 immunoreactivity and AP histochemistry. The epithelial layer neighboring the neurohypophysis was alternately labeled, giving the impression of a longitudinal pattern. In the anterior lobe some scattered labeling was seen for SSEA1 and AP.

The shift of SSEA1 during development from lateral to medial in the MBH of the rat is thought to represent a differentiation gradient. The appearance of a striped pattern in the pars intermedia of the pituitary is unique.

238.7 DISTRIBUTION OF ACETYLCHOLINESTERASE AND SUBSTANCE P IMMUNOREACTIVITY IN THE DORSAL THALAMUS OF THE DEVELOPING RAT. Cheryl L. Lieu*, Richard T. Robertson and Jen Yu. (SPON: E.A. Davis, Jr.) Departments of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

We have reported that the primary sensory relay nuclei of the dorsal thalamus in the developing rat display transient patterns of acetylcholinesterase (AChE) activity in the first two postnatal weeks of life (Robertson et al., Neurosci. Abstr., 1986). AChE activity in the ventrobasal complex (VB), dorsal nucleus of the lateral geniculate body (dLGN), and ventral nucleus of the medial geniculate body (vMG) appears shortly after birth, reach peak intensity at about 5-9 days of postnatal age (DPN) and decline to adult levels during the third postnatal week. The function of the transient AChE is unknown, but several possibilities exist. In addition to its hydrolytic role for acetylcholine, AChE has also been shown to act as a catabolic enzyme for substance P (Chubb et al., Neurosci., 1980). The present study is a comparison of the distribution of AChE and Substance P in the developing rat dorsal Subjects were laboratory born Long-Evans or Sprague-Dawley rats of 0-60 DPN. Animals were anesthetized and perfused with 0.9% saline at 37°C, followed by 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4 and at 4°C. Frozen sections of 20-40 um were taken through the dorsal thalamus. Series 1 was processed for immunocytochemical localization of substance P, series 2 was processed for AChE histochemistry using a modification of the technique of Koelle, and series 3 was processed for a Nissl stain.

Intense patterns of AChE activity were characteristic of VB, dLGN and vMG, and also of the anterior dorsal nucleus. Substance P immunoreactivity was absent in each of these regions, the nuclei appearing markedly pale against light to moderately stained neuropil in the surrounding thalamic regions. Absence of Substance P immunoreactivity probably was not due to Substance P catabolism by AChE; animals treated with the AChE inhibitor DFP showed the same pattern of Substance P immunoreactivity as did normal animals. Thus, the pattern of Substance P immunoreactivity appears as a reverse image of the pattern of AChE histochemical reactivity in the dorsal thalamus of the developing rat.

238.8 POSTNATAL DEVELOPMENT OF SEROTONIN-IMMUNOREACTIVE FIBERS OF THE SPINAL CORD OF THE RAT. R.B. Aramant[1] and L.T. Giron, Jr.[1,2]. [1] Neurology Dept., University of Kansas, Kansas City, KS and [2] Spinal Cord Research Lab., VAMC, KC, MO 64128.

Early studies of the development of serotonergic fibers employed fluorescence of native indoleamines. More recent immunocytochemical studies either examined adult anatomy or else concentrated on development of ascending serotonergic connections. The present study employs sensitive serotonin immunocytochemical means to describe the postnatal development of serotonergic fibers in the rat spinal cord.

The distribution of serotonin-immunoreactive (SI) fibers of rat spinal cord was studied in the cervical, thoracic and lumbar regions at birth, 6, 14, 30 and 90 days of age. At birth, SI fibers were widely distributed in all regions of the cord.

In gray matter, extensive networks of fine beaded fibers were present throughout except in dorsal zones of the dorsal horn. Regions surrounding alpha-motor neurons were densely innervated at all levels; in the zona intermedia of the thoracic cord, fibers innervating the dorsal autonomic nucleus, the intermediolateral cell column (IML) and the transverse connections between them were periodically organized. With maturation, staining became concentrated in the latter regions; but, elsewhere in the gray matter, both the density and extent of networks of fine fibers diminished except in laminae I and II of the dorsal horn.

In the white matter at birth, SI fibers were distributed in lateral and ventral funiculi, and also radiated from the IML and the ventral horn. With maturation, the majority of fibers in the lateral funiculus assumed a marginal location and, together with the dense fibers near the ventral sulcus, receded markedly in number; but fibers radiating from the IML to white matter became more numerous and more densely stained. In contrast, pargyline treatment of a 90-day animal revealed marginal-pial fibers whose distribution recalled that at 6 days.

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238.9 ONTOGENY OF TYROSINE HYDROXYLASE AND NEURAL-SPECIFIC PROTEIN 4 (NSP4) IMMUNOREACTIVITY IN THE MESENCEPHALON FROM MOUSE EMBRYOS. U. di Porzio, D.B. Murphy* and J.L. Barker. Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md; Institute of Protein Biochemistry and Enzymology, CNR, Naples, Italy.

The ontogeny of tyrosine hydoxylase (TH)-like immunoreactivity was studied during the early development of the mouse embryo in order to detect the earliest appearence of catecholamine neurons in the midbrain. In contrast to others we found that TH could be first detected immunocytochemically in brain sections at E 8.5, in perikaria localized in the caudal periventricular zone. At E 10 TH-like immunoreactivity was detectable in perikaria and axons of mesencephalic neurons (putative dopamine neurons) exibiting an anatomical distribution similar to that seen in the adult substantia nigra. At this stage of development, no TH-positive fibers could yet be visualized in the striatum. Sparse TH+ fibers appeared in the striatum at E 13 and E 15. The early TH+ neurons (E 8.5) did not express the neural-specific protein NSP4, an antigen recognized by a rat monoclonal antibody against mouse cerebellar membranes. The NSP4-immunoreactivity appeared first at E 10. It was not restricted to TH+ neurons or the mesencephalic region alone, although it colocalized with TH+ neurons. We studied NSP4 distribution and developmental expression in mouse embryonic brain sections and dissociated cell suspensions. Previously (di Porzio et al. Neurosci. Abstr. 11:978, 1985) we showed that NSP4 recognized in vitro a surface glycoprotein (mw 270-300 K) expressed on the intact external membrane (unfixed cells) of a restricted neuronal subpopulations (15-20% of the neurons from 1-day to 3-week-old mesencephalic cultures). It was not detectable in glial cells (GFA+) or oligodendrocytes (galactocerebroside+). Flow cytometric analysis showed that the NSP4+ population accounts for 20-25% of freshly dissociated cells from mesencephalon, basal ganglia and other areas of the neuraxis, at the various stages of embryonic development; the antigen is present on the surface of living cells. Flow cytometric analysis was also applied to examine the uprise of TH-like immunoreactivity in the mesencephalon and to quantify the ontogeny of TH+ neurons from individual mouse embryos throughout the early embryonic development, using fixed cell suspensions and anti-TH indirect immunofluorescence.

238.10 CELLULAR RELATIONSHIPS OF TYROSINE HYDROXYLASE IMMUNOREACTIVE NEURONS WITHIN THE DEVELOPING RAT ARCUATE NUCLEUS. M. Piotte*, A. Beaudet and J.R. Brawer* (SPON: S. Gauthier). Departments of Anatomy and Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

The fine structure and cellular relationships of tuberoinfundibular dopaminergic (TIDA) neurons were examined by electron microscopic immunocytochemistry in neonatal, 15 and 30 day old, and adult Wistar female rats, by means of the PAP method, using anti-tyrosine hydroxylase (TH) antibodies (Tong H. Joh, Cornell University, NY). In all age groups, 3 types of immunoreactive profiles are visible in the arcuate nucleus: neuronal perikarya, dendrites, and axon terminals. TH immunoreactive perikarya are contacted by labeled and unlabeled axon terminals, as well as by labeled and unlabeled dendrites. Appositions between labeled terminals and labeled perikarya are rare, and seen only in the adult. The frequency of unlabeled axon terminals synapsing on immunoreactive perikarya is 3X higher in the adult than in the neonate. Finally, labeled perikarya are contacted by unlabeled dendrites in neonatal, 15 and 30 day old rats, but not in the adult.

TH immunoreactive dendrites, in all age groups, are synaptically contacted by unlabeled axon terminals. However, the frequency of these contacts is much higher in the adult (17%) than in the neonatal (10%) rats. Labeled varicosities in contact with labeled dendrites are scarce, and never seen in the adult. Labeled dendrites are also directly apposed to one another, to unlabeled dendrites, and to both labeled and unlabeled perikarya. They are also frequently juxtaposed to, or partly ensheathed by, tanycytic processes. The proportion of dendro-somatic and dendro-dendritic appositions involving 2 labeled elements shows little variation between birth and adulthood being, respectively, 2% and 2% in the neonate, 1% and 3% in all other age groups. In contrast, the proportion of labeled dendrites adjacent to unlabeled perikarya decreases from 8% in the neonate, to 2% in 15 day old rats, and 1% in the 30 day old as well as in the adult rats. The percentage of labeled dendrites observed in apposition to unlabeled ones undergoes an even more dramatic decrease from 24% in the neonate, to 10% in the 15 day old, 6% in the 30 day old, and 2% in the adult rats. The present results suggest that TIDA neurons establish extensive contacts with one another early during development, and that these connections, which are potentially operative at birth, are likely to be retained until adulthood. On the other hand, quantitative maturational changes in contacts involving labeled and unlabeled elements suggest that there is a rearrangement, in time, of the connections between the TIDA neurons and other neuronal systems of the arcuate neuropil.

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238.11 GLYCOCONJUGATES AND THE DEVELOPMENT OF THE CEREBROSPINAL FLUID OUTFLOW PATHWAY IN THE MOUSE. J.Herman*,D.G.McLone*,R.G. Higbee*,W.Goossens* and P.A.Knepper*(Spon:A.Farbman).Division of Neurosurgery,Children's Memorial Hospital,Northwestern Univ.Med.Ctr.,Chicago,IL 60614.

The development of the cerebrospinal fluid (CSF) outflow pathway -- the superior sagittal sinus, the adjacent dura mater and arachnoid -- was studied in C-57 mice on postnatal days 1,4,6,8,10 and 14 by light and electron microscopy and by fluorescein-labeled lectin staining of plastic (lum) sections. Using methods which prevent loss of fluorescence (0.2% phenylenediamine, pH 9.0), the lectin staining patterns were determined by video image analysis and computer-aided densitometry. On days 1 through 6, the CSF outflow pathway was characterized by the presence of loosely arranged cells, occasional macrophages, and an extracellular matrix that exhibited intense areas of concanavalin A (recognizes α-D-mannose and α-D-glucose), and ricinus communis agglutinin lectin staining (B-galactose). By day 8, the CSF outflow pathway, particularly the inner-arachnoid and glial limitans, was characterized by positive staining with other lectins, wheat germ agglutinin (N-acetyle-glucosamine and sialic acid) and limulus polyphemus agglutinin (sialic acid).

These results suggest: (1) The composition and/or confirmation of the glycoconjugates on cells and extracellular matrix changed as the CSF outflow pathway differentiated into a functional tissue. (2) The use of FITC lectins as biological markers for studies of CSF outflow pathway provided information on the potential role of glycoconjugates in the development of the normal CSF outflow pathway. (3) Modifications in the time of appearance, type, amount and distribution of glycoconjugates may provide a basis for understanding certain cellular mechanisms of abnormal development of the CSF outflow pathway, e.g., congenital hydrocephalus.

238.12 IN VITRO REASSOCIATION OF NEURONS FROM THE PATCH COMPONENT OF THE RAT STRIATUM. L.A. Krushel*, J.A. Connolly and D. van der Kooy. Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8.

The rodent striatum can be divided into two cellular compartments, the patches and matrix, defined on the basis of different neurotransmitter phenotype, neurotransmitter receptors, and neuroanatomical connections. Neurons destined to form the adult patch compartment leave the mitotic cycle before neurons that form the matrix compartment (van der Kooy and Fishell, submitted). Specifically, a large proportion of the neurons labeled with H^3-thymidine injected on embryonic day (E) 13-15 end up in the adult patch compartment, whereas a majority of the neurons labeled on E18-20 end up in the adult matrix compartment. The H^3-thymidine labeling technique was used here to observe whether patch cells would aggregate together when removed embryonically, dissociated, and cultured. Alternatively, if the matrix cells are embryonically labeled at E18 will there be clumping or a random dispersal of labelled cells in culture? Timed pregnant Wistar albino rats were injected with 1 mCi H^3-thymidine (specific activity 52 Ci/mmol) on either E13 or E18. Two days later, under sterile conditions, the striatum was dissected, dissociated into single cells, and plated on collagenized coverslips at a cell density of approximately 1 x 10^6 per plate. After five days in culture, the tissue was fixed in 4% paraformaldehyde in PBS, dried and dipped in Kodak NTB2 nuclear track emulsion. After a 6-8 week exposure the emulsion was developed and the tissue was dehydrated and counterstained with thionin or cresyl violet. Initial results revealed that single dissociated striatal neurons in culture reaggregate into multicellular clumps. Many individual clumps were found to contain numerous neurons that were labeled with H^3-thymidine on E13 (>5 labeled cells per clump). Other clumps contained only rare H^3-thymidine labeled neurons. Within the heavily labeled aggregates individual labeled neurons were more often associated with each other than with unlabeled cells. This is in direct contrast with cells labeled with H^3-thymidine on E18, dissected and dissociated two days later and cultured for five days. In this case cells were evenly dispersed between and within cell aggregates. The results suggest that the embryonic neurons of the patch compartment possess some inherent adhesion factor that may allow them to organize in vivo.

238.13 THE EFFECT OF FOCAL BETA RADIATION LESIONS ON THE DEVELOPMENT OF CEREBELLAR FOLIA IN RATS. James A. Pulliam. Dept. of Anatomical Sciences, Meharry Medical College, Nashville, TN 37208.

This investigation tested the hypothesis that the role of cells in the development of the cerebellum could be detected by selectively destroying these cells and analyzing the effects of the lesion.

Lesions were made in the cerebellum of 36 rat fetuses that ranged in age from the 17th to the 20th day of development. At this stage, the cells which will differentiate into granular layer neurons are located in the most superficial layer of the cerebellum. Attenuated focal lesions were made by using a 2mm diameter beam of beta radiation from ^{90}Sr--^{90}Y. It is known that 61% of this radiation is absorbed within 1mm of soft tissue and 99% of it absorbed within 5mm of soft tissue. Laparotomy was performed on anesthetized pregnant rats and the fetal cerebellums were irradiated through the uterine wall, extraembryonic membranes, skin and skull. Unirradiated littermates were used as controls.

Irradiated litters revealed tremor, leg dragging, and motor incoordination. When the irradiated rats were one month old, they and their controls were autopsied and their brains were removed for histologic study. Cerebellar folia normally parallel each other in the horizontal direction, but at the site of the lesions folia were oriented sagittally or diagonally. The cell population reduced most by the irradiation was the granule cells. Since the cerebellar parallel fibers are axonal branches of granule cells,these data suggest that the reduction in granule cell numbers, also reduced parallel fiber numbers which, in turn, prevented the normal folial horizontal alignment. Consequently, the folia became aligned in the sagittal plane in conjunction with the dendrites of Purkinje neurons. These data also suggest that the functional disorders were caused by an insufficiency of granule cells and an abnormal alignment of parallel fibers. *Supported by Grant No. USAEC-W-7401-Eng-49 from the Atomic Energy Commission, Grant No. MH-8034 from the National Institute of Mental Health, NSF Grant GY 2410, AEC Grant AT (30-1) 3725 and a Meharry Interim Research Award from Grant #5 S01 FR05422-08.

238.14 OXYGEN FREE-RADICALS IN FETAL BRAIN MACROPHAGES. C.J.D'Amato, K.S.O'Shea, S.E.G.Fligiel*, J.Varani* and S.P.Hicks. Depts. of Pathology and Anatomy and Cell Biology, Univ. of Michigan Medical Center, Ann Arbor, MI 48109.

The white cells of the mammal defend it against microorganisms, and foreign substances, and they scavenge dead cells. They are also now known to do harm to the host's normal cells by producing oxygen free-radicals: for example, in adult lung infections. Whether fetal macrophages, by producing oxygen free-radicals, aggravate malformative effects of injury, such as killing primitive cells by X-rays, is not known.

As a first step to answer the question, we have irradiated 16 days pregnant rats with 200R whole body and removed the fetuses after killing the animal quickly with ether 4, 8, 12, or 24 hours after irradiation. Up to three quarters of fetal neuroepithelial and mesenchymal cells were necrotic by 4 to 6 hours in many populations, and macrophages had materialized and begun to engulf them. Control unirradiated fetuses were obtained in the same way. The fetal brains were dissected into PBS, minced, triturated in F10 Ham's nutrient mixture with 10% fetal calf serum. The brain cells were examined for the production of superoxide anion. A portion of the cells in each group was put into cell culture and a day later they were stained for non-specific esterase activity. Light and electron microscopy were used to study the morphology and distribution of the macrophages in the brain and other tissues.

Measurable amounts of superoxide anion were found at 12 and 24 hours after irradiation. The amounts were low but support the conclusions that the macrophages produced superoxide anion. The esterase studies indicated that there may have been as many as $5x10^4$ esterase-positive macrophages per culture dish. Expressed per cell, the superoxide generated was comparable to that measured in adult tissue systems. (Supported by USPHS NS 19825)

238.15 INTERRUPTIONS OF EXTERNAL GRANULE LAYER LEAD TO ABNORMAL CEREBELLAR FOLIATION INDUCED BY METHYLAZOXYMETHANOL IN PERINATAL RATS. S. Chen and D. E. Hillman. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Our previous studies found that perinatal injections of methylazoxymethanol (MAM) into rats produced aberrant cerebellar foliation giving rise to numerous small blebs resembling a cauliflower head. The abnormal appearance emerged only following injections between gestational day 21 and postnatal day 2. Numerous speculations on the mechanism of foliation have been suggested: (a) a buckling force generated by a five times differential gain between the area of the cortical lamina and the white matter from birth to maturity, (b) a faster growth at the depth of fissures than the distal end of folia, and (c) a genetically controlled rate and timing for the proliferation of the external granule layer (EGL). The finding that MAM affected foliation within a short period, relative to EGL genesis, suggests that some other factors may be critical to the folial deformation.

We have studied the subsequent development of EGL on series of coronal and sagittal sections of the cerebellum treated with MAM during the critical period. The primary target of MAM is on dividing cells. Neonatal administration of MAM produced partial to subtotal destruction of EGL and resulted in small patches of EGL within a week. There was a greater effect in the anterior than in the posterior vermis of the cerebellum. In severely affected rats, anterior lobules developed even without an EGL. However, in moderate and mildly affected rats, blebs in various sizes occurred on lobules interrupting the folial pattern. Most remarkable was the occurrence of large blebs which mushroomed to form islands of cortex in sagittal sections. Reconstruction showed a narrow connection to the central core of the cerebellum. Interruptions in the EGL, as tiny gaps without EGL cells or markly thinned zones, corresponded to the depth of the folds at the base of the blebs. These irregular convolutions of lobules have not been observed in developing control animals.

We conclude that the transient interruption of EGL following MAM injections results in the formation of blebs which seem to play a significant role in abnormal cerebellar foliation. Blebs are likely derived from (a) an exponential multiplication of surviving progenators of the EGL and (b) a greater buckling force produced by expanding the Purkinje cell mass and protrusion of the central fiber core. Supported by USPHS grant NS20349 from NINCDS.

238.16 EFFECTS OF EARLY CALLOSAL AND THALAMIC LESIONS ON DIFFERENTIATION OF CORTICAL CYTOARCHITECTURE. M.S. Windrem,* S.M. Jan de Beur* and B.L. Finlay. Department of Psychology, Cornell University, Ithaca, N.Y. 14853

Columns from functionally distinct cortical areas vary in cell number, connectivity, and relative proportions of cell types. Through lesions which manipulate cortical afference and target, and labeling of cell cohorts by birthday, we are investigating the relative contributions of afference, target, and time of generation to the determination of cortical cytoarchitecture.

Prior experiments have shown that early thalamic lesions reduce cortical area, cell number in a unit column, and proportion of stellate-like cells in a column, and increase neonatal cell death compared to the non-lesioned side. Specifically, cortical areas neonatally disconnected from the thalamus had no layer IV in the adult (Windrem and Finlay, 1985). In order to determine whether the loss of stellate-like cells in these areas is due to the selective loss of a particular birthday cohort, pregnant hamsters were injected with 3H thymidine on embryonic days 14 and 15, the days on which the cells of layers IV (E14), and II-III (E15) are generated. The results of the E14 injections are reported here. The total number of cells in layer II-III in a unit column on the lesioned side is unchanged while the number of cells in layer IV is sharply reduced. The number of labeled cells, however, is unchanged in layer IV and greatly increased in layer II-III. Thus, the cohort that preferentially forms layer IV on the unlesioned side is selectively spared and over-represented in the later-formed layer III on the lesioned side. Since there are fewer cells overall in the upper layers of a unit cortical column following thalamic lesions, yet there are more E14 cells, the selective loss of layer IV must be due to an increased loss in the last-generated, E15 cells. This hypothesis is currently under investigation. The apparent reassignment of E14 cells to layer II-III suggests that cell type is determined not in the ventricular zone but by afference upon arrival at the cortex.

To further study the role of afference on the specification of cell type in the upper layers, a second experiment was performed. The corpus callosum reciprocally connects layers II-III and V of selected cytoarchitectonic areas of the two hemispheres. On the day of birth in hamsters, the cerebral hemispheres were separated with barriers of foil or bone wax, thereby preventing the development of the corpus callosum. Acallosal and callosal projection zones were compared, area by area, with age-matched controls. Total cell number, laminar distribution, and cell area were the same in comparable samples from lesioned and age-matched control animals. The apparent preservation of normal cytoarchitecture in callosally lesioned animals suggests that neurons which would have projected callosally have retained intrahemispheric connections. In summary , the early presence of thalamic, but not callosal axons, specifies aspects of cell number and morphology in the developing neocortex.

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239.1 NATURAL, INDUCED AND REGULATIVE CELL DEATH IN THE TECTO-PARABIGEMINAL SYSTEM OF DEVELOPING RATS. R. Linden and L.G.P. Piñon* Inst. Biofisica UFRJ, R. Janeiro, Brazil

The parabigeminal nucleus of the midbrain (Pb) connects reciprocally with the superior colliculus (SC) in mammals. In rodents the Pb has 3 divisions: the dorsal (Pb_d) and the ventral (Pb_v) divisions project to and receive afferents from the ipsilateral SC; the middle division (Pb_m) receives afferents from the ipsilateral SC and projects to the contralateral SC. This pathway is present in newborn rats. Unilateral lesions of the SC produce the expected retrograde and anterograde degeneration in the Pb, detected as a net cell loss in adult rats operated at different ages after birth (Linden & Perry, JCN 218:270, 1983). Such lesions made on the day of birth, however, failed to elicit a net retrograde cell loss in the contralateral Pb, which then forms an aberrant projection to the thalamus. We investigated the relationship between natural cell death in the Pb and the cell death induced by tectal lesions made at birth. Lister hooded rat pups were anaesthetized by hypothermia and the upper layers of the left SC were aspirated. Operated pups and unoperated controls were perfused at postnatal days (P) 1-10 and 15 and degenerating cellswere counted in sections of the Pb stained with cresyl-violet. Natural cell death in the normally developing Pb has already started at birth, was maximal at P3 and proceeded with a declining rate up to P8-10 in all 3 divisions. The SC lesion changed this pattern. In the ipsilateral Pb_m the rate of cell death was increased above normal from P1 to P10, with a maximum effect at P3-6. In the contralateral Pb_m cell death was increased at P1-2 and decreased below normal at P3-5. In the ipsilateral Pb_d and Pb_v cell death was increased in 2 peaks at P1-2 and at P4-6. The results suggest that: (a) cell death induced by axotomy and by deafferentation proceed with distinct time courses; (b) the 2 modes of degeneration are additive when axotomy and deafferentation occur simultaneously; (c) the Pb regulates through a reduction in the rate of natural cell death after an early depletion of neurones caused by axotomy; (d) trophic factors related both to the target/axon and to the afferent supply concur for the control of developmental cell death.

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239.2 TIME COURSE OF RECOVERY AND DISAPPEARANCE OF SYNAPTIC TRANSMISSION IN HIPPOCAMPUS FOLLOWING CEREBRAL ISCHEMIA

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Transient cerebral ischemia leads to necrosis of the pyramidal neurons in the CA1 region of hippocampus and polymorphic neurons in the hilus of dentate gyrus. The damaged neurons are manifested in the light microscope as shrunken and acidophilic, and appear in the hilus of dentate gyrus less than 24 hours, and in the CA1 region 48-72 hours postischemia. Synaptic transmission, notably synaptic release of glutamate, has been implicated as an important factor in the development of ischemic neuronal damage (for ref see T.Wieloch, Prog Brain Res 63:69-85).

The present experiments were designed to correlated synaptic transmission in the hippocampus with morphological changes induced by ischemia.

Male Wistar rats were subjected to 20 minutes of transient cerebral ischemia, induced by bilateral common carotid occlusion combined with hypotension, and allowed to recover for various periods of time (1 day, 2 days and 7 days).

The electrophysiological experiments were performed under thiopental anesthesia. Constant current stimulation of the angular bundle was accomplished by a stainless steel electrode and synaptic responses recorded with glass electrodes stereotactically placed to obtain maximal responses in the CA1 region and dentate gyrus. Recurrent inhibition was assessed by paired pulse inhibition of the population spike recorded in the dentate gyrus.

Our results show that evoked, trisynaptic responses recorded in CA1 recover and remain active up to 24 hours, but not 2 days, postischemia. Thus failure of synaptic transmission precedes gross necrosis of CA1 pyramidal neurons. In spite of extensive neuronal necrosis in the hilus of dentate gyrus recurrent inhibition was not grossly affected by the ischemic insult.

The data demonstrate that neuronal hyperactivity due to loss of recurrent inhibition in dentate gyrus does not seem to be a factor influencing the outcome of the insult. Loss of synaptic transmission in CA1 during ischemia is reversible and retained for up to 24 hours postischemia. During this time interval postischemic pharmacological interventions to protect the brain against progressing neuronal damage can be envisaged.

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239.3 GLYCOGEN DISTRIBUTION IN GERBIL CORTEX AND HIPPOCAMPUS AFTER CEREBRAL ISCHEMIA. D.v.Lubitz* and G.Goping* (Spon .Nabil A.Azzam), NINVDS/NIADDK/NIH ,Bethesda,Maryland,MD 20205

Ultrastructural distribution of glycogen in gerbil cerebral cortex and hippocampus was studies following 5 min ischemia induced by bilateral carotid occlusion.After ischemia the animals were allowed to survive for 6 hrs,1,2 and 7 days.They were then perfused with glutaraldehyde/paraformaldehyde for 30 min and the brains were then processed for E.M. Staining of sections for glycogen was done by means of the "reduced osmium" technique (Karnovsky,1971).

Glycogen presence followed both spatial and temporal pattern.

CONTROLS. Glycogen was present both in the cortex and in the hippocampus in the end feet of the perivascular astrocytes only.

6 HRS SURVIVAL. Considerable elevation of glycogen concentration was seen in the astrocytes and occasionally in the dendrites of the cortex.In the hippocampus,the CA4/fascia dentata(F.D) had glycogen only in the astrocytes.In the CA2/3 the granules were also observed in several dendrites.In the CA1 glycogen was scarce(astrocytes only.)

1 DAY SURVIVAL. The cortical concentration of glycogen decreased. In the hippocampus the granulae were confined to the astrocytic cytoplasm in the CA4/F.D. In CA2/3 the granulae were absent from the dendrites and scarce in the astrocytic processes.In CA1 small numbers of glycogen granulae were present only in the region of Schaeffer's collaterals.

2 DAYS SURVIVAL. In the cortex the contents of astrocytic glycogen decreased even further.Astrocytes of CA4/F.D. contained very little glycogen but the granulae were conspicuous in the damaged dendrites.The same was true of CA2/3 although the amount of glycogen was even less than in the preceding region.In CA1 glycogen was present in the area of Schaeffer's collaterals and in the trunks of the damaged dendrites.

7 DAYS SURVIVAL. The cortical distribution of glycogen was similar to that of the controls.The same was true of CA4/F.D. in the hippocampus.In CA2/3 the granulae were still present but in smaller numbers than at shorter reperfusion periods.In CA1,where the damage was most severe,glycogen was seen throughout the area both in the astrocytes and in the proximal dendrites of the pyramidal cells.

Ultrastructural damage observed in this study was similar to that described by other authors.Our results indicate that the cortical neurons are probably capable of postischemic recovery.In the hippocampus on the other hand,the damage progresses like a wave from fascia dentata/CA4 towards the CA1.It appears to be irreversible in the latter sector,whereas CA4/fascia dentata and CA2/3 are probably capable of at least some degree of recovery.

239.4 MATURATION OF NEURONAL DAMAGE IN HIPPOCAMPUS FOLLOWING REVERSIBLE FOREBRAIN ISCHEMIA. J.K. Deshpande*, L.A. Paul*, T. Wieloch* (SPON: M.C. Rogers). Laboratory for Experimental Brain Research, University of Lund, S-22185, Lund, Sweden.

Irreversible neuronal damage following cerebral ischemia evolves over many days during the recovery period. A rat model has been developed which allows long term recovery after reversible forebrain ischemia. The density and distribution of cell death as seen on Nissl stain after ischemia and one week recovery in this model has been described (Acta Neuropath. (Berl.) 64:319, 1984). We previously reported that Golgi studies in ischemic animals allowed several days' recovery revealed the presence of dendritic swellings on CA_1 - subiculum neurons, which may have inhibitory function. The present study was undertaken to characterize the development of neuronal necrosis and to correlate the findings with changes observed in Golgi studies of the same groups. Wistar-SPF rats (Mollegaards Avlslab., Copenhagen) were anesthetized with halothane. Catheters were inserted in the jugular vein for subsequent withdrawal of blood, and in the tail artery for monitoring blood gases. Both common carotid arteries were exposed and loose sutures placed around them. Needle electrodes were placed into the temporalis muscle to monitor EEG. The rats were paralyzed, mechanically ventilated on $N_2O:O_2$, and the halothane discontinued. Ischemia was induced by the infusion of trimetaphan (Arfonad^R) and blood withdrawal until mean arterial pressure reached 50mm Hg. Vascular clamps were then applied to both carotid arteries. Isoelectricity was observed within fifteen seconds in all cases. At the end of the 10 minute ischemia period, blood was reinfused, vascular clamps removed, and $NaHCO_3$ was given. Animals were extubated (45-60 minutes) when they resumed spontaneous ventilation. They were placed in cages with access to water and food, and allowed to recover for varying times. Following formalin perfusion, brains were removed and stained either with celestin blue-acid fuchsin or with a variant of the rapid Golgi technique. The early and intermediate recovery groups (3-48 hr) had well-known but reversible ischemic changes (pyknosis, cytoplasmic clearing and microvacuoles in CA_1 and CA_4). However, significant irreversible changes (acidophilia) were noted only in the longer recovery groups (72 and 96 hr). The most remarkable findings in the Golgi stains of ischemic animals were large round dendritic swellings in CA_1 cells. The morphologic characteristics suggest that these are probably inhibitory interneurons. The enlargements were first evident in the 24-48 hr groups, thus preceding the Nissl findings. Morphological changes of inhibitory cells prior to the appearance of neuronal death further implicate the imbalance of inhibition and excitation in producing neuronal damage.

239.5 POST-ANOXIC TREATMENT WITH ANTICONVULSANTS: ELECTROPHYSIOLOGICAL AND ANATOMICAL STUDIES OF CA1 PYRAMIDAL CELLS. K.S. Lee, G. Sancesario, W. Tetzlaff and G.W. Kreutzberg. Dept. of Anatomy, Jefferson Medical College, Philadelphia, PA 19107 and Dept. of Neuromorphology, Max Planck Inst. for Psychiatry, 8033 Martinsried, W. Germany.

Pyramidal cells in the hippocampal CA1 region are among the most sensitive neurons in the brain to transient anoxia. The basis for this selective vulnerability is unclear, but recent evidence suggests that post-anoxic hyperexcitability contributes to neuronal loss in this region. While the use of anticonvulsive agents for the attenuation of stroke-related damage has met with only partial success, such agents might be appropriate for treatment of the CA1 region if administered subsequent to the anoxic event (i.e. during the period of pyramidal cell hyperexcitability).

The anticonvulsive drugs, phenytoin (25-100 mg/kg), clonazepam (0.2-0.5 mg/kg) and phenobarbital (20-50 mg/kg) were therefore tested for their abilities to protect against the loss of CA1 pyramidal cells following a brief period of transient anoxia. Gerbils were subjected to bilateral clamping of the carotid arteries for 10 minutes and drugs were first administered 15 minutes following the removal of arterial clamps. Drugs were subsequently administered once or twice per day for 3 days and animals were sacrificed 4 days following surgery. Brains from control animals (i.e. saline-injected) and anticonvulsant-treated animals were rapidly removed and, 1) sectioned with a cryostat and stained with cresyl violet or, 2) the hippocampus was removed and in vitro hippocampal slices were prepared. As judged by cresyl violet staining of coronal brain sections, each of the drugs tested was capable of attenuating the post-anoxic loss of CA1 pyramidal cells at 4 days following surgery. This is a post-operative interval at which operated, saline-injected animals show an almost total loss of CA1 pyramidal cells. In addition, correlative electrophysiological studies utilizing hippocampal slices indicated that the CA1 cells were responsive to synaptic activation in phenobarbital-treated animals but unresponsive in the saline-treated animals. Specifically, the orthodromically-evoked field EPSP in the stratum radiatum could be easily obtained in the slices from phenobarbital-treated animals while similar responses were small or absent in slices from saline-injected animals.

At present, it is unclear whether anticonvulsant treatment provides an actual blockade of the post-anoxic degenerative process or merely delays the loss of pyramidal cells. It is conceivable that such treatment could reduce hippocampal hyperactivity without actually affecting the mechanism(s) which causes the cellular instability. Nonetheless, the sustained viability of CA1 pyramidal cells following treatment with the various anticonvulsive agents further implicates post-anoxic hyperexcitability as a critical factor contributing to post-anoxic loss of CA1 pyramidal neurons.

239.6 DIFFERENTIAL DEGENERATION OF THE MEDIODORSAL THALAMIC NUCLEUS AFTER NEONATAL OR ADULT CEREBRAL HEMISPHERECTOMY. F. Benedetti*, D.A. Hovda and J.R. Villablanca (SPON: W. Wyrwicka). Mental Retardation Research Center, Departments of Anatomy and Psychiatry, School of Medicine, University of California, Los Angeles, 90024.

The issue of whether neonatal brain injury results in reduced or enhanced neuronal degeneration relative to lesions inflicted in adulthood is undecided. For the thalamic ventrobasal complex we reported greater sparing in the cats with neonatal vs adult cerebral hemispherectomy (Brain Res., 368: 211, 1986). Here we extend that study to a thalamic nucleus with substantial targets not destroyed by the lesion. Using computerized morphometric methods, we measured cell and tissue degeneration in the mediodorsal n. (MD) in cats surviving over 1 year after ablation of the left telencephalon either as neonates (5-12 days of age; N=5) or adults (N=5). Intact adults (N=5) were controls. Formaline-fixed brains were frozen sectioned (50μm) coronally and thionine-stained. With a camera lucida, the outline of the MD was drawn at 6X and cellular structures were drawn at 75X. Drawings were digitized on a magnetic tablet interfaced with a microcomputer. Measurements were, bilaterally: a) total area at coronal planes A8.0 and A9.0; b) counts and soma areas of glia cells-small neurons (<35μm^2) and of larger neurons (>35μm^2). For the MD ipsilateral to the ablation: a) in adult-hemispherectomized MD area was decreased by 53% ($p<.01$) whereas neonatal-hemispherectomized showed only a 37% decrease ($p<.01$); adult-lesioned vs neonatal-lesioned, $p<.05$. b) larger neuron counts revealed no significant differences between groups however, both lesion groups showed loss of the largest cells (>200μm^2) and this was more pronounced for adult-lesioned cats. Both lesion groups showed a decrease in glial cells-small neurons in both hemithalami. In conclusion, atrophy and large cell loss were more marked in the MD for adult vs neonatal-hemispherectomized animals. Also, as expected, the overall degeneration was less marked for MD than for ventrobasal complex (previous study) for both age groups. The reduced retrograde degeneration seen in neonatal-lesioned cats may help to understand the greater behavioral recovery reported for these animals vs adult-lesioned cats (Behav. Brain Res., 19: 205, 1986). USPHS Grants HD 05958, HD 04612 and HD 0732.

239.7 RED NUCLEUS NEURONS ONE YEAR AFTER T-9 SPINAL CORD TRANSECTION IN ADULT RATS. R.L. McBride, J.N. Pruitt II*, and E.R. Feringa. Veterans Administration Medical Center and Medical College of Georgia, Augusta, GA 30910.

Spinal cord transection in adult rats results in decreased numbers of neurons in sensory-motor cortex, Clarke's column and red nucleus. The change occurs after a time delay of at least five weeks and may be a result of loss of target tissue. We have studied the red nucleus of five female rats whose spinal cord was transected at T-9 at six weeks of age and were sacrificed 52 weeks after surgery. Five isogeneic female non-transected age-matched rats served as controls. The brains were embedded in paraffin, sectioned at 7μm and stained with hematoxylin and eosin. All red nucleus neurons were counted at 250x on every fourth section. Because we had previously determined that caudal red nucleus neurons, which project primarily to the lumbar cord, were most at risk from transection, the area of each neuron was determined on every fourth section in the caudal 250μm of the nucleus using the Bioquant Image Analysis System.

Total neurons decreased 28% ($p<.05$) in the whole nucleus compared with controls and in the caudal portion by 38% ($p<.001$). The mean area of neurons in the caudal portion increased by 4% ($p<.05$) compared with controls; this was due to a slight increase in percent of larger neurons and a slight decrease in percent of smaller neurons measured.

While it has been hypothesized that neurons in adult animals may shrink rather than die as a result of section of the axon far from the soma, our data suggest that red nucleus neurons of all sizes die as the result of axotomy.

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239.8 TARGET RELATED CELL DEATH IN CEREBELLAR GRANULE CELLS; ARE EARLY GENERATED CELLS AT A COMPETITIVE ADVANTAGE?. M.W. Vogel and K. Herrup. Dept. of Human Genetics. Yale Med. School, New Haven, CT 06510.

The period of naturally occurring cell death may represent a mechanism for quantitatively matching the size of presynaptic and postsynaptic populations. In staggerer-wild type mouse chimeras, there is a linear relationship between granule and Purkinje cell numbers. However, in lurcher (+/Lc)-wild type (+/+) chimeras the adult granule to Purkinje cell ratio is increased up to 4.5 fold at low Purkinje cell values. +/Lc Purkinje cells make transient, morphologically normal granule cell synapses before they die. In +/Lc-+/+ chimeras, therefore, +/Lc Purkinje cells may help the +/+ Purkinje cells to "stabilize" early born granule cells, but they then degenerate. We have analyzed the relative survival of early and late born granule cells in +/Lc-+/+ chimeras to determine if early generated granule cells are at a competitive advantage compared to late generated granule cells.

Wild type and +/Lc controls and +/Lc-+/+ chimeras were injected at either P4 or P13 with tritiated thymidine to label early or late born granule cells. The number of granule cells, labeled granule cells and Purkinje cells were counted in saggital sections of adult animals. In +/+ animals, approximately 2.0 to 3.0% of the total granule cell population is born at either P4 or P13. In +/Lc animals, over 35% of the surviving granule cells are born at P4, while only 1.0% or fewer are born at P13. This suggests that early born granule cells must be at a tremendous temporal advantage in the mutant, even though Purkinje cell numbers in the +/Lc and +/+ animals are equal until P7.

Analysis of chimeric animals reveals that developmental interactions among granule cells are complex. The presence of relatively few Purkinje cells (as few as 5 to 8% of wild type numbers) dramatically reduces the percentage of surviving P4 labeled granule cells, compared to +/Lc values. The percentage of surviving P13 granule cells only increases modestly in response to higher Purkinje cell numbers, compared to +/Lc values. In chimeras with similar numbers of Purkinje cells, the percentage of surviving P4 granule cells is higher than that of surviving P13 granule cells. However, the total number of surviving P4 granule cells is reduced compared to P4 +/Lc values, while the number of surviving P13 granule cells is increased with respect to P13 +/Lc values. Our results will be discussed in terms of a two phase model for competitive interactions, the first phase an acute process requiring the establishment of early Purkinje cell contacts and the second phase a chronic competitive interaction among granule cells of all ages.

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239.9 CELL DEATH IN THE INFERIOR OLIVE AS A FUNCTION OF CEREBELLAR PURKINJE CELL NUMBER: A QUANTITATIVE STUDY IN LURCHER CHIMERIC MICE. Lisa Panzini* and Karl Herrup, Yale Med. Sch., New Haven.

Target related cell death is a common feature of many developing neuronal cell populations. Although the developmental "logic" behind its existence may be multifaceted, one hypothesis is that it serves to bring a population of nerve cells into numerical balance with its post-synaptic target population. This numerical matching function has been recently demonstrated to apply to the cerebellar granule:Purkinje cell circuit in staggerer chimeric mice. We have now completed a quantitative study of a second major Purkinje cell input, the neurons of the inferior olive (IO). Lurcher mutant mice (+/Lc) lose 100% of their cerebellar Purkinje cells (PC) as a direct consequence of gene action. Eighty percent of the IO neurons are also lost (between postnatal 7 and 90), but these deaths are not directly attributable to the expression of the Lc gene. They are presumably caused instead by the loss of their primary synaptic target, the PC.

Lurcher chimeric mice represent an ideal model system to examine numerical matching in this circuit since analysis of sufficient animals makes it possible to observe the effects of increasing the number of surviving normal (+/+) PCs across a broad range of values, from zero to wild type. The numbers of IO neurons and PCs were counted in left and right brains of +/Lc and +/+ controls as well as 9 +/Lc <--> +/+ chimeras. IO neuron counts were plotted as a function of contra-lateral PC counts. If the relationship between IO and PC numbers were linear then all of these values should fall on a straight line connecting the mean control values (+/+ and +/Lc). Instead, the resulting points all fall significantly above this hypothetical line. This finding has four possible explanations. First, it may be that the cooperation of the wild type PCs plus the transient presence of the +/Lc cells rescues more IO cells than the +/+ PCs could on their own. Second, it could be that the collaterals of the IO neurons (to deep nuclear neurons or ipsilateral cortex) buffer these cells from target-related cell death. Third, it could be that the retraction of the well known multiple innervation of PC by IO neurons is either delayed or abandoned so that a given number of PCs offer an effectively larger synaptic target than normal. Finally, it could be that numerical matching in this focussed system is either not rigorous (i.e., there is true developmental plasticity in the interaction) or it is not linear. Analyses of several additional strains of wild type mouse may help to distinguish among at least some of these attenatives.

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239.10 PREVENTION OF TRANSNEURONAL DEATH OF NEURONS IN THE SUBSTANTIA NIGRA BY CHRONIC INFUSION OF THE GABA AGONIST MUSCIMOL. M. Saji* and D.J. Reis. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Neurons in the substantia nigra pars reticulata (SNr) die following destruction of intrinsic neurons of the caudate nucleus (CN) by ibotenic acid (IBO) (Saji and Reis, Neurosci. Abs., 1985). Since fibers of the striatonigral pathway contain the inhibitory neurotransmitter GABA and excessive excitation of neurons can lead to neuronal death, conceivably degeneration of SNr neurons may be due to disinhibition consequent to loss of an inhibitory GABAergic input. To test the hypothesis we assessed whether: a) there is a topographic correspondence between loss of SNr neurons initiated by IBO lesion of CN and loss of the GABAergic innervation; b) death of SNr neurons can be prevented by the chronic administration of the GABA agonist muscimol.

IBO was stereotaxically injected unilaterally into 3 sites (4 ug/0.4 ul, each) in the CN of 45 anesthetized male rats. Controls received saline. In some animals, in the same operation, a cannula was implanted into the lateral ventricle contralateral to the lesion for subsequent intraventricular injection of muscimol. At various days thereafter, the animals were anesthetized and perfused with 4% paraformaldehyde. The brains were removed, sectioned and stained with thionin or with antiserum to GABA. The extent of neuronal death in the SNr was assessed as a function of the reduced density of neurons.

IBO destroyed about 90% of CN neurons within 5 days. By 20 days after IBO-lesion 20% of the total number (10,200) in SNr ipsilateral, but not contralateral, to the lesion had disappeared ($p < .05$). Associated was a marked gliosis, retraction of TH dendrites, and volumetric shrinkage. The loss of SNr neurons was not homogeneous. The greatest loss of neurons (53-57%, $p < .01$) was concentrated in the central core of SNr, while neuronal loss was observed in the lateral (28%, $p < .05$) but not medial (16%, not significant) areas. GABA immunoreactivity was reduced in all areas ($p < .05$). The greatest loss (82%) was in the dorsal central area followed by loss in the ventral (68%), lateral (61%) and medial (43%) areas, demonstrating thereby the close topographic correspondence between the loss of GABA terminals and neruon death.

In other animals (n=24), muscimol was infused (0.5 ul/h) continuously over 20 days after the lesion by osmotic pump. Such treatment completely prevented the loss of SNr neurons. Treatment with muscimol, however, did not alter the gliosis, dendritic retraction nor volumetric loss. The response was dose-dependent between 0.01 to 100 ng/ul.

The present study indicates that the mechanism for anterograde transneuronal degeneration of neurons in the SNr by lesions of CN is disinhibition as a result of the removal of inhibitory GABAergic projection within the striatonigral pathway. Loss of inhibition may be a general mechanism of transneuronal degeneration.

239.11 COMPARISON OF VASOACTIVE INTESTINAL PEPTIDE, VIP(10-28), PHI-27, SECRETIN, AND GRF FOR EFFECTS ON NEURONAL SURVIVAL IN DEVELOPING SPINAL CORD CULTURES. D.E. Brenneman. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, Maryland 20892

Vasoactive intestinal peptide has been shown to increase the survival of developing spinal cord neurons grown in dissociated cultures (PNAS 83: 1159, 1986). This effect was evident when spontaneous electrical activity was blocked with tetrodotoxin. The specificity of this neurotrophic action of VIP was investigated by examining the effects of other peptides that have significant sequence homology to VIP.

Neuropeptides were tested on dissociated spinal cord cultures obtained from 10-12 day old fetal mice. Cultures were maintained in defined medium supplemented with 5% horse serum. Prior to treatment, a complete change of nutrient medium was performed to remove endogenous conditioning substances that have been shown to affect neuronal survival during electrical blockade (Dev. Brain Res. 15: 211, 1984). Neuropeptides were added to the cultures on day 9 in vitro and treatment was continued for 5 days. To assay for neuronal survival, the neurons were immunocytochemically identified with antibodies to neurofilament or neuron specific enolase and then counted from predetermined coordinates. Neuronal survival after peptide treatment was examined in electrically active cultures and in cultures that were electrically blocked with tetrodotoxin. Each peptide was studied at concentrations ranging from 10^{-13} to 10^{-7} M.

The survival of spinal cord neurons was decreased significantly (30-35%) after incubation with PHI-27, VIP(10-28), or growth hormone releasing factor (GRF). These neuron-depleting effects were evident at threshold concentrations ranging from 10^{-13} (VIP (10-28) to 10^{-11} M (PHI-27 or GRF) with the effect of all the neuropeptides being attenuated at higher concentrations (>10-8 M). The maximum amount of neuronal cell death produced by these peptides never exceeded the amount of neuronal cell death resulting from tetrodotoxin treatment alone. The mechanism of peptide-mediated neuronal cell death is unknown. Our hypothesis is that these substances may be interfering with the action of endogenous VIP. Secretin had no detectable effect on neuronal survival at any of the concentrations tested. In tetrodotoxin-treated cultures, PHI-27 and GRF prevented neuronal cell death produced by tetrodotoxin, but concentrations greater than 10^{-7} M were required for this action. Similar studies conducted with VIP have shown that 10^{-11} M VIP significantly increased neuronal survival in the presence of tetrodotoxin. These studies indicated that the survival-promoting effect of VIP on cultured spinal cord neurons is very structurally specific and that other closely homologous peptides either have no such action or produce decreases in neuronal survival during this period in development.

239.12 ECDYSTEROID-DEPENDENT NEURONAL DEATH IN ADULT MANDUCA SEXTA MOTHS: INTERACTION OF HUMORAL SIGNALS WITH NEURAL INPUT. S.E.Fahrbach and J.W.Truman. Dept. of Zoology, Univ. of Wash., Seattle, WA 98195.

The emergence of the adult Manduca sexta moth is followed by the death of almost half of this insect's abdominal motoneurons and interneurons. The spatial and temporal pattern of this cell death has been described (Truman, J.W., J. Comp. Neurol., 216: 415, 1983) and it has been determined that the same cells die in a fixed sequence in every moth. The death of these neurons has been shown to be dependent upon the normal decline of the steroid hormone, 20-hydroxyecdysone, that occurs at the end of metamorphosis (Truman, J.W. and Schwartz, L.M., J. Neurosci., 4: 274, 1984). A variety of experimental manipulations can advance, delay, or interrupt the normal sequence of this cell death, but they cannot change the order in which the neurons die.

The mechanism for the ordering of time of neuronal death in this system is not known. One possibility is that other factors act in concert with the steroid signal, so that the decline in ecdysteroid produces a state of neuronal readiness to die, but that degeneration of a particular cell does not begin until another signal triggers the start of the death process.

This hypothesis is supported by our finding that the death of an identified pair of motoneurons in the third abdominal ganglion (A3), the MN-12 cells, is prevented by abdominal isolation late in adult development. These motoneurons are the last cells to start to die within the ganglion, with the first signs of degeneration normally appearing about 40 h after adult ecdysis. By 96 h after ecdysis, no living MN-12 cells are found in A3. We typically assessed the effects of manipulations on MN-12 survival in ganglia stained with toluidine blue at this time. The effect of abdominal isolation on MN-12 survival was tested at a range of times around adult ecdysis. The MN-12 neurons in A3 were effectively preserved by this procedure through 26 h after ecdysis, with the 50% survival point occurring at 30 h. The effect of abdominal isolation was replicated by cutting the ventral nerve cord above A3 in otherwise intact animals, indicating that the effect is due solely to disruption of descending neural pathways. The sparing effect of abdominal isolation was persistent, with 60% of the MN-12 cells in A3 still surviving 8 days after treatment. Removal of the brain, subesophageal ganglion, and the first thoracic ganglion did not save the cells, suggesting that the fused pterothoracic ganglion is the source of the input that must be present for the death of MN-12 to occur.The transganglionic nature of the signal to MN-12 provides an easily-manipulated experimental system for the study of extrinsic factors regulating the timing of developmentally-programmed neuronal death.

239.13 ANTIBODIES TO SKELETAL MUSCLE EXTRACTS ENHANCE EMBRYONIC MOTONEURON SURVIVAL IN THE CHICK LUMBAR LATERAL MOTOR COLUMN IN VIVO. GUY J. PETRUZZELLI* and W.F. HUGHES* (SPON: S.K. JACOB). Dept. of Anatomy, Rush Medical College, Chicago, IL.

Previously, we have reported that a crude extract of embryonic avain skeletal muscle reduces motoneuron death in the chick lumbar lateral motor column (LMC) in vivo (Petruzzelli & Hughes, Soc. Neuro. Abs.; 11, 75.9 (1985). The present study determined the effects on embryonic motoneuron survival of a polyclonal antiserum raised against this extract. Crude immune serum was produced in a female New Zealand white rabbit by immunization with muscle extract in Freund's adjuvant; control sera were obtained from normal rabbits. The total protein concentrations of the two sera were 5.0 gr/dl and 6.4 gr/dl respectively. Sera were diluted 3:1 with Minimal Essential Medium (MEM; Sigma Co.) and a total volume of 350 ul was applied to the vascularized chorioallantoic membrane (CAM) of embryos on embryonic days E 5,6,7,8,9,10. Embryos were fixed at E 12, processed for light microscopy, and the population of the lumbar LMC was calculated (Hamburger, '75). The mean of the untreated LMC populations was consistant with values in the literature. Re: 12,532 (± 200, n=10). As we have reported, embryos receiving muscle extracts had a mean LMC population of 14,077 (± 272, n=9) which represented an increase in survival of 11.4%. Paradoxically, embryos receiving immune serum had a mean LMC population of 15,508 neurons (± 461, n=5), an increase of 27.4% over untreated controls. Control embryos treated with normal rabbit serum and MEM had LMC populations of 12,727 (± 171, n=4) and 12,462 (± 137, n=5) respectively. Immune serum treated animals differed significantly from controls with a p value of < 0.002. Comparison of immune serum and extract treated embryos demonstrated significance at only the $p < 0.02$ level. These antibodies bind to the sarcolemmal surface and may act in the same manner as pharmacologic denervation which prevents functional synaptogenesis yet increases cell survival. Indirect immunofluorescence with FITC congugated polyvalent anti-rabbit serum revealed anti-muscle extract antibodies on sarcolemmal surfaces. Further studies are needed to identify more clearly the location of these antibodies and the nature of the muscle antigens. (This work was supported by Basic Sciences Research Grant No.37242 awarded to G.J.P).

239.14 PERIPHERAL NERVE AND MUSCLE CHARACTERISTICS AFTER EXPERIMENTAL MODIFICATION OF NEURONAL DEATH IN THE CHICK CILIARY GANGLION. S.D. Meriney, M.J. Ford, H.J.L. Fryer,* B.S. Wautlet and G. Pilar. Department of Physiology and Neurobiology, The University of Connecticut, Storrs, Ct 06268.

The interaction of the ciliary neuron population of the ciliary ganglion with its targets, the iris and ciliary body, have been hypothesized to regulate normal neuronal death. We have examined characteristics of ciliary nerve and muscle after chronic target muscle activity blockade, which is known to increase ciliary neuron survival. Ultrastructural examination of aBTX treated irises after cell death reveals a dramatic decrease in sarcomeric organization of contractile filaments compared to controls. Chronic aBTX treatment also increases both ciliary nerve conduction velocity and axonal myelination, suggesting an increased neuronal maturation compared to controls.

Increased survival and maturation of neurons suggests increased neuronotrophic support from the target is available to developing ciliary neurons in response to target muscle activity blockade. One possibility is an overall increase in neuronotrophic factor production. This however, does not occur in aBTX-treated embryos. In fact, the soluble ciliary neuronotrophic activity from the eye tissues of aBTX-treated embryos was consistently lower than in control embryos throughout the neuronal death period.

Although soluble trophic activity in the eye decreases, neurons may acquire more trophic support by increasing terminal size or number. SV2 is an antigen associated with synaptic vesicle membranes (Buckley and Kelly, J.C.B. 100: 1284, 1985). Accordingly, we have examined the distribution of SV2 in the iris at the end of the neuronal death period. Interestingly, SV2 staining distribution is not significantly different between control and aBTX treated embryos.

These observations indicate that changes in pre- and post-synaptic elements induced by target activity blockade may regulate neuronal survival in a more complex manner than previously hypothesized.

This work was supported by NIH 10338, NSF-BNS 840581, and The University of Connecticut Research Foundation.

239.15 PATTERN OF NEURONAL CELL DEATH WITHIN THE MEDIAL MOTOR COLUMN(MMC) OF A THORACIC NEURAL TUBE TRANSPLANTED TO THE BRACHIAL REGION(THOR-BR)OF CHICK EMBRYOS. P. Cauwenbergs, E. Cosmos & J. Butler* Dept. Neurosci. McMaster University, Hamilton, Ontario L8N 3Z5.

Nerves derived from a thoracic neural tube(NT)transplanted at day 2E initially establish functional(wing motility)and structural contact with brachial muscles of chick embryos. Beyond day 10E, however, this union uncouples, wing motility declines and muscles deteriorate due to an apparent withdrawal of innervation (JEEM 95: 1, 1986). Since preliminary HRP retrograde labelling analyses revealed that the primary source for motor innervation of experimental brachial muscles is the MMC of the foreign thoracic NT, we investigated the fate of MMC motoneurons(MN)during the period of functional interaction and the subsequent uncoupling phase. Both unoperated embryos and embryos receiving a transplanted brachial NT (Br-Br) served as controls. Histological examination of thionin-stained cross-sections revealed that all transplanted NT developed according to their site of origin. Thus, the Thor-Br NT exhibited a small, intermittent lateral motor column(LMC), a large MMC and the column of Terni, similar to an in situ thoracic NT. The Br-Br NT developed a large LMC and no column of Terni, similar to an in situ brachial NT. A quantification of the number of MN in the MMC of experimental and control embryos was performed between days 6E-16E. Whereas neurothanasia was greatest between days 8E-12E (55%) in all control NT (Br-Br, and in situ Br, Thor NT), cell death was not initiated until day 10E in the Thor-Br NT. In the latter, between days 10E-12E, 85% of the MN degenerated; a total of 1000 MN survived. In contrast, 4500 MN remained in all control NT. Thus, the delay in the onset of neurothanasia within the MMC of the Thor-Br NT reflects a positive response to an enlarged peripheral target (wing muscles as opposed to axial muscles which are the usual target for MN of the MMC). Likewise, the abrupt, accelerated rate of neuronal cell death noted between days 10E-12E reflects the actual uncoupling or withdrawal of intramuscular nerves from experimental Thor-Br muscles.

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239.16 A POSTNATAL REORGANIZATION IN THE DISTRIBUTION OF LOCUS COERULEUS NEURONS WHICH PROJECT TO THE SPINAL CORD IN RATS IS DUE TO COLLATERAL ELIMINATION RATHER THAN CELL DEATH. Karen S. Chen and Brent B. Stanfield. Dept. of Neurosciences M-008, Univ. of California (San Diego), La Jolla, CA 92093 and The Salk Institute, La Jolla, CA 92037.

It is now well established that in several instances a change in the tangential distribution of cortical projection neurons occurs during development and that this redistribution which results in the definitive adult pattern is achieved through a process involving the selective elimination of some of the initially extended long axonal collaterals. While it is not known to what extent a similar process is involved in the sculpting of fiber systems which arise subcortically, a recent study by Cabana and Martin (Dev. Brain Res. 15 (1984) 247-263) which showed that in the opossum locus coeruleus (LC) the neurons which project to the spinal cord are more widely distributed early-on than in the adult, raises the possibility that the phenomenon of early collateral withdrawal may be fairly widespread during CNS development. To examine this further we have studied in rats the distribution of labeled LC cells following injections (0.2-0.5 µl of a 2% solution) of Fast Blue (FB) or Diamidino Yellow (DY) into either the rostral forebrain or the spinomedullary junction and made at various ages.

One to four days following spinomedullary injections made on the day of birth, retrogradely labeled neurons are distributed virtually throughout the LC. However, similar injections, but made at four weeks of age, result in the labeling of cells primarily confined to the ventral LC. In contrast, we see no unequivocal difference in the distribution of labeled cells following forebrain injections made at these two ages.

In order to examine the fate of those dorsal LC cells which initially extend a spinal collateral, we again injected FB into the spinomedullary junction on the day of birth, but in these experiments we allowed the animals to survive until the fourth postnatal week - i.e. after the restriction in the distribution of the spinal projection cells has occurred. As in animals killed during the first postnatal week, in these long-term survival animals, FB labeled neurons are spread throughout the LC. Further, in additional cases in which FB was injected into the spinomedullary junction on the day of birth and a similarly placed injection of DY was made four days prior to perfusion during the fourth postnatal week, FB labeled cells are again found throughout the LC, while DY labeled cells (many of which are FB labeled as well) are largely confined to the ventral LC. Thus a restriction of the distribution of LC neurons which project to the spinal cord occurs during the postnatal development of the rat and this reorganization is achieved largely through a process of collateral elimination rather than being due to cell death. Supported by NIH Grant NS-18506.

240.1 INFLUENCE OF NIGRAL SEROTONIN ON STRIATAL DOPAMINE METABOLISM. S.L. Rubinstein* and K. Gale (SPON: D.M. Stoff). Dept. of Pharmacol. Georgetown Univ. Schs. of Med. and Dent., Washington DC 20007.

The substantia nigra (SN) is densely innervated with serotonergic terminals originating from the midbrain raphe nuclei. We are investigating the influence of serotonin (5-HT) receptor stimulation locally within the SN on dopamine (DA) metabolism in the terminals of the nigrostriatal DA projection. 5-HT HCl (47 nmoles in 0.5 ul saline) was microinjected (0.2 ul/min.) through indwelling guide cannulae unilaterally into SN of freely moving, unanesthetized 350 g male Sprague-Dawley rats. Rats were sacrificed by decapitation at 20 or 45 min after injection. Rats sacrificed at 45 min received a supplemental microinjection of 47 nmoles 5-HT in 0.5 ul saline at 20 min prior to decapitation. Controls received microinjections of saline in place of 5-HT. Striata were dissected, rapidly frozen on dry ice and stored at -70° C until time of assay. Levels of DA and the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striata were assessed by reversed-phase ion-pair HPLC/EC analysis of perchloric acid extracts of the striatal tissue.

DA metabolism was elevated in the striata ipsilateral to the nigral 5-HT infusions as compared to that in the striata of rats receiving nigral saline microinjections. A small increase (16%) in DOPAC was measured at 20 min after 5-HT microinjection; at this time no elevation in HVA or DA was obtained. At 45 min, DOPAC was increased by 40%, HVA was increased by 22% and DA was increased by 14% (all elevations at 45 min significant at $p < .05$ level).

The effect of intranigral microinjections of the 5-HT agonist 1-(m-trifluoromethylphenyl)-piperazine (TFMPP) on striatal DA metabolism was also investigated. At 20 min following treatment with TFMPP (30 nmoles in 0.5ul saline) DOPAC in striata ipsilateral to the TFMPP infusion was significantly elevated ($p < .05$) by 29% compared to that in striata of saline microinjected controls. Striatal HVA and DA levels were not significantly altered this time point.

Our findings are not consistent with those of James and Starr (J. Pharm. Pharmacol. 32:196, 1980) who reported decreases in striatal HVA levels 20 min following a comparable intranigral dose of 5-HT. The fact that James and Starr made their microinfusions in anesthetized animals may account for the discrepancy in results. Our current research is aimed at determining 1) if observed effects of 5-HT agonists involve activation of 5-HT receptors and 2) whether, in fact, the observed elevation in DA metabolite levels reflects a stimulation of DA neurons by 5-HT.

240.2 MEDIOLATERAL DISTRIBUTION OF DOPAMINE METABOLITES IN RAT STRIATUM FOLLOWING ACUTE CHLORPROMAZINE. J.A. Childs and K. Gale. Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Numerous biochemical and histological techniques have been applied to examine the distribution of dopamine (DA) terminals, content and receptors in the striatum. The distribution of DA metabolites, DOPAC and HVA, has received less attention. Our laboratory recently reported (Rubinstein et al., Soc. Neurosci. Abst. 11:206, 1985) a rostrocaudal gradient in DA metabolites in the striatum, a gradient which is markedly enhanced by systemic treatment with chlorpromazine (CPZ), a DA antagonist known to increase DA turnover. The difference in DOPAC levels in striatal sections of CPZ-treated rats vs. vehicle-treated rats was 53% higher in rostral than in caudal sections. HVA measured in the same sections showed a CPZ-induced increase that was more than twice as high in rostral as compared to more caudal sections. The present experiment examined whether a mediolateral gradient in DA metabolite accumulation might also exist.

Adult male rats were treated with CPZ (20 mg/kg i.p.) or with an equal volume of saline 45 min prior to decapitation. Freshly dissected striata (tail removed) were sectioned mediolaterally in parasagittal sections (500 uM). Concentrations of DA, DOPAC and HVA were determined by HPLC. In controls, the ratio of HVA:DA was 25% higher in lateral sections than in medial sections, while DA and DOPAC were uniformly distributed. The difference in HVA in striatal sections of CPZ-treated rats vs. those of controls was 36% higher in lateral vs. medial sections; there was no mediolateral gradient in the CPZ-induced increase in DOPAC levels (see Table).

Net increase 45 min after CPZ (pmol/mg prot)

	Medial		→			Lateral
HVA	52	65	76	81	79	80
DOPAC	203	255	300	291	256	259

These results are of particular interest in light of the distinct rostrocaudal and mediolateral gradients for dopamine (D_2) receptors within rat striatum (Altar et al., J. Neurosci. Meth. 10:173, 1984; JPET 233:527, 1985). These authors describe an enrichment of D_2 receptors in rostral (rostral 53% greater than caudal) and lateral (lateral 193% greater than medial) regions of striatum. While it appears that D_2 receptors and accumulation of DA metabolites tend to distribute in the same general direction, we have found the rostrocaudal gradient for DA metabolites more pronounced than the mediolateral gradient, while the converse was described for D_2 receptors. Although there may be a topographical significance to the fact that the DA receptor density is highest in regions containing DA terminals that have relatively high rates of DA turnover, there does not appear to be a direct quantitative correlation between these variables.

240.3 COMPENSATORY CHANGES IN THE ACTIVITY OF NIGRAL DOPAMINE CELLS IN RESPONSE TO PARTIAL DOPAMINE-DEPLETING LESIONS. J.R. Hollerman*, T.W. Berger and A.A. Grace, Depts. of Psychology & Psychiatry, Center for Neuroscience, Univ. Pittsburgh, Pittsburgh, PA 15260.

Previous studies have shown that akinesia in response to 6-hydroxydopamine (6-HDA) lesioning of the nigrostriatal dopamine (DA) system does not occur until 95% or more of the DA input to the striatum is removed (Zigmond & Stricker, 1972). Furthermore, the firing rate of striatal neurons does not increase until a lesion of similar extent is produced (Orr et al, Soc. Neurosci. Abs. 1986). These experiments suggest that the nigrostriatal system is capable of undergoing compensation for rather extensive destruction of the DA projection. In this study, we examined whether or not one such compensatory change involves alterations in the firing rate or pattern of nigrostriatal DA neurons.

Zivic-Miller albino male rats, following DMI pretreatment, received bilateral intraventricular injections of 6-HDA. Two doses of 6-HDA (100 ug & 250 ug) were used to produce 80% DA depletion and 95% DA depletion, respectively, as verified by subsequent analytical measurements. Recordings were made from identified nigral DA neurons (Grace & Bunney, 1983) one week after 6-HDA administration. Three parameters of DA cell activity were measured: 1) the number of spontaneously active DA neurons counted in 12 electrode passes through a predefined region of the substantia nigra (Bunney & Grace, 1978), 2) firing rate, and 3) percentage of spikes occurring in bursts (Grace & Bunney, 1984).

Initial results indicate that one week following 100 ug 6-HDA administration, there was an increase in the average number of DA cells encountered per electrode track (from 0.8 cells/track in controls to 1.3 cells/ track post 6-HDA), an increase in average firing rate (from 4.6 Hz to 6.1 Hz), and an increase in the degree of burst firing. This change was similar to that observed after acute administration of haloperidol to control rats. Since DA neurons had not as yet undergone significant retrograde degeneration at this time point (Onn et al, in press), these results are most likely related to 6-HDA blockade of impulse flow prior to cell degeneration. In contrast, following larger 6-HDA injections, almost no spontaneously active DA neurons were found in the substantia nigra (0.02 cells/track); however, the cells displaying spontaneous activity were firing at very rapid rates (9.5 Hz). Since these recordings also were made at post-injection times too soon to allow for DA cell degeneration, it is unclear if this effect is due to a more toxic action of the higher dose of 6-HDA, or may instead be related to another factor, such as development of depolarization block (Grace & Bunney, 1986). Further experiments on rats one week postlesion as well as at 4-6 weeks postlesion (after cellular degeneration is complete) are currently in progress. (Supported by NS19608).

240.4 L-DOPA INCREASES DOPAMINE RELEASE IN STRIATUM: IN VIVO AND IN VITRO STUDIES. R.W. Keller, W.G. Kuhr, G.L. Snyder, R.M. Wightman, E.M. Stricker, and M.J. Zigmond. Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, and Dept. of Chemistry, Indiana University, Bloomington IN 47401.

L-Dihydroxyphenylalanine (DOPA) has been shown to be effective in the treatment of deficits caused by damage to the dopamine (DA)-containing projections of the nigrostriatal bundle (NSB). The mechanism by which DOPA acts remains poorly understood. We wished to determine whether DOPA increased DA release and, if so, whether DA neurons played any role in this increase.

Rats were injected with 6-hydroxydopamine (6-HDA, 200 ug, ivt.) reducing striatal DA by 70-90%. Two months post-lesion rats were anesthetized, Nafion-coated carbon fiber electrodes (Kuhr et al., J. Neurochem. 43: 560-569 1984) were lowered into the striatum and stimulus-evoked changes in extracellular DA levels were monitored using fast scan cyclic voltammetry. A 10 sec stimulus (60 Hz, 100 uA) was delivered to the NSB before and 30-60 min after ip administration of DOPA (200 mg/kg) and carbidopa (100 mg/kg). In intact rats electrical stimulation resulted in an acute rise in extracellular DA of 49.1 $\pm$ 0.6 uM. When the stimulus was terminated, extracellular DA levels returned to basal levels at a rate of 11.1 uM/sec. After administration of DOPA, stimulation caused extracellular DA levels to rise to 143 $\pm$ 9.5 uM without any change in time course. In 6-HDA-lesioned rats no release was observed before DOPA was given. After DOPA administration the peak response to stimulation was 5.4 $\pm$ 0.1 uM, 11% of the response due to DOPA in intact animals. In addition, the rate of the removal of DA was decreased to 2.5% of the control value. Kinetic analyses indicated that this difference in time course was the result of a decrease in the number of uptake sites for DA rather than a change in the affinity of existing sites.

In parallel experiments, striatal slices (350 um) were prepared and incubated in Kreb's bicarbonate buffer. Endogenous DA efflux was examined under basal conditions and during field depolarization (bipolar, 10 mA, 4.5 Hz for 15 min). Dopa (10 uM) increased basal and stimulated efflux 21-fold and 11-fold, respectively. Both components of efflux were markedly attenuated in slices prepared from 6-HDA-lesioned animals.

These results suggest that (a) DOPA increases DA efflux under basal conditions and in response to depolarization, (b) a large proportion of this increase comes from DA neurons, and (c) once released, this DA is dependent on terminals of DA neurons for inactivation. Hence, after partial injury to the NSB, the DA neurons that remain as well as the relative absence of sites for DA uptake may both play important roles in the capacity of DOPA to restore extracellular DA levels toward normal. Supported by USPHS NS19608 & MH29670 and NSF BNS83-20510.

240.5 ULTRASTRUCTURE OF THE DOPAMINE NIGROSTRIATAL SYSTEM IN THE RAT FOLLOWING STRIATAL INJECTION OF IBOTENIC ACID. D.E. Smith, V.M. Pickel, M. Saji*, T. Joh and D.J. Reis. Dept. of Anatomy, LSU Med. Sch., New Orleans, LA 70112, and Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

When ibotenic acid (IBO) is injected stereotaxically into the rat striatum, light microscope (LM) studies show destruction of intrinsic and projection neurons of the striatum and shrinkage of the dopaminergic (DA) neurons in the substantia nigra (SN). In order to investigate the ultrastructural changes as they relate to the DA neurons of the nigrostriatal system, a series of IBO injected rats were perfused at 7,30 and 60 days postinjection. Representative sections from the striatum and substantia nigra were processed for immunocytochemical labeling of tyrosine hydroxylase (TH), the catecholamine synthesizing enzyme.

At 7 days, the striatum ipsilateral to the injection is edematous and contains multivesicular bodies, myelin figures and dissociated organelles. Terminals containing TH reaction product are present, but none are seen in contact with a postsynaptic structure; astrocytic processes are prominent. In the ipsilateral SN, some neurons containing TH reaction product display large cytoplasmic vacuoles. In SNr (reticulata), degenerating myelinated axons are observed and one sees fewer small dendritic branches containing TH reaction product. By 30 days, TH reaction product is still evident in enlarged terminals in the striatum. Multivesicular bodies, swolled mitochondria and fragmented membranes are also present. Dendrites in SNr have an irregular shape and are often surrounded by a watery glial envelope. By 60 days, myelinated axons are again seen in the striatum. TH labeled terminals and neuroglia are also present. The SNr is atrophied and dendrites containing TH reaction product extend only a short distance into the neuropil.

Detection of immunoreactivity for TH in axon terminals without speciallized junctions are visible up to 60 days following IBO lesions of the target neurons. This suggests that these terminals may assume a neuromodulatory function with undamaged neurons and glia. Furthermore, diminution of the TH labeled dendritic arbor in SN provides morphological support for postsynaptic alteration in DA neurons of SN following chemical lesions of the striatonigral path. (Supported by NS07780, Smith; NSF 8320120,NIMH 00078, Pickel)

240.6 PARTICIPATION OF DOPAMINE IN THE EXCITABILITY CHANGES INDUCED IN THE STRIATAL DOPAMINE-CONTAINING TERMINALS. M. García-Muñoz, P. Patiño*, L. Chávez-Noriega*, G.W. Arbuthnott* and A. Ryman*. Institute of Cellular Physiology. U.N.A.M. P.O. Box 70-600. México, D.F. 04510

It has been demonstrated that dopamine terminals in the striatum are susceptible to excitability changes due to autoreceptor activation (Groves, P.M. et al, Brain Res., 221: 425, 1981). On the other hand we have previously shown that the cortico-striatal input decrease the excitability of the dopaminergic terminal field (Chávez-Noriega, L. et al, Brain Res., 1986). The present experiments were carried out in an attempt to elucidate the participation of autorreceptors in the excitability changes induced by the cortico-striatal pathway.

Male Wistar rats where anesthetized with halothane and fixed to a stereotaxic instrument. The striatum was stimulated with a bipolar stainless-steel electrode, monophasic square wave pulses (1000-1500 uA/0.5 msec) were delivered at 1 Hz. Extracellular activity was recorded in substantia nigra; all cells were antidromically activated as confirmed by collision with spontaneous action potentials. The striatal stimulating current was adjusted to threshold defined as the minimun current necessary for antidromic stimulation of the cell on a 100% of non-collision trials. Once the threshold was determined, a train of monophasic square wave pulses (500 uA, 0.3 msec) were delivered to the frontal cortex through a bipolar stimulating electrode.

A group of 7 rats received a pretreatment of alpha-methyl-para-tyrosine (AMPT) (20 mg/kg, i.p.) 12 and 3 hrs before the acute experiment. Another group received reserpine (200 mg/kg, i.p.) 24 hrs before the recording session in addition to AMPT.

In 15 control rats frontal cortex stimulation produced an increase in the threshold for antidromic stimulation of 31 $\pm$ 9.9% compared to its control. After AMPT no change (1.6 $\pm$ 0.8 %) was seen. After reserpine and AMPT treatment only a 3.3 $\pm$ 0.2 % increase was observed.

It is proposed that the changes in excitability seen in the dopaminergic terminal field in the striatum are due to dopamine being released as a consequence of frontal cortex stimulation.

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240.7 TRANSMITTER-SPECIFIC PROJECTIONS FROM THE BASAL GANGLIA TO THE TEGMENTUM IN PIGEONS. A. Reiner. Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109.

In birds, neurons of the striatum contain substance P (SP), dynorphin (DYN), enkephalin (ENK) and GABA (Reiner, Brain Res., '86), while neurons of the pallidum contain GABA and the neurotensin-related hexapeptide LANT6 (Reiner, Anat. Rec., '86). Some of these striatal and pallidal neurons may contain more than one of these substances. For example, SP and DYN co-occur in many striatal neurons (Reiner, Brain Res., '86). To determine whether populations of striatal and pallidal neurons containing the above substances project to tegmental dopaminergic (DA) neurons, the effects of knife cuts of the descending basal ganglia efferents on the distribution of the tegmental fibers containing these substances was examined using immunohistochemical techniques.

SP+ fibers were identified with a monoclonal antibody against SP (obtained from Accurate Chemical Co.), DYN+ fibers with an antiserum specific for the C-terminus of Dynorphin A (1-17)(provided by L. Terenius), ENK+ fibers with an antiserum specific for methionine-enkephalin-arg6-phe7 (provided by G.J. Dockray), GABAergic fibers with an antiserum against glutamic acid decarboxylase (provided by I.J. Kopin) and LANT6 with an antiserum specific for LANT6 (provided by R.E. Carraway).

Each of the substances under investigation was found to be present in fibers that were densely clustered within the tegmental regions that are rich in DA neurons. Fourteen days after unilateral stereotaxic knife cuts at mid-diencephalic levels of the fiber bundle composed of striatal and pallidal axons, SP+ fibers and DYN+ fibers were dramatically reduced in the tegmental DA neuron-rich regions, GABAergic fibers and LANT6+ fibers were substantially reduced (but not as much as the SP+ and DYN+ fibers) and ENK+ fibers were reduced but less than the others examined.

Previous studies have shown that SP and DYN co-occur extensively in neurons within the portion of the striatum projecting to the tegmentum (Reiner, Brain Res., '86). Since the present results show that major SP+ and DYN+ striatotegmental projections are present in pigeons, it seems very likely that a population of striatal neurons containing both SP and DYN is the source of a major projection to tegmental DA neurons. The present results suggest that a small number of ENK+ striatal neurons also project to tegmental DA neurons. Finally, since GABA and LANT6 have been shown to co-occur in pallidal neurons (Reiner, Anat. Rec., '86), the reduction in the numbers of LANT6+ and GABAergic fibers in the tegmental DA neuron-rich regions is consistent with the possibility that a population of pallidal neurons containing both LANT6 and GABA projects to the tegmentum. Thus, it appears likely that a number of different transmitter-specific neuronal populations of the basal ganglia influence tegmental DA neurons. Supported by NS-19620.

240.8 DISTRIBUTION AND MORPHOLOGICAL CHARACTERISTICS OF DOPAMINE IMMUNOREACTIVE NEURONS IN THE MIDBRAIN OF THE SQUIRREL MONKEY. M.-Y. Arsenault, A. Parent, P. Séguéla* and L. Descarries. Lab. de Neurobiol., Fac. Méd., Univ. Laval, Québec, and Centre de recherche en sci. neurol., Fac. Méd., Univ. de Montréal, Qué., Canada.

The distribution of midbrain dopamine (DA) neurons was investigated in the squirrel monkey (Saimiri sciureus), using a highly specific antiserum raised against DA-glutaraldehyde-lysyl-protein conjugate and the peroxidase-antiperoxidase immunohistochemical method. DA-immuno-stained neurons were located in four contiguous areas: (1) the substantia nigra, pars compacta (SNc), (2) the ventral tegmental area (VTA), (3) the retrorubral area (RRA) and (4) the periaqueductal gray (PAG). The SNc comprised the vast majority of midbrain DA neurons; most of its immunoreactive cells were relatively large (mean diameters: 36 x 13 um), they varied in shape from fusiform to polygonal but a few smaller-sized (14 um) globular reactive neurons were dispersed among them. The DA-positive neurons were particularly abundant in the caudal two-thirds of the SNc, where they formed several distinct cell columns impinging deeply upon the underlying pars reticulata (SNr). Some of these trabeculae surrounded large oval sectors mostly devoid of immunoreactivity within which a few unstained SNr neurons were present. The long dendritic processes of the SNc DA cells formed prominent bundles oriented dorsoventrally which arborized along the dorsal surface of the cerebral peduncle. In the VTA, most positive neurons were regrouped in a well-delineated triangular zone dorsal to the interpeduncular nucleus and medial to the oculomotor fibers. These DA cells were of medium size (20 x 13 um), globular or fusiform in shape, and usually showed one or two thick primary dendrites oriented dorsoventrally. The immunoreactive cells in the RRA laid in continuity with the caudalmost DA elements of the SNc, but could be distinguished by their smaller size (28 x 13 um), shorter and more profusely branched dendrites and darker immunostaining. These DA neurons were characteristically scattered among fibers of the lateral lemniscus, and a few could be observed as far caudally as the pedunculopontine nucleus. In the PAG, immunostained neurons were seen at all rostrocaudal levels of the midbrain. These cells were of medium size (28 x 10 um) and prevailed within the ventral half of the PAG. Some came close to the ventricular lining but they did not intermingle with dorsal raphe neurons caudally. Preliminary electron microscopic observations of the SNc revealed that its DA neurons are endowed with spiny dendrites synaptically contacted by numerous immunonegative axon terminals. Many axons presumably arising from these cells were myelinated and no immunoreactive axonal varicosities or terminal boutons were visible. These data provide a basis for further comparative analyses in primates. (Supported by MRC and FRSQ).

240.9 IMMUNOHISTOCHEMICAL LOCALIZATION OF GABA-CONTAINING NEURONS IN BASAL GANGLIA OF THE SQUIRREL MONKEY. Y. Smith, A. Parent, P. Séquéla* and L. Descarries. Lab. de Neurobiologie, Fac. Méd., Univ. Laval, Québec, and Centre de recherches en Sciences Neurol., Univ. Montréal, Montréal, Qué., Canada.

The distribution of γ-aminobutyric acid (GABA) immunoreactivity in basal ganglia of the squirrel monkey (Saimiri sciureus) was studied by means of the indirect peroxidase-antiperoxidase method and a highly specific antiserum raised against GABA-glutaraldehyde-lysil-protein conjugate. In neostriatum, GABA-immunoreactive cells represented approximately 20-30% of the total neuron population. The vast majority of these somata were of medium size (average diameter: 10 um), but some larger ones (25 um) were also observed. Neostriatal GABA-immunoreactive cells displayed no obvious clustering but were nevertheless significantly more numerous in caudate nucleus than in putamen and more abundant at caudal than rostral levels of the striatum. Their numerical density amounted to 62 and 47 cells/mm^2 in the rostral third of the caudate nucleus and putamen, respectively; the corresponding values for the caudal third of these regions were 110 and 72 cells/mm^2. Positive axon terminals were also seen in moderate number and appeared rather homogeneously distributed. In ventral striatum, a moderate number of GABA-immunoreactive cells and axon terminals were present within the nucleus accumbens and in deep layers of the olfactory tubercle, whereas, in the islands of Calleja, many terminals but no cell bodies were positive. In globus pallidus, almost every cells were GABA-immunoreactive and the neuropil comprised a multitude of GABA-positive terminals. In substantia innominata, aggregates of small, globular GABA-immunoreactive somata were scattered among clusters of larger non-immunoreactive neurons belonging to nucleus basalis. The entire substantia innominata showed a low to moderate spread of GABA-positive terminals. In subthalamic nucleus, the cells were generally surrounded by several GABA-positive terminals but did not themselves show GABA-immunoreactivity. In substantia nigra, GABA-immunoreactive somata predominated in the pars lateralis (SNl) and progressively decreased in number along the latero-medial axis of the pars reticulata (SNr). In the pars compacta (SNc) and in the ventral tegmental area (VTA), they were few in number and of small size, and dispersed among larger, unreactive neurons. In both SNl and SNr, GABA-positive terminals were abundant and their distribution was rather uniform; a smaller number of terminals was visible in SNc and VTA. These findings indicate that GABA is widely and heterogeneously distributed in the various components of basal ganglia in the squirrel monkey. This neurotransmitter may thus play a crucial role in the functional organization of these forebrain structures in primates. (Supported by MRC and FRSQ).

240.10 A 2-DEOXYGLUCOSE STUDY OF THE EFFECTS OF DOPAMINE D-1 AND D-2 AGONISTS IN RATS WITH UNILATERAL NIGRAL LESIONS. J.M. Trugman and G.F. Wooten (SPON: J.E. Greenlee). Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908.

The in vivo effects of dopamine D-1 and D-2 specific agonists were studied by ^{14}C-2-deoxyglucose (2-DG) autoradiography in rats with unilateral substantia nigra (SN) lesions. Male Sprague-Dawley rats with effective 6-hydroxydopamine SN lesions were studied 3-5 weeks post-lesion. Drugs were administered intravenously and followed one minute later by ^{14}C-2-DG (25 μCi/0.4 ml saline i.v.). Film autoradiographs were analyzed with a microdensitometer and RCGU data were expressed as optical density ratios of gray matter/corpus callosum. Dunnett's test was used to compare the experimental groups to lesioned-controls.

Administration of the selective D-1 agonist, SKF-38393 5.0 mg/kg, resulted in contralateral facial grooming, no rotational behavior, and RCGU increases in the ipsilateral entopeduncular nucleus (EP, ↑75%) and SN pars reticulata (SN$_r$, ↑125%). The percentage increases are expressed relative to the same structure in the control group. SKF-38393 25 mg/kg produced contralateral turning (mean 7 rotations/min) and more prominent RCGU increases in the ipsilateral EP (↑220%) and SN$_r$ (↑270%). Administration of the selective D-2 agonist, LY-171555 0.1 mg/kg, elicited facial grooming and minimal turning with no RCGU changes in the EP and SN$_r$. LY-171555 1.0 mg/kg produced contralateral turning (mean 3/min), bilateral equivalent RCGU increases in the EP (↑70%) and a mild RCGU increase in the contralateral SN$_r$ (↑30%). Both SKF-38393 and LY-171555 increased RCGU in the subthalamic nucleus, parafascicular thalamus, and deep layers of the superior colliculus, and decreased RCGU in the lateral habenula ipsilateral to the SN lesion.

In summary, D-1 agonist-induced contralateral rotation was accompanied by large RCGU increases in the ipsilateral EP and SN$_r$, producing a RCGU pattern qualitatively and quantitatively similar to that produced by apomorphine and L-dopa in this model. In contrast, D-2 agonist-induced turning is not accompanied by pronounced RCGU asymmetries in the EP and SN$_r$. These results are consistent with prior behavioral and lesion studies which have suggested that D-1 and D-2 agonists mediate rotation via anatomically distinct striatal efferent pathways (Herrera-Marschitz and Ungerstedt, Brain Res., 323:269-278, 1984).

Supported by the United Parkinson Foundation.

240.11 A TOPOGRAPHY OF DOPAMINE RELEASE IN RAT STRIATUM. B.K. Yamamoto and N. Leidenheimer. Dept. Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

The neostriatum is heterogeneously organized both biochemically and anatomically. Dopamine (DA) content and turnover is highest in the rostral caudate and lowest caudally, while D-2 receptors follow a lateral-to-medial gradient. We studied whether regional DA release, measured by in vivo voltammetry, follows a topographic distribution similar to that reported for DA content. In addition, a release topography was also investigated in relation to the heterogeneous distribution of D-2 receptors. Male Sprague-Dawley rats were anesthetized with urethane. Stearate-modified carbon paste electrodes were implanted into 5 areas of the striatum: anterodorsal medial (ADM) (1.2;1.5;-5.0), anterodorsal lateral (ADL) (1.2;3.5;-4.5), nucleus accumbens (NA), post rodorsal medial (PDM) (-0.8;2.7;-4.5) and the posteroventral lateral (PVL) (-0.8;4.8;-7.2). Recordings were every 5 min. by linear sweep voltammetry from -0.2V to +0.4V. Amphetamine or the D-2 antagonist, haloperidol were injected i.p.. Data are expressed as percent baseline.

	0 min	60 min	120 min
	Amphetamine (1.25 mg/kg)		
ADL	98.8 ± 0.5	134.4 ± 6.3	95.2 ± 1.4
ADM	101.1 ± 0.4	136.6 ± 5.1	103.7 ± 1.7
NA	100.7 ± 0.4	140.3 ± 4.6	109.8 ± 2.0
PVL	99.6 ± 1.0	118.4 ± 1.7*	99.0 ± 3.1
PVM	100.3 ± 0.5	120.2 ± 2.5*	108.6 ± 2.2
	Haloperidol (0.5 mg/kg)		
ADL	97.7 ± 1.1	165.5 ± 8.3*	163.2 ± 13.1*
ADM	99.2 ± 1.0	130.1 ± 5.1	128.3 ± 5.3
NA	98.3 ± 2.2	143.7 ± 6.3	144.8 ± 5.8
PVL	100.0 ± 0.3	181.7 ± 13.7*	177.0 ± 12.6*
PVM	101.6 ± 1.0	140.6 ± 6.6	145.7 ± 7.8

*Significantly different from other regions ($p<0.05$). Results with the specific D-2 antagonist (-)sulpiride were similar to haloperidol. Therefore, amphetamine-induced DA release correlates with the rostral-caudal distribution of DA content while release following D-2 receptor blockade corresponds to the medial-to-lateral gradient of D-2 receptors. In conclusion, striatal DA release is topographically organized and is dependent on the receptor subpopulation and the degree of dopaminergic innervation of specific subregions.

240.12 FUNCTIONAL LINK BETWEEN SUPERSENSITIVE STRIATAL D-1 AND D-2 RECEPTOR AFTER UNILATERAL NIGRAL 6-OHDA LESION. A.C. Rossi, M. Buonamici*, C. Caccia*, M. Carpentieri*, R. Maj* and L. Pegrassi*. R.& D., C.N.S., Farmitalia Carlo Erba, 20014 Nerviano, Italy.

Rats bearing unilateral lesions of the nigrostriatal pathway by 6-OHDA (Ungerstedt, U. and Arbuthnott, G.S., Brain Res., 24:485, 1970) showed the expected unidirectional circling behavior, contralateral to the lesioned side, in response to direct or indirect dopamine (DA) agonists. This contralateral turning has been attributed to supersensitivity of the denervated striatal DA receptors (Creese, I. and Snyder, S.H., Europ. J. Pharmacol., 56:277, 1979). Three months after the lesion the striatal binding characteristics were contemporarily determined for striatal D-1 (labeled by ^{3}H-flupentixol) and D-2 (labeled by ^{3}H-spiperone) receptors in turning rats. No differences between lesioned and non-lesioned sides did appear for K_D values, but B_{max} values resulted consistently increased, more for D-1 (+31%) than for D-2 (+16%) receptor subtypes, in the lesioned side. The functional significance of the D-1 and D-2 supersensitivities was explored administering to a parallel group of lesioned rats several selected DA agonists or precursors, after pretreatment with the D-1 antagonist SCH 23390 or the D-2 antagonist l-sulpiride, following a latin square design. The D-1/D-2 ratio for DA-receptor stimulation was assessed in vivo for each tested compound on circling behavior. Surprisingly the specific block of D-2 or D-1 receptors enhanced the turning-inducing effects of D-1 or D-2 agonists, respectively. This finding is clearly suggestive of a functional link between striatal D-1 and D-2 receptor subtypes.

240.13 INTERCONNECTIONS OF THE MESENCEPHALIC DOPAMINE CELL GROUPS. A. Y. Deutch, P.W. Kalivas[2], M. Goldstein[3] and R.H. Roth. Departments of Pharmacology & Psychiatry, Yale Medical School, New Haven, CT 06510; [2]Washington State Univ., Pullman, WA; [3]NYU Medical Center, NY, NY.

The dopamine (DA) cell groups of the mesencephalon give rise to the mesocortical, mesolimbic, and nigrostriatal DA systems. On the basis of ontologic and hodologic studies it has been suggested that these forebrain DA innervations comprise a single mesotelencephalic DA system. Recent studies indicate that perturbations in the functional activity of a given forebrain DA projection area effect alterations in the activity of other telencephalic DA systems, suggesting that the various DA systems may function in an integrated manner. Projections from DA terminal field areas onto midbrain DA cell body regions and other DA terminal field regions have been thought to represent the anatomical basis of such integrated functional activity. We now report that there are also interconnections between the three mesencephalic dopamine cell groups.

Combined anterograde tracer (PHA-L)-immunohistochemical (tyrosine hydroxylase, TH) and retrograde tracer (WGA-HRP)-TH methods were used to assess interconnections between the A8 (retrorubral field, RRF), A9 (substantia nigra, SN), and A10 (ventral tegmental area, VTA) DA cell groups. PHA-L injections of the RRF and SN resulted in heavy anterograde filling of axons which funneled through the SN and into the VTA before turning rostrally to ascend to the forebrain. Axons could also be traced into the contralateral VTA, SN, and RRF. Terminal varicosities could be observed in the VTA and in the SN and RRF, although the majority of axons appeared to represent fibers of passage. Occasional pericellular axon terminal arrays could be seen to surround TH-positive neurons in the VTA. PHA-L injections into the VTA also revealed projections to the lateral SN and RRF; these efferents were much less extensive than the medially-directed projections. Discrete iontophoretic deposits of WGA-HRP into the VTA resulted in retrograde labeling of both DA and non-DA neurons in the SN and RRF. Numerous retrogradely-labeled neurons in the RRF were noted, less than half of which were TH-positive. In contrast, few labeled A9 neurons were observed. WGA-HRP injections in the RRF also resulted in the retrograde labelling of neurons in the VTA; these were predominantly non-dopaminergic.

These data indicate that there are extensive interconnections between the mesencephalic dopamine cell groups. It is unclear to what degree these projections represent axon collaterals from DA neurons projecting to telencephalic or brainstem sites. These interconnections may allow the integrated function of the forebrain DA projection systems, and suggest that heterogeneities in DA efferents may be embedded within a larger integrated mesotelencephalic system.

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240.14 EFFECTS OF UNILATERAL DOPAMINE LESIONS OF SUBSTANTIA NIGRA ON TRAINED CIRCLING IN THE RAT. M. Reite, J.B. Richards* and C.R. Freed, Depts. of Psych., Med., and Pharm., Univ. Colo. Health Sciences Center, Denver, CO 80262.

Using the trained circling paradigm developed in our laboratory (Yamamoto and Freed, Nature 298: 467, 1982), Dunnett and Bjorklund (Neurosci. Lett. 41: 173, 1983) have shown that unilateral dopamine (DA) depletion impaired the rats" ability to circle in the direction contralateral to the lesion. They concluded that the primary motor deficit of the rats was the initiation of movement. Using the circling rat model we have studied the effect of unilateral lesions of the nigrostriatal system. Twelve water deprived rats were trained to circle for a 10% sucrose solution using a computer automated training apparatus. Each rat was trained to circle in both left and right directions on alternate days. For example, if a rat were rewarded for turning left on day one the same rat was rewarded for turning right on day two. Two different training chambers were used and the reward for turning in a given direction was always associated with a particular chamber. After performance had reached a maximum, the rats were lesioned with unilateral injections of 6-hydroxydopamine hydrobromide (8 ug in 2 ul of 0.2 mg/ml ascorbate-saline) aimed at DA fibers in the medial forebrain bundle (AP. 4.4 behind bregma; L. 0.9 from midline; V. 7.5 below dura; nose bar set at 2.3 below interaural line). The effectiveness of lesions was tested by injecting 1.0 mg/kg amphetamine and observing the circling response without reward. All 12 rats circled ipsilateral to the lesion at an average rate of 4.63 rpm. In rewarded circling, we found that unilateral DA lesions resulted in decreased turning rates both ipsilateral and contralateral to the lesion. The rate of ipsilateral turning was reduced from 8.56 rpm to 4.87 rpm (43%). The rate of contralateral turning was even more reduced to 1.01 rpm (89%). In some rats contralateral turning for reward was abolished. Further analysis of the rats" turning deficit revealed two separate effects. First, in agreement with Dunnett and Bjorklund, we observed an increase in the pause after completing a turn and starting the next turn. Second, there was a decrease in the speed at which the rats actually turned. We interpret these results as indicating that rats with unilateral DA depletion show a complex motor deficit influencing both contralateral and ipsilateral turning.

240.15 ULTRASTRUCTURAL EVIDENCE OF DOPAMINERGIC INPUT TO ENKEPHALIN, SUBSTANCE P AND GABAERGIC NEURONS IN RAT NEOSTRIATUM. Y. Kubota*, S. Inagaki, S. Kito, J.-Y. Wu., and E. Senba 3rd Dept. of Int. Med., Hiroshima Univ. Sch. of Med, Kasumi, Hiroshima, 734 Japan, Dept. of Physiol, Pennsylvania State Univ., Hershey Med. Ctr., Hershey, PA 17033, Dept. of Neuroanatomy, Osaka Univ. Med. Sch., Kita-ku, Osaka, 530 Japan.

The synaptic relationships between the neuronal structures reacting with antibodies to tyrosine hydroxylase (TH), and leu- or met-enkephalin (ENK), substance P or glutamate decarboxylase (GAD),were studied by the electon microscopic " mirror technique" in adjacent sections of the rat neostriatum.

ENK-immunoreactive (ENK-IR) neurons showed medium sized perikarya (10-15 um in diameter) with round unindented nuclei, suggesting that they belonged to the medium size spiny category of striatal output neurons. They corresponded to strio-pallidal ENK neurons as previously described. ENK-IR neurons always received TH-IR axonal boutons on their surface and frequently received simultaneous ENK-IR terminlas.

SP-IR neurons showed fusiform or oval shaped medium size perikarya (10-15 um in diameter) with unindented nuclei, suggesting that they belonged to the mediumsize spiny category of striatal output neurons, and corresponded to striatonigral substance P neurons. Conterparts of all 33 SP-IR neurons were close to TH-IR axonal boutons.

GAD-IR neurons were divided into three groups. The first type cell was medium in size (12.7 $\pm$ 1.88 um, n=12) with an unindented nucleus, and thus it was suggested to belong to the medium spiny catgory that posseses a long axon. The second type of GAD-IR neuron was medium in size (13.9 $\pm$ 1.25 um, n=11) with nuclear indentations, suggesting this type was medium aspiny neuron with a short axon. The third type neuron was large in size (20.3 $\pm$ 2.96 um, n=11), but slightly larger than the first type neuron, with deep nuclear indentations. All types of GAD-IR neurons received symmetrical synapses from TH-IR axons and very frequently simultaneous inputs from GAD-IR axons. The findings of this study provided ultrastructural evidence that TH-IR axons, presumably originated from dopaminergic nigrostriatal neurons, can influence a large variety of striatal neurons, such as ENK, SP and GABAergic neurons.

240.16 GABA RECEPTOR CHANGES IN THE CAT MOTOR THALAMUS AFTER IPSILATERAL DEAFFERENTATION FROM THE BASAL GANGLIA. J. Fogarty*, B. Hughes*, I. Ilinsky and K. Kultas-Ilinsky.(SPON: I. Bruce).Dept. of Anatomy, University of Iowa College of Medicine, Iowa City, IA 52242.

The ventral anterior and ventral medial thalamic nuclei of the cat receive massive GABAergic input from the substantia nigra pars reticularis (SNr) and entopeduncular nucleus (EPN). In the current series of experiments stereotactic injections of kainic acid (1.5 µg/µl) in phosphate buffer pH 7.4 were made ipsilaterally in the SNr and EPN. Animals were sacrificed at 1 week and at 3, 6, and 8 months postlesion. Using ^{3}H-muscimol as a ligand, receptor binding assays were performed on 10 µm serial fresh frozen sections. After incubation the sections and Amersham tritium standards were exposed against LKB ultrofilm. The autoradiographs of the sections (in triplicate for each ligand concentration) were digitized and averaged using the Computer Image Analysis System at LONI, Dept. of Neurology, Washington University, St. Louis. Two types of analyses were performed on the images. In one, the optical density readings were converted into concentrations of radioactivity and specific regions of the motor nuclei were measured. Saturation curves and Scatchard plots were then constructed for each of the regions. In the other, a linear regression analysis program (Toga et al., J. Neurosci., 1986) was used to determine Bmax and K_D for each series of computer averaged images of specific binding. The two types of analyses correlated well, and both demonstrated a complex nature of lesion-induced GABA receptor changes in the thalamic motor nuclei. These changes were bilateral and consisted of a gradual increase in Bmax and a slight decrease in K_D in the basal ganglia projection zone over the time period studied. Additionally, non-linear Scatchard plots obtained from both lesioned and control animals seem to indicate the presence of two or more GABA receptors in the motor thalamus. The findings suggest the involvement of these receptors in lesion-induced reorganization in the adult motor thalamus.

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240.17 AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-BACLOFEN BINDING IN THE CAT THALAMUS. B. Hughes*, J. Fogarty*, A. Toga, I. Ilinsky and K. Kultas-Ilinsky. (SPON: A. Afifi) Dept. of Anatomy, University of Iowa College of Medicine, Iowa City, and LONI, Dept. of Neurology, Washington University School of Medicine, St. Louis.

The basal ganglia projections to the thalamus are known to be GABAergic and are distributed to the ventral medial (VM) and ventral anterior (VA) nuclei. However, binding studies with $GABA_A$ receptor ligands such as muscimol and flunitrazepam have not demonstrated a correlation between the distribution of the binding sites and the known basal ganglia projection zones in the thalamus. The purpose of the present study was to determine whether the distribution pattern of $GABA_B$ receptors more closely resembled the distribution of GABAergic basal ganglia afferents in the thalamus. ^{3}H-baclofen, an agonist for $GABA_B$ receptors, was used as a ligand. The binding assays were carried out on 10 µm fresh frozen serial sections which were at first preincubated in 0.05 M Tris-HCL buffer, pH 7.4, containing 190 mM sucrose and 2.5 mM $CaCl_2$ for 50 min at room temperature. The assay medium contained varying concentrations (10-120nM) of (-)^{3}H-baclofen in the same buffer. The incubation was carried out for 30 min at 37°C and was followed by 3 x 2 min buffer rinses at 0°C. The nonspecific binding was determined under the same conditions in the presence of 10 mM of cold GABA or lioresal. The sections and Amersham autoradiographic tritium standards were then apposed to LKB ultrofilm. The resulting autoradiographs were digitized using the LONI Computer Image Analysis System. The computer was used to create images of specific binding by subtracting unspecific from total binding. The distribution of the unspecific binding (40% of total) was relatively uniform as opposed to specific binding where a number of nuclei showed high binding intensity. The specific binding was the highest in the VA and VM as well as in limbic nuclei and the lateroposterior nuclear complex, whereas it was very low in the VL and practically absent in the VB. Furthermore, the binding intensity was very uniform in the VA and VM which is different than the distribution pattern of muscimol binding sites in these nuclei. The latter usually shows low binding in the zone of overlap of pallidal and nigral projections (VA and lateral VM) in comparison to the high intensity of binding in medial VM. Our results indicate that the distribution of ^{3}H-baclofen binding sites corresponds better with the basal ganglia projection zones than other GABA receptor ligands studied. However, the presence of high binding intensity in several thalamic nuclei without extrinsic GABAergic inputs makes it difficult to conclude that there is a definite association of $GABA_B$ receptors with the basal ganglia thalamic pathways. Supported by NINCDS grant RO11928.

240.18 GABAERGIC SPINY NEURONS IN THE NEOSTRIATUM OF THE RAT. Lorraine Dubé* and Laurent Descarries (SPON: J.Y. Montplaisir). Centre de recherche en sciences neurologiques, Faculté de médecine, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Immunocytochemistry was combined with Golgi impregnation (Somogyi et al., Neurosci. 9:475, 1983) to investigate the morphology of putative GABA-containing neurons in the neostriatum of adult rat. Thick sections (70 µm) were immunostained using a highly specific antiserum against GABA-glutaraldehyde-lysyl-protein conjugate (Séguéla et al., Proc. Natl. Acad. Sci. 81:3888, 1984) revealed by the peroxidase-antiperoxidase method. They were then impregnated by the section-Golgi procedure (Gabbott and Somogyi, J. Neurosci. Methods 11:221, 1984). Many neuronal somata showing GABA immunoreactivity were visible throughout the neostriatum, in increasing number rostrocaudally. In keeping with earlier results based on glutamate decarboxylase immunoreactivity or [^{3}H]GABA uptake (Bolam et al., J. Comp. Neurol. 213:121, 1983; 237:1, 1985), a significant fraction of the GABA-immunostained nerve cell bodies exhibited deeply indented nuclei and small to large perikarya of variable shape, all features typical of striatal interneurons. There were however a considerable number of GABA positive somata (2/3) which displayed the characteristics of medium size (10-20 µm) spiny neurons, i.e. a round, centrally located nucleus surrounded by a thin rim of cytoplasm. These neurons showed no visible nuclear invagination in the light microscope. Among the numerous Golgi impregnated neurons in this material, some exhibited a dense Golgi staining of their dendritic tree but only partial impregnation of their parent cell body, thus allowing concomitant GABA immunoreactivity to be seen. Out of 15 such GABA positive cells so far examined, 4 had a deeply invaginated nucleus and a perikaryon giving rise to smooth elongated dendrites. The 11 others were endowed with densely spinous dendrites emerging from medium size somata containing a spherical, not visibly indented nucleus. The dendrites of these cells were initially smooth, divided into secondary branches at a distance of 10-30 µm from the nerve cell body, and then became progressively laden with spines. In 1 such case, the axon could also be followed: it arose from a primary dendrite, gave rise to collaterals branching profusely within the dendritic field of its parent neuron, and bore small enlargements spaced along its whole length. Numerous immunostained punctate axon terminals were also visible throughout the neuropil, some of which came close to the somata and dendrites of the densely spinous GABA-reactive neurons. These data convincingly demonstrate that GABA is present in medium size densely spiny neurons of the rat neostriatum. This conclusion has far reaching implications since neurons of this morphological type have been shown to be the major source of neostriatal efferents. (Supported by FRSQ and MRC).

240.19 ORGANIZATION OF HIPPOCAMPAL AND THALAMIC AFFERENTS OF THE NUCLEUS ACCUMBENS IN THE RAT. RELATION WITH INHOMOGENEITIES IN ENKEPHALIN-LIKE (ENK) IMMUNOREACTIVITY.
H.W.Berendse* and H.J.Groenewegen, Dept. of Anatomy, Vrije Universiteit, Amsterdam, The Netherlands. (SPON: European Neuroscience Association).

The aim of the present study was to determine the topographical organization of the hippocampal and thalamic afferents of the nucleus accumbens in the rat, and to relate the projection pattern of these afferents to the distribution of enkephalin-like (ENK) immunoreactivity. The anterograde transport of the lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) was used to mark the hippocampal and thalamic fibers in the nucleus accumbens. This technique is pre-eminently suited to distinguish between terminal labeling and staining of passing fibers. The results of the tracing experiments demonstrate that there exists a distinct topography in the projections from the subiculum to the nucleus accumbens. The ventral subiculum projects primarily to the caudomedial part of the nucleus accumbens and in decreasing intensity to rostromedial parts of the nucleus, whereas progressively more dorsal portions of the subiculum send fibers to successively more rostral and lateral parts of the nucleus accumbens. The termination area of the most dorsal subicular fibers occupies the extreme rostrolateral part of the nucleus accumbens surrounding the anterior commissure. The hippocampal terminal fibers are arranged in an uneven, patchy pattern. A patchy way of termination is even more pronounced following injections of PHA-L in the rostromedial thalamus. Also between the nucleus accumbens and these rostral thalamic nuclei a topographical arrangement exists such that the anterior paraventricular nucleus projects most heavily to the caudomedial nucleus accumbens, whereas fibers from rostral parts of the paracentral and central lateral nuclei terminate predominantly in the rostrolateral part of the nucleus.

ENK immunoreactivity is distributed in the nucleus accumbens in a rather irregular but, through all brains studied, constant pattern. Rostrolaterally, densely stained patches are embedded in a moderately stained matrix. Caudomedially, the matrix has a similar staining activity but is now interrupted by areas of lighter staining. Comparison of adjacent sections stained for ENK immunoreactivity and PHA-L reveal that in the rostrolateral nucleus accumbens the projections from the thalamus avoid the densely stained patches and concentrate within the matrix. In the caudomedial nuceus accumbens the thalamic fibers likewise occupy the moderately stained compartment but are absent in the lighter areas. As regards the hippocampal projections a relation of its heterogeneous pattern of termination with the distribution of ENK is less clear.

240.20 COEXISTENCE OF DYNORPHIN B-LIKE AND SUBSTANCE P-LIKE IMMUNOREACTIVITY IN STRIATAL NEURONS IN THE CAT. M.-J.Besson*, A.M.Graybiel and B. Quinn, Whitaker College, Mass. Institute of Technology, Cambridge, MA 02139 (SPON: E.V. Sullivan)

In the cat's striatum, both neurons expressing dynorphin B-like immunoreactivity (DYNB-LI) and neurons expressing substance P-like immunoreactivity (SP-LI) are concentrated in striosomes. Neurons exhibiting met-enkephalin-like immunoreactivity (mENK-LI) are present mainly in the extrastriosomal matrix unless colchicine pretreatment is given (Graybiel and Chesselet'84). The similarity in spatial distribution of DYNB- and SP-positive neurons suggested that these peptides might coexist in a subpopulation of striosomal neurons, as in some neurons of the sauropsid striatum (Reiner '85).

To test this hypothesis and to look for coexistence of mENK with these peptides, we analyzed the occurrence of SP-LI, DYNB-LI and mENK-LI in neurons of the cat's caudate nucleus. We first used the double-bridge PAP method with rat anti-SP (Sera-Lab) and rabbit anti-DYNB (E.Weber) or anti-mENK (R.Elde) on serial 3-4 µm cryostat sections cut from blocks treated with buffered 2.5% polyethylene glycol and 15% sucrose. We also tested for colocalization in single sections by the double anti-rabbit FITC/anti-rat rhodamine histofluroescence method.

In the caudate nucleus of colchicine-treated cats (intraventricular, 3 day survival) intense SP-LI and DYNB-LI was observed in striosomal medium-sized neurons. SP-LI and DYNB-LI coexisted in nearly all (>95%) of these neurons as viewed in triplicates of PAP-reacted sections or in single FITC/rhodamine-treated sections. Comparable coexistence of SP-LI and DYNB-LI was seen in neurons of the extrastriosomal matrix. There were striking instances in which the different levels of expression of SP-LI in individual striosomal neurons seemed to match the levels of DYNB-LI expression.

These findings raise the question of whether separate or common mechanisms regulate the synthesis of these two peptides, products of different mRNAs. Furthermore, the finding of colocalization of SP-LI and DYNB-LI in striatal neurons may account for the similarity in distributions of SP-LI and DYNB-LI in the pallidal and nigral targets of striatal efferent projections. If the two peptides are co-released in these targets, their mutual action might influence receptor activation.

In contrast to the nearly invariable coexistence of SP-LI and DYNB-LI, colocalization of these peptides with mENK-LI was not the rule, and mENK-LI was almost always weak in neurons (either in or out of striosomes) in which coexistence with SP-LI or DYNB-LI (or both) was found. Estimates of such colocalization (15-30% of the SP/DYNB neuronal population) varied with the method of demonstration (PAP % higher), colchicine dose and proximity to the level of colchicine injection.

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241.1 SPINAL MODULES FOR WALKING MOVEMENTS REVEALED IN FICTIVE LOCOMOTION EXPERIMENTS. L. M. Jordan, R. M. Brownstone, D. J. Kriellaars*, and B. R. Noga*. Dept. of Physiol., Univ. of Manitoba, Winnipeg, MB R3E 0W3.

Through the use of simultaneous electroneurogram (ENG) and intracellular recordings from alpha motoneurons of the lumbar spinal cord during fictive locomotion, it has been possible to determine some of the subsets of neurons, or "modules" of neuronal organization, which must be involved in the control of locomotion at the spinal level.

A comparison of the locomotor drive potentials (LDPs) of lateral gastrocnemius (LG) motoneurons with the ipsilateral tibialis anterior (TA) ENG during apparent periods of "uncoupling" between the two motoneuron pools revealed that failure of a burst in TA was accompanied by absence of the inhibitory phase of the LG LDP. This finding is strong evidence in favor of the conclusion that one of the modules for control of locomotion is a group of interneurons which provides both excitation of the agonist motoneuron pool and inhibition (presumably through interposed inhibitory interneurons) of the antagonist pool. Quantitative analysis of the times of onset and termination of depolarization and hyperpolarization in the LDPs of flexor and extensor motoneurons revealed strict reciprocity between the LDPs of antagonist motoneurons.

A second module is suggested by a comparison of the ENGs of LG and TA bilaterally. This analysis revealed that both groups display an equally strong relationship between burst duration and cycle duration, in contrast to previously published findings (Grillner and Zangger, Exp. Brain Res.,34:241-261, 1979). These authors concluded that the duration of flexor discharges during fictive locomotion vary less with the cycle duration than the extensor discharges. An explanation for the discrepancy is that the previous study evaluated flexor and extensor ENGs unilaterally. Our results show that a constant burst duration of the TA ENG over cycles of variable lengths is accompanied by a comparable constant burst duration of the contralateral LG, while increasing burst durations of LG for increasing cycle lengths are accompanied by similarly lengthened TA burst durations contralaterally. This suggests a spinal locomotor module consisting of excitatory interneurons on one side of the spinal cord with symmetrical excitatory linkages to homologous interneurons impinging on contralateral motoneuron pools.

Our results are consistent with the half-centre organization described by Jankowska, et al (Acta Physiol. Scand., 70:389-402, 1967) and with the excitatory and inhibitory pathways from the mesencephalic locomotor region previously demonstrated (Shefchyk and Jordan, J. Neurophysiol, 53:1345-1355, 1985).

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241.2 FACTORS DETERMINING THE PATTERN OF FIRING IN LUMBAR ALPHA-MOTONEURONES DURING FICTIVE LOCOMOTION. R.M. Brownstone, L.M. Jordan, D.J. Kriellaars*, B.R. Noga*, and S.J. Shefchyk, Depts. Physiol. and Int. Med., Univ. Manitoba, Winnipeg, Canada R3E 0W3.

A prevailing theory in mammalian neurobiology is that the pattern of firing in motor nerves is a consequence of the intrinsic properties of the motoneurone with the inter-spike interval (ISI) determined by the afterhyperpolarisation (AHP). Many investigators claim that synaptic activation and current injection simply add algebraically in the cell, resulting in a frequency-current (f-I) relationship parallel to the control f-I curve. This has led to the conclusion that only the "net driving current" is important in the production of motoneurone firing patterns (Schwindt and Calvin, Brain Res. 59, 1973). We have previously shown that the AHP is reduced in amplitude and duration during fictive locomotion evoked by stimulation of the mesencephalic locomotor region (MLR) (Jordan and Shefchyk, Soc. Neurosci. Abstr. 10:184.7, 1984). MLR stimulation produces rhythmic membrane potential (Em) oscillations, or locomotor drive potentials (LDPs), coincident with the step cycle. We have investigated the mechanisms controlling the repetitive firing often seen in the depolarised phases of the LDPs. The inter-spike trajectories observed here do not display the typical scoop and ramp components seen when a similar firing rate is produced by current injection. Furthermore, during fictive locomotion similar ISIs occur in spite of great variations in the inter-spike trajectories. f-I curves obtained during fictive locomotion do not parallel those obtained in the absence of locomotor activity. This suggests that there is not a prominent role of "net driving current" in determining the motoneurone firing pattern during fictive locomotion. Also, in some "anomalous" step cycles the hyperpolarising phase of the LDP "drops out" and the cell remains depolarised for more than one step cycle. The motoneurone, however, still exhibits bursts of firing at appropriate times in the step cycle. Contrary to what is seen with current injection, there are also cases during fictive locomotion where firing frequency increases at the end of a depolarised phase. If this were a result of an increased step depolarisation, one would expect to see both an increased membrane depolarisation during the ISI and an increased voltage "threshold" (cf. Schwindt and Crill, J. Neurophysiol. 48, 1982). However, neither of these phenomena are seen. We conclude that a mere step depolarisation of the cell is not sufficient to account for the pattern of spiking. Thus, mechanisms extrinsic to alpha-motoneurones determine the firing pattern of these cells during fictive locomotion.

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241.3 EFFECTS OF THE INFERIOR GLUTEAL NERVE ON CAT MOTONEURONS. D.J. Kriellaars*, L.M. Jordan and S. Shefchyk (SPON: R.M. Jell). Depts. of Physiology and Internal Medicine, University of Manitoba, Winnipeg, Canada, R3E 0W3.

Afferents from the hip area have been demonstrated to have potent phase dependent effects on locomotor central pattern generator (CPG) output in acute and chronic spinal cats. It has been demonstrated that unilateral small amplitude mechanical oscillations of the femur in the flexion/extension plane entrain the locomotor rhythm within a range of frequencies (Andersson and Grillner, Acta Physiol Scand., 113, 1983). In addition, hip position has been shown to be critical for the initiation of hindlimb flexion phase (Grillner and Rossignol, Brain Res., 146, 1978). In both of these studies extensive denervation of the hindlimb failed to show the key hip structures (muscular or capsular) which mediated the effects on the step cycle. Other studies have shown that mechanoreceptors of the hip joint capsule are not sensitive to flexion and extension movements (Rossi & Grigg, J. Neurophysiol., 47, 1982; Carli et. al., J. Neurophysiol., 45, 1981, ;Dee, Ann. R. Coll. Surg. Engl., 45, 1969) indicating that these hip are probably not responsible for the effect of hip movement on locomotion.

The aim of this research was to determine the postsynaptic potentials (PSPs) produced by specific hip afferents in motoneurons and to further use these afferents as a tool to examine components of the CPG. In chloralose anaesthetised cats, PSPs in lumbar motoneurons were evoked by electrical stimulation of the inferior gluteal nerve (supplying hip extensors and hip abductors). Electrical stimulation 2 to 5 times threshold produced inhibitory PSPs (IPSPs) in 12 of 14 motoneurons examined (4 med. gastrocnemii, 3 lat. gastrocnemii, 4 post. biceps/semitendinosus, 1 semimembranosus/ant. biceps, and two unidentified). In two cells no evoked PSPs were observed. At 2T the mean segmental latency of the IPSP was 1.7 msec (Range: 1.2 - 2.4 msec) with a mean peak amplitude of 1.3 mV (Range: 0.6 - 2.5 mV). The peak amplitude of the IPSPs increased slightly with 5T stimulation. The latency was consistent with a disynaptic linkage and the inhibitory response to 2T stimulation was attributed to group I & II afferents (Jankowska, McCrea and Mackel, J. Physiol., 316, 1981). In light of the profound effect of hip movement on locomotor activity and the substantial inhibitory connections to hindlimb flexor and extensor motoneurons from the inferior gluteal nerve observed in this study, it is hypothesized that the inferior gluteal afferents are important in modification of the CPG and its output. Further examination of these hip afferents is warranted.

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241.4 A COMMON PATHWAY FOR MLR EVOKED PSPs AND THE INITIATION OF LOCOMOTION. B.R. Noga*, D.J. Kriellaars*, R.M. Brownstone, B. Mallory* and L.M. Jordan. (SPON: J.A. Paterson) Dept. of Physiology, University of Manitoba, Winnipeg, Canada, R3E 0W3.

In the cat, the pathway through which the mesencephalic locomotor region (MLR) activates the spinal cord central pattern generator (CPG) for locomotion remains unclear. The MLR is said to relay through the pontomedullary locomotor strip and then descend via a polysynaptic pathway in the dorsal half of the spinal cord (Selionov and Shik, Neurosci. 13, 1984). Evidence has also been presented demonstrating that the MLR relays via the medial reticular formation (MRF) and then descends the spinal cord in the ventral lateral funiculus (VLF) to the CPGs (Steeves and Jordan, Brain Res. 307, 1984). The fact that stimulus-locked short latency postsynaptic potentials (PSPs) have been recorded intracellularly from hindlimb motoneurons (MNs) during MLR evoked fictive locomotion (Shefchyk and Jordan, J. Neurophysiol. 53, 1985) supports the latter pathway.

In order to establish the pathway by which the MLR produces locomotion, dorsal hemisections of the spinal cord one centimeter in length were made at the C2 level in precollicular postmamillary decerebrate cats to disrupt the proposed polysynaptic pathway. Treadmill locomotion induced by stimulation of the MLR was monitored by electromyograms recorded from fore- and hind-limb muscles. Following dorsal hemisection, MLR stimulation produced both hindlimb and forelimb locomotion at stimulation strengths comparable to control trials.

In "fictive " locomotion experiments reversible cooling of either the MRF (synaptic block) or the unilateral VLF (fiber block) at the T12-L1 spinal cord level was used to establish the relationship between the MLR evoked PSPs and the locomotor drive potentials (LDPs) in MNs. Hindlimb locomotion was monitored by electroneurograms and from intracellular records of identified hindlimb MNs. Unilateral VLF cooling abolished MLR evoked hindlimb locomotion ipsilaterally. MRF cooling reversibly abolished MLR evoked hindlimb locomotion bilaterally. As locomotion diminished with cooling there were concurrent decreases in amplitude of both the LDP and the short-latency PSPs. Slightly longer latency PSPs were sometimes revealed upon cooling the VLF. Occasionally, the hyperpolarized phase of the MN LDP and the burst activity of the antagonist ENG disappeared with VLF or MRF cooling.

It is concluded that the MLR projects to the limb CPG for locomotion through the MRF-VLF pathway and not via a polysynaptic chain of cells located in the dorsal half of the spinal cord. Furthermore, evidence is provided suggesting that the MLR evoked PSPs are related to the production of LDPs and locomotion.

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241.5 RESPONSES IN MUSCLES AND IN THE DESCENDING CORTICAL PATHWAY FROM MICROSTIMULATION IN THE FELINE MOTORCORTEX DURING DIFFERENT POSTU -RES AND LOCOMOTION. C.I.PALMER Institute of Physiology, Fribourg University, Switzerland.

Muscle (EMG) responses from intracortical microstimulation (ICM S) are variable.Some factors which may account for this are variat -ions in the descending cortical volley, the excitability of inter -neuronal circuits and the excitability of the motorneuronal pool.

Systematic variations in these responses were sought in 4 awake intact cats during locomotion, while they sat quietly and while standing. Each was chronically implanted with between 8 to 11 tefl -on insulated 25µm d. PT/Ir wires in the motor cortex for ICMS (10 to 30µA,40ms trains,350Hz,0.2ms pulses). The descending corti-cospinal volley was recorded in one cat with a monopolar electrode in the pyramidal tract at P12 and in another from the dorsolateral funiculus at C3. Each animal was chronically implanted with 7 to 14 EMG electrodes in the distal (6) and proximal (5) forelimb, shoulde -r (4) and hindlimb (1), in muscles activated from ICMS.

ICMS responses were obtained from 16 cortical sites. The latencies of the responses ranged from 6 to 20ms n82 mean 13.7 (measured from averaged records). At 8 cortical sites some of the ICMS resp -onses were found only when the muscle was active during the step cycle or during the different postures. At 9 cortical sites ICMS responses occurred in muscles with two bursts of activity during the step cycle (once in stance and once in swing). These included both shoulder and distal forelimb muscles. ICMS responses from 7 of these cortical sites only occurred when the muscle was used in swin -g and during lying even though the muscles were active during stan -ce and some were tonically active on standing. At 2 cortical sites ICMS gave either inhibition or excitatory responses in the same mu -scle each occurring with the same short latency. The type of resp -onse depended on which phase of the step cycle or which posture was occurring during microstimulation. When the ICMS current was increased responses spread to a greater number of muscles and were characteristic in their time of occurrence during movement and their form to that particular cortical site.

The descending volley from ICMS at 3 cortical sites was recorded from the pyramidal tract and from another site, from the dorsolateral funiculus. During phases of the step cycle or movements when ICMS EMG responses were strongest the waves of descending activity did not summate with succeding stimuli in the train (10ms,100Hz,0.2 ms pulses). The volley after the first stimulus was not sig. great -er from that produced by the 5th stimulus. During other phases of these movements each stimulus in the train gave a successively lar -ger descending volley.

In conclusion results from this study show that although the sa -me muscle can be activated from many different cortical sites, th -se effects may operate under different motor contexts.

241.6 SYNERGIES IN THE BIOMECHANICAL MOTOR PATTERNS IN NORMAL HUMAN GAIT. D.A. Winter. Dept. Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Based on extensive moment of force analyses on a large number of normal subjects walking a range of cadences, and on intra and inter-subject correlations, we concluded the following:

1(a) A consistent total extensor (support) moment pattern is seen during stance with minor variations in shape as cadence changes.

(b) The kinematics of the foot, leg and thigh are extremely consistent for any given subject over repeat trials.

(c) In spite of the consistency noted in (a) and (b), there is considerable variability in the moment patterns at the hip and knee.

(d) The variability in these joint moments is not random; there is considerable coupling (covariance) between the moments at the hip and knee (89%) and between the knee and ankle (76%).

(e) The tight coupling between the moments at the knee and hips results from a one-for-one trade-off of moment of force between these joints such that the net extensor pattern remains relatively undisturbed. On one stride, for example, the average knee extensor moment might decrease by 10 N.m while the hip extensor moment increased by 10 N.m. There is a similar but less pronounced trade-off between knee extensors and ankle plantarflexors.

(f) This flexible anterior-posterior trade-off appears to be related to stride-to-stride regulation of posture and balance of the upper body. During single support, the hip moments are seen to be highly correlated with the angular accelerations of the trunk. It thus appears that the hip muscles not only contribute to the support of the lower limb but also are the prime controller of the trunk, and the response of the knee and ankle muscles is such as to keep the total support pattern almost the same.

2(a) The averaged intersubject moment patterns at slow to fast cadences are extremely similar in shape for the summed hip and knee moments. The ankle pattern has subtle cadence-related changes.

(b) Tonic control of the moment patterns is seen at the hip and knee, and selective increases and decreases are evident at the ankle as walking speed increases.

3. Evidence is presented from patients with surgery to their periphery (amputees, joint replacement) that shows very drastic alterations to the motor patterns at all joints. Such information does not support the presence of a dominant hard-wired central pattern generator.

241.7 AN ANALYSIS OF BACKWARD WALKING IN HUMANS. J. A. Vilensky,* E. Gankiewicz* and G. Gehlsen* (SPON: S. W. Carmichael). Dept. of Anatomy, Indiana Univ. Sch. of Med., Ft. Wayne, IN 46805 and Biomechanics Laboratory, Ball State Univ., Muncie, IN 47306.

Grillner (1981) suggested that backward walking could be achieved by switching the neural circuitry controlling hip-knee interactions from excitatory to inhibitory; such a change would act to reverse hip-knee coordination by a phase shift of .5 (hip flexion accompanied by knee extension). This is not the case for humans. Rather, in many respects human backward walking is quite different from predictions based either on Grillner's hypothesis or on forward walking in general. In our study, four adults were filmed (at 60.2 frames/sec) walking forward and backward on a treadmill at the same three speeds in each direction. Subsequently, temporal and angular parameters were derived from the films for each form of walking and compared.

At each speed, the absolute temporal parameters (cycle, swing, stance and double support durations) were generally shorter during backward walking. On the contrary, relative temporal parameters (percent of cycle duration) were virtually identical. The range of hip movements was less during backward walking, primarily as a result of less extension. Knee movements were also usually reduced during backward walking, primarily as a result of less flexion. Ankle movements were characterized by greater plantarflexion during forward walking and greater dorsiflexion during backward walking. The difference in ankle plantarflexion was especially noticeable at the toe-off/toe-on position. The decreased plantarflexion at toe-on in backward walking facilitated weight acceptance on the phalanges.

With regard to joint timings and interactions, it would be expected (according to Grillner's hypothesis) that during backward walking hip extension would be initiated during stance. Instead, hip extension did not begin until well into swing phase. Similarly, the relationships between the knee and ankle would be expected to be unchanged during backward walking. On the contrary, during backward stance the movements of the knee and ankle are primarily in phase while during most of forward stance they are out of phase. The differences between predicted and actual backward walking reflect the different functional demands placed on the system during the two types of gait. Clearly, backward walking is not achieved by a simple phase shift in hip-knee interactions.

241.8 THE DEVELOPMENT OF MOTOR CONTROL IN STEPPING. A. Beuter, H. Flashner* and A. Arabyan. Kinanthropology, University of Québec in Montréal, P.Q., H3C 3P8.

The kinematics of stepping are quasi-organized at birth and form the basis of future goal-directed movement sequences. In agreement with this, a hierarchical model of stepping, as a pre-planned trajectory defined by a small number of parameters stored in the CNS, and combining open and closed loop control logics has been proposed (Beuter et al., Biol. Cybern., 53: 273-284, 1986). The purpose of this paper is to expand the previous results and propose a model for the development of motor control in stepping. During the learning stage, the interactive unit (closed loop) solves the optimization problem defined by $\min_{Q} J = P(X_p, Q, \theta)$ subject to imposed, kinematic and dynamic constraints, where X_p is the candidate trajectory, Q is the control torque, θ denotes the vector of joint coordinates and P is the trajectory of a point on the leg. The learning process is explained as follows: the trajectory generator sends a candidate trajectory to the forward control subsystem. Using the equations of the kinematic and dynamic constraints the joint angles and the control torques are calculated. A performance index is computed and stored in memory. During learning, the task is repeated with different candidate trajectories yielding different values for the performance index. Comparison of performance indices of the various trajectories yields the best control strategy for the control torques. We reformulate the problem as a finite dimensional optimization defined by $\min_{h} \min_{f \in F} P(\theta, Q, X_p)$ where h is the vector of relevant parameters and F is a finite set of know functions corresponding to the hardwired movement segments observed in newborn babies. The model presented does not require learning (repeating the optimization procedure) for each particular obstacle and therefore it is more realistic and efficient than Raibert's Model (1978). We suggest ways to implement the model on a robot and to simulate the process of learning.

241.9 EMG WAVEFORMS IN CAT LOCOMOTION. J.A.Hodgson*, A.Garfinkel*, V.R.Edgerton, R.R.Roy and R.J.Gregor Kinesiology Department and Brain Research Institute, UCLA, Los Angeles, CA 90024.

We have assessed quantitative aspects of emg activity in the soleus (SOL), medial gastrocnemius (MG) and vastus lateralis (VL) muscles of cats walking on a treadmill over a range of speeds (1-4m/s) and gaits.

Standard intramuscular techniques were used to record emg data. Digital techniques were used to analyse data from several consecutive steps (>10) at each speed of locomotion. For each trial, mean emg amplitudes, variance and coefficients of variation, phase portraits and joint probability density distributions of emg amplitudes in muscle pairs were calculated.

We found that the same waveforms and patterns of variability recur in emg data from recording sessions separated by several days in individual animals. Moreover, similar waveforms were observed in all animals studied. EMG waveforms changed predictably with speed of locomotion (Fig 1). Waveforms from SOL remained constant across all speeds of locomotion in the E_1 phase of the step cycle, so that most changes in the emg waveform with speed of locomotion occurred after paw contact (PC in Fig 1). Phase portraits between SOL and MG and MG and VL also showed consistent patterns, as did joint probability distributions.

These results suggest that emgs reflect magnitude in addition to timing of command signals to skeletal muscles and that some significance may be attached to emg waveform. The phase portrait and joint probability density findings indicate specific, speed dependent relationships between amplitudes in different skeletal muscles.

This information may be used to make deductions about control strategies used by the CNS to control hindlimb locomotion.

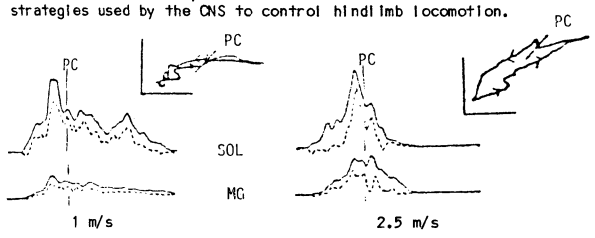

Figure 1. Mean (-SD) emg amplitudes for SOL and MG at 1 and 2.5 m/s and their corresponding phase portraits (MG vert., SOL horiz.). Supported by NIH Grant NS-16333

241.10 EFFECT OF TASK ON PERTURBATION RESPONSES DURING RHYTHMIC MOVEMENT IN HUMANS. Aftab E. Patla, Neural Control Lab, Department of Kinesiology, University of Waterloo, Waterloo, Ontario.

The responses to perturbation applied during various phases of treadmill walking show a complex organization (Belanger & Patla, Neurosci. Lett., 1984). Locomotion is a complex task which involves asynchronous movements of the joints. Besides, treadmill locomotion imposes physical and strategy constraints on the subject. Hopping, a relatively simple movement, provides an unique alternative to study how the task affects response organization.

In this study subjects (N=6) were asked to hop on the force plate at their preferred frequency. Processed EMG signals from tibialis anterior, soleus, medial gastrocnemius, vastus lateralis, rectus femoris and biceps femoris were monitored along with the footswitch signal and the ground reaction force. An electrical stimulus (20 ms train of 10-1 ms pulses, 4-5 x threshold) was applied unexpectedly at two different points in the cycle: ground contact and in air. Response to perturbation was determined by subtracting averaged perturbed signal (N=5) from averaged normal signal.

When the stimulus was applied at ground contact, excitatory responses were observed in TA (49 ms) and BF (79 ms) and an inhibitory response in SO (56 ms). Thus distal muscles (TA & SO) generate a withdrawal response. The enhanced BF activity represents an extensor response at the hip and knee joint (by backward rotation of the thigh). This conclusion is arrived at as follows. The area under the force time curve which represents the impulse was not altered even though the time of force application was reduced ($p < .05$). If BF activity produced a flexor torque at the knee, it would cause a reduction in reaction force and hence alter the area under the force time curve.

In response to the stimulus applied during free flight phase, excitatory responses were observed in TA (59 ms), SO (74 ms), and BF (68 ms). The footswitch data revealed an increase in the free flight phase duration. In contrast, the force/time curve showed no change in the free flight phase. Thus the response represents withdrawal of the ipsilateral limb, with the contralateral limb making ground contact at regular time.

In contrast to walking, the response show a very simple organization. Same set of muscles are activated, with only the magnitude of the response showing phase dependent modulation. A functional response reversal is seen. The characteristics of the response provide evidence for the task dependency of the responses to perturbation applied during rhythmic movement in humans.

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241.11 MODULATION OF PERIPHERAL AFFERENT INFORMATION TO RETICULOSPINAL CELLS DURING LOCOMOTION IN INTACT CATS. T. Cabana*, S. Rossignol and T. Drew. Centre de recherche en sciences neurologiques, Univ. de. Montréal, Qué, CANADA.

It has been demonstrated that during locomotion the amplitude of cutaneous reflexes of the fore- and hindlimbs are dependent upon the phase of the step cycle in which stimulation is applied. In the present study we have examined whether the responses induced in reticulospinal cells by cutaneous inputs are also modulated during locomotion in a phase dependent manner.

Experiments were carried out during treadmill locomotion in 2 intact cats chronically implanted with a chamber through which a microelectrode could be advanced into the medullary reticular formation. In addition, microwires were implanted into the spinal cord (L2) to identify reticulospinal neurones; electrodes were inserted into flexor and extensor muscles of all four limbs, and stimulating electrodes, held in a polymer cuff, were implanted around the superficial radial and peroneal nerves on both sides.

Of the 43 reticulospinal cells recorded during locomotion, 28 could be held long enough to test their response to cutaneous stimulation of all four limb nerves: 10 of these cells, including both modulated and unmodulated neurones, were excited by stimulation of all four cutaneous nerves, 9 responded to stimulation of one or more limb nerves and the remaining 9 cells did not respond to stimulation of any of the nerves.

For cells excited by the nerve stimulation of more than one limb, it was found that the response evoked from any one nerve was maximal during the swing phase of the respective limb and minimal during its stance phase. This was true both for modulated and unmodulated neurones and suggests that the excitatory responses evoked in these cells were probably independent of their discharge pattern during locomotion. It was also noticeable that the responses observed during stance in 31/35 cases occurred even though no reflex activity was observed in the limb musculature. In 27/31 cases the discharge of the cell to limb nerve stimulation was greater when the cat was at rest than at any phase of the locomotor cycle suggesting that there may also be a tonic modulation of cutaneous afferents during locomotion.

The fact that the excitatory responses evoked in the cells by cutaneous nerve stimulation were more dependent upon the time of the stimulation during the locomotor cycle of the stimulated limb than to the discharge pattern of the cell further suggests that at least part of the modulation of the sensory input observed in reticulospinal cells may be subserved by the same pathways that mediate the phase dependent responses observed in segmental cutaneous reflex pathways.

(Funded by the FRSQ and the Canadian MRC. T. Cabana is a Herbert Jasper fellow and T. Drew is a FRSQ scholar).

241.12 FORELIMB CUTANEOUS REFLEXES DURING FICTIVE LOCOMOTION IN DECORTICATE CATS. P. Saltiel*, J.-P. Gossard*, T. Drew and S. Rossignol. Centre de recherche en sciences neurologiques, Univ. de Montréal, Montréal, Québec, Canada.

In intact cats, stimulation of the Superficial Radial (SR) nerve during the swing phase of locomotion induces short latency responses (9-11 ms) in elbow flexors such as Brachialis (Br) which is active during that period as well as in elbow extensors such as the Lateral (TriL) and Long head (Tri) of Triceps which are silent in that phase. These are followed by a longer latency response in flexors (35-50 ms). During stance, the short latency responses are virtually absent in all muscles although subsequently a more complex sequence of inhibition (14 ms) and excitation (24-31 ms) is observed in extensor muscles. As part of a further investigation on the origin of this elaborate reflex sequence, we have studied the effects of similar stimuli during fictive locomotion.

Cats were anaesthetized with steroids, decorticated by suction, paralyzed with gallamine and spinalized at T13. Nerves to SR, TriL, Tri and Br were dissected and mounted on Ag/AgCl electrodes in a warm paraffin oil pool for recording or stimulation. A well organized locomotor rhythm (0.8 to 1.3 Hz) lasting for several hours was obtained in a few preparations. SR was stimulated with single pulses of 0.2 ms (4 times threshold for activating the largest fibers) at different moments in the locomotor cycle.

Stimuli during Br activity evoked in Br nerve an excitation at 7-8 ms which was sometimes followed by an inhibition at 14 ms. There was no longer latency response as in the intact cat. Further, there was no out-of-phase response in TriL and only an occasional excitation at 9 ms in Tri.

Stimulation during the period of Br inactivity caused only a very small excitation of Br. In TriL/Tri, the response pattern was complex. There were two excitatory responses at 8-9 ms and at 19-21 ms which could merge or be separated by a trough at 14 ms. In addition, an inhibition at around 37 ms and a longer latency excitation at 60 ms could at times occur in TriL.

The largely similar latencies of the responses seen in real and fictive locomotion suggest that the same pathways are recruited but differently controlled in the two conditions. In particular, the out-of-phase responses in TriL/Tri seen consistently during the swing phase of real locomotion are virtually absent during fictive locomotion. Whether this difference can be attributed to the lack of cyclical afferent feedback and/or lack of cortical input remains to be determined. (Funded by the MRC. P. S. and J.-P. G. are supported by the MRC. T. D. is an FRSQ Scholar).

241.13 THE STUDY OF LOCOMOTION AND CUTANEOUS REFLEXES IN THE SAME CAT BEFORE AND AFTER SPINALIZATION. M. Bélanger, T. Drew, J. Provencher* and S. Rossignol. Centre de Recherche en Sciences Neurologiques, Dept de Physiol., Univ. de Montréal, Montréal, Québec, Canada, H3C-3J7.

Adult cats can recuperate the ability to walk following spinalization at T13 (Rossignol et. al. Soc Neurosci Abstr. 8, 168, 1982). The present study set out to detail the locomotor activity in the same chronically implanted cat before and after spinalization.

Electromyograms (EMGs) were recorded from flexor and extensor muscles of the hindlimbs. Synchronized video recordings were made while the cat walked on a treadmill (V=0.3-0.6 m/s) with either all four limbs or with the hindlimbs only (the forelimbs being placed on a board about 2 cm above the treadmill belt). Post-spinalization recordings were made as in the latter case. For reflex studies, electrical stimulation (2xT) was applied to the skin of the dorsum of the cat's paw or to the superficial peroneal nerve with chronically implanted electrodes.

The EMGs and joint excursion patterns of the pre- and post-spinalized walking were remarkably similar at comparable velocities. However, the firing of the Semitendinosus (St), which discharged at the beginning of swing before the spinalization, was delayed so that knee flexion was retarded and some dragging of the foot was observed. The Sartorius (Srt), which discharged only as a flexor before spinalization, showed an additional large burst of activity in the stance after spinalization. This could, in part, be attributed to walking with the hindlimbs only, since such stance related activity could be seen, to a lesser degree, in the pre-spinalization period under similar walking conditions. Similarly, this interpretation could also apply to the observation that the Vastus Lateralis activity trailed the discharge in Gastrocnemius Lateralis after spinalization.

The post-spinalization reflex threshold was reduced to approximately half of the pre-spinalization values for both the dorsum and superficial peroneal nerve. However, the latencies and amplitudes of the pre- and post-spinalized reflex responses were similar. Flexors were facilitated during the swing phase whereas the response in the extensors consisted in an inhibition followed by an excitation during the stance phase. Excitatory reflex responses were found in the Srt during both phases of the step cycle after spinalization.

In conclusion, the chronically implanted preparation allows a rigorous comparison of locomotion under normal and spinal conditions in the same cat. The initial findings show that the EMG and joint excursion patterns as well as the reflex responses are not grossly altered after spinalization. (Funded by the Canadian MRC, T.D. funded by FRSQ and M.B. supported by NSERC).

241.14 THE PYRAMIDAL TRACT MODULATES SPINAL REFLEXES IN THE IN VITRO HAMSTER SPINAL CORD. J. Keifer and K. Kalil. Neurosciences Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

The pyramidal tract (PT) of the hamster projects from the sensorimotor cortex to all contralateral spinal cord segments within which it innervates interneurons in dorsal horn lamina V and VI. Based on a kinematic analysis of locomotion of normal hamsters and of those with lesions of the PT, we proposed that the PT regulates the stiffness of the limb through the modulation of sensory inputs during movements in which sensory feedback is continually variable, for example, during locomotion on rough terrain (Soc. Neurosci. Abs. 11:1030, 1985). In support of the hypothesis that the PT regulates sensory inputs in the spinal cord, we now present physiological evidence that the PT modulates spinal reflexes in an in vitro hamster medulla-spinal cord preparation.

The in vitro medulla-spinal cord preparation from hamsters up to three weeks of age consisted of the medulla cut caudal to the inferior colliculus, the cervical enlargement, and attached dorsal roots (DR) and ventral roots (VR). The cord was hemisected, superfused with oxygenated saline at 30°C, and remained viable for at least six hours. Suction electrodes were used to stimulate a DR and record from the VR of the same segment. Two reflexes could be evoked in this manner: the large monosynaptic spike, and a higher threshold polysynaptic volley. Stimulation of a DR while recording a DR immediately rostral to it produced a volley of action potentials characteristic of the dorsal root reflex (DRR). Since the PT lies on the ventral surface of the medulla, a stimulating suction electrode could be placed on the contralateral PT. The effect of PT stimulation on reflex transmission was tested by stimulating the PT (400 Hz, .3 msec duration pulses, 50 msec train) at varying delays prior to evoking a reflex. The results show that the amplitude of the monosynaptic reflex is progressively diminished when preceded by PT stimulation at shorter delays. Similarly, PT stimulation also inhibits and eventually blocks the polysynaptic volley. However, in contrast, the PT facilitates the DRR by lowering its threshold slightly. Furthermore, repeated stimulation of the PT alone may also induce either tonic or phasic antidromic action potentials in the dorsal roots. The modulation of spinal reflexes by the PT in the in vitro spinal cord provides evidence that the PT makes the appropriate synaptic connections consistent with a role in the regulation of sensory inputs in the spinal cord. Intracellular experiments are currently in progress to determine whether the PT exerts its influence upon sensory inputs by a presynaptic or a postsynaptic mechanism.

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241.15 DIFFERENTIAL MOTOR UNIT RECRUITMENT IN THE PRIMARY DOWNSTROKE MUSCLE OF THE PIGEON (COLUMBA LIVIA) DURING FLIGHT. K. P. Dial*, F. A. Jenkins, Jr.*, G. E. Goslow, Jr.*, S. R. Kaplan*. (SPON: R. A. Satterlie). Biology, Northern Arizona Univ., Flagstaff, AZ 86011 and MCZ, Harvard Univ., Cambridge, MA 03128.

The pigeon pectoralis muscle constitutes 25-35% of the bird's total body weight but is usually regarded as mechanically simple in that it is the primary depressor of the wing during flight. Architecturally the muscle is complex and visually is divided into an anterior and posterior portion by the alignment of its constituent muscle fibers. These regions are each innervated by distinct primary nerve branches. In four birds, glycogen depletion methods of nerve stimulation (up to nine hrs) revealed each nerve branch to be associated with fibers primarily located in the anterior and posterior regions. Muscle fibers were depleted in the anterior compartment in two experiments and the posterior compartment in two experiments. Endurance capabilities of the pectoralis fibers necessitated a varied stimulation paradigm coupled with muscle ischemia. Histochemical analyses of myofibrillar ATPase activity (alkaline preincubaton, at pH 10.4) and oxidative enzyme activity (NADH-D) revealed the presence of fast, red fibers (FOG) and fast white fibers (FG).

Electromyographic activity (EMG) was measured in each of the two compartments during takeoff, sustained flight, and landing. Either silver wire (100um) or teflon coated, 15 stranded stainless steel wire (254um) was surgically implanted in 10 adult pigeons trained to fly along a 100m corridor. EMGs were telemetered in five experiments. EMGs coupled with cinematography (64 - 200 fps) revealed differential activity in the two pectoralis compartments during flight. Both compartments were most active during takeoff and landing, when presumably power requirements are greatest. During sustained flight EMG amplitudes diminished significantly in both compartments. Activity levels within the posterior compartment, however, showed relatively more reduction. These data support differential recruitment patterns within two compartments in a single muscle and further, as trunk orientation relative to the axis of the wing changes, motor unit activation patterns within these two compartments change differentially. Supported by NSF Grant BSR 8511867.

241.16 IS THE BASIC OUTPUT OF THE LOCOMOTOR CPG TO FLEXOR AND EXTENSOR MUSCLES SYMMETRICAL? EVIDENCE FROM WALKING, SWIMMING AND EMBRYONIC MOTILITY IN CHICKS. A. Bekoff. EPO Biology Dept., University of Colorado, Boulder, CO 80309.

In a wide variety of animals, asymmetric duration versus period relationships are seen for flexors and extensors during walking. That is, extensor burst duration tends to increase with increasing period, while flexor burst duration stays relatively constant. It has been suggested that this feature is a characteristic of the central pattern generator (CPG) for locomotion. However, recent evidence from several preparations, including an analysis of walking and hatching in deafferented chicks (Bekoff, 1982, Soc. Neurosci. Abstr. 8: 738; Bekoff et al., submitted) have suggested that these relationships can become symmetrical or even reversed after sensory input from the limb is removed or altered.

The present study was undertaken to evaluate a possible role for sensory input related to load detection in regulating burst duration versus period relationships during chick locomotion. For this purpose, EMG electrodes were implanted in a variety of leg muscles in 0 to 1 day old chicks. Recordings were made during free walking down a runway, a situation in which cyclical loading and unloading of the legs occurs. These were compared to recordings made during swimming, a situation in which buoyancy of the chick in the water eliminates cyclical loading and unloading of the legs. In contrast to the results seen during walking, during swimming the flexor burst durations were found to vary as much as, or more than, extensor burst durations. Another situation in which the legs are not loaded is found during embryonic motility, which occurs while embryos float in the amniotic fluid. EMG recordings in 9-day old embryos produced results similar to those found during swimming.

Taken together with the results of the deafferentation studies, these results suggest that the basic output of the locomotor CPG results in symmetrical duration versus period relationships for flexors and extensors. Furthermore, it appears that sensory information related to load normally plays a role in regulating burst durations during walking, resulting in asymmetrical duration versus period relationships for flexors and extensors.

This research has been generously supported by a John Simon Guggenheim Fellowship, NSF grant 79-13826 and NIH grant NS 20310.

241.17 CONTRAVERSIVE CIRCLING ELICITED FROM THE INTERNAL CAPSULE AND VENTRAL TEGMENTUM: EVIDENCE FOR A CONTINUOUS FOREBRAIN PATHWAY FOR LATERAL MOVEMENT. E.J. Tehovnik and J.S. Yeomans, Department of Psychology, University of Toronto, Toronto, Ontario, Canada, M5S 1A1.

Electrical stimulation of the anteromedial cortex produces contraversive eye movements, head turns, and circling (Ferrier, 1874; Sinnamon and Galer, 1984; Tehovnik and Yeomans, in prep.). Moreover, these movements can be elicited from many diencephalic sites, such as the internal capsule, fields of Forel, and the ventral tegmentum (Hess, 1957). Although much is known about how these sites are interconnected anatomically, there is little information available on how they are interconnected for the production of the lateral movements (Bender and Shanzer, 1964; Hess, 1957).

We used the double-pulse technique (Shizgal et al., 1982) to determine whether axons pass between the internal capsule medial to the caudate (IC) and ventral tegmentum (VT) for the elicitation of contraversive circling. In rats, paired pulses (conditioning pulses 'C' and test pulses 'T') were presented concurrently to these substrates while holding behavioral output (one 360 degree body turn in 10 s) constant. The intrapulse-pair (C-T) interval was traded off against frequency of paired pulses. It was found that at short C-T intervals (0.2 to 1.0 ms), high frequencies were required to produce one body turn, whereas at long C-T intervals (4.0 to 10.0 ms) low frequencies were required. This suggests that collision of stimulation-elicited action potentials occurred at short but not at long C-T intervals. Between 30 and 80% collision was found. These data support the contention that axons projecting between the IC and VT mediate contraversive circling.

In addition, refractory periods and conduction velocities were determined for these axons. The refractory periods ranged from 1 to 7 ms, which are similar to refractory periods of axons in the anteromedial cortex (Tehovnik and Yeomans, in prep.). The conduction velocities ranged from 1 to 5 m/s, which are much slower than those found for axons mediating circling in the medial pons (Yeomans and Linney, 1984). Collision between the anteromedial cortex and IC or VT has not yet been found.

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241.18 RECOVERY OF FUNCTION FOLLOWING BRAIN INJURY IN RAT AND CAT: BENEFICIAL EFFECTS OF PHENYLPROPANOLAMINE. M.J. Chen*, R.L. Sutton, and D.M. Feeney. Departments of Psychology and Physiology, UNM, Albuquerque, NM, 87131.

Both amphetamine (AMP) and phentermine (PHENT) accelerate recovery of locomotor ability in rats and cats with unilateral sensorimotor or frontal cortex ablations (1,2,3). Since phenylpropanolamine (PPA) is both structurally and functionally similar to AMP, the effect of this drug on recovery was tested in rats with unilateral sensorimotor cortex ablations. Rats were trained to traverse a narrow elevated beam, surgery was performed, and 24 hr later they were injected (IP) with PPA at doses of 10 (n=11), 15 (n=10), or 20 (n=11) mg/kg or saline (SAL; n=10). Tests on the beam were given hourly for 6 hr and at 24 hr postinjection and then every other day for 15 days. Only the 15 & 20 mg/kg doses significantly ($p<.05$) accelerated recovery compared to saline controls, with the 15 mg/kg dose being most effective. Subsequent to this study the 15 mg/kg dose of PPA was tested in cats. These animals were tested for beam-walking ability and forelimb tactile placing (TP) responses for 1 week prior to surgical removal of the right frontal cortex. After surgery behavioral tests were conducted on days 4, 6, and 8. After a predrug test on days 10, 14, 18, 22, 26, and 30 cats were administered PPA (n=4) or SAL (n=4) and additional tests were conducted at 1, 2, 3, 6, 24 and 48 hr postinjection. Further tests on the beam and TP were conducted on days 31, 35, 40, 50, and 60. This dose of PPA did not reinstate TP responses in the forelimb contralateral to injury as both AMP (4) and PHENT (3) have been shown to do in cats. Although a trend for improved recovery in beam-walking ability was evident in PPA-treated cats from the 2nd to 5th injections, recovery levels were not statistically different from SAL controls over the entire 60 days of the study due to the large variablity of responses within each of the groups. Further research with PPA and other catecholamine agonists seems warranted given the paucity of rational pharmacotherapies for inducing recovery of function following brain damage.

1. Feeney, et al., Science, 217, 855, 1982.
2. Feeney & Hovda, Psychopharm., 79, 67, 1983.
3. Hovda & Feeney, Brain Res., 298, 358, 1984.
4. Hovda, et al., FASEB Abstracts, 42, 1983.

(Supported by Grant #DHHS 3 S06 RR08139 and funds supplied by Thompson Medical Company).

241.19 BILATERAL COORDINATION OF STEPPING MOVEMENTS OF 7-MONTH-OLD HUMAN INFANTS ON A SPLIT-BELT TREADMILL. E. Thelen, D.B. Ulrich* and D. Niles*. Department of Psychology, Indiana University, Bloomington, IN 47405.

Seven-month-old human infants normally perform few, if any, stepping movements when held upright. However, when supported on a treadmill, these infants show well-coordinated, alternating stepping movements, suggesting that elements of locomotor pattern generation are in place before the behavior is voluntarily performed. (Thelen, E. Dev. Psychol., in press).

Here we investigate the bilateral coordination of elicited stepping using a split-belt treadmill where the speeds could be independently varied. When mesencephalic or spinal cats (Kulagin, A.S. & Shik, M.L. Biophys., 15:171, 1970; Forssberg, H., et al, Acta Psyiol. Scand., 108:283, 1980) are placed on treadmills with two belt speeds, hindlimbs will maintain a common step frequency at a cycle length intermediate between the fast and slow speeds. Adjustments are made in both swing and stance phases.

Subjects were normal seven-month-old infants who were held supported over a treadmill in 4 conditions: both belts at .1 m/s ("slow"); both belts at .2 m/s ("fast"); and two conditions where one belt was at the slow and the other at the fast speed ("mixed"), with right and left alternating. Each foot was maintained on a separate belt. Leg movements were analyzed by a WATSMART optical-electronic digitizing system which detects infrared light-emitting diodes taped to the infants' joints. Sampling rate was 100 Hz. Analysis used the excursions of the foot in the saggital plane.

Infants performed a high proportion of single, alternating steps in all conditions (slow, .76; fast, .90; mixed, .74, .78). Step cycle durations depended on belt speed for the slow (2.4s) and fast (1.8s) conditions. Cycle durations for the 2 mixed conditions were exactly intermediate (2.1 and 2.0 s). Initiation of left steps occured at 47% and 56% of the right cycle in the slow and fast conditions. In the mixed conditions, the left leg initiated swing at 43% of the right leg cycle when the right was on the slower belt, but at 58% of the cycle when the right belt was the faster. Cycle duration adjustments were made in both the swing and stance phases as to maintain a consistent phase proportion in all conditions.

Like cats, human infants can maintain precise bilateral coordination in response to varying external conditions apparently without voluntary control. This suggests that the pattern-generating processes are sensitive to dynamical context and self-correct to maintain goal-appropriate behavior.

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242.1 DESCENDING SUPRASPINAL PATHWAYS NECESSARY FOR HINDLIMB LOCOMOTION IN BIRDS G.N. Sholomenko and J.D. Steeves. Dept. of Zool., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 2A9

The control of voluntary locomotion is dependent on information transfer from supraspinal nuclei to locomotor pattern generators present in the spinal cord. Current evidence indicates that these pathways arise in the brainstem and traverse the ventral funiculi of the spinal cord in many vertebrate species (Eidelberg et al., Prog. Neurobiol. 17:185-202, 1981). We have examined these pathways to the hindlimb in the bird using a series of selective spinal cord lesions. The bird is an interesting animal for the study of locomotion for two reasons. First, it is a true biped whose walking resembles that of human locomotion. Second, birds do not possess the corticospinal tract which has been implicated in mammalian motor control.

Two types of preparations were examined. In the first, selective lesions were made in the low thoracic spinal cord in chronic surviving animals. The lesions included; 1)dorsal cord 2)hemisection 3)dorsal and ventromedial cord 4)dorsal and bilateral ventrolateral cord 5)section of the entire cord except one ventral quadrant and 6)complete transection. In the second preparation, the above spinal cord lesions were performed in acute decerebrate animals in which locomotion was evoked by brainstem stimulation (Steeves et al., in press, 1986). Locomotion was monitored in both preparations using electromyographic (EMG) activity in hindlimb flexors and extensors (Weinstein et al., Can. J. Zool. 62:1596-1604, 1984).

Our results showed that lesions 1-4 (above) had little affect on bilateral walking ability in either the chronic or acute preparations. The lesion leaving only a single ventral quadrant intact (5) prevented overground locomotion in the chronic animals. However the birds were capable of standing and taking a few steps. When floated on water, they were able to paddle in a normal alternating pattern voluntarily. Acute birds with the same lesion showed severely decreased force production on the side contralateral to the intact side but bilateral stepping was again present. Chronic birds with a complete transection showed no voluntary locomotion but did demonstrate "spinal stepping". The stepping, which was not weight supporting, was spontaneous or could be elicited by noxious perianal or footweb stimulation. Transected acute decerebrate birds showed no elicited stepping following the lesion but wing flapping could be evoked at higher stimulation intensities.

These results indicate that the same descending supraspinal projections, the reticulospinal and possibly vestibulospinal pathways which travel in the ventral funiculus, play a major role in the control of locomotion in birds as in mammals and other lower vertebrates.

242.2 ORIGIN OF AVIAN SUPRASPINAL PATHWAYS PROJECTING TO THE SPINAL CORD. D.M.S. Webster and J.D. Steeves, Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C., V6T 2A9.

To complement studies which have demonstrated that focal electrical stimulation of discrete brainstem area evokes locomotion (walking and flying) in decerebrate birds, the origins of descending spinal axons were determined by spinal cord injections of a retrograde tracer in white Pekin ducks and Canada geese. True blue (TB) dye (5%) was injected bilaterally (5-20 L) into either the cervical or lumbar spinal cord. After a survival period of 10-20 days, transverse or sagittal tissue sections were examined under a fluorescent microscope.

TB labelled cell bodies were never found within the telencephalon. Preliminary evidence indicates that a lack of telencephalic-spinal projections is also characteristic of highly prehensile parrots (Steeves et al., in prep.). In the brainstem, the distribution of TB labelled cells was similar to that previously described for the pigeon (Cabot, et al. Prog. Brain Res. 57: 79-108, 1982). Labelled cells, after a lumbar injection of TB, were found in all the brainstem nuclei labelled after a cervical injection, including, gigantocellular, magnocellular and parvocellular reticular formation, lateral and descending vestibular, raphe, red nucleus, interstitial, subtrigeminal, locus coeruleus, subcoeruleus and lateral reticular nucleus, except the nucleus intercollicularis.

In further experiments, TB was injected into more restricted areas of the cross-sectional lumbar spinal cord. To prevent retrograde transport along specific spinal cord funiculi, the spinal cord was lesioned (sub-totally) at least 1.0 cm rostral to the injection site. Labelled TB cells were mapped following either sectioning the dorsal half of the spinal cord, a contralateral hemisection, or a three-quarter lesion sparing only the ipsilateral ventral quadrant. Results indicate that axonal projections include, raphe-spinal in both dorsal and ventral funiculi, crossed rubrospinal in dorsolateral funiculi, bilateral cerulospinal, vestibulospinal in the ventral funiculi and bilateral reticulospinal primarily in the ventral funiculi.

The position of the labelled reticulospinal cells correspond with those brainstem areas where locomotion can be evoked and they may be one of the major direct descending projections for the activation of spinal motor networks.

242.3 THE MESENCEPHALIC LOCOMOTOR REGION. I. PROJECTIONS TO RETICULOSPINAL NEURONS. E. Garcia Rill and R. D. Skinner. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205

Previous anatomical studies in our laboratories have established that a major descending projection of the mesencephalic locomotor region (MLR) travels to the medioventral medulla (Brain Res. Bull. 10, 1983). Subsequently, we reported that electrical and chemical activation of this medullary region produced locomotion in the precollicular-transected cat (Anat. Rec. 211, 1985). The present studies were designed to determined if MLR afferents to the medioventral medulla influence reticulospinal neurons. After halothane induction, a precollicular-postmamillary brainstem transection was performed in adult cats. The MLR was physiologically identified by the ability to induce controlled locomotion on a treadmill using high frequency (20-60Hz), low amplitude current (15-60μA) pulses (0.5-1.0 ms). Extracellular recordings (n=233) then were carried out in the medioventral medulla. Criteria for antidromic identification of reticulospinal cells following ventrolateral funiculus stimulation (C2), included high frequency following (>300Hz), invariant latency (±0.2 ms) and collision. A large proportion (47%) of all the neurons sampled in the medioventral medulla (P 6.5-9.5, L 1.0-2.0, H -7.0--9.0) were found to respond orthodromically to MLR stimulation at short latency (3.06±1.49 S.D. ms). Of these, 32% received input from both MLRs. Over one third (34%) of medioventral medulla neurons were found to project to the spinal cord through the ventrolateral funiculus (antidromic latency 1.56±1.09 S.D. ms). One half of these reticulospinal cells (17% of total population) were found also to respond orthodromically to MLR stimulation. In some cases, the threshold for evoking orthodromic responses (one pulse) was similar to the threshold for inducing locomotion (high frequency). The regional distribution of the cells studied corresponds with the area found to receive descending projections from the MLR in previous anatomical experiments. In some of the present experiments, locomotion was induced following stimulation of the homologous point on the side of the medulla contralateral to the recording site(s). These findings demonstrate that descending MLR projections influence a large number of medioventral medulla cells, some of which have direct spinal projections, and suggest that this area is a primary relay in the manifestation of MLR function.

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242.4 THE MESENCEPHALIC LOCOMOTOR REGION. II. CHOLINERGIC NEURONS IN THE VICINITY OF LOCOMOTION-INDUCING SITES. R. D. Skinner, C. R. Houser and E. Garcia-Rill. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205 and Brain Research Institute, UCLA, Los Angeles, CA 90024

Studies in several laboratories have reported that locomotion-inducing stimulation sites [physiologically identified as the mesencephalic locomotor region (MLR)] in the precollicular transected preparation are located in proximity to the pedunculopontine nucleus (PPN). We reported the presence of pallidal and nigral projections to the MLR, areas known to project to PPN. Recent immunocytochemical findings by several groups have demonstrated the presence of choline acetyltransferase (ChAT) containing neurons in PPN. In order to determine if the PPN forms part of the MLR, we carried out the physiological identification of the MLR in the same brains subjected to ChAT immunocytochemistry. Rats were anesthetized with halothane and a precollicular transection performed. Semimicroelectrodes (40μ tip, 250 KΩ resistance) were stereotactically introduced into the posterior midbrain until controlled locomotion on a treadmill could be elicited. Only one drop on each side of the brain was made and only the lowest threshold point marked by DC lesion (2-5μA for 15-30 sec). Perfusion and immunohistochemical processing were carried out according to published procedures (Houser et al, J. Comp. Neurol. 234, 1985). Stimulation sites which produced controlled locomotion at low thresholds (31.9±13.1 S.D. μA) were located in and around ChAT labeled cell bodies. In several cases, the marking lesion destroyed a number of labeled cells as evidenced by necrotic areas surrounded by labeled cells. There was a correlation between threshold and the distance away from ChAT labeled cell groups. Drops which missed the PPN medially or laterally invariably showed higher thresholds, with the lowest threshold at the level of the nucleus. Drops through the PPN showed very low thresholds within the nucleus, and lowest at the ventral edge of the PPN. These findings provide evidence suggesting that stimulation in the area of the PPN (as delineated by its ChAT labeled population) leads to controlled locomotion on a treadmill in the precollicular preparation. This provides further support for to the suggestion that the PPN is part of the MLR.

Supported by NIH grant NS 20246.

242.5 THE MESENCEPHALIC LOCOMOTOR REGION. III. MORPHOMETRY OF CHAT LABELED CELLS IN THE VICINITY OF LOCOMOTION-INDUCING STIMULATION SITES. C. Conrad*, C. Campbell*, R. D. Skinner, C. R. Houser and E. Garcia-Rill (spon. T. W. Schoultz), Dept. of Anatomy, Univ. of Arkansas for Med. Sci., Little Rock, AR 72205 and Brain Research Institute, UCLA, Los Angeles, CA 90024.

A companion study demonstrated the relationship between locomotion-inducing sites of the mesencephalic locomotor region (MLR) and choline acetyltransferase (ChAT) labeled cells in the pedunculopontine nucleus (PPN). Tissue processed immunocytochemically for ChAT was used for morphometric analysis of labeled cells. Sagittal sections through the area were found to yield the best view of the PPN. The somata of ChAT labeled cells were drawn and the area and ratio of long versus short axis measured using a digitizing tablet. For comparison, ChAT labeled cells in the motor nuclei of cranial nerves V and VII in the same sections also were measured. In addition, cells in the area of PPN which were retrogradely labeled with HRP were measured. HRP was injected into the CM-Pf area in a different series of animals and cell body sizes as well as axial ratios were measured in PPN for comparison with ChAT labeled cells. (The PPN is known to project to CM-Pf and at least a portion of this system is thought to be cholinergic.) ChAT labeled cells (n=2433) in the PPN had a mean area of 300.88 ±99.18 S.D. sq. μm, and an axial ratio of 2.16±0.74 S.D. Distributions of cell areas indicated a flattened, normal distribution around the mean with long tails on each side of the median. Overall, more cells were located in the posterior third of the nucleus and these were generally smaller in area. There was also a tendency for larger labeled cells to be located laterally in PPN. Axial ratios in the sagittal plane were smaller posteriorly (rounded cells) and larger anteriorly (spindle cells). HRP labeled cells (n=984) in the PPN had a mean area of 254.12 ±97.87 S.D. sq. μm, and an axial ratio of 3.14±1.30 S.D. Like ChAT labeled cells, these were located in greater numbers posteriorly. Such observations suggest that cholinergic neurons in PPN project to CM-Pf. The differences in mean area and ratio, while not statistically significant, indicate that HRP labeled cells may include non-cholinergic components which also project to CM-Pf. Distributions of cell areas were similar to those of ChAT labeled cells. ChAT labeled cells in nuclei of V (n=400) and VII (n=574) showed mean areas similar to one another (490.66±197.50 S.D. sq. μm and 497.27±143.44 S.D. sq. μm, respectively) but the distributions were markedly different, with VII cells having a narrow distribution about the median and V having a flattened distribution. Cell ratios in nuclei of V and VII were 1.75±0.55 and 1.51±0.37 respectively, emphasizing their rounded nature.

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242.6 ELECTROPHYSIOLOGICAL INVESTIGATION OF A DESCENDING PATHWAY FROM THE CUNEIFORM NUCLEUS TO ALPHA MOTONEURONS IN CATS. S.J. Shefchyk and D.A. McCrea. Depts. Internal Medicine and Physiology, Fac. of Medicine, University of Manitoba, Winnipeg, Canada R3E 0W3.

The cuneiform nucleus (CN) has been described as the anatomical correlate of the mesencephalic locomotor region (MLR), a site which when stimulated produces locomotor movements (see Shik and Orlovsky., Physiol. Rev. 56:465, 1976). Shefchyk and Jordan (J. Neurophysiol., 53:1345, 1985) have described postsynaptic potentials (PSPs) mediated by lumbar interneurons in motoneurons time locked to the MLR stimulus producing fictive locomotion. While the identity of interneurons mediating the MLR PSPs during fictive locomotion remains unknown, a group of interneurons which have been described recently (Edgley and Jankowska, in prep.) are candidates. Located in laminae VII and VIII, and in segments L3-L5, these interneurons receive strong group II input and project to motoneurons in L6 and L7. These interneurons also receive PSPs from the CN (mean latency 4 ms), with segmental delays suggesting monosynaptic activation by the descending pathway at the level of the spinal cord (Edgley, Jankowska and Shefchyk, in prep.). In the present experiments we examined the latency of PSPs evoked in alpha motoneurons produced by stimulation of the CN with parameters comparable to those used in the study of the midlumbar interneurons. This was in an effort to determine whether the PSPs observed in the chloralose and decerebrate walking preparation are comparable and to evaluate the possibility that the evoked PSPs could be mediated by the L3-L5 interneurons with group II input.

Chloralose anaesthetized cats were used, and a variety of hindlimb leg nerves were prepared for stimulation. Intracellular records from lumbar motoneurons were used to determine the effects produced by stimulation of the CN. The CN was stimulated with a monopolar electrode placed stereotaxically into the CN (P1,L4,HØ) using trains of 2 to 10 stimuli (300 Hz) at strengths not exceeding 500 uA. The location of the stimulation was verified histologically.

To date, PSPs produced by trains of stimuli to the CN have been observed in all of the 19 motoneurons examined. EPSPs were observed in 12 motoneurons, IPSPs in 5 motoneurons and mixed PSPs in 2 motoneurons. The latency from the stimulus artifact associated with the effective shock to the onset of the PSPs ranged from 4.5-10 ms (mean=6.5 ms) for EPSPs and 7-17 ms (mean=10.7 ms) for IPSPs. Thus the latencies of PSPs seen in the present experiments are comparable to the MLR evoked PSPs observed in motoneurons during fictive locomotion. The data is also consistent with their being mediated by the interneurons in midlumbar segments with group II input. Further assessment of the pathway is underway.

This research was supported by the Medical Research Council of Canada and the Seller's Research Foundation.

242.7 LOCALIZATION OF BRAINSTEM COMMAND NEURONS FOR THE INITIATION OF LOCOMOTION IN THE LAMPREY. A.D. McClellan and P.A. Getting. Dept of Physiol & Biophys, U of Iowa, Iowa City, IA 52242

An *in vitro* brainstem/spinal cord preparation of the lamprey CNS has been developed for studying the brainstem command system which activates spinal pattern generators to initiate locomotion (McClellan, *Brain Res.* 302: 151, 1984). Transections through various levels of the brainstem indicate that brainstem neurons below the rostral border of the mesencephalon are important for the initiation of locomotion. Previous evidence obtained from this preparation suggested that the descending pathways from this command system consist of moderately long axons. In the present study, anatomical and physiological techniques were used to determine the location of the brainstem locomotor command neurons, particularly the neurons which descend into the spinal cord.

Rostral spinal cord lesions which spare either the lateral tracts or the ventrolateral tracts do not abolish the initiation of locomotion, turning or withdrawal response, although there is some deficit in equilibrium. Retrograde transport of HRP through the spared parts of the spinal cord labels brainstem neurons in the medial (mrrn) and to a lesser extent the posterior (prrn) rhombencephalic reticular nuclei. Thus, at least some of the neurons in the mrrn with moderately long descending axons in the ventrolateral spinal tracts (McClellan and Grillner, *Brain Res.* 300:357, 1984) are probably involved in initiating locomotion. In contrast, partial lesions in the rostral spinal cord that spare the medial tracts abolish both spontaneous or sensory evoked locomotion, as well as withdrawal and turning responses. Retrograde transport of HRP through the spared parts of the spinal cord labels some of the larger reticulospinal neurons in the mesencephalic tegmentum (mt), and in the anterior (arrn) and medial (mrrn) rhombencephalic reticular nucleus. Presumably these neurons with medial descending axons do not play a significant role in initiating locomotor responses.

Ionophoretic chemical stimulation in the brainstem with excitatory amino acids (0.5 M DL-glutamate or DL-aspartate) or their agonists (0.1 M N-methyl-DL-aspartate or 0.1 M kainate) has been used to activate cell bodies of neurons in the command system. Chemical stimulation (-100 nA, 100 ms pulses at 5 Hz) in the vicinity of the dorsally located reticulospinal nuclei, including the mrrn, can elicit well coordinated locomotor patterns. Modifications of the chemical stimulation technique are presently being used to stimulate only the descending neurons in the command system.

Intracellular recordings indicate that some of the neurons in the mrrn with descending axons in lateral spinal tracts fire tonically during an episode of locomotion, but the frequency of spiking is correlated with the phase of the locomotor rhythm. Other reticulospinal neurons may either fire briefly at the onset of motor activity or be inactive; in some cases, oscillations of the membrane potential are correlated with the phase of the locomotor rhythm. (Supported by NIH grant NS23216, SCRF grant NBR 501-5, and IAS grant ISF 85-3 to ADM and by NIH grant NS17328 to PAG)

242.8 REVERSAL OF INHIBITION OF EDGE CELLS DURING FICTIVE LOCOMOTION IN THE LAMPREY BY CHLORIDE INJECTION OR HYPERPOLARIZATION. T.L. Williams* & S. Alford*, (SPONSOR: ENA), Physiology Department, St. George's Hospital Medical School, University of London, SW17 ORE, U.K.

Edge cells are propriospinal stretch-receptor neurones with receptive fields in the lateral margins of the spinal cord, which are rhythmically stretched and compressed during locomotion (Grillner, McClellan & Sigvardt, 1982; Grillner, Williams & Lagerback, 1984).

We have recorded intracellularly from these primary sensory neurones during fictive locomotor episodes induced by mechanical stimulation of the tailfin (McClellan & Grillner, 1983).

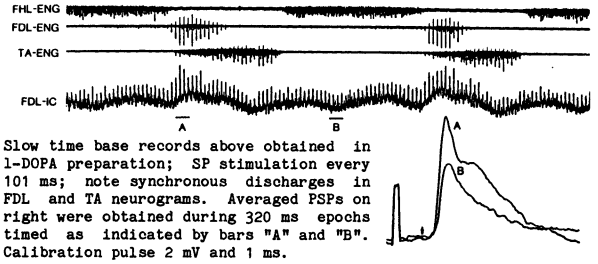
Recordings were made from a contralateral pair of ventral roots in a preparation (Lampetra fluviatilis or Petromyzon marinus) consisting of spinal cord, notochord, and a small portion of innervated tailfin. The fictive locomotor episodes consisted of between one and five cycles of alternating bursts of ventral root activity. Every edge cell tested showed numerous putative IPSPs and a net hyperpolarization, both of which persisted throughout the locomotor episode and for some few seconds following. When a tail fin stimulation failed to excite a locomotor episode, the edge cell showed no such response.

Both the net hyperpolarization and the IPSPs were reversed by injection of negative current during a locomotor episode (electrode filled with 0.3 M Lithium acetate & 5% Lucifer yellow) or by the injection of chloride ions prior to an episode (as negative current, electrode filled with 1 M KCl).

Thus we have evidence that edge cells are inhibited during locomotion, by activation of a synaptic channel with a reversal potential negative to the normal resting potential. Furthermore, the synaptic current must be carried at least partly by chloride ions. Such a centrifugal control would allow maximum sensitivity of these proprioceptors when the body is at rest, and may also allow precise regulation of their activity at different speeds of locomotion.

242.9 MODULATION OF SHORT LATENCY EPSPs FROM CUTANEOUS AFFERENTS TO FDL MOTONEURONS DURING FICTIVE LOCOMOTION IN THE CAT.
B.J.Schmidt, D.Meyers, M.Tokuriki*, J.W.Fleshman, and R.E.Burke. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20892.

The distinctive activity pattern exhibited by the flexor digitorum longus muscle (FDL) during normal locomotion is preserved during 'fictive' stepping (Fleshman et al., Exp. Brain Res. 54: 133, 1984), presumably driven by a spinal central pattern generator (CPG). In order to study CPG organization, we examined modulation of the short latency EPSPs produced by low threshold superficial peroneal (SP) nerve afferents in FDL motoneurons during fictive stepping. Fictive locomotion was induced in decerebrate (precollicular-postmammillary), paralysed cats by stimulation of the mesencephalic locomotor region or by administration of nialamide and L-DOPA after spinalization at the T13 spinal cord level. Intracellular records were obtained from FDL motoneurons during fictive locomotion while the SP nerve was stimulated at 10-70 Hz and about twice threshold, along with electroneurograms (ENG) from various nerves including flexor hallucis longus (FHL), FDL, and tibialis anterior (TA) nerves. As shown on a slow time base below, depolarizing oscillations of FDL membrane potential (FDL-IC) were synchronized with FDL neurogram activity during fictive stepping (note timing in relation to alternating activity in extensor (FHL) and flexor (TA) nerves). SP EPSPs (10 Hz stimulation; sharp upward deflections in the FDL-IC trace) were enhanced during periods of FDL neurogram activity. SP PSPs averaged (32 responses each) during FDL bursts (e.g., "A") showed marked enhancement of early EPSP components, as compared to PSPs during extensor phases (e.g., "B"). This enhancement of SP EPSP components suggests that excitatory drive from the CPG projects to interneurons in the pathway from low threshold SP afferents to FDL motoneurons. These interneurons could relay excitation from CPG to FDL motoneurons, or be part of the CPG.



Slow time base records above obtained in l-DOPA preparation; SP stimulation every 101 ms; note synchronous discharges in FDL and TA neurograms. Averaged PSPs on right were obtained during 320 ms epochs timed as indicated by bars "A" and "B". Calibration pulse 2 mV and 1 ms.

242.10 **INTRACELLULAR RECORDING OF QUADRICEPS FEMORIS ALPHA MOTONEURONS OF AWAKE, INTACT CATS DURING LOCOMOTION.**
L.L. Glenn, J.F. Whitney*, J.A. Salamone*, and S.A. Mariash*. *Department of Physiology, Ohio College of Podiatric Medicine, Cleveland, Ohio 44106.*

Spinal motoneurons have been recorded intracellularly in awake, resting cats (Glenn and Dement, J. Neurophysiol., 46:839, 1981). Intracellular studies of motoneurons during motor activity such as locomotion have been conducted in pharmacologically-treated decerebrate or spinal preparations (Shefchyk and Jordan, J. Neurophysiol., 54:1101, 1985), but not intact and awake cats. The present communication reports a method for intracellularly recording from motoneurons during treadmill locomotion in lumbar-restrained cats.

The following transducer were implanted in pentobarbital-anesthetized cats: (1) a saline-in-tubing strain gauge was anchored to the ischial tuberosity and the distal femur for the recording of the ischiofemoral angle; (2) bipolar patch-style electromyogram electrodes were sutured to the ventralis medialis m., sartorius m., rectus femoris m., and the ventralis lateralis m.; (3) a cuff electrode was placed around the femoral n.; and (4) vertebrae L3-L5 were mechanically fused by an implantable vertebral fixation assembly.

After recovery from surgery, cats were gradually adapted to lumbar restraint and trained to locomote on a belt moving under the lower limbs. During recording sessions, quadriceps femoris alpha motoneurons (membrane potential more negative than -50 mV) were identified on the basis of antidromic activation from femoral nerve stimulation and crosscorrelation between motoneurons spikes (evoked by depolarizing currents) and each of the quadriceps electromyograms. Episodes of voluntary stepping were then promoted by belt displacement at 0.4 m/s for 8 s over an distance of 3.2 m.

The foregoing method is promising. In the first cat, only one motoneuron was successfully recording before, during, and after an episode of locomotion in three experimental sessions. In the second cat, 10 motoneurons were recorded in two experimental sessions with an average of 2.3 episodes of locomotion each. In conclusion, the above method can provide data on the electrophysiological properties of quadriceps motoneurons before, during and after locomotion, and as a function of the phase of the step cycle.

242.11 **INFLUENCE OF PENTOBARBITAL ON MOTONEURON FUNCTION IN AWAKE, INTACT CATS.**
J. F. Whitney* and L.L. Glenn (Spon: J.W. Fleshman), *Dep. of Physiology, Ohio College of Pod. Med., University Circle, Cleveland, Ohio 44106.*

A method for obtaining intracellular records from spinal motoneurons of awake, intact-restrained cats has recently been developed (Glenn et. al., Brain Res., 163:328,1979; Morales and Chase, Expt. Neurol., 62:821, 1978). Using this we have been studying the influence of general anesthetics on the electrophysiological properties of alpha motoneurons that innervate the hindlimbs. Our goals are to establish baseline data concerning normal motoneuron function since all previous work involved modified nervous systems (through use of anesthetics, transections, slice preparations, etc.) and to develop a model system to directly study the influence of anesthetics on neural tissue. Four cats, under pentobarbital anesthesia, were implanted with cuff electrodes around the femoral n., obturator n., and tibial and common peroneal branches of sciatic n. for antidromic identification of motoneurons. Vertebra L3-L5 were fused with a stainless steel plate that was later attached to a recording table. Depth of anesthesia was determined by testing corneal, pinna, and forelimb flexion reflexes. We have found that pentobarbital significantly influences the resting membrane potential, synaptic activity, and spike properties of motoneurons.

MEASURE	AWAKE		ANESTHETIZED	
Membrane Pot.(mV)	-53.78+1.24	(6)	-58.81+1.54	(8)1
Input Res. (M)	1.67+0.22	(2)	1.52+0.04	(7)
Synap. Activ. (V/s)	1.89+0.34	(6)	1.34+0.06	(7)2
Spike Amp. (mV)	51.03+1.94	(7)	56.74+2.71	(7)2
1/2 Amp. Dur.(us)	678.57+18.31	(7)	870.00+53.45	(7)3

1=p<0.25,2=p<.05,3=p<.005; 1-tailed, indep. t-tests.

From the preliminary results shown above, it is clear that pentobarbital significantly influences motoneuron properties and that the criteria used for awake recording entail modification of existing, anesthetized criteria. We are presently examining the effects of TEA and TTX in our preparation in an attempt to reveal the ionic conductances involved in the influence of anesthetics on neural tissue.

242.12 EFFECTS OF BRAINSTEM ACTIVATION ON CORTICALLY INDUCED RHYTHMICAL JAW MOVEMENTS IN THE GUINEA PIG.
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The reticular formation of the brainstem has been implicated in the control of motor behaviors such as locomotion, respiration, eye movements, and oral–facial movements among others. With respect to the control of jaw movements, there have been numerous studies which have investigated the supraspinal influences on oral–facial reflex pathways. There are few studies, however, which have examined the brainstem influences on centrally generated rhythmical jaw movement activity. The purpose of the present study was to further elucidate, with the use of microstimulation techniques, the influences of brainstem loci on the neuronal networks responsible for cortically induced rhythmical jaw movements in the anesthetized guinea pig and to establish if these brainstem loci are capable of modulating the timing of occurrence of rhythmical EMG activity as well as its amplitude during the rhythmical jaw movement cycle.

In ketamine anesthetized guinea pigs, fine wire bipolar recording electrodes were inserted into the jaw opener digastric(DIG) muscles for recording EMG activity. For some experiments the movements of the jaw were monitored by a photoelectric position sensor placed in front of a small tungsten light source fixed to the inferior surface of the mandible. Rhythmical jaw movements were elicited by repetitive stimulation(40Hz) of the masticatory area of the cortex. To determine the effects of stimulation of different brainstem loci on RJMs, the stimulus to the brainstem consisted of either repetitive trains(RT) of long duration (10sec, 50Hz)) or short pulse trains(SPT:50-100msec, 50-100Hz) delivered at various times after the onset of a DIG burst within the cycle. Cycle by cycle measurements of RJM cycle duration (CD, DIG onset to onset), burst duration (BD) and integrated EMG (I–EMG) prior to and during brainstem stimulation were obtained.

It was found that RT stimulation of widespread areas of the brainstem reticular formation suppressed ongoing cortically induced RJMs. Prior to complete suppression, the CDs were increased while the burst durations and I–EMG were reduced. During SPT stimulation of rostral pontine and midbrain suppressive sites, the subsequent onset of a DIG burst following the stimulus was always delayed. This delay was related to the phase of the cycle the SPT was delivered. For medullary and caudal pontine sites, SPT stimulation delivered early in the RJM cycle advanced the onset of the subsequent DIG burst while SPT stimulation occurring at later times delayed the onset of the subsequent DIG burst. For all areas of brainstem examined, the durations of the cycle following the SPT stimulus were unchanged from control suggesting that a true phase resetting of the cycle had occurred. These data suggest that widespread areas of the brainstem are capable of suppressing ongoing RJM activity and are capable of controlling the excitability of the central circuits responsible for cycle oscillation and timing as well.

This research was supported by NIH grants DE 06193 and DE4166.

242.13 RECOVERY OF THE VOMITING REFLEX FOLLOWING AREA POSTREMA ABLATION IN SQUIRREL MONKEYS. S. Elfar‡ K. Brizzee, R. Fox, M. Corcoran‡ N. Daunton, J. Coleman*. San Jose State Univ., San Jose CA, Delta Regional Primate Center, Covington LA, and NASA Ames Research Center, Moffett Field CA.

The role of the area postrema (AP) in motion-induced emesis has been re-assessed recently in several different species (e.g. Borison et al., 1984; Corcoran et al., 1985; Wilpizeski, 1986). In a few of these studies, the role of the AP in motion-induced conditioned taste aversion (CTA) has also been addressed (e.g. Sutton et al., 1983; Corcoran et al., 1985). The purpose of the present study was to extend this comparative study to the squirrel monkey, to evaluate further the role of AP in vomiting, and to investigate the dynamics of the recovery process.

The AP was ablated bilaterally in 7 motion-susceptible squirrel monkeys which previously had been characterized in terms of their responses to various motion sickness-inducing stimuli. After recovery from surgery all animals were tested at 30-day intervals for a period of 11 months to determine the effects of AP ablations on susceptibility to the same sickness-inducing conditions. In addition, the effectiveness of motion in producing CTA was evaluated. All pre-ablation motion tests involved stimulation for 30 min., while post-lesion tests were 60 min. in duration.

All animals showed significant increases in latencies to vomiting after AP ablations. However, the latencies tended to decrease with time after the ablation. All but one animal vomited on at least one of the 10 motion tests occurring after ablation of AP. In addition, CTA was produced by motion in animals which continued to vomit after AP ablation, whether or not vomiting occurred during the 30 min. of motion used in the conditioning sessions. These results suggest that structures other than AP, and processes other than those mediated through AP, may play an important role in motion-induced emesis.

242.14 ORGANIZATION OF THE SENSORY INNERVATION OF CRURAL AND COSTAL REGIONS OF THE CAT DIAPHRAGM. J.R. Holtman, Jr., W.R Revelette* and D.F. Speck. Departments of Pharmacology and Physiology, Coll. of Med., Univ. KY, Lexington, KY 40536.

Previous work has demonstrated that there is a somatotopic organization of the motor innervation to different regions of the cat diaphragm (Duron et al., Neurosci. Lett. 14: 159, 1979). The purpose of the present study was to determine whether a similar somatotopic organization exists for the sensory innervation of the diaphragm. To address this question, we have used neuroanatomical techniques employing a retrogradely transported fluorescent dye (fast blue).

Cats (2-4.5 kg) of either sex were anesthetized with pentobarbital (40 mg/kg, I.P.). Fast blue (5%) was injected (4x10 µl) into either the costal region or crural region of the diaphragm in different animals. The cats were allowed to recover for seven days to allow transport of dye to dorsal root ganglion (DRG) cell bodies. At this time, cats were deeply anesthetized with pentobarbital (70 mg/kg, I.P.) and perfused through the ascending aorta initially with 2 L of 0.1 M phosphate buffered saline (pH=7.4) followed by 2 L of 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.4). The DRG from the fourth, fifth and sixth cervical spinal cord segments were removed bilaterally. The ganglia were postfixed (60 min) and then placed in 10% sucrose in 0.1 M phosphate buffer (pH=7.4) for at least 24 hours. The ganglia were cut (16 µm thickness) and viewed under epifluorescence illumination. Cell bodies containing fast blue in each DRG were counted and their mean diameters determined. Only cell bodies with an identifiable nucleus were counted for this study.

Injection of fast blue into the crural region of the diaphragm resulted in the labelling of cell bodies in the fourth, fifth and sixth DRG. The majority of the labelled cell bodies were located in the fifth and sixth DRG. Labelled cell bodies were found bilaterally, however, the largest number of cell bodies were present ipsilateral to the injection. Cell bodies in the fourth DRG ranged in size from 20-35 µm. Cell bodies in the fifth DRG ranged from 18-80 µm while those in the sixth DRG were 18-76 µm in size. Injection of fast blue into the costal region of the diaphragm resulted in the labelling of cell bodies in the fifth and sixth DRG. The cell bodies were present predominantly ipsilateral to the injection. Cell bodies in the fifth DRG ranged from 18-80 µm while those in the sixth DRG were 18-76 µm in size.

The results of this study indicate that there may be a somatotopic organization of the sensory innervation of the diaphragm. [Supported by HL 36050 (JRH), and UKMC Research Fund (DFS)].

242.15 SHORT LATENCY EFFECTS OF DIAPHRAGMATIC AFFERENTS ON MEDULLARY NEURONS AND PHRENIC MOTOR DISCHARGE. D.F. Speck, W.R. Revelette* and D.T. Frazier. Dept. of Physiology, University of Kentucky Medical Center, Lexington, KY 40536

In a recent report (Physiologist 28: 308, 1985) cervical phrenic nerve stimulation (CPNS) excited 25% of the inspiratory-modulated neurons in the dorsal respiratory group (DRG) but only 10% of those in the ventral respiratory group. Additional experiments have been performed to examine the three issues described below. Cats were anesthetized with pentothal or chloralose-urethane, paralyzed and artificially ventilated. The C5 and/or C6 phrenic rootlets and the thoracic phrenic nerve were isolated for recording and/or stimulation. Microelectrodes were inserted into the DRG for extracellular recording of spontaneous and evoked neuronal discharges. To determine if the neuronal responses could be due to nondiaphragmatic afferents in the cervical phrenic nerve, we examined the neuronal responses to both CPNS and thoracic phrenic nerve stimulation (TPNS). 90% of the DRG units activated by CPNS were also activated by TPNS. The difference in onset latency of neuronal excitation with CPNS vs. TPNS was used to calculate the peripheral conduction velocity of the afferents involved. Conduction velocities ranging from 1.8 to 30 m/sec suggest that this supraspinal facilitation of respiratory (and non-respiratory-modulated) neurons is due to activation of Group III afferents.

Because of the known projections of phrenic nerve afferents to the cortex and cerebellum, we determined if these structures were necessary intermediates in the production of the brainstem responses to phrenic nerve stimulation. After aspiration of the cerebellum and a midcollicular decerebration, DRG neuronal responses to phrenic nerve stimulation were still evident. Similarly, although phrenic nerve afferent pathways have been reported in the dorsal columns, transection of the dorsal columns at C2 did not alter the DRG neuronal responses to CPNS.

A single shock (0.2 msec, 0.01 - 10 mA) of the phrenic nerve elicits a transient attenuation of the phrenic motor discharge (Fed. Proc. 45: 520, 1986). The almost synchronous occurrence of a brainstem excitatory neuronal effect and a reduction in phrenic motor discharge suggests that at least two different reflexes must be elicited by phrenic nerve afferents - a supraspinal facilitation and a spinal inhibition. The hypothesis of an inhibitory phrenic-to-phrenic reflex is supported by preliminary data which demonstrate that in a C2 spinalized cat, the excitatory intercostal-to-phrenic reflex can be reduced by simultaneous stimulation of the phrenic nerve. (Supported by HL-34568 and the UKMC Research Fund).

242.16 LOCATION AND PROJECTIONS OF RESPIRATORY INTERNEURONS OF THE THORACIC SPINAL CORD. P.A. Kirkwood, J.B. Munson, T.A. Sears* and R.H. Westgaard**. Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London WC1N 3BG England.

Extracellular recordings have been made of single unit activity in the ventral horn of the thoracic spinal cord (segments T3, T4, T6, T7) of cats anesthetized with pentobarbital, paralyzed with gallamine and artificially ventilated with an air/02/C02 mixture so as to produce moderate hypercapnia. Microelectrode tip position was determined by reference to the antidromic field potentials from stimulation of the intercostal nerves and the dorsal spinal ramus, confirmed by dye marks.

Spontaneous single unit activity with respiratory modulation (always at a slower rate than the ventilator rate, 52/min) was readily found in all segments investigated. Units were located among or just dorsal to the dorsal ramus motoneurons. Units identified as motoneurons by antidromic activation or spike-triggered averaging of the efferent activity in muscle nerves were all located close to the appropriate field potential maxima. Units in the more dorsal group and many others within the motor nuclei could not be identified in this way and are considered likely to be interneurons. Some of these fired at high rates, up to 300/sec. 35 of the units (12 expiratory, 23 inspiratory) were proven to be interneurons by antidromic activation from other segments, 1 to 5 segments caudally. 9 of their axons were identified ipsilaterally, 24 contralaterally and 2 bilaterally. The proportion of units with ipsilateral axons may be low because of the frequent presence of short latency (ca. 1 ms) orthodromic activation which could occlude or obscure antidromic invasion. Inspiratory and expiratory units were apparently not spatially segregated.

About 60% of the identified interneurons were located among the motoneurons. This emphasizes that anatomical demonstrations of terminal projections within the motor nuclei should not be taken alone as evidence for monosynaptic projections to the motoneurons.

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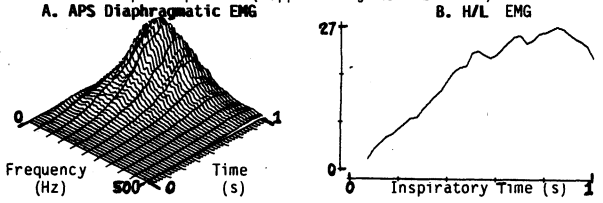
242.17 **HIGH/LOW POWER RATIOS (H/L) OF PHRENIC NERVE AND DIAPHRAGM EMG POWER SPECTRA INCREASE DURING THE INSPIRATORY PHASE IN DECEREBRATE CATS.** Charles A. Richardson. Cardiovascular Research Institute, Depts Anesthesia and Physiology, University of California, San Francisco, CA 94143-0542.

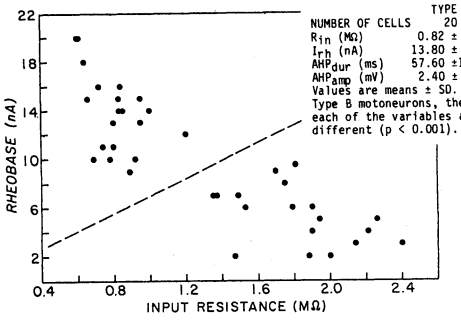
A change in power spectra of skeletal muscle electromyogram accompanies muscle fatigue: the ratio of high frequency power to low frequency power (H/L) decreases. Similarly, as the diaphragm fails as a pressure generator, H/L decreases. However, when a fatiguing inspiratory load is applied, H/L decreases within seconds, long before the diaphragm fails as a pressure generator (Gross, J Appl Physiol 46:1-7 1979). When the load is removed, H/L increases quickly, long before the muscle regains its full force. I propose that the central respiratory pattern generator can contribute to changes in the power spectrum of the diaphragmatic EMG. By computing the coherence spectrum between the phrenic nerve and the contralateral diaphragm EMG, I detected frequency components contributed by the central respiratory pattern generator. I recorded inspiratory activity from the central end of the cut left phrenic nerve and from two pairs of stainless steel wires sewn into the right diaphragm in 4 decerebrate cats. The bare ends were sewn into the muscle perpendicular to the muscle fibers, one wire near the costal margin, the other 8mm centrally. I computed the auto-power spectra (Fig A) for all three individual signals and the coherence spectra for all three possible pairs of signals. I computed H/L (low=20-44Hz; high=140-640Hz) for power spectra shifted through the inspiratory phase. There was a major peak in the APS of the phrenic nerve activity at 80, 86, 90, and 110 Hz for the 4 cats. The coherence between the left phrenic nerve activity and the contralateral diaphragm EMG was significant at frequencies below 120, 105, 135, and 140 Hz respectively. The H/L increased as a function of inspiratory time (Fig B) suggesting that H/L may drop if inspiratory time shortens in response to an applied load. I conclude that changes in the power spectra of the diaphragmatic EMG may be an indication of a smart controller adjusting to a large perturbation in its controlled system rather than of the immediate failure of its power plant. (Supported by NIH HL-26176)



242.18 TWO SUBPOPULATIONS OF PHRENIC MOTONEURONS REVEALED BY RHEOBASE-INPUT RESISTANCE MEASUREMENTS. J.S. Jodkowski*, F. Viana*, T.E. Dick and A.J. Berger. Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

The electrical properties of lumbosacral motoneurons correlate strongly with mechanical properties of muscles fibers they innervate. One can predict with high accuracy the type of motor unit (Type F - fast, Type S - slow) by determining motoneuronal input resistance (R_{in}) and rheobase (I_{rh}) (1). We measured these two variables, as well as afterhyperpolarization duration (AHP_{dur}) and amplitude (AHP_{amp}) for 38 antidromically identified phrenic motoneurons (membrane potential at least -60 mV) in anesthetized, paralyzed and partially deafferented cats during hypocapnic apnea. A plot (see Figure) of I_{rh} against R_{in} for phrenic motoneurons revealed two subpopulations: Type A (high I_{rh}, low R_{in}) and Type B (low I_{rh}, high R_{in}). We superimposed the line (I_{rh}=7xR_{in}) separating Type F and Type S lumbosacral motoneurons (1) onto the plot and this line unambiguously demarcated the two subpopulations of phrenic motoneurons. Table 1 presents the electrophysiological properties of these two types of phrenic motoneurons. Since the diaphragm contains both F and S motor units (2), and if we assume that the properties of diaphragmatic motor units are similar to those of motor units supplied by lumbosacral motoneurons, then we would predict that Type A phrenic motoneurons supply Type F muscles fibers, and Type B supply Type S muscle fibers. (Supported by NS 14857, a Parker B. Francis Fellowship to T.E.D., and CCHNCCE to F.V.)

(1) Zengel et al., J. Neurophysiol., 53: 1323-1344, 1985.
(2) Sieck et al., Soc. Neurosci. Abstr. 11: 211, 1985.



	TYPE A	TYPE B
NUMBER OF CELLS	20	18
R_{in} (MΩ)	0.82 ± 0.15	1.83 ± 0.31
I_{rh} (nA)	13.80 ± 3.2	5.30 ± 2.4
AHP_{dur} (ms)	57.60 ±10.7	79.40 ±19.9
AHP_{amp} (mV)	2.40 ± 0.9	4.40 ± 1.97

Values are means ± SD. For Type A versus Type B motoneurons, the mean values of each of the variables are significantly different ($p < 0.001$).

242.19 MAINTENANCE OF COMPLEX CIRCUIT FUNCTION DURING PERFUSION OF THE MAMMALIAN BRAIN. G.B. Richerson* and P.A. Getting. (SPON: R.B. Felder) Dept. of Physiology & Biophysics, University of Iowa, Iowa City, IA 52242.

We have developed an *in situ* perfused guinea pig brain preparation which preserves normal electrical function of complex circuits for prolonged periods. Adult guinea pigs were anesthetized with pentobarbital and Innovar-vet, and the brainstem was exposed by aspiration of the cerebellum. The rostral half of the body was perfused via the ascending aorta at 37°C with a defined artificial blood containing the oxygen carrier perfluorotributylamine. The venous effluent was collected, dialyzed against Ringer's, reoxygenated and then recirculated. The anesthetic pentobarbital (40-50 μM) was added to the blood and the dialysate. The electrocorticogram (ECoG) was monitored to assess the anesthetic level. The motor output from the brainstem respiratory network was recorded with a cuff electrode on the phrenic nerve. The membrane potential of brainstem respiratory neurons was recorded using glass microelectrodes (3M KCl, 40-80 megohms).

The ECoG was preserved for as long as 7 hours with no change in amplitude compared to the ECoG recorded before perfusion. The frequency content of the ECoG was typical of barbiturate anesthesia. The respiratory motor output pattern displayed a regular rhythm and a normal rate and waveform for an average of 6.2 hours (± 0.95 hrs STD; n=9). Systematically increasing the PCO_2 of the artificial blood resulted in an increase in the amplitude of phrenic activity with a slight increase in inspiratory duration. Electrical stimulation of the vagus nerve resulted in a decrease in inspiratory duration and a decrease in amplitude of phrenic activity with no change in the rate of rise. These are the normal responses of the respiratory system to PCO_2 and pulmonary stretch receptor feedback. The use of nonpulsatile perfusion resulted in increased mechanical stability, which permitted stable intracellular recordings from brainstem neurons with ongoing respiratory activity.

Perfused brain preparations have been used in the past, but it has been difficult to maintain complex electrical function. Although normal biochemistry, histology, and cellular electrophysiology have been preserved for extended periods, the electroencephalogram has only been sustained unchanged from control for 1-2 hours. Although we have previously recorded respiratory activity during perfusion (Neurosci Abst, 10:745, 1984), normal motor output from a complex neural circuit has never been clearly demonstrated. We have increased the duration of normal cortical activity during perfusion, and have demonstrated for the first time normal function of a complex neural network and its major afferent and efferent pathways. We are now using this preparation to study the electrophysiology of brainstem respiratory neurons. This approach may also be generally useful for electrophysiology throughout the CNS, and for other fields including brain biochemistry, neuropharmacology and neurology.

Supported by NIH grants GM07337 & MH15172 to GBR & NS15350 to PAG

243.1 3-HYDROXYANTHRANILIC ACID OXYGENASE: PURIFICATION FROM RAT LIVER AND IMMUNOHISTOCHEMICAL ANALYSIS IN RAT BRAIN. E. Okuno, C.Köhler*[1] and R. Schwarcz. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228 and [1]ASTRA-Research Labs., Dept. Pharmacology, S-151 85 Södertälje, Sweden.

The endogenous excitotoxin quinolinic acid (QUIN) has been speculatively linked to the pathogenesis of human neurodegenerative disorders (Life Sci., 35, 19, 1984). Its biosynthetic enzyme, 3-hydroxyanthranilic acid oxygenase (3HAO), converts 3-hydroxyanthranilic acid to α-amino-β-carboxymuconic semialdehyde, which in turn spontaneously rearranges to form QUIN. 3HAO is present in mammalian (including human) brain and can therefore be envisioned to constitute an important factor in the regulation of cerebral QUIN function and/or dysfunction.

3HAO has now been purified to homogeneity from rat liver following a seven-step procedure including sequential elutions from DEAE-Sephadex, CM-cellulose, hydroxylapatite and Sephadex G-150 resins. Since enzyme activity was found to rapidly decrease after the DEAE-Sephadex step, bovine serum albumin (10 µg) was routinely added to the incubation mixture in order to increase activity and stabilize the protein. Homogeneous liver 3HAO has a molecular weight of 37000 and is not composed of subunits, as shown by SDS gel electrophoresis. Its motility when subjected to polyacrylamide gel electrophoresis was 0.37. Kinetic analysis revealed a K_m for 3-hydroxyanthranilic acid of 3 µM. All these physico-chemical properties (as well as pH-optimum, heat stability and behavior during partial purification) were shared by partially purified rat brain 3HAO, thus tentatively demonstrating the identity of the two enzymes. Further proof was provided by Ouchterlony double-diffusion experiments, using anti-rat-liver 3HAO antibodies (center well) against both liver and brain 3HAO. Fusion occurred between the precipitates caused by the liver and the brain enzyme.

The homology of liver and brain 3HAO allowed the use of anti-liver 3HAO antibodies for immunohistochemical analysis of the brain. 3HAO-immunoreactivity was shown to be preferentially contained in small glial cells throughout the brain. However, sporadic neuronal localization was noted in several brain areas including hippocampus, pyriform cortex, thalamus and striatum. Overall, the regional distribution of 3HAO-immunoreactivity in the brain as well as the morphological characteristics of 3HAO-containing cells were reminiscent of those noted in similar studies using antibodies directed against quinolinic acid phosphoribosyltransferase, QUIN's catabolic enzyme (Soc. Neurosci. Abstr., 11, 240.8, 1985). The availability of pure 3HAO and anti-3HAO antibodies can be expected to constitute valuable tools for future studies on the brain's QUIN system.

Supported by USPHS grants NS 16102 and 20509.

243.2 EFFECTS OF LIMBIC LESIONS ON QUINOLINIC ACID METABOLISM IN THE RAT BRAIN. C. Speciale and R. Schwarcz. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228.

A pathologic overabundance of the brain metabolite quinolinic acid (QUIN) has been hypothetically linked to the etiology of seizure disorders in man (Exp. Neurol., 84, 1, 1984). In view of the prominent involvement of limbic brain structures in the initiation and propagation of seizure phenomena, it seemed prudent to examine the effects of both limbic seizures and experimentally induced limbic nerve cell degeneration on the brain's QUIN system. To this end, the latter was assessed by measurements of the enzymes immediately responsible for QUIN biosynthesis (3-hydroxyanthranilic acid oxygenase; 3HAO) and degradation (quinolinic acid phosphoribosyltransferase; QPRT).

In the first set of experiments, kainic acid (12 mg/kg,i.p.), causing a reproducible pattern of limbic seizures and neuronal necrosis (Neuroscience, 14, 375, 1985), was administered to adult male rats. Animals were killed in the acute (3 hours) or subacute (7 days) phase following kainate. No changes in the activities of 3HAO, QPRT and glutamate decarboxylase (GAD; a marker for neuronal integrity) were noted at the time of peak seizure activity (3 hours) in the pyriform cortex, hippocampus or striatum (assessed as a non-limbic control area). In contrast, both 3HAO and QPRT activities were increased markedly 7 days following kainate application in hippocampus (344±62 and 213±26% of control; N = 9) and pyriform cortex, i.e. areas of nerve cell loss. No significant changes were observed in simultaneously assessed striatal tissue.

In a second study, complete hippocampal nerve cell loss was induced by an intrahippocampal microinjection of the excitotoxin ibotenic acid (20 µg/1µl). While no changes were noted in hippocampal 3HAO and QPRT activity 2 days after surgery (GAD: 62±2% of control), substantial increases in both QUIN-metabolic enzymes, limited to the injected hippocampus, were measured between 7 and 60 days after the lesion (peak effects at 14 days; 3HAO:452±48%; QPRT:221±28% of control; N = 5). No significant changes in hippocampal 3HAO or QPRT activity were found 7 or 60 days after cholinergic deafferentation by fornix-fimbria transection.

Taken together, our data indicate that the limbic QUIN system is significantly activated in response to primary or seizure related nerve cell loss (cf. also Schwarcz et al., this meeting). In contrast, no such increase in QUIN metabolism appears to be associated with seizure activity itself.

Supported by USPHS grant NS 16102.

243.3 BASAL GANGLIA LESIONS: BIOCHEMICAL EXAMINATION OF QUINOLINIC ACID METABOLISM. R.Schwarcz, R.J.White* and E. Okuno. Maryland Psychiatric Research Center, P.O.Box 21247, Baltimore, MD 21228.

The selective nerve cell loss observed in disorders such as Parkinson's disease and Huntington's disease may be mediated by a pathologic overabundance of endogenous excitatory amino acids ("excitotoxins"). For a comprehensive assessment of the chemical neuropathology of basal ganglia disorders of possible excitotoxic origin, it is important to examine the consequences of selective neuronal degeneration on potential endogenous pathogens. We have therefore, in the rat, investigated the effects of experimentally induced basal ganglia lesions on the metabolism of quinolinic acid (QUIN), the most potent endogenous excitotoxin discovered so far. Lesions were made in adult male rats according to established procedures. Neuronal degeneration in the striatum was caused by a local injection of ibotenic acid (IBO; 40 µg/2 µl); dopaminergic cell loss in the substantia nigra was elicited by intranigral application of 6-hydroxydopamine (6OHDA; 8 µg/2 µl); and striatal deafferentation was accomplished by suction of the overlying cortex (cortical ablation). All procedures were performed unilaterally and their success monitored biochemically. For all measures, contralateral homotopic structures were used as controls since they did not show differences from naive tissue in any of the parameters examined. Animals were killed seven days after surgery and their striata and substantiae nigrae rapidly dissected. The QUIN system was assessed by measurement of the biosynthetic enzyme, 3-hydroxyanthranilic acid oxygenase (3HAO) and the degradative enzyme, quinolinic acid phosphoribosyltransferase (QPRT).

Lesioned Area	Area of Analysis	3HAO (% of control)	QPRT (% of control)
Striatum (IBO)	Striatum	439 ± 44**	227 ± 38**
"	S. Nigra	188 ± 8**	142 ± 20*
S. Nigra (6-OHDA)	S. Nigra	101 ± 3	not done
"	Striatum	207 ± 13**	131 ± 12**
Cortex (ablation)	Striatum	215 ± 17**	244 ± 20**

Data are the mean ± SEM. N=5-6/group. **P<0.01, *P<0.05 vs controls;

These data demonstrate a pronounced increase of QUIN metabolism in response to both cell body and axon terminal degeneration. Preliminary results from immunohistochemical and developmental studies indicate that this phenomenon may not be related to the proliferation of reactive glial cells. Supported by grant NS 20509 and a grant from the American Parkinson's Disease Association.

243.4 BIOCHEMICAL AND BEHAVIORAL EFFECTS OF ADENOSINE DEAMINASE INHIBITION BY DEOXYCOFORMYCIN. J.L. Lewis*, V.S. Westerberg*, J.I. Nagy & J.D. Geiger. Dept. of Pharmacol., Univ. of Manitoba Fac. of Med., Winnipeg, MB. R3E 0W3.

Adenosine deaminase (ADA), a major degradative enzyme for adenosine, is associated with specific neural systems in rat CNS as demonstrated immunohistochemically (Nagy et al., Science, 224: 166, 1984) and biochemically (Geiger and Nagy, J. Neurosci. 1986, in press). If a functional relationship exists between ADA activity and adenosine, then enzyme inhibition may affect responses thought to be regulated through adenosine. Therefore, administration of 2'-deoxycoformycin (DCF), a potent inhibitor of ADA, may help to elaborate the functional significance of this enzyme.

DCF was administered at 3 dosage levels (0.5,1, and 5 mg/kg ip). The decrease and time-course of recovery of ADA activity were examined in whole brain and intestine of rats killed 2 hrs, 24 hrs, and 4, 12, 16, and 20 days after DCF injection. In both tissues, 5 mg/kg was the most effective dose with maximum decrease occurring at 2 hours (8% of control activity in intestine, no detectable levels in any brain samples). Intestinal ADA activity was 75% of control values by 4 days and returned to control levels by 12 days. Brain activity, however, was 17% by 4 days, and only 41% of control values at 20 days.

To examine behavioral effects, we administered DCF (5 mg/kg, ip) to rats acclimated for 8 days in cages with free access to activity wheels. Running behavior was then monitored for the next four days and compared to a matched group of rats receiving only vehicle. No significant change in daily running was seen. One-half of the rats in each group were then challenged with adenosine (30 mg/kg, ip). Rats given adenosine exhibited locomotor depression and this response was potentiated in rats previously treated with DCF. A separate acute activity experiment has suggested that this potentiation was due to a prolongation of the duration of action of adenosine in ADA-inhibited rats. Additional studies are ongoing to assess the effect of ADA inhibition on nociception and temperature regulation.

JLL is a Student of and JDG and JIN are Scholars of the Medical Research Council of Canada (MRCC). VSW is a Fellow of St. Boniface Res. Fdn. This work was supported by MRCC.

243.5 INTRINSIC CHEMOSENSITIVITY OF RABBIT SUPERIOR CERVICAL GANGLIA. B. Dinger, G. Hanson A. Obeso,* C. Gonzalez and S. Fidone (SPON: C. Eyzaguirre). Dept. Physiology, School of Medicine, Univ. of Utah, Salt Lake City, UT 84112.

The sympathetic nervous system controls the functional state of diverse neuroeffector organs in the periphery and thereby determines essential homeostatic and behavioral responses. Classical views hold that postganglionic sympathetic activity is dominated by outflow from central preganglionic axons. In the present investigation, we have tested the hypothesis that sympathetic ganglia contain intrinsic mechanisms which respond to physiological stimuli independent of central outflow.

The preganglionic trunk of the superior cervical ganglion (SCG) was sectioned unilaterally in adult rabbits. Fourteen days following surgery, the animals were exposed in a flow chamber to either air or 5% O_2 in N_2 for two 30 min periods, with 20 min interim (our previous studies showed that hypoxia reduced SP levels in SCG of unoperated rabbits; Hanson et al., J. Neurobiol. 17: 51-54, 1986). Selected tissues were surgically removed for RIA determination of substance P (SP). In separate experiments, the glucose consumption of normal and decentralized rabbit SCG was determined in vitro under normoxic (100% O_2-equilibrated media) or hypoxic (20% O_2 in N_2 media) conditions with ^{3}H-2-deoxyglucose (^{3}H-2DG, 2 μM).

SP levels were unaffected by decentralization, but hypoxia reduced SP by 36% ($p<0.05$) in normal, and 41% ($p<0.025$) in operated ganglia. Comparative studies of nodose ganglia and corpus striata showed that the SP content of these tissues was unchanged in hypoxic animals. Confirmation of SCG intrinsic sensitivity to hypoxia was obtained in preliminary experiments in vitro where the levels of SP and a related tachykinin, substance K, were reduced following two 20 min exposures to low O_2 superfusion media. Also, exposure for 5 min to low O_2 superfusion media approximately doubled the rate of ^{3}H-2DG uptake by normal SCG ($p<0.001$), and this effect was still present 48 hrs following decentralization. In contrast, ^{3}H-2DG uptake by nodose ganglia was unaffected by hypoxia.

The low O_2-induced reductions in SP and increase in glucose consumption in the SCG parallel changes in peptide content and intermediary metabolism following low O_2 exposure of the carotid body, a well recognized chemosensitive organ (Hanson et al., Soc. Neurosci. Abst. 9: 143, 1983). Thus, both our in vivo and in vitro studies suggest that internal mechanisms in the SCG are uniquely sensitive to hypoxia and can respond to physiological stimuli independent of the CNS or circulating hormones.

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243.6 SUBSTRATE SPECIFICITY OF RABBIT BRAIN CHOLINE KINASE. R.W. Guynn, C.-F. Tsai* and D. K. Merrill*. Department of Psychiatry and Behavioral Sciences, University of Texas Medical School, Houston, TX. 77225.

Choline kinase is a widely distributed and relatively active enzyme in brain. The enzyme competes with the cholinergic system for available choline and confounds both the understanding of the dynamics of that system in the whole animal and the development of choline analogues which might impact it selectively. Very little is known about the substrate specificity of choline kinase from any source let alone brain. Therefore we have undertaken a study to begin to supply the information.

Rabbit brain choline kinase was prepared from rabbit brains using a modification of an affinity media suggested by Brophy and Vance (FEBS Lett. 62:123-125, 1976). The enzyme was assayed spectrophotometrically at pH 7.5 using pyruvate dehydrogenase and lactate dehydrogenase as indicator enzymes. The relative rates of the reaction with the analogues was compared to choline at relatively high substrate concentrations (10 mM).

Rather surprisingly, the specificity of the brain enzyme is qualitatively different from that of the enzyme from yeast, also studied for comparison. Unexpectedly, the brain enzyme, in fact, shows less specificity. For example, whereas successive replacement of the methyl groups of choline with ethyl groups results in decreasing substrate activity with the yeast enzyme the same modification results in increasing activity with the brain enzyme. N,N,N-triethylethanolamine is 37% as active as choline with yeast choline kinase but 292% as active as choline with the brain enzyme. The brain enzyme also shows more tolerance for single long chain alkyl substitutions. For example, N-octyl-N,N-dimethylethanolamine still retains 17% of the activity of choline with brain choline kinase but is non-reactive with the yeast enzyme. Likewise cyclic analogues of choline such as 3-hydroxy-1,1-dimethylpiperidine and 4-hydroxy-1,1-dimethylpiperidine, which show little or no reactivity with yeast choline kinase, give rates 99% and 264% of that of choline, respectively, with the brain enzyme. Even the caged structure N-methyl-3-quinuclidinol is partially (33%) reactive with the latter preparation.

The commonality between the two enzymes, however, is the non-reactivity of analogues carrying aromatic rings attached to the nitrogen atom such as N-benzyl-N,N-dimethylethanolamine and N-(2-naphthyl)-N,N-dimethylethanolamine or with any of the dicholine analogues tested [of the general structure $HOCH_2CH_2N(CH_3)-_2(CH_2)_nN(CH_3)_2CH_2CH_2OH$ where n = 2-14]. It is planned in subsequent work to compare the specificities of brain choline kinase with that of choline acetyltransferase from the same tissue looking for analogue structures having relative specificity for either system. (The work is supported by NIMH grant R01 MH39603).

243.7 DETERGENT RELEASED CHOLINE ACETYLTRANSFERASE FROM MEMBRANE FRACTIONS OF RAT AND HUMAN BRAIN. G. Bruce*, C. White III*, and L.B. Hersh (SPON: M.R. Morrison). Departments of Biochemistry and Pathology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Subcellular fractionation was performed on rat and human brain tissue. Following the extraction of soluble choline acetyltransferase (ChAT) the resultant membrane fraction was subjected to hypo-osmotic shock and subsequently washed in high and low salt buffers. The ChAT activity associated with the washed pellet fraction was small in relation to the total soluble activity recovered in the first supernatant and the washes (3.7% for rat and 5.2% for human). However the addition of 1% triton X-100 to this pellet fraction resulted in the significant increase in ChAT activity (4.9-fold for rat and 8.7-fold for human). The triton released ChAT and the soluble ChAT from both species were efficiently purified by immuno-affinity chromatography. The molecular weights of these purified forms of ChAT were determined by SDS-PAGE followed by immunoblotting. Three different anti-ChAT antibodies all revealed an identical single molecular weight of 68K for the soluble and triton released ChAT from both species. A more detailed comparison of these two enzyme forms from human brain was carried out by epitope mapping. An identical pattern of immunologically reactive peptides were produced from a limited proteolytic digestion of both soluble and triton released ChAT from human brain.

As it was possible that the triton released ChAT might simply represent occluded soluble enzyme the distribution of the cytosolic enzyme lactate dehydrogenase (LDH) was also studied during the above subcellular fractionations. As with ChAT most LDH activity was recovered in the first supernatant. The LDH activity associated with the washed pellet fraction was small in relation to that recovered in the first supernatant and the washes (7.0% for rat and 5.9% for human). The addition of 1% triton X-100 to this pellet fraction resulted in an increase in LDH activity (5.9-fold for rat and 6.7-fold for human) comparable to that observed for ChAT. This action of triton X-100 is consistent with it disrupting membrane vesicles and releasing occluded soluble enzymes.

It is concluded that triton released ChAT from rat and human brain membrane fractions is identical to soluble ChAT and is so because it simply represents occluded soluble ChAT as opposed to a distinct membrane form of the enzyme.

243.8 MOLECULAR FORMS OF ACETYLCHOLINESTERASE: BRAIN REGIONAL DIFFERENCES IN SENSITIVITY TO PHYSOSTIGMINE. F.P.Huger, G. Robertello* and W. Petko* Dept. of Biochemistry, Hoechst-Roussel Pharmaceuticals Inc. Somerville, NJ 08876

Acetylcholinesterase (AChE, EC 3.1.1.7) from rat striatum and frontal cortex was separated into two major molecular forms (10S and 4S) by continuous sucrose gradient centrifugation after solubilization in 10 mM phosphate buffer, pH 7.1, containing 1 M NaCl and 1.0% Triton X-100. Under these conditions, the 10S and 4S forms constituted approximately 80% and 10%, respectively, of the total cholinesterase activity of the fractions, with no real differences in relative amounts between brain regions. Current literature suggests that the 10S form may be developmentally and functionally more important.

Enzyme inhibition studies and kinetic analysis were done using 4S and 10S AChE derived from cerebro-cortical and striatal homogenates to investigate possible brain regional differences. Physostigmine was significantly more potent as an inhibitor of both 4S and 10S AChE from striatum than from cortex, whereas, BW-284C51 and tacrine showed only slight differences. No significant differences in inhibition of the 10S and 4S forms from the same region were found. The butyrylcholinesterase inhibitor, iso-OMPA did not inhibit activity and likewise, no enzyme activity could be detected using 5 mM butyrylthiocholine as a substrate, indicating measurement of true cholinesterase activity.

Enzyme inhibition studies were then done in various preparations of striatal and cortical tissue, to determine whether differences in sensitivity to physostigmine existed at all steps in the resolution of the molecular forms. These experiments revealed that physostigmine was somewhat more potent in all preparations of striatal AChE than cortical AChE, but BW-284C51 and tacrine again showed no brain regional selectivity.

243.9 A NEURONAL CELL CULTURE AS A MODEL SYSTEM TO STUDY ACETYLCHOLINESTERASE. R. Ray, F.M. Cowan* and O.E. Clark*. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

Cholinesterase (ChE) activity in the clonal neuroblastoma-glioma hybrid NG108-15 cell line was studied, and the results were compared with those observed in the mammalian brain in other laboratories. The ChE activity of NG108-15 cells is almost totally (>90%) inhibited by the specific acetylcholinesterase (AChE) inhibitor BW284C51 (IC_{50} = $3x10^{-8}$M), indicating that the enzyme activity is predominantly AChE as it is reported to be in the brain. Specific AChE activity (nmoles ACh hydrolyzed/min/mg protein) increases dramatically in NG108-15 cells, differentiated with 1 mM dibutyryl cAMP (Bt_2cAMP), as is also reported in the developing brain. Sucrose density gradient sedimentation analysis was employed to characterize the molecular forms of AChE in NG108-15 cells with respect to their developmental changes, subcellular localization, and release into the culture medium. Two molecular species of AChE with sedimentation coefficients of approximately 5S and 11S, comparable to brain AChE molecular forms, are present in NG108-15 cells. During development of NG108-15 cells in culture in the presence of 1 mM Bt_2cAMP, the relative proportions of the 5S and the 11S AChE molecular forms change from a high 5S/low 11S (80% 5S/20% 11S) in a 3 day old poorly differentiated culture to the converse (45% 5S/55% 11S) in a 13 day old highly differentiated culture, similar to the changes reported in the developing rat brain. As in the brain, the 11S AChE in NG108-15 cells is mostly membrane-bound, and secreted extracellularly. Based on these similarities of AChE in NG108-15 cells and in the brain, the NG108-15 cells may be used as a potential neuronal model system to study AChE with respect to biosynthesis, polymorphism, properties, and effects of chemical agents that interfere with these processes.

243.10 USE OF A RADIOMETRIC ASSAY FOR BUTYRYLCHOLINESTERASE TO CHARACTERIZE THE ENZYME FROM HUMAN CSF. James B. Rand and F. Jacob Huff, Dept of Zoology, U. of Wisconsin, Madison, WI 53706 and Alzheimer's Disease Research Center, U. of Pittsburgh, Pittsburgh, PA 15213

We have developed a single-vial biphasic extraction assay for butyrylcholinesterase (BuChE) which is based on the acetylcholinesterase assay of Johnson and Russell (Analyt. Biochem. 64:229). The procedure involves incubation of the enzyme with H^3-butyrylcholine and buffer in a 5-ml glass vial. The reaction is terminated by the addition of concentrated buffer at pH 3 and organic scintillation fluid containing 10% isoamyl alcohol. The vial is then capped and shaken, forming a two-phase system. At pH 3, the tritiated butyrate (formed by enzymatic hydrolysis of the substrate) is fully protonated, and is efficiently extracted into the organic phase, where it is counted. Unreacted butyrylcholine, being charged, remains in the aqueous phase, where the weak beta-decays cannot reach the fluors in the organic phase.

Under comparable assay conditions, measurements of BuChE activity using the radiometric method are directly comparable to spectrophotometric assays using Ellman's protocol (Biochem. Pharmacol. 7:88). However, the radiometric method becomes much more sensitive at very low substrate concentrations: under such conditions, 1 µl of a human CSF sample provides a signal that is as much as 5-fold above blank (30-min assay at room temperature).

Because of the nonlinear cooperative kinetics observed with BuChE (Augustinsson et al., Biochem. J. 141:825), as well as the large effects of salt concentration on kinetic parameters, measurements of BuChE activity using any method may be subject to considerable variability due to differences in buffer, substrate and salt concentration. To avoid this problem, we extrapolate from the linear (low-substrate) portion of an Eadie-Hofstee plot to define the "equivalent V_{max}" for the enzyme.

We have observed that human BuChE is relatively inefficient at utilizing acetylcholine as a substrate. This property of the enzyme, which is accentuated at low substrate concentrations, has allowed us to determine AChE and BuChE activities independently in CSF, without requiring the use of specific inhibitors. We have also observed considerable differences between human BuChE (from either serum or CSF) and BuChE from horse serum, with respect to pH optima, kinetic parameters, and effects of inorganic ions. We therefore conclude that the horse serum enzyme may not always be an appropriate control for neurochemical and pharmacological studies of human BuChE.

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243.11 ULTRASTRUCTURAL LOCALIZATION OF PSEUDOCHOLINESTERASE TO BRAIN SYNAPSES. M. C. Bundman and C. Gorenstein. Department of Pharmacology, University of California, Irvine, CA 92717.

We have previously shown that at the light microscope level pseudocholinesterase (E.C. 3.1.1.8) was localized in neuronal cell bodies as well as the neuropil. Electron microscopic studies of the areas demonstrating perikaryal staining showed that pseudocholinesterase was exclusively localized in the nuclear envelope and the granular endoplasmic reticulum. The present study was undertaken to study the localization of pseudocholinesterase in areas where neuropil staining was prominent.

The histochemical localization of pseudocholinesterase was studied in the rat *anterior ventral nucleus of the* thalamus (AV) at the electron microscope level. We employed the ionic fixation technique described by Tsuji and Fournier (*Histochemistry*, 80:19, 1984). Propionylthiocholine was used as the substrate and produced a fine, electron dense precipitate at the sites of enzymatic activity. Acetylcholinesterase activity was inhibited with 10^{-4}M BW284c51 ; control reaction mixtures also contained 10^{-4}M iso-OMPA.

Pseudocholinesterase activity was observed in neuronal perikarya within the nuclear envelope and in the cisternae of the granular endoplasmic reticulum. Other organelles in the perikaryal cytoplasm did not contain reaction product.

Examination of the tissue at high magnification revealed that a significant fraction of the reaction product was found in close association with small to medium size synaptic terminals impinging on the perikarya and dendrites of thalamic neurons. In these areas the reaction product could frequently be observed filling the synaptic cleft. The synaptic terminals, filled with spherical vesicles, were free of reaction product. These results indicate that pseudocholinesterase is localized post-synaptically in AV.

To determine the origin of the terminals which form synapses with pseudocholinesterase-containing neurons in AV, we iontophoretically labeled neurons in the cingulate cortex and hippocampus with the biotinylated, anterograde tracer lectin, PHA-L. Terminals in AV labeled with biotin-PHA-L were visualized after treatment with an avidin-colloidal gold complex. Electron microscopic studies revealed populations of terminals double-labeled with both pseudocholinesterase and PHA-L.

The results obtained indicate that in the brain, pseudocholinesterase is intimately associated with synapses and synaptic terminals. The strategic postsynaptic localization of pseudocholinesterase suggests that it participates in the metabolism of neurotransmitter substances.

Supported by NS18994

243.12 CHEMICAL MODIFICATION AND SPECTRAL CHANGES OF CYTOCHROME b561 IN CHROMAFFIN-VESICLE GHOSTS. P.M. Kelley* and D. Njus* (SPON: H. Normile). Dept. of Biological Sciences, Wayne State University, Detroit MI 48202.

Cytochrome b561 is an integral membrane protein found in a variety of secretory vesicles including the adrenal medullary chromaffin vesicles and peptide amidating vesicles in the pituitary. We have suggested that this cytochrome catalyzes electron transport across the membranes of these vesicles. The electron transport is thought to regenerate ascorbic acid which is needed for the synthesis of norepinephrine and for the amidation of neuropeptides. Although many compounds have been tested, none have been found to inhibit the oxidation or reduction of cytochrome b561. We have recently found that chemical modification with acetic anhydride slows the rate of cytochrome reduction, while leaving the extent of reduction unaffected. The involvement of the cytochrome in electron transfer is confirmed by changes in its redox state observed as changes in the absorption spectrum. In ascorbate-loaded chromaffin-vesicle ghosts, cytochrome b561 is nearly completely reduced and exhibits an absorption maximum at 561 nm. When ferricyanide is added to a suspension of these ghosts, the cytochrome becomes oxidized as indicated by the loss of the absorption peak at 561 nm. If a small amount of ferricyanide is added, it becomes completely reduced by electron transfer from intravesicular ascorbate and cytochrome b561 returns to its reduced state. If an excess of ferricyanide is added, the intravesicular ascorbate is exhausted and the cytochrome remains oxidized. The kinetic spectrum of these absorbance changes correlates with the difference spectrum (reduced-oxidized) of cytochrome b561. These results indicate that cytochrome b561 can transfer electrons across the chromaffin-vesicle membrane. Cytochrome b561 becomes transiently oxidized when ascorbate oxidase is added to a suspension of ascorbate-loaded ghosts. Since dehydroascorbate does not cause any changes in the spectrum of cytochrome b561, it is likely that this spectral change is caused when ascorbate, available in the external medium, is oxidized to semidehydroascorbate by ascorbate oxidase. This suggests that cytochrome b561 can reduce semidehydroascorbate and supports our hypothesis that the function of cytochrome b561 in vivo is to transfer electrons into chromaffin vesicles to reduce semidehydroascorbate and regenerate intravesicular ascorbate. This work was supported by NIH grant GM 30500.

243.13 EFFECT OF ANTIDEPRESSANTS ON RAT BRAIN PHOSPHATIDYLINOSITOL SYNTHETASE ACTIVITY. P.P. Li, J.J. Warsh and N.Z. Stanacev*, Dept. of Clinical Biochemistry & Psychiatry, University of Toronto, Toronto, Ontario, Canada.

Considerable evidence indicates that phosphatidylinositol (PI) plays an important role in a variety of membrane-mediated functions. PI is synthesized de novo from myo-inositol and CDP-diglyceride by the enzyme PI synthetase. However, myo-inositol can also be incorporated into PI in the absence of CDP-diglyceride catalyzed by a PI:myo-inositol exchange enzyme. Recent findings indicate that the metabolism of PI may be altered by antidepressant drug treatment in rat brain (J. Neurochem. 44:401, 1985). The present study examined the in vitro effect of antidepressants on PI synthetase and PI:myo-inositol exchange enzymes in rat brain subcellular membranes. PI synthetase activity was determined by the incorporation of ^{3}H-myo-inositol into PI in the presence of CDP-dipalmatin and Mn^{++}. The activity of PI exchange enzyme was assayed in the same manner as PI synthetase except that CDP-dipalmatin was omitted.

Preliminary results indicated that bovine serum albumin (5% w/v) and Triton X-100 (0.1-0.3%) significantly inhibited PI synthetase activity. In subsequent studies, intact subcellular membranes without detergent were employed. In rat brain subcellular fractions, PI synthetase occurred exclusively in the microsomal fractions. Moreover, the activity of CDP-diglyceride independent PI:myo-inositol exchange enzyme was low (3%) in comparison with PI synthetase activity in the microsomal fractions. Addition of 1 mM of desipramine, amitriptyline, imipramine, iprindole, clomipramine and mianserin significantly inhibited rat brain microsomal PI synthetase activity (-30 to 65%), whereas the same concentration of lithium, zimelidine and fluoxetine had no effect. None of the above drugs were effective in inhibiting the PI synthetase activity at concentrations less than 1 mM.

The mode of inhibitory action of these drugs on PI synthetase activity was studied by varying concentrations of CDP-dipalmatin in the presence and absence of imipramine. At low CDP-dipalmatin concentrations (0.1-0.2 mM), PI synthetase activity was stimulated by imipramine, whereas the enzyme activity was inhibited at higher liponucleotide concentrations (0.3-1.0 mM). In contrast, imipramine had no effect on the PI:myo-inositol exchange enzyme. These observations suggest a complex interaction occurs between the liponucleotide and imipramine.

The above results suggest that in vitro synthesis of PI is inhibited by some antidepressant drugs. However, in view of the high concentration of the antidepressants required, the significance of this inhibitory action with respect to their therapeutic effect would be doubtful.

P.P. Li is an OMHF Research Fellow.

243.14 DECREASED AUDIOGENIC SEIZURE ACTIVITY FOLLOWING SUBCUTANEOUS ZINC INJECTIONS. G.A. Howell, J. Morton*, D. Doninni*, C.J. Frederickson, Lab for Neurobiology, University of Texas at Dallas, Richardson, TX 75080.

A role of zinc in epileptiform disorders has been repeatedly suggested. For instance, Chung & Johnson (Proc. R. Soc. Lond, 221:145,1984) have shown that intracerebral injections of ZnCl (plus IP pyridoxal-5-phosphate) increase audiogenic seizure susceptibility in mice. In contrast, Sterman (Kindling III, ed. Wada, 1985) has shown that dietary zinc deprivation increases susceptibility to kindled seizures while dietary zinc loading decreases susceptibility in cats.

We now report that subcutaneous injections of ZnCl (100 mg/kg) in 25- 28 day old Jackson DBA/2J audiogenic seizure prone mice 15 min prior to noise exposure (100, 110, 115 dB) causes substantial decreases (P .05-.0001) in the frequency of (1) running fits,(40% decrease), (2) clonic seizures (83% decrease), (3) tonic seizures (81% decrease), and (4) a 100% decline in deaths. There was 33% mortality among controls (given equimolar Cl as NaCl) and zero mortality among experimentals. Lower doses of ZnCl (20 mg/kg) produced similar but smaller declines whereas 200 mg/kg produced essentially the same results as the 100 mg dose. In all experiments the mice were run in parallel sets with the observers blind to the drug condition.

Prior research from this laboratory (Howell et al, Nature 308:736, 1984) has shown increased turnover of zinc in hippocampal slices during electrical stimulation. Therefore it might be expected that zinc utilization would be augmented during seizures. In fact, depletion of brain zinc has been found immediately after prolonged seizure activity (Sloviter, Brain Res 330:150, 1984) whereas an increase in zinc has been found one day after cessation of seizure activity (Kasarskis et al. Epilepsia 26:513, 1985) suggesting a compensatory increase in zinc uptake. Furthermore, DBA/2J mice have been shown to have a slightly higher zinc content in the whole brain (2.8%), and in the hippocampus (8.3%; Chung op cit). Perhaps this elevation of CNS zinc in previously-seized and seizure prone animals is due to increased utilization of zinc during seizure activity. The present findings suggest that such zinc utilization may actually impede seizure development.

243.15 DEPLETION OF HIPPOCAMPAL ZINC DURING SEIZURES: APPARENT POST-ICTAL REPLETION. J.D. Morton*, J.F. McGinty, M.D. Hernandez*, A.J. Cooper*, and C.J. Frederickson, Dept. Anat., E. Carolina Med. Sch. Greenville, NC 27834 and Lab. for Neurobiol. Univ. Texas at Dallas, Richardson, TX 75080.

Prior studies of mossy fiber (MF) zinc changes during seizures have produced mixed results. Sloviter, using a post mortem zinc stain, found that MF zinc was depleted after stimulation-induced seizures (Brain Res. 330:150 1984), but Frederickson et al., using intravital zinc staining, found a modest rise in MF zinc after kainic acid (KA) seizures (Epilepsia 26:512, 1985).

We first tested whether the method of zinc staining might account for the discrepancy between prior results. We repeated the experiment of Frederickson et al., but used post mortem, instead of intravital staining. Rats were given KA (10 mg/kg, s.c.), allowed to seize for three hours, then brains were cryotomed and stained for zinc by quinoline fluorescence (Frederickson et al., J. Neurosci. Meth. -submitted). Compared to controls, the KA-treated rats had clearly reduced staining: CA3a and CA3b were nearly devoid of zinc fluorescence. Thus, with Sloviter, we find a loss of MF zinc after seizures.

We now seek the mechanism by which intravital staining can reverse the loss of zinc which accompanies KA seizures. We noted that giving the intravital stain (dithizone) stops behavioral seizures, and thus imposes a 17 min seizure-free period (the staining period) prior to sacrifice. Accordingly, we have treated rats with KA, allowed them to seize for 2-3 hrs, then given anticonvulsants (diazepam, pentobarbital), sacrificing the rats after brief, seizure-free "recovery" periods. Like dithizone-treated animals, these animals show no net depletion of MF zinc at sacrifice. These results suggest that the MF zinc which is lost during seizures may be rapidly repleted during post-ictal recovery.

Parallel immunocytochemical studies of opioid peptides indicate that both dynorphin and enkephalin are also lost from the MF during KA seizures; enkephalin appears to be lost more rapidly than dynorphin. (Supported by DA 03982 to JFM and NIH-BSRG 807-RR07133 to CJF.)

244.1 TIME COURSE OF PHYSOSTIGMINE AND ITS METABOLISM AND ChE ACTIVITY IN PLASMA OF DOG AFTER I.V. ADMINISTRATION. M. McIlhany*, M. Downen, M. Hallak*, A. Khalique*, S. Somani* and E. Giacobini. Depts. Pharmacol. & Surgery, Southern Illinois University School of Medicine, Springfield, IL 62708

Administration of physostigmine (Phy), a tertiary amine carbamate anticholinesterase drug, appears to temporarily improve memory function in Alzheimer patients. This study reports the time course of Phy concentration (conc) and metabolism and cholinesterase (ChE) and acetylcholinesterase (AChE) activity in plasma and different brain regions of beagle dogs after i.v. administration. ^{3}H-Physostigmine (100 ug/kg) was administered to three beagle dogs into the cephalic vein. Blood samples were collected at various time intervals (1, 2, 3, 5, 7, 10, 15, 30 and 45 min) and plasma was immediately separated by centrifugation. Following the last blood sample (45 min), the dogs were sacrificed and the brain was removed from the skull and dissected into seven regions. ^{3}H-Physostigmine and its metabolites (eseroline, M_1 and M_2) concentrations were determined by high performance liquid chromatography (Somani and Khalique, J. Ann. Toxicol., 9:71-75, 1985; Fund. Appl. Toxicol., 6:327-334, 1986). Cholinesterase and AChE activities were measured in plasma and various brain regions. Semilogarithmic plot of plasma Phy concentration versus time showed a biphasic decline. The mean half-life of the distribution phase $t_{1/2(\alpha)}$ and the elimination phase $t_{1/2(\beta)}$ were 3.5 and 23.0 min, respectively. The elimination rate constants were $Ke_{(\alpha)}$ .23 min^{-1} and $Ke_{(\beta)}$ .032 min^{-1}. The mean volume of distribution (Vd) and clearance (Cl) were 1720 $ml \cdot kg^{-1}$ and 54.11 $ml \cdot kg^{-1} min^{-1}$. The time course of ^{3}H-Phy metabolism in plasma indicates that Phy is rapidly metabolized to eseroline and to two other unidentified metabolites, M_1 and M_2. Radioactivity (RA) due to M_1 and M_2 consistently increased from 2 to 52% and 2 to 26% respectively in plasma from 2 to 45 min. Eseroline accounted for 5-6% of the total RA for up to 15 min then declined to 3.5% of the total RA at 45 min. Cholinesterase activity in plasma decreased to a minimum of 24% of baseline at 2 min, then slowly increased up to 41% at 45 min corresponding to high (124 ng/ml) and low (16 ng/ml) conc of Phy. By plotting percent ChE inhibition versus time, the rate of recovery of ChE activity in plasma was found to be .0059 min^{-1}. Half-time for recovery of ChE in plasma was 118 min compared to half-life [$t_{1/2(\beta)}$ 23 min] of Phy. Acetylcholinesterase acitvity at 70 + 5 min was not significantly different from controls in any of the brain regions examined. However, Phy conc at this time was maximal in the HC (28) followed by CX (24), CxT (22), Cer (16), Md (13), St (13) ng/g. These results indicate that the half-time for recovery of ChE in plasma is longer than the half-life of Phy in plasma. (Supported in part by the S.I.U. Alzheimer Research Fund)

244.2 EFFECTS OF O-CRESYLBENZODIOXAPHOSPHORIN OXIDE (CBDP) PRETREATMENT ON SOMAN-INDUCED CHOLINESTERASE INHIBITION IN THE RAT CENTRAL NERVOUS SYSTEM. V.R. Jimmerson, T.-M. Shih, D.M. Maxwell,* T. Koviak,* S. Harris,* K. Henry,* and Y. Hodge.* Basic Pharmacology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

Pretreatment with CBDP, the cyclic metabolite of tri-o-cresylphosphate, has been shown to potentiate the lethality of certain organophosphorus cholinesterase (ChE) inhibitors, including soman (GD). In rats receiving GD alone, the LD50 was 110 ug/kg s.c., whereas with CBDP it was 13 ug/kg s.c. This potentiation is due to CBDP's preferential and irreversible binding to, and inhibition of, carboxylesterase (CaE) "detoxification" sites for GD, which presumably increases GD's availability to the more critical ChE sites (Toxicol Appl Pharmacol, 20:474-479, 1971). In this study, CBDP was used to block CaE sites, and GD-inhibited ChE's were measured. Male rats (250-300g) were divided into 2 groups: group 1 received CBDP (16 mg/kg, s.c.) 60 min prior to s.c. injection of saline, 0.3 LD50 (3.9 ug/kg) or 0.6 LD50 (7.8 ug/kg) of GD; group 2 received vehicle (VEH: 10% ethanolic propylene glycol) 60 min prior to injection of saline, 0.3 LD50 (33 ug/kg) or 0.6 LD50 (66 ug/kg) of GD. Thirty min after the injection of saline or GD, brains were obtained and dissected into brainstem, cortex, hippocampus, midbrain, cerebellum, striatum and spinal cord for ChE assay. VEH treatment alone did not affect brain regional ChE activity, whereas CBDP treatment alone inhibited ChE ranging from 28-41%, depending on brain regions examined. In CBDP-pretreated rats, each increment of 0.3 LD50 of GD (3.9 ug/kg) inhibited ChE by an average of 24%. In VEH-pretreated rats, increments of 0.3 LD50 of GD (33 ug/kg) inhibited ChE by an average of 16%. The calculated GD dose ratio of CBDP- to VEH-pretreated rats for equivalent brain ChE inhibition averaged 1:5.6. The data indicate that after CBDP pretreatment 82% less GD is required to induce the same extent of ChE inhibition in the central nervous system. Our data are consistent with the assumption that CBDP makes CaE sites unavailable to GD, thereby allowing GD to reach critical ChE sites, and potentiating lethality at lower GD doses.

244.3 RELATIONSHIP BETWEEN SOMAN-INDUCED ELEVATION OF BRAIN REGIONAL ACETYLCHOLINE CONCENTRATIONS AND LETHALITY. T.-M. Shih, S. Harris*, K. Henry*, T. Koviak* and V.R. Jimmerson. Basic Pharmacology Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

Organophosphorus cholinesterase (ChE) inhibitors such as soman (GD) can cause a progression of signs including convulsions and death. This is due presumably to ChE inhibition and the subsequent increase in acetylcholine concentration (ACh) in the central nervous system. However, the time course of increased brain ACh and its relationship to GD-induced lethality is unclear. We have previously reported that, in rats, HI-6 (125 mg/kg, ip), supposedly a peripherally acting reactivator of GD-inhibited ChE, delayed the time to death and provided a protective ratio of 2.5 (Soc. Neurosci. Abst. 10: 1184, 1984) when injected immediately after GD. Therefore, we utilized this compound in the present study to increase animal survivability in order to examine the time course of brain ACh accumulation. In an initial study, male rats (250-300 grams) were treated with a lethal dose (1.8 LD_{50}; 200 ug/kg, sc) of GD. The "time to onset" of convulsions and "time to death" averaged 1.97 min and 10.1 min respectively. In subsequent studies, rats were divided into two groups for ACh assay. Group 1 received GD alone, while group 2 received GD immediately followed by HI-6 (125 mg/kg, i.p.). All animals were killed by microwave irradiation at 1, 2, 3, 5, 9, or 15 min (Group 2 only) after GD. Brains were dissected into brainstem (B), cortex (C), hippocampus (H), midbrain (M), cerebellum (R) and striatum (S), and ACh was determined by a gas chromatograph/mass spectrometric method. In rats treated with GD alone, ACh progressively increased in all brain areas and at 9 min post-GD reached 5.5, 65.0, 45.5, 14.5, 14.0 and 12.5% above control in B, C, H, M, R, and S respectively. Treatment with HI-6 resulted in a time course of ACh elevation similar to that obtained with GD alone during the first 9 min in each brain region. The "time to onset" of convulsions was identical in both groups, indicating that HI-6 did not prevent GD-induced convulsions. Furthermore, treatment with HI-6 resulted in all animals surviving at least 15 min, at which time ACh was elevated in B, C, H, M, R, S, by 12.5, 98.5, 55.0, 35.5, 30.5 and 45.5% above control respectively. These data showed that in surviving HI-6 treated rats, ACh was higher than in rats receiving GD alone. Thus, elevated central ACh does not correlate with the lethality resulting from GD poisoning.

244.4 RELEASE OF ENDOGENOUS AND RADIOLABELLED ACETYLCHOLINE BY 3,4-DIAMINOPYRIDINE OR POTASSIUM FROM QUINOLINIC ACID INJECTED STRIATUM. S.R. El-Defrawy*, K. Jhamandas, R.J. Boegman, R.J. Beninger, and M. Bailik*. (SPON: J.V. Milligan) Departments of Pharmacology and Toxicology, and Psychology+, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Aminopyridines are known to increase presynaptic release of acetylcholine (ACh) evoked by nerve impulses in peripheral tissue. In the CNS, 4-aminopyridine has been found to induce endogenous ACh release from striatal slices and to support cortical ACh release under pharmacological conditions known to depress this release. The present experiments were designed to determine whether aminopyridines can support the release of ACh from brain tissue that is partially deficient in cholinergic neurons. We investigated the action of 3,4-diaminopyridine (DAP) and high potassium on endogenous and radiolabelled ACh release from normal and quinolinic acid (QUIN) lesioned striata. Rats received a unilateral injection of quinolinic acid (100 nmoles) into the striatum. Seven days post-injection, striatal slices were prepared from the uninjected (control) and injected hemisphere. The spontaneous and evoked release of ACh was measured using the brain slice superfusion technique. Release was evoked by exposing the tissue to 35 mM potassium or 1 mM DAP for 5 or 10 minutes. Endogenous ACh was measured by gas chromatography-mass spectrometry.

In the endogenous release experiments, 6.38 ± 0.52 nmoles ACh/g wet wt was released by DAP from the control side and 3.29 ± 0.26 nmoles ACh/g wet wt from the QUIN injected side. Potassium-induced release of endogenous ACh was comparable to that produced by DAP. The reduction in ACh release paralleled the decrease in choline acetyltransferase activity observed in the damaged striatum (176.55 ± 12.51 nmoles/mg protein/hr) when compared to control (375.18 ± 32.53 nmoles/mg protein/hr).

In radiolabelled release experiments, DAP induced a 10.24 ± 0.81 total percent release of ^{3}H-ACh over baseline from control striatum and 8.16 ± 0.90 from the lesioned tissue. In contrast, potassium induced a 13.79 ± 0.63 total percent release of ^{3}H-ACh over baseline from the control side and this value was reduced to 6.91 ± 1.48 on the lesioned side.

These results show that DAP can induce ACh release from striatum that is deficient in cholinergic neurons. In addition, DAP appears to have a differential effect on the release of ACh synthesized from radiolabelled choline and the release of endogenous ACh.

(Supported by the Ontario Mental Health Foundation and the Canadian Geriatrics Research Society)

244.5 MODULATION OF ACETYLCHOLINE SYNTHESIS IN RAT HIPPOCAMPAL SLICES BY VASOACTIVE INTESTINAL PEPTIDE. P.A. Lapchak and B. Collier. Dept of Pharmacol. & Ther., McGill University, McIntyre Medical Bldg., Montreal, Quebec, Canada, H3G 1Y6.

Vasoactive intestinal peptide(VIP) has been suggested to be a modulator of cholinergic function in both the central and peripheral nervous systems. VIP and choline acetyltransferase (ChAT) have been reported to coexist in the hippocampal regio superior. VIP activates choline acetyltransferase (ChAT) in hippocampal homogenates. Then we confirmed that VIP can increase ChAT activity in homogenates of hippocampal tissue, and the present study was undertaken to determine whether VIP has an effect on cholinergic function in intact preparations of rat hippocampus.

First, we studied the effect of varying concentrations of VIP on the amount of ^{3}H-acetylcholine(ACh) synthesized from ^{3}H-choline in hippocampal slices using a 10 min preincubation period. When slices were incubated in normal medium, VIP enhanced ^{3}H-ACh synthesis maximally at 10^{-8} M VIP(43.0 $\pm$ 8.1 %, n=7); increasing the concentration of VIP to 10^{-6} M decreased ^{3}H-ACh production to 82.6 $\pm$ 6.1 % (n=4) of control values. When slices were incubated in a high K^{+} medium(50 mM), VIP also increased the production of ^{3}H-ACh. At 10^{-6} M VIP there was a 37.0 $\pm$ 12.0 %(n=5) increase in ACh production over the control K^{+} stimulated production. To test whether the enhanced synthesis of ACh is dependent upon increased choline uptake we determined the kinetic parameters for the high affinity choline uptake system using a hippocampal synaptosome preparation. Neither the (K_m) nor the (V_{max}) for choline were altered by incubation with 10^{-8} M VIP. At 1.0 uM choline the control uptake of choline was 152.28 $\pm$ 12.54 umol/gm/hr compared to 188.76 $\pm$ 10.02 umol/gm/hr in the presence of 10^{-8} M VIP (n=3). To test whether the apparent increase in ^{3}H-ACh resulted from altered ACh release, we determined whether VIP(10^{-8}) could alter the basal release of either endogenous ACh or newly synthesized ^{3}H-ACh: there was no significant effect of the peptide on the basal release of ACh from hippocampal slices. The basal release in the presence of VIP was not significantly different from controls (incubated with 200 uM bacitracin). ACh release was 9.26 $\pm$ 0.74 nmol/gm/hr and 10.64 $\pm$ 1.03 nmol/gm/hr in the presence and absence of VIP, respectively.

In conclusion, these results support the existence of a VIP-cholinergic interaction in the hippocampus. Furthermore, the modulation of ACh synthesis by VIP may be mediated by presynaptic VIP receptors on cholinergic nerve terminals and appears to result from changed ChAT activity rather than altered choline delivery or altered ACh release. (Supported by MRC, Canada and FCAR, Quebec.)

244.6 PRESYNAPTIC REGULATION OF ACETYLCHOLINE RELEASE FROM A SYMPATHETIC GANGLION BY ALPHA-ADRENERGIC AND OPIATE RECEPTORS: ROLE OF CALCIUM. D.M. Araujo* and B. Collier. Dept. Pharmacol. & Ther., McGill University, McIntyre Medical Bldg., Montreal, Quebec, Canada, H3G 1Y6.

Presynaptic alpha-adrenoceptors (Araujo and Collier, Eur. J. Pharmacol.: in press) and opiate receptors (Araujo and Collier, Soc. Neurosci. Abst., 15: 574, 1985) can regulate acetylcholine (ACh) release in the cat superior cervical ganglion. This was shown by experiments which tested the effects of norepinephrine (NE) and of met-enkephalin-arg-phe (MEAP), on ACh release from the perfused ganglion: both depressed evoked (Ca^{2+}-dependent) ACh release, but did not affect spontaneous (Ca^{2+}-independent) ACh release. This result suggested that the depression of ACh release from the cat superior cervical ganglion by presynaptic alpha-adrenoceptors and opiate receptors might be mediated by a Ca^{2+}-sensitive mechanism. The present study tested this hypothesis.

First, the Ca^{2+}-dependence of evoked ACh release was measured in the presence and in the absence of either NE (1 nM) or MEAP (100 nM). When ganglia were perfused with medium containing varying concentrations of Ca^{2+} (2.4, 1.2, 0.6, or 0.2 mM), evoked ACh release was depressed by both agonists and the inhibition increased with reduced levels of extracellular Ca^{2+}; this was especially evident when Ca^{2+} in the medium was reduced to 0.2 mM. Second, the effect of NE (1 nM) or of MEAP (100 nM) on Ca^{2+} influx into presynaptic nerve terminals was determined by measuring the accumulation of ^{45}Ca by ganglia in the presence or absence of either drug. Both agonists reduced the stimulation-induced (20 Hz) increase in ^{45}Ca accumulation. The reduction in Ca^{2+} influx by NE was the result of activation of alpha-adrenoceptors, since it was mimicked by clonidine (1 µM) and was blocked by the alpha-antagonists, yohimbine and phentolamine. The decreased ^{45}Ca accumulated by ganglia in the presence of MEAP was due to activation of presynaptic opiate receptors, since the effect was blocked by naloxone (10 µM). Furthermore, D-Ala-met-enkephalinamide (DAEA), an analogue of met-enkephalin that does not inhibit ACh release in this model, did not alter Ca^{2+} influx into preganglionic nerve terminals during stimulation, suggesting that the effect of MEAP to reduce Ca^{2+} influx may be mediated by a receptor for which the heptapeptide is specific.

It is concluded that activation of presynaptic alpha-adrenergic and opiate receptors in the cat superior cervical ganglion alters ACh release by a similar mechanism, i.e. by reducing Ca^{2+} influx during preganglionic nerve terminal depolarization. (Supported by MRC, FCAC, and McGill University, Canada).

244.7 COMPARISON OF THE TIME COURSE OF BuChE AND ChE ACTIVITY AND PHYSOSTIGMINE CONCENTRATIONS IN PLASMA, BRAIN AND MUSCLE OF RAT AFTER I.V., I.M. AND ORAL ADMINISTRATION. S.M. Somani and E. Giacobini (Spon. Wm. H. Cline, Jr.), Dept. of Pharmacol., Southern Illinois Univ. Sch. of Med., P.O. Box 3926, Springfield, IL 62708.

Although physostigmine (Phy) has been in use for more than a century, the relation between the time course of ChE inhibition and drug concentration (conc) in plasma (Pl), brain (B) and muscle (M) after different routes of administration is not known. Rats were administered ^{3}H-Phy 100 µg/kg, i.v., 650 µg/kg, i.m., or 650 µg/kg, oral and sacrificed at 2, 3, 5, 7, 10, 15, 22, 30, 45, 60 and 120 min. Blood was collected, B and M were removed and frozen immediately until analyzed. ^{3}H-Phy and its metabolites, eseroline, M_1 and M_2, were determined by HPLC (Somani and Khalique, Fund. Appl. Toxicol. 6:327-334, 1986).

After i.m. administration, Phy conc in Pl peaked at 5 min (582 ± 4 ng/ml) and then continuously declined to 36 ± 4 ng/ml at 60 min. Plasma BuChE activity was maximally inhibited to 53% of control at 7 min then recovered to 80% at 120 min. Phy conc in B peaked at 5 min (537 ± 51 ng/g) and continuously decreased to 42 ng/g at 60 min. Brain ChE activity was reduced to 40% of baseline at 2 min, 22% at 5 min, then recovered to 63% at 60 min.

After i.v. administration, Phy conc in plasma was highest (83.3 ± 11 ng/ml) at 2 min, declined to 9.2 ± 1.5 ng/ml at 45 min. At these times, BuChE activity was 38% and 70% of control. Phy conc in B peaked (128.5 ± 6.1 ng/g) at 3 min then steadily declined to 3.3 ± 4 ng/g at 60 min. Brain ChE activity was 38% of control at 5 min then recovered to 82% at 60 min. Phy conc in M was 34.7 ng/g at 2 min,then declined to 3.34 ng/g at 60 min. ChE activity was 75% of control at 2 min then recovered to about 90% at 30 min.

After oral administration, Phy was rapidly absorbed; its conc in plasma was 1.96 ± .37 ng/ml at 5 min. It peaked (2.89 ± .47 ng/ml) at 15 min and then declined to .67 ± .35 ng/ml at 60 min. BuChE activity in Pl did not change significantly up to 10 min; however, it was 95%, 70% and 85% of control at 15, 30 and 45 min, respectively. Phy conc in brain was .89 ± .26 ng/g at 5 min peaked to 4.57 ± 2.6 ng/g at 22 min and then declined to .33 ± .11 ng/g at 60 min. ChE activity at the corresponding times was 96%, 71%, and 83% of control. Phy conc in M was .16 ± .07 ng/g at 5 min peaked to 1.9 ± 1.2 ng/g at 22 min and then declined to .08 ± .04 ng/g at 60 min. At these times, ChE activity was 85%, 75%, and 76% of control. These results indicate that both the Pl and tissue conc of Phy and ChE activity were extremely low after oral administration compared to i.m. and i.v. administration. These results also suggest on the basis of pharmacokinetics that BuChE and ChE have much prolonged activity in Pl and B than is reflected by Phy conc. (Supported by U.S. Army Contract No. DAMD 17-83-3195.)

244.8 PHARMACOLOGY AND TURNOVER OF HIGH AFFINITY CHOLINE UPTAKE AND ACETYLATION IN PRIMARY RAT BRAIN CELL CULTURES. M.C. Kelley, D. Koppenaal‡ M.K. Raizada‡ and E.M. Meyer. Depts. of Pharmacology and Physiology, Univ. Florida, Gainesville, FL 32610.

High affinity choline transport is closely coupled to and rate limiting for acetylcholine (ACh) synthesis at resting membrane potentials. We have studied some of the pharmacological characteristics and the celllular regulation of this transporter in primary brain cell cultures that are enriched with either neurons or glia. Neuronal cultures were prepared from 1-day old rats by treating them with cytosine arabinoside to kill dividing glia; glial cultures received no cytosine arabinoside. Little or no hemicholinium 3 (5 uM)-sensitive uptake and acetylation of [^{3}H]-choline (1 uM) was observed after a 30 min incubation at 37° in glial cultures 14 days after plating. In contrast, neuronal cultures retained hemicholinium-3 sensitive uptake and acetylation of [^{3}H]-choline under these conditions. Neuronal choline-acetylation increased in cultures as they aged, reaching a peak between 12-15 days post-plating. The irreversible choline uptake blocker AF64A (30 uM, 60 min, 37°) also inhibited this neuronal cholinergic activity. Recovery from choline-uptake and acetylation-inhibition occurred in a biphasic manner within several days of drug-washout in younger cultures (7-9 days post-plating), while older cultures (12 days post-plating) never recovered hemicholinium-3 sensitive choline uptake. Choline acetyltransferase activity and total protein levels were not reduced by this AF64A treatment, arguing against a neurotoxic action. Both hemicholinium-3 and AF64A were found to be more potent in rat brain synaptosomes compared to neuronal cultures: the former 2-fold more so and the latter 15-20-fold more so. The affinity of the hemicholinium-3 sensitive choline uptake process was similarly reduced in the cultures compared to synaptosomes. These results demonstrate: 1) the potential of primary brain neuronal cultures for studying the turnover of high affinity choline transporters under various physiological conditions; and 2) that pronounced tissue-related differences in the pharmacological characteristics of the transporter should be considered, particularly when using AF64A.

244.9 HEMICHOLINIUM-3 BINDING TO BRAIN MEMBRANES RELATIVE TO CHOLINERGIC FUNCTION. A.C. Swann, L.O. Hewitt*,and R.W. Guynn. Department of Psychiatry, University of Texas Medical School and Mental Sciences Institute, Houston, TX. 77030.

Hemicholinium-3 (HC-3) binding may provide an index of presynaptic cholinergic activity. We have examined the effects of treatments altering presynaptic acetylcholine release on HC-3 binding in membranes from cerebral cortex, hippocampus, and corpus striatum. These were compared to effects on high affinity choline uptake and muscarinic receptor (QNB) binding. Initial experiments showed that high affinity (K = 3 nM) HC-3 binding was increased by NaCl and that soaking of filters with 0.5% polyethylenimine was necessary to reduce appreciable saturable binding to filters. Treatment for 3 days with physostigmine (1 mg/kg/day) or with arecoline (2 mg/kg/day) reduced HC-3 binding, while binding was increased by atropine (10 mg/kg/day). These treatments had similar effects on high affinity choline uptake measured in freshly prepared P2 fraction, and on QNB binding. Acute treatment resulted in similar, but smaller, changes in HC-3 binding. The treatments changed Bmax, but not kB. These data suggest that the number of HC-3 binding sites can provide an indicator of acetylcholine release in vivo.

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244.10 LOW-LEVEL MICROWAVE IRRADIATION AFFECTS CENTRAL CHOLINERGIC ACTIVITY IN THE RAT. H. Lai*, J. Zabawska*, C.K. Chou*, A. Horita* and A.W. Guy* (SPON: K. Chan). Departments of Pharmacology, and Psychiatry and Behavioral Sciences, and the Center for Bioengineering, University of Washington School of Medicine, Seattle, WA 98195.

Male Sprague-Dawley rats were irradiated acutely (45 min) with low-level 2450 MHz pulsed (2 μs, 500 pps) or continuous-wave microwaves in cylindrical waveguides. The power density of irradiation was 1 mW/cm^2, which gives a whole body specific absorption rate of 0.6 W/kg. Sodium-dependent high-affinity choline uptake was determined in different regions of the brain immediately after exposure.

Pulsed microwave irradiation decreased choline uptake in the hippocampus and frontal cortex, but did not significantly affect that in the striatum, hypothalamus, and inferior colliculus. The effect of microwaves on hippocampal choline uptake was blocked by pretreatment with either naloxone or naltrexone (1 mg/kg, IP), whereas the effect on frontal cortical choline uptake was not affected by treatment with narcotic antagonist.

Continuous-wave microwave irradiation decreased choline uptake in the frontal cortex, but had no significant effect on the uptake in the hippocampus, striatum, and hypothalamus. Furthermore, the effect on frontal cortex was not significantly affected by pretreatment with narcotic antagonist.

Thus, low-level pulsed and continuous-wave microwaves of the same power density have different patterns of effect on cholinergic activity in the brain of the rat. The effect of pulsed microwaves on cholinergic activity in the hippocampus is probably mediated by endogenous opioids.

244.11 HIPPOCAMPAL RSA IN RELATION TO BEHAVIOR FOLLOWING ETHYLCHOLINE MUSTARD AZIRIDINIUM ION (AF64A) TREATMENT: EFFECTS OF ATROPINE AND URETHANE. D. J. Stewart, S. M. Leventer, I. Hanin and C. H. Vanderwolf, Dept. of Psychology, Univ. Western Ontario, London, Ontario Canada N6A 5C2 and WPIC, Univ. Pittsburgh Sch of Med., Pittsburgh, PA 15213.

Ethylcholine mustard aziridinium ion (AF64A) has recently been used to produce selective lesions of the cholinergic system in vivo. Biochemical studies show a marked reduction in cholinergic markers in the hippocampus following intracerebroventricular (ICV) injection of AF64A. A functional dimension was added to this work by the study of the cholinergic projection from the medial septal and diagonal band region known to be involved in the generation of the atropine-sensitive form of hippocampal rhythmical slow activity (RSA). The effects of ICV injections of AF64A on the pattern of hippocampal electrical activity were studied in freely moving and urethane anesthetized rats. Male Sprague-Dawley rats were injected with either AF64A (3nmol/3μl/side) or a similar volume of vehicle solution. Bipolar recording electrodes were then implanted in the dorsal hippocampus. AF64A treated rats showed a significantly smaller increase in 6-12 Hz RSA with struggling in the no drug condition in comparison to the vehicle injected rats. However, neither AF64A treatment nor a control injection abolished the presumed cholinergic form of hippocampal RSA that is present during urethane anesthesia. Systemic injection of atropine in waking rats did not significantly alter RSA in either the AF64A or vehicle injected rats. Histological sections revealed extensive damage to the fimbria-fornix, CA3 of the hippocampus, corpus callosum, neocortex and striatum. Acetylcholinesterase staining of the remaining hippocampus appeared normal in the AF64A treated rats. The data indicate that the depletion of cholinergic markers in the hippocampus following ICV administration of AF64A is not sufficient to disrupt the cholinergic form of RSA. Further, it is possible that AF64A may produce its apparent cholinoselective effects in the hippocampus primarily due to the amount of damage it produces in the fimbria-fornix system, the major route by which the cholinergic cells in the septal region reach the hippocampus. While other neurotransmitter systems such as noradrenaline or serotonin show little or no change following AF64A this may be due to anatomical considerations. These systems reach the hippocampus largely via a supracallosal route that would presumably not be affected by ICV injection of AF64A.

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244.12 CLONIDINE PREVENTS NORADRENALINE DECREASE IN RESPONSE TO CHOLINERGIC HYPOFUNCTION FOLLOWING TREATMENT WITH ETHYLCHOLINE MUSTARD AZIRIDINIUM ION (AF64A). H.Hörtnagl*, P.E.Potter*, and I.Hanin, Dept. of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

AF64A is a potent irreversible inhibitor of high affinity choline transport in vitro, and causes a marked reduction in cholinergic markers in vivo when injected intracerebroventricularly (i.c.v.) or into selected brain areas (Fisher, A. & Hanin, I., Ann. Rev. Pharmacol. Toxicol 26:161-81,1986). Moreover, i.c.v. injection of 2 nmoles of AF64A/side produces both a long-lasting reduction in hippocampal acetylcholine (ACh) content, and a transient decrease in noradrenaline (NA) levels, which may result from loss of tonic inhibitory cholinergic input.

In the present investigation we have examined the effects of pharmacological manipulations of NA release, by using the α_2-adrenoceptor agonist clonidine and the α_2 antagonist yohimbine, on levels of ACh and NA after i.c.v. injection of AF64A.

AF64A (2 nmoles/3ul) or 3 ul of vehicle were injected into each lateral ventricle of male Sprague-Dawley rats (8 rats in each group). Clonidine (0.2 mg/kg ip), yohimbine (2 mg/kg ip), or saline was injected 1 h prior to AF64A administration. The rats then received clonidine (0.2mg/kg) or saline ip every 8 h, or yohimbine (2mg/kg) or vehicle every 12 h for 3 days. Animals were killed on day 4 by head-focussed microwave irradiation, and ACh and NA levels were determined in hippocampus and cortex by HPLC with electrochemical detection.

In both brain regions, clonidine prevented the transient reduction in NA content (hippocampus,35%; cortex,25%) following AF64A treatment. However, clonidine did not significantly attenuate the 52% reduction in hippocampal ACh content following AF64A. In rats which had received vehicle i.c.v., clonidine treatment had no effect on NA or ACh levels. Similarly, yohimbine had no effect, other than that the loss in NA after AF64A treatment tended to be more pronounced in yohimbine treated rats.

These results indicate that the transient decrease in hippocampal and cortical NA levels in response to AF64A results from increased release rather than from a direct toxic effect of AF64A on noradrenergic neurons. They also imply that a loss of cholinergic input initiates multiple changes in interacting neuronal systems including, e.g., an increased release of NA. This, in the long run, might lead to a loss of noradrenergic neurons such as we have observed in rats treated with 5 nmol AF64A, and more importantly is also apparent in patients with Alzheimer's disease. The prevention of the loss of NA in AF64A treated animals by clonidine might thus provide an experimental basis for new possibilities in the treatment of Alzheimer's disease.

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244.13 COMPARISON OF HIGH-AFFINITY CHOLINE UPTAKE IN HIPPOCAMPUS AND STRIATUM FOLLOWING DIFFERENT IN VITRO TREATMENTS. I. Vincent and J.R. Simon. Depts. of Biochemistry and Psychiatry, Institute of Psychiatric Research, Indiana Univesity School of Medicine, Indianapolis, IN 46223.

The differential sensitivity of the cholinergic system in the hippocampus and striatum to various in vivo and in vitro treatments has been appreciated for several years. In this regard, the effects of in vitro treatments on sodium-dependent high-affinity choline uptake (HAChU) into synaptosomes prepared from rat hippocampus and striatum have been compared. Choline uptake was examined in normal Krebs medium following preincubation under various conditions. Preincubation in high potassium-Krebs buffer (47.5 mM K^+) resulted in an approximate two-fold increase in HAChU in the hippocampus. The uptake in the striatum was much less sensitive under these conditions, exhibiting an increase of only 20%. Pre-exposure to a sodium-free medium produced an approximate 50% increase in subsequent HAChU uptake in both regions. Thus, while hippocampal uptake is sensitive to pretreatment with both high potassium and sodium-free media, uptake in the striatum appears considerably more sensitive to sodium omission than to high potassium pre-treatment. The sensitivity of HAChU to hemicholinium-3 (HC-3) was compared in the hippocampus and striatum and was found to be the same (IC_{50}=1 x 10^{-8}M). In addition, the K^+-stimulated uptake in the hippocampus was also found to be sensitive to HC-3 with an IC_{50} equal to that obtained when synaptosomes were preincubated in normal medium. Preincubation in a calcium free/EGTA medium with subsequent uptake in calcium-containing medium resulted in a significant decrease (50-70%) in both hippocampus and striatum. This suggests that tissue calcium might be important in maintaining high-affinity choline uptake. Additional experiments will be required to determine whether this, and/or other biochemical mechanisms, might play a role in the differential sensitivity of the hippocampal and striatal cholinergic systems.

244.14 CHOLINOTOXICITY OF AF64A: IN VITRO AND IN VIVO CORRELATIONS. Z. Pittel*, A. Amir*, A. Fisher, A. Shahar*, R. Brandeis* and E. Heldman* (SPON: E. Hazum), Israel Institute for Biological Research, Ness-Ziona, Israel.

Ethylcholine aziridinium ion (AF64A) is a cytotoxin which produces selective cholinergic hypofunction when injected icv to rats. It has been postulated that the toxin uses the high-affinity choline transport system (HAChT) to enter the cholinergic cells and destroy them upon its accumulation within their terminals. We studied the interaction between AF64A and the HAChT system in isolated synaptosomes and correlated the results with the cholinotoxicity obtained in cultured neurons and rats in vivo. In isolated synaptosomes, AF64A, at concentrations up to 10μM, induced reversible inhibition of the HAChT, while at higher conc. (>50μM) it induced mostly irreversible inhibition. The inhibition was not elicited in the presence of choline, suggesting that AF64A and choline compete for the same site on the carrier. Under conditions in which the HAChT was blocked but the carrier was not occupied (Na^+-free medium), AF64A still inhibited the transport process. The HAChT was more sensitive to AF64A than the low-affinity choline transport (LAChT). Based on these results, we propose a kinetic model in which AF64A, at low concentrations, is used as a substrate for the choline carrier, while at higher conc. it alkylates the carrier at its outward position and inactivates it irreversibly. Thus accumulation of AF64A in cholinergic neurons (by transport via HAChT), and consequent selective cholinotoxicity, should occur at low conc. of the toxin. At higher conc., the toxin could be transported via the LAChT and induce non-selective cell lesion. This hypothesis was examined in primary neuronal cultures from fetal rats. In these cultures, AF64A, in conc. up to 50μM, caused significant reduction in the number of cholinergic neurons (AChE histochemistry) but only slightly affected dopaminergic neurons (^{3}H-dopamine autoradiography and measurement of dopamine uptake and release); at 100μM, some dopaminergic parameters (uptake and release) were also affected and various stages of general cytotoxicity were evident. In vivo studies showed that the minimal dose (icv) which produced cholinergic hypofunction (reduction in HAChT and ChAT) was 3 nmoles/side/8 min (maximal cumulative ventricular conc. calculated is 240μM, but could be lower due to clearance). The hippocampus was more affected than the striatum, suggesting that the distribution of the toxin within the brain is uneven, thus local tissue conc. cannot be evaluated properly. An alternative route of administration, which might result in a better distribution and in lower initial conc. of the toxin, is intracarotid injection combined with an opening of the blood-brain barrier. Preliminary results obtained with such unilateral injection showed that AF64A produced 50% reduction of ChAT in the ipsilateral hemisphere.

244.15 EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF TWO HETEROCYCLIC HEMICHOLINIUM-3 ANALOGS ON CHOLINERGIC SYSTEMS. C.E. Tedford*, J.R. Flynn*, J.G. Cannon* and J.P. Long* (SPON: W.J. Steele). Departments of Pharmacology and Medicinal Chemistry, University of Iowa, Iowa City, IA 52242

Previous work in our laboratory has demonstrated that, in vitro, tertiary amine (A-4) and quaternary amine (A-5) 4-methyl piperidine analogs of hemicholinium-3 are potent inhibitors of sodium-dependent high affinity choline uptake, reduce ACh levels in rat caudate slices and inhibit neuromuscular transmission upon high frequency stimulation in vivo. Systemic administration of (A-4), the tertiary amine analog, was effective in selectively reducing ACh levels in several brain regions, whereas HC-3 and A-5 were not effective centrally after systemic administration. The above studies have shown that the quaternary amine (A-5) was equipotent to HC-3 and the tertiary amine was approximately 100-1000-fold less potent than its quaternary analog. Since the quaternary amine compounds are ineffective in crossing the blood brain barrier and unable to reduce cholinergic function centrally, these studies investigated and compared the effects of intraventricular administration of A-4 and A-5 with HC-3 on ACh, choline, dopamine, norepinephrine, epinephrine and serotonin levels in the rat striatum.

Male Sprague-Dawley rats (200-300 grams) were anesthetized and a polyethylene cannula was stereotaxically implanted into the right ventricle. Animals were allowed to move freely about their cages during infusion of the experimental compounds and were sacrificed at various times by head-focused microwave irradiation (2.0 sec.). Both the contralateral and infused sides of the brain were dissected and assayed by HPLC-EC.

Results demonstrated that HC-3 was effective in reducing striatal ACh levels in a dose-dependent fashion. Significant reductions in ACh levels were seen in the striatum at HC-3 doses of 0.1, 1.0, and 10 ug. 100 ug of HC-3 produced maximal reductions in ACh levels (>80%), elevation in choline levels, and prominent behavioral effects and toxicity. The effect of HC-3 on striatal ACh levels was selective for cholinergic neurons as no significant changes in other neurotransmitters were demonstrated. The results following intraventricular administration of the quaternary analog (A-5) were similar to the effects seen after HC-3 administration in producing a significant reduction in striatal ACh levels. The intraventricular administration of the tertiary amine (A-4) produced no significant reduction in ACh levels after infusion of 100 or 200 ug of A-4.

This work demonstrates that replacement of the choline moiety in HC-3 with the 4-methyl piperidine moiety maintains HC-3-like activity in the quaternary amine analog. This work was supported in part by DOD Cont. No. DAMD-17-83-3010.

244.16 MAINTENANCE OF ACETYLCHOLINE SYNTHESIS AND RELEASE IN STIMULATED STRIATAL SLICES IS DUE TO AN ACCELERATED LIBERATION OF CHOLINE FROM PHOSPHOLIPIDS. R.J. Wurtman, C. Mauron* and J.K. Blusztajn*. Dept. of Brain & Cognitive Science, M.I.T., Cambridge, MA 02139.

When rat striatal slices are superfused with a choline-free medium containing eserine and stimulated electrically for 30 min., they continue to release acetylcholine (ACh) at a constant and higher rate than at rest. However the total amount of ACh + choline (Ch) in the superfusate exceeds the total decrease in the amount of ACh + Ch in the tissue (Maire, J-C, & Wurtman, R.J., J. Physiol. Paris, 80:189, 1985). This suggests that the source of Ch for ACh synthesis in the slices was Ch-containing membrane phospholipids [phosphatidylcholine (PC), lysophosphatidylcholine, sphingomyelin]. To test this hypothesis, total membrane phospholipids (PL) were measured in striatal slices before and after stimulation, and correlated with ACh release. Slices (0.3 μm) from rat striatum, were superfused with a physiological medium and stimulated (15 Hz, 30 V) for 30 min. The superfusate was assayed for ACh during 4 min. intervals; ACh was also assayed in samples of the superfusate taken during the 12 min. preceding and 18 min. following stimulation. Total and individual PL were determined in tissue samples taken before and after the collection period.

	Rest	Stimulation		
	No Ch	No Ch	20 μM Ch	No Ch, TTX (0.5 μM)
PL	104±5	77±6	106±4	102±2
ACh release	9±1	32±3	54±4	9±1

PL are expressed as % of the amount present before the beginning of the collection period (which was 15±1 μg phosphorus/mg protein). ACh release is expressed as pmol/mg protein/min.

Depolarization increased ACh release fourfold, and concomitantly decreased PL by 23% in the slices; PC, phosphatidylethanolamine and phosphatidylserine were all decreased stoichiometrically. The ACh release was further increased, and PL degradation totally prevented, when exogenous Ch (20 μM) was added. To determine whether PL degradation was due to depolarization of striatal cells (and not an artifact of electrical stimulation) tetrodotoxin [(TTX) which blocks sodium channels] was added to the superfusion medium: It inhibited ACh release (possibly by blocking action potentials) and prevented PL degradation.

We conclude that depolarization of striatal slices decreases membrane PC (and other PL), concurrently making Ch available for ACh synthesis. Moreover, this decrease (which might affect membrane functions) can be blocked by Ch, in levels that also enhance ACh synthesis.

244.17 ACETYLCHOLINE SYNTHESIS IN A HUMAN NEUROBLASTOMA CELL LINE. J.D. Schein*[1], J.L. Biedler*[2], and P. Davies*.[1,3] (SPON: L. Brown). Departments of Pathology[1] and Neuroscience[3], Albert Einstein College of Medicine, Bronx, New York, 10461, and the Laboratory of Cellular and Biochemical Genetics[2], Memorial Sloan-Kettering Cancer Center, Rye, New York, 10580.

Acetylcholine synthesis was studied in a human cholinergic neuroblastoma cell line designated MC-IXC. Cultured cells were incubated in the presence of various radiolabeled potential precursors for the acetyl moiety and the labeled acetylcholine formed was measured according to the method of Peterson and Gibson (J. Pharmacol. Exp. Ther.; 222: 576, 1982). Synthesis from ^{14}C-glucose was linear over a period of 2 hours with an apparent Km of 180μM and an apparent Vmax of 5.4 nanomoles/hr/mg protein. By comparison, the rate of acetylcholine synthesis by homogenized MC-IXC cells was 77 nanomoles/hr/mg protein in the presence of saturating concentrations of acetyl CoA and choline.

To determine whether the rate of synthesis in intact cells is limited by the availability of choline, the dependence of acetylcholine synthesis on choline concentration was examined. The apparent Km for choline is 30μM. With higher concentrations of choline, acetylcholine synthesis rates reached a plateau at only 4.8% of the rate measured in homogenates of these cells. Thus choline availability does not appear to be rate limiting.

Synthesis rates were also determined using ^{14}C-β-hydroxybutyrate and ^{14}C-acetate as potential acetyl group donors yielding apparent Km values of 90μM and 100μM and apparent Vmax values of 2.9 and 4.8 nanomoles/hr/mg protein respectively. In the presence of saturating concentrations of both ^{14}C-glucose and ^{14}C-β-hydroxybutyrate synthesis rates were greater than in the presence of either substrate alone. Furthermore, using ^{14}C-pyruvate as the acetyl group donor the rate of synthesis reached 44.8 nanomoles/hr/mg protein, a value approaching that obtained for cell homogenates. These results indicate that acetyl CoA, not choline, is the rate limiting substrate in acetylcholine synthesis in cultured MC-IXC cells, and that the rate determining step is likely to involve one of glycolytic enzymes, probably phosphofructokinase.

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244.18 INDUCTION OF CHOLINE ACETYLTRANSFERASE IN A HUMAN CHOLINERGIC NEUROBLASTOMA CELL LINE. D. Casper[1], J. L. Biedler*[2], and P. Davies*.[1,3] Departments of Pathology[1] and Neuroscience[3], Albert Einstein College of Medicine, Bronx, New York, 10461, and the Laboratory of Cellular and Biochemical Genetics[2], Memorial Sloan-Kettering Cancer Center, Rye, New York, 10580.

The human neuroblastoma clone, MC-IXC, was used to investigate the effects of several agents on the regulation of choline acetyltransferase (ChAT) activity. This cell line, a subclone of the line SK-N-MC, expresses basal levels of two enzymes of cholinergic metabolism, choline acetyltransferase and acetylcholinesterase. The ChAT activity is increased approximately ten-fold (from 4.8 to 55 nmol/hr/mg pr) when cells reach high density. This effect is reported as a function of protein content per flask as the protein correlates linearly with cell number. The induction of ChAT appears to be mediated by cell-cell contact and is reversible on dissociation of the cells in culture either by trituration or trypsinization.

Retinoic acid can also increase ChAT activity up to five-fold in a dose-dependent manner. This induction of ChAT activity appears independent of cell density as retinoic acid has no effect on either protein content or cell number, and is obtainable in both low and high density cultures. The effects of retinoic acid on ChAT activity are not reversible at 4 days after removal of this compound from the medium.

1mM dibutyryl cyclic AMP can also increase the specific activity of ChAT up to five-fold in culture after a 7 day incubation. This induction could not be obtained using forskolin, despite prolonged elevation of intracellular cAMP concentrations. Other agents, such as DMSO, dexamethazone and estradiol do not induce ChAT activity in these cells. None of the agents tested were able to induce or inhibit the activity of acetylcholinesterase, suggesting that the regulation of acetylcholine synthesis and degradation is not coordinately controlled.

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244.19 PHYSICAL DIFFERENCES BETWEEN SOLUBLE AND IONICALLY MEMBRANE-BOUND CHAT. M. Badamchian, K.J. Morrow, Jr.* and P.T. Carroll, Depts. of Pharmacol. and Biochem., Texas Tech Univ. Health Sci. Center, Lubbock, TX 79430

Choline-O-acetyltransferase (EC 2.31.6; ChAT) was prepared from synaptosomal fractions (P_2) of mouse and rat brain in the presence of proteolytic inhibitors by the method of Gray & Whittaker (1962) as modified by Salehmoghaddam & Collier, 1976. The P_2 fraction was hypo-osmotically shocked with glass distilled water and centrifuged to separate the cytosolic (S_3) and crude vesicular (P_3) fractions. Fraction S_3 was saved for ChAT assay and compared with that ChAT fraction eluted from the P_3 by salt at a pH 7.4 or by detergent (Benishin & Carroll, 1983). These three ChAT fractions were then compared by molecular weights, isoelectric points, immunoblotting with monoclonal or polyclonal antibodies, and hydrophobicity. The results show that the S_3 ChAT fraction has a molecular weight of 66 Kd, whereas the ionically bound fraction of ChAT has a molecular weight of 73-78 Kd. SDS-PAGE of these two ChAT fractions followed by immunoblotting revealed the presence of two immunoreactive bands at 28-29 Kd and 50-51 Kd for the ionically bound ChAT fraction. Conversely, none of these antibodies immunostained any protein bands for the S_3 ChAT fraction even though one monoclonal antibody (MB 16) had been prepared against this ChAT fraction and bound the native S_3 ChAT fraction in a co-precipitation assay. The S_3 fraction has only one isoelectric point at pH 7.8, whereas the ionically bound and detergent soluble ChAT fractions have two isoelectric points at pH 8.1-8.15 and 7.45-7.5. The S_3 ChAT fraction is also less hydrophobic then the other two ChAT fractions. These differences between the S_3 and salt soluble ChAT fractions were not obviated by addition of detergent and thus could not be attributed to the association of lipids with either of the fractions. Veratridine depolarization of hippocampal minces caused a Ca^{2+} dependent stimulation of both the S_3 and detergent soluble ChAT fractions but not the salt soluble fraction. We conclude that the water soluble fraction of ChAT in central nerve terminals differs in its physical properties and subcellular location from that which ionically binds to membranes (supported in part by a Tarbox postdoctoral Fellowship to MB and NINCDS 2121289-02 to PTC).

245.1 THE EFFECTS OF ACETYLCHOLINE ON SPONTANEOUS AND TONE EVOKED NEURONAL DISCHARGE IN AUDITORY CORTEX OF THE CAT. T.M. MCKENNA, J.H. ASHE, G.K. HUI*, and N.M. WEINBERGER. Center for the Neurobiology of Learning and Memory, Univ. California, Irvine,CA 92717.

Two sources of acetylcholine (ACh) have been identified in cerebral cortex, one of subcortical origin and the other intrinsic in origin. The function of ACh in auditory cortex is of particular interest because manipulations of cholinergic systems have been shown to affect auditory perception, and complex cognitive processes. Furthermore physiological plasticity is rapidly induced in auditory cortex during learning (Weinberger et.al.; Diamond & Weinberger, Behav. Neurosci.,1984,98,171-210), and cholinergic processes have been repeatedly implicated in various forms of plasticity.

The present studies are aimed at characterizing cholinergic actions on the rate and pattern of discharge of single neurons. Action potentials were recorded extracellularly in primary auditory cortex of waking cats, and the cholinergic agonists, ACh and methacholine (MCh), and antagonists, atropine, and scopolamine, were applied by microiontophoresis or pressure ejection.

To date, data have been collected from 135 neurons, of which 58 have been analyzed quantitively. Cholinergic agonists, ACh or MCh, significantly modified spontaneous rate in 67% of the neurons. Increases in spontaneous activity were detected in 36% and decreases in 31%. These agents also effectively modified neuronal responses to acoustic stimuli (tone bursts) in 65% of the cells. Increases were observed in 24%, and decreases in 41%. Differential actions on different components of the pattern of response to tone, i.e., poststimulus time histogram, were noted. The effects of a given agonist on spontaneous and evoked disharges were often different for the same cell.

Evidence is provided that muscarinic receptors are involved in mediating these effects since (1) the muscarinic agonist MCh exhibited the same spectrum of actions as ACh, (2) muscarinic antagonists were capable of modifying both spontaneous and tone-driven activity, (3) the direct effects of antagonists could be opposite to those of agonists in the same cell, and (4) antagonists were able to suppress the effects of the agonists.

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245.2 NICOTINIC ACETYLCHOLINE RECEPTOR DISTRIBUTION IN THE SONGBIRD BRAIN: AUTORADIOGRAPHIC STUDIES. Thomas R. Podleski*, James T. Watson, Elizabeth Adkins-Regan, Jon M. Lindstrom, and Paul Whiting. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, and Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138.

We have undertaken localization of nicotinic acetylcholine receptors in the zebra finch (*Peophila guttata*) brain. We were particularly interested in whether the identified song system nuclei, or other nuclei which concentrate steroid hormones, are nicotinic. We have used three 125I-labelled ligands for the identification of nicotinic receptors: alpha bungarotoxin (BGT), and two monoclonal antibodies, one produced against the acetylcholine receptor(AChR) from electric organ of *Electrophorus electricus*, mAb 35, (Swanson, *et al.*, Proc. Nat. Acad. Sci., 80: 4532, 1983) and one which has been produced against a recently isolated nicotinic AChR from the chick brain, mAB 270 (Whiting & Lindstrom, J. Neurosci. in press).

Intact adult male and female zebra finches, aged 11-16 months, were heavily anesthetized and decapitated. Their brains were cryostat sectioned at 14 µm, sections were freeze-dried according to the method of Herkenham and Pert (J. Neurosci., 2: 1129, 1982). Each set of dried sections was then incubated with one of the nicotinic ligands: 20 nM BGT (males: N=5, females: N=4), mAB 35 (males: N=2, females: N=1) or mAB 270 (males N=2, females: N=1). Specificity of binding was determined in the case of BGT by including 1 mM carbamylcholine in the incubation mix, which reduced the binding of BGT to background levels and no regional variation was seen. Inclusion of unlabeled antibody blocked the binding of labeled antibody. Labeled sections were fixed and applied to LKB Ultrofilm™. Following LKB autoradiography, selected slides were coated with Kodak NTB-2™ emulsion and overexposed to confirm the neuroanatomical localization of labelled regions.

^{125}I BGT heavily labeled telencephalic and mesencephalic visual areas, the hippocampus, the steroid-binding nuclei in the song system, and the diencephalic steroid-binding nuclei. As has been described in other birds and fish (reviewed by Oswald and Freeman, J. Neurosci., 6: 1-14), BGT heavily labeled the optic tectum (OT). In addition, we observed areas of dense labeling in the hippocampus (Hp) and at the border of *hyperstriatum dorsale* (HD) and *hyperstriatum accessorium* (HA). Of the song control regions, *nucleus magnocellularis* of the anterior neostriatum (MAN), *hyperstriatum ventrale, pars caudale* (HVc) and *nucleus intercollicularis* (ICo), were heavily labeled, nucleus *robustus archistriatalicus* (RA) was not specifically labeled, and area X were far less labeled than immediately surrounding areas. As has been reported in the rat (Clark *et al.* J. Neurosci. 5:1307, 1985), the diencephalic nuclei *preopticus medialis* (POM), *ventromedialis hypothalami* (VMH), and *infundibuli* (Inf) had high toxin binding. We found the binding patterns in HVc and area X are sexually dimorphic, i.e., they conform closely to the contours of the respective nuclei, which are much larger in males. There was no marked sexual dimorphism in BGT binding in other nuclei. We are examining the influence of gonadal steroids on BGT-binding levels in the zebra finch brain.

The binding pattterns of the two antibodies were similar but not identical. Neither antibody specifically labeled MAN, HVc, RA or Inf. Both labelled HV, the OT, and the *nucleus lateralis spiriformis*. The major differences in the binding of the two antibodies were in *lobus parolfactorius* (LPO), Hp, POM, VMH, and ICo which are labeled by mAB 270 and not by mAB 35. Although mAB 270 labeled LPO heavily, the paucity of labeling in area X seen in the BGT autoradiograms was not observed. Supported by NSF BNS84-12083 and the Cornell Biotechnology Program which is sponsored by the NY State Science and Technology Foundation and a consortium of industries.

245.3 EFFECTS OF BODY TEMPERATURE ON PENTOBARBITAL-INDUCED NARCOSIS AND DEPRESSION IN CHOLINERGIC ACTIVITY. E.S. Swearengen*, H. Lai* and A. Horita* (SPON:C. Chavkin). Departments of Pharmacology, and Psychiatry & Behavioral Sciences, Univ. of Washington, School of Medicine, Seattle, WA 98195.

Pentobarbital induces narcosis, hypothermia, and depression of cholinergic activity in the septohippocampal pathway in rats. Previous pharmacological studies have suggested that the duration of pentobarbital-induced narcosis is negatively correlated with cholinergic activity of this pathway. In the present study we investigated the effects of body temperature on the duration of narcosis and depression of cholinergic activity induced by pentobarbital. Body temperature of pentobarbital-anesthetized rats was adjusted to three different levels by placing them in different ambient temperatures. Body temperature was measured with a probe inserted into the animals' colon; cholinergic activity was determined by measuring sodium-dependent high-affinity choline uptake in hippocampal and frontal cortical synaptosomal preparations.

Analysis of body temperature response during pentobarbital-induced anesthesia revealed signifigant differences among the groups of animals in different ambient temperatures. However, the duration of narcosis (from the time of pentobarbital injection to restoration of the righting reflex) was not affected by differences in body temperature. We found that body temperature was positively correlated ($r = 0.96$, $p < 0.02$) with cholinergic activity of the septohippocampal but not the cortical pathway. Body temperature had no effect on whole brain levels of pentobarbital. These results suggest that cholinergic activity of the septohippocampal pathway is not a sufficient condition in the control of the duration of pentobarbital-induced narcosis, since temperature dependent changes in cholinergic activity were not accompanied by changes in the duration of narcosis. Our data also suggest that septohippocampal cholinergic neurons are sensitive to changes in body temperature.

245.4 BIOCHEMICAL AND BEHAVIORAL CHARACTERISTIC OF A MUTANT STRAIN OF WISTAR RATS (Han-WIST: spa/spa). L. Turski, T. Honore*, N.N. Osborne*, M. Schwarz*, T. Klockgether* and K.-H. Sontag* (SPON: U. Kuhnt). Max-Planck-Inst. Exp. Med., D-3400 Göttingen, FRG, A/S Ferrosan, Res. Division, DK-2860, Soeborg, Denmark and Nuffield Lab. Ophth., Univ. Oxford OX2 6AW, UK.

The mutant strain of Wistar rats (Han: WIST; spa/spa) carries an autosomal recessive gene defect and is characterised by a sequence of progressively developing behavioral alterations including hyperexcitability, tremor, olfactory and gustatory automatisms, bradykinesia, ataxia, rigidity, paresis and cachexia. The synthesis and release of dopamine in vivo in the striata of the mutants is decreased, while the choline acetyltransferase (CAT) activity is greatly enhanced. These rats exhibit also a lower activity of glutamic acid decarboxylase (GAD) in synaptosomal fractions of the substantia nigra. The analysis of the behavior of mutants underlines deficient functioning of the basal ganglia in these animals. In this study, we sought to determine the functional state of the limbic forebrain in mutant rats. To accomplish this, we examined a) the sensitivity of mutant rats to seizures induced by pilocarpine, a cholinergic agonist, b) the activity of CAT and GAD in the hippocampus, c) the binding of ^{3}H-amino-3-hydroxy-5-isoxazole propionic acid (AMPA), ^{3}H-kainate (KA) and ^{3}H-glutamate in the presence of quisqualate (QA) (measurement of N-methyl-D-aspartate receptor) in the hippocampus and d) morphological structure of the hippocampus by light microscopy. The threshold for seizures induced by pilocarpine was significantly lower (4-8 times) in mutant compared to control rats. Pilocarpine hydrochloride, 25-100 mg/kg (doses which did not induce seizures in control rats), administered systemically in mutant rats elicited limbic seizures and status epilepticus, which was followed by widespread damage to limbic forebrain including hippocampus, thalamus, amygdala, neocortex and substantia nigra. Mutant rats exhibited higher activity of CAT in the hippocampus, whereas the activity of GAD remained unaltered. The ^{3}H-glutamate binding in the presence of QA was significantly reduced in hippocampal membranes, which suggests alteration of N-methyl-D-aspartate receptors. ^{3}H-AMPA and ^{3}H-KA binding was unchanged. Morphological analysis of the hippocampus with light microscopy revealed marked loss of pyramidal cells in the subfields CA3 and CA4 of the dorsal hippocampus in mutant rats. These findings indicate derangements in the function of limbic system in mutant Han: WIST rats. Supported by a grant from the Deutsche Forschungsgemeinschaft (So 136/2-2.

245.5 ANIMAL MODELS OF CHOLINOMIMETIC THERAPY. E. Giacobini and M. Hallak*. Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708 USA

Recent clinical trials have shown that oral and intravenous (i.v.) administration of physostigmine (Phy) can temporarily influence memory tasks in Alzheimer dementia patients. Cognitive improvement following Phy therapy is believed to be related to inhibition of central nervous system (CNS) acetylcholinesterase (AChE) activity. No pharmacokinetic data are available to guide Phy therapy in humans. We have studied the effect on brain of two prototypes of reversible and irreversible, centrally and peripherally acting cholinesterase (ChE) inhibitors. Three routes of administration were compared: i.m., i.v. and intracerebral ventricular (i.c.v.). Intraventricular administration of ChE inhibitors might provide for long-lasting and sustained brain concentrations permitting a slow, intracerebral dosage. With i.c.v. administration of Phy in beagle dogs, over 90% inhibition of CSF AChE activity was reached within 5 min at all dosages (10-120 ug). At the lowest dosage (10 ug), 50% of CSF enzyme activity was still present at 90 min. Plasma ChE activity was only minimally inhibited. The opposite response was obtained by i.v. administration (250-100 ug). Striatum and hippocampus were maximally inhibited at 5 min after i.c.v. administration. Following i.c.v. administration (4 ug) of Phy in the rat, ChE activity was maximally inhibited in hippocampus (83%) at 15 min. Physostigmine concentration was also highest in this region. With metrifonate i.c.v. (2.5 mg), ChE activity was maximally inhibited in hippocampus at 30 min. These effects of Phy and metrifonate were found to be different for duration, distribution and percent acetylcholine elevation. Side effects were also different for various drugs and routes of administration. These experiments in animals have provided useful guidelines for clinical trials.

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245.6 THE EFFECT OF CHOLINERGIC STIMULATION OF THE NUCLEUS ACCUMBENS ON LOCOMOTOR BEHAVIOR. M.C. Austin and P.W. Kalivas. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

It has been reported that muscarinic and nicotinic receptors are located in the nucleus accumbens (NAc). It is well documented that dopamine and dopamine agonists produce locomotor activation when infused into the NAc. Therefore, we investigated the role of cholinergic stimulation of the NAc on locomotor behavior. Male S.D. rats were implanted with chronic bilateral injection cannulae into the NAc. One week after surgery, rats were adapted to photocell cages for one hour, microinjected with the cholinergic agonist carbachol or saline vehicle (0.25 µl/side over 60 sec) and placed back into photocell cages for 120 min. Carbachol produced a dose-dependent increase in horizontal and vertical photocell counts with a minimum effect dose of 0.1 µg/side. A similar dose-response curve was produced by carbachol injected into the DBB, and no effect at a dose of 0.33 µg/side was produced in the striatum.

To determine the type of cholinergic receptor carbachol was stimulating in the NAc, rats received an i.p. injection of either a muscarinic antagonist, atropine sulfate (1 mg/kg), or a nicotinic antagonist, mecamylamine (5 mg/kg), followed by infusion of carbachol (0.33 µg/side) into the NAc. The carbachol-induced locomotor activation was significantly reduced by mecamylamine, and atropine sulfate did not attenuate the locomotor hyperactivity. To evaluate whether the carbachol induced locomotor response involved dopaminergic transmission, rats were pretreated with the dopamine antagonist, haloperidol (0.1 mg/kg i.p.). Haloperidol significantly reduced the locomotor response to carbachol.

Dopamine-induced locomotor activity in the NAc is believed to be mediated via inhibition of a GABAergic projection to the substantia innominata (SI). It was shown, that while pretreatment with muscimol (5ng) into the SI completely abolished the motor effect of dopamine injection into the NAc, it did not alter the response to carbachol.

Our results indicate that cholinergic stimulation of the NAc by carbachol produces a dose-dependent motor stimulant response which can be reduced by the nicotinic antagonist, mecamylamine and the dopamine antagonist, haloperidol. It appears that dopamine may have a permissive role in the NAc, thus allowing carbachol to produce a locomotor response. However, this locomotor activation does not appear to be mediated by dopamine and may involve some alternate pathway other than the SI circuit.

245.7 EFFECTS OF MUSCARINIC CHOLINERGIC AGENTS ON SPONTANEOUS SWIMMING IN RATS. J.P. Symons, M. Smith*, J.G. Marriott and R.E. Davis. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105

Muscarinic anticholinergics, such as scopolamine, in a dose-dependent manner increase locomotor activity of rats in an open field (Geyer, 1985). In other environments such as a maze, rats become trapped at the end of a runway and exhibit decreased activity after treatment with anticholinergics (Schallert, et al, 1980). It is clear from this and other work that environmental factors affect the behavioral response to muscarinic agents in rodents. The demands of the task may also affect their response to drug. To characterize further the behavioral effects of muscarinic agents, we studied their effects in rats swimming in an open pool.

Male Long-Evans rats were tested for a single 5 min trial in a 122 x 122 x 60 cm deep white acrylic plastic tank filled to a depth of 35 cm with tap water (20-22^{0} C) made opaque by the addition of powdered milk. The pattern of activity and distance traveled during the 5 min trial was recorded for each animal using computer-controlled video-tracking equipment.

Untreated animals swam rapidly during the first minute of the test then gradually decreased their activity over the remainder of the session. Initially, they swam around the periphery of the tank. However, over the 5 min test they altered their swimming pattern and swam throughout the pool, exploring all areas.

A variety of muscarinic agents--agonists, antagonists, and anticholinesterases--were administered (SC or PO) generally 30 min prior to testing. It was found that: 1) anticholinergics (scopolamine and atropine) produced an increase in swimming and a profound thigmotaxia; 2) peripherally active agents, such as neostigmine and methylscopolamine, did not affect swimming activity; 3) the muscarinic agonists, arecoline and oxotremorine, and the anticholinesterase, physostigmine, decreased swimming activity; and 4) the effects of scopolamine on both the distance and pattern of swimming was reversed by centrally active cholinergic agents, such as oxotremorine, arecoline and physostigmine, and vice versa.

These results support and extend earlier studies of muscarinic cholinergic drug effects upon behavior in rodents, showing (1) an increase both in locomotor activity and stereotyped behavior following anticholinergics and (2) a decrease in activity following muscarinic agonists and anticholinersterases. The effects of muscarinic anticholinergics upon spontaneous swimming behavior may be related to the well-known, deleterious effects of these agents upon maze learning. In addition, these procedures provide a simple, rapid, and somewhat specific method to identify centrally-acting muscarinic agents in a whole animal preparation.

245.8 CENTRAL CHOLINERGIC PHARMACOLOGY OF A SERIES OF CHOLINESTERASE INHIBITORS. R.E. Davis, L.L. Coughenour*, J.G. Marriott, W.H. Moos*, R.D. Schwarz, J.P. Symons, and A.J. Thomas*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105

The short duration of action and poor therapeutic ratio of the anticholinesterase, physostigmine, has limited its utility in treating patients suffering from cholinergic deficiencies. Recently, a series of acetylcholinesterase inhibitors (ACHE-I) has been described by Weinstock-Rosen (RA series) with a potential for decreased toxicity and increased duration of action relative to physostigmine. We have examined the effects of this series in a variety of 'in vitro' biochemical and 'in vivo' behavioral tests which are designed to characterize central cholinergic function.

All compounds from the RA series exhibit higher affinity for muscarinic cholinergic agonist sites labeled by cis-methyldioxolane (CMD) than for sites labeled by the muscarinic antagonist, QNB. The IC-50's of these compounds for displacing ^{3}H-CMD is directly correlated to their potency for inhibiting cholinesterase activity (r^2 = 0.92). CMD displacement and cholinesterase activity of this series also are directly related to bulk parameters such as lipophilicity and molar refractivity. A similar relationship holds with respect to the ability of these compounds to decrease the K+-stimulated presynaptic release of acetylcholine from brain slices. However, these RA series compounds do not stimulate the turnover of phosphoinositides (PI). The order of potency for displacing CMD, inhibiting cholinesterase activity and decreasing ACH release is RA-2 > RA-10 > RA-6 > RA-7 $\geq$ RA-8.

A similar order of potency holds for the ability of these compounds to decrease spontaneous swimming and to reverse scopolamine-induced increases in swimming activity of rats. However, RA-6 does not reverse scopolamine-induced increases in swimming activity.

Taken together these data suggest that the ability of these compounds to displace CMD binding but not QNB binding may be related to their ability to inhibit cholinesterase activity. ACHE-I activity also is directly related to the ability of these compounds to decrease the presynaptic release of ACH from brain slices, decrease spontaneous swimming activity and reverse scopolamine-induced swimming activity. This pattern of effects also is seen with the known anticholinesterase, physostigmine.

245.9 ONLY CERTAIN ANTICONVULSANT DRUGS PROTECT RATS AGAINST SEIZURES INDUCED BY PILOCARPINE. W.A. Turski*, E.A. Cavalheiro*, C. Coimbra*, M.P. Berzaghi*, C. Ikonomidou-Turski* and L. Turski (SPON: P.L. Herrling) . Dept. of Pharmacol., Med. Sch., PL-20090 Lublin, Poland and Dept. of Neurol. Neurosurg., Lab. of Exp. Neurol., Escola Paulista Med., CEP-04023 Sao Paulo, SP, Brazil.

Seizures produced in rats by systemically administered pilocarpine provide a model for studying the initiation, spread and generalisation of convulsive activity in the forebrain. Pilocarpine, 380 mg/kg, induces a sequence of behavioural and electroencephalographic alterations indicative of motor limbic seizures and status epilepticus which is followed by widespread damage to limbic forebrain resembling that occurring subsequent to prolonged intractable seizures in humans. The present study was undertaken to determine whether clinically utilised antiepileptic drugs share an ability to suppress seizures and brain damage elicited by pilocarpine in rats. Clonazepam, ED_{50} 0.35 mg/kg (0.25-0.49), phenobarbital, 23.4 mg/kg (18.5-29.6), and valproic acid, 286 mg/kg (202-405), prevented the build-up of limbic seizures and protected from seizure-related brain damage. Pretreatment with trimethadione, 179 mg/kg (116-277), resulted in moderate protection against pilocarpine-induced seizures, whereas carbamazepine, 10-50 mg/kg, and diphenylhydantoin, 10-100 mg/kg, blocked neither convulsions nor brain damage produced by the drug. Surprisingly, ethosuximide, 196 mg/kg (141-272), lowered the threshold for seizures induced by pilocarpine and converted non-convulsant dose of pilocarpine, 200 mg/kg, into a convulsant one. These results indicate that only certain anticonvulsant drugs elevate the threshold for pilocarpine-induced seizures and prevent the occurrence of seizure-related brain damage. The resistance of seizures produced by pilocarpine in rats to antiepileptic drugs reaffirms the clinically obvious lack of effective treatments for limbic convulsions. The substantia nigra modulates propagation of seizure activity in the rat forebrain. Inhibition of nigral output neurons by microinfusions of GABA agonists or excitatory amino acid antagonists into the pars reticulata protects rats from electrically and chemically induced convulsions and suppresses amygdala-kindled and pilocarpine-induced seizures. Wasczak et al. (Soc. Neurosci. Abstr., 11: 41, 1985) showed that in rats clonazepam, phenobarbital, valproic acid and trimethadione, drugs which protected against pilocarpine-induced seizures, inhibit firing of non-dopaminergic nigral neurons. Carbamazepine and diphenylhydantoin, drugs which offered no protection against pilocarpine seizures, did not alter firing of nigral cells. Interestingly, ethosuximide, a drug which acted as a proconvulsant in the pilocarpine model of seizures, markedly elevated firing rate of nigral non-dopaminergic neurons. These findings suggest that the efficacy of protective action of antiepileptic drugs against seizures induced by pilocarpine correlates well with their action on neuronal activity in the substantia nigra pars reticulata.

245.10 NIGROSTRIATAL DOPAMINE REGULATES THE SEIZURE THRESHOLD OF THE LIMBIC FOREBRAIN. E.A. Cavalheiro* and L. Turski (SPON: B.B. Lee). Dept. of Neurol. Neurosurg., Lab. of Exp. Neurol., Escola Paulista Med., CEP-04023 Sao Paulo, SP, Brazil and Dept. of Pharmacol., Med. Sch., PL-20090 Lublin, Poland.

Seizures produced by pilocarpine have been found to be useful for studying preventive and therapeutic approaches to temporal lobe epilepsy and optimal in delineating morphological substrates of the brain induced in the spread and termination of convulsions. There is experimental evidence that the basal ganglia influence initiation and expression of generalised and of limbic seizures. However, precise pathways in the basal ganglia and their specific neurotransmitters determining the spread of seizures have not been identified. The substantia nigra has been proposed as a site at which anticonvulsant activity related to -aminobutyrate-mediated inhibition is operative. Blockade of excitatory neurotransmission in the substantia nigra also raises the threshold for seizures and curtails their motor expression. Dopamine (DA) is the principal neurotransmitter of the nigral efferent pathway terminating in the striatum. We have recently observed that rats infused with N-methyl-D-aspartate (NMDA), an excitatory amino acid, into specific subregions of the ventral mid-striatum are protected from convulsant action of pilocarpine. We now present evidence that DAergic pathway linking the substantia nigra pars compacta and striatum regulates the seizure threshold of the limbic forebrain in rats. Microinjections of NMDA, 200 pmol, bilaterally, into the substantia nigra pars compacta protected rats against seizures and seizure-related brain damage produced by pilocarpine hydrochloride, 380 mg/kg. Microinjections of apomorphine hydrochloride, 5-50 pmol, bilaterally, into the striatum conferred protection against behavioral, electrographic and morphological sequelae of pilocarpine-induced seizures. Lesioning the nigrostriatal DAergic pathway (by bilateral intrastriatal microinjection of 6-hydroxydopamine, 25 nmol, in rats pretreated with pargyline hydrochloride, 50 mg/kg, and desmethylimipramine, 25 mg/kg) converted a subconvulsant dose of pilocarpine, 200 mg/kg, into convulsant one. Focal microinjections of haloperidol, 2.5-10 nmol, into the striatum moderately lowered the threshold for seizures produced by pilocarpine, 200 mg/kg. The present results demonstrate that an increase in the DAergic transmission in the rat striatum results in a profound anticonvulsant effect. Microinjections of apomorphine into the striatum not only blocked the motor expression of seizures induced by pilocarpine but also prevented the evolution of electrographic activity in the hippocampus and cortex as well as totally prevented the seizure-related brain damage. These observations indicate that DAergic neurotransmission within the striatum modulates the seizure threshold of the limbic system and define the striatal output pathways that are responsible for this effect.

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245.11 CHOLINERGIC FUNCTION IN ACQUISITIONAL AND POSTICTAL INVESTIGATIONS OF RAT OLFACTORY BULB KINDLING. C.R. Lupica* and R.F. Berman. (SPON: J.A. Benjamins). Dept. Psychology and Neuroscience Program Wayne State University, Detroit, Mi.

The cholinergic system has been implicated in the kindling process by a variety of studies, including dietary (McCann, Cain, and Philbrick, 1983), Pharmacological (Arnold, Racine, and Wise, 1973), and electrophysiological (Burchfield, Duchowny, and Duffy, 1979). In a series of three experiments we have examined the involvement of the cholinergic system in the establishment and maintenance of kindled seizures in the rat olfactory bulb (OB). The importance of acetylcholine (ACh) at the kindled focus and in the development of OB kindling was investigated using unilateral ibotenic acid injections into the lateral preoptic area (LPO). These lesions resulted in a partial disruption of the major ACh projection to OB, as assessed by acetylcholinesterase staining, but did not significantly alter the rate of development of kindling in these animals when compared to controls. However, in a second study, atropine sulfate (25 mg/kg, i.p.) given one hour prior to OB stimulation significantly delayed the development of kindled seizures. This finding has also been demonstrated with the amygdaloid kindling model (Arnold, Racine, and Wise, 1973) and in cortical kindling (Racine, Burnham, and Livingston, 1979). The contrasting results of the above experiments may be due to the possibility that peripherally administered atropine sulfate, which delays the development of OB kindling, does not act directly at the seizure focus, but rather in some other neural region. Alternatively, the level of ACh depletion in the OB following LPO lesions may not have been sufficient to alter the rate of kindling.

A third study was performed to investigate the influence ACh may have on posticta1 events in OB fully kindled rats. Atropine sulfate (25 mg/kg, 5 mg/kg, 1 mg/kg, or 0.2 mg/kg) or sterile saline was administered intraperitoneally, one half hour prior to electrical stimulation. When compared to saline the two highest drug doses significantly prolonged behavioral depression, defined as the absence of movement following a seizure, while the two low doses did not. In addition, atropine sulfate at the two highest doses resulted in a significant potentiation of postictal spiking frequency (spikes/min.) after the third minute following a seizure when compared to saline, while the low doses did not. These findings indicate a role for ACh in the suppression of neuronal excitability during the postictal phase following a kindled seizure, but do not support the contention that ACh is central in the development of a kindled seizure focus.
(Supported by NIH Grant RR08167-08)

245.12 CHOLINERGIC METABOLISM AND PHARMACOLOGICAL CHARACTERIZATION OF STATUS EPILEPTICUS INDUCED BY LITHIUM AND PILOCARPINE. R.A. Morrisett, R.S. Jope and O.C. Snead, Depts. of Pharmacology and Pediatric Neurology, University of Alabama at Birmingham, Birmingham, AL 35294.

We recently described (Exp. Neurol. 91:471, 1986) a model of generalized convulsive status epilepticus (GCSE) induced in rats by administration of lithium and pilocarpine. These reproducible and long-lasting seizures are (1) initiated by stimulation of muscarinic receptors, (2) dependent upon presynaptic cholinergic activity, (3) capable of recruiting noncholinergic neurons and (4) blocked by pretreatment with diazepam. We have characterized this model of GCSE more completely and investigated the effects of lithium and seizures on cholinergic neurochemistry.

Further EEG results include: (1) lithium potentiates cholinergic agonists differentially, (2) the EC_{50} of lithium is 1.5-3.0 mEq/kg when the pilocarpine dose is 30 mg/kg, (3) chronic administration of lithium also potentiates the convulsant dose of pilocarpine and the EC_{50} of the agonist is 10 mg/kg, (4) frequency analysis of seizures reveals a 100% increase in the percentage of time spent in frequency band 2 (3-6 Hz) in every region.

Since adenosine agonists are potent anticonvulsants we are studying the ability of l-phenylisopropyladenosine (l-pia) to inhibit the initiation and propagation of GCSE. Administration of l-pia (2 mg/kg, i.p.) prior to pilocarpine treatment prevents GCSE. Ongoing GCSE is not altered by the same dose of l-pia. The anticonvulsant effect of l-pia is prevented by theophylline which also decreases the latency of GCSE.

Neurochemical determinations include assays of pre- and postsynaptic cholinergic processes. Prior to seizure initiation acetylcholine (ACh) levels fall 30% but during GCSE ACh content rise to maximum levels 300% and 500% of control in hippocampus and cortex, respectively, after 2 hr of seizures. High affinity choline transport (HAChT) is not altered in any region tested by any drug treatment. Since HAChT is thought to be the rate limiting step of ACh synthesis, disruption of regulatory mechanisms controlling ACh synthesis may occur during GCSE caused by lithium and pilocarpine.

We are interested in the role of muscarinic stimulated phosphatidylinositol turnover in the potentiation of pilocarpine induced GCSE by lithium. From EEG studies we have shown that lithium potentiates partial muscarinic agonists much more effectively than full agonists. Lithium may alter the inositol response to pilocarpine and therefore potentiate the effectiveness of the agonist. We are presently investigating these interactions in rats treated chronically with lithium.
Supported by NIMH grant MH38752.

245.13 OSCILLATION OF ACETYLCHOLINE CONCENTRATION IN MOUSE BRAIN REGIONS 0.4 TO 7 SECONDS AFTER ELECTROSHOCK. A. Sawa*, W.B. Stavinoha*, and A. Modak. Dept. of Pharmacol., Univ. of Texas HSC at San Antonio, San Antonio, TX 78284.

To study the response of cholinergic neurons during tonic convulsions following electroshock (ES), the acetylcholine (ACh) concentration in mouse brain regions was measured. Male ICR mice, weighing 25-35 g were placed in a plastic holder, inserted into the chamber of the microwave apparatus and the instrument was tuned to the mouse. The mouse was then given ES at 85 mA and 150 Hz with 0.8 msec. duration for 0.4 sec. This produced tonic convulsions for about 10 sec. followed by clonic convulsions for about 40 sec. The mouse was sacrificed 0.4 - 6.9 sec. after ES by microwave irradiation at 6.0 kw for 0.29 - 0.33 sec. using a NJE 2603, 10 kw, 2450 MHz, New Japan Radio Co., Ltd. instrument. Control animals were sacrificed without ES. The mouse brain was dissected into frontal cerebral cortex, parietal cerebral cortex, hippocampus, neostriatum, olfactory bulb, basal forebrain part 1 which contains globus pallidus and basal magnocellular nucleus, basal forebrain part 2 which contains medial septal nucleus and nucleus of the ventral limb of the diagonal band, fimbria hippocampi, thalamus and hypothalamus, and midbrain and medulla-pons. ACh in the tissue was extracted and measured by pyrolysis-GC.

ACh concentration in all the brain regions studied following ES showed a repeating sine-like waveform as a function of time. ACh concentration decreased immediately after ES to the trough which was 46 to 76% of control value depending on the region, then increased to the peak which exceeded in most regions the mean ACh concentration in control animals. ACh synthesis and release in the neurons in all regions was synchronized with a period of 2.0 - 2.6 seconds. The synthesis rate of ACh as calculated from the slope of the rising phase was several fold higher than the generally accepted choline acetyltransferase activity measured in vitro.

It is possible that each cholinergic neuron oscillates in constant cycles of synthesis and release of ACh which are normally not apparent because they are asynchronous, and strong ES may synchronize the cycles for a time, and/or the cycle of release and synthesis of ACh in cholinergic neurons after ES may coincide with the brain seizure caused by ES.

245.14 PONTINE MICROINJECTION OF CARBACHOL PRODUCES A DOSE DEPENDENT INCREASE IN DESYNCHRONIZED (D) SLEEP SIGNS. H.A. Baghdoyan, R. Lydic, C.W. Callaway and J.A. Hobson. Lab. of Neurophysiology, Harvard Medical School, 74 Fenwood Rd., Boston, MA 02115.

Cholinoceptive/cholinergic mechanisms of the pontine reticular formation (RF) have been hypothesized to play a crucial role in generating D sleep. Microinjection of carbachol into the pontine RF produces a state (D-carb) which is behaviorally and electrographically similar to physiological D sleep. The present study sought to futher characterize D-carb by examining the dose dependent effects of carbachol on D sleep signs in the cat.

Six doses of carbachol (0.01, 0.04, 0.1, 0.4, 1.0, 4.0 micrograms) were administered in 250 nl saline through chronically implanted guide tubes aimed at the rostral pontine RF. Because of the site-dependent nature of the cholinoceptive response, all injections were made into the same site. There were 3 injections per dose and 3 saline control injections, for a total of 21 injections. The two dependent measures were latency to onset of the first D-carb episode (D latency), and percentage of total recording time (4 hrs) spent in the D sleep-like state (D percentage).

The shortest D latency (mean ± S.D.=10.4 ± 2.3 min) was obtained by microinjecting the highest dose of carbachol. D latency doubled (23.6 ± 4.8 min) with the middle dose (0.1 ug), but still remained well below control levels (87.1 ± 21.6 min). The lowest dose had no effect on D latency (77.6 ± 10.0 min). The highest D percentage (80.8 ± 7.9%) was evoked by 4.0 ug of carbachol, and the lowest D percentage (6.7 ± 6.4%) occurred following the lowest dose of carbachol. The middle dose (0.1 ug) doubled D percentage (34.7 ± 1.6%) as compared with control (14.8 ± 7.3%).

The time course of the cholinoceptive response also was dose dependent. Following the two high doses of carbachol D percentage reached its peak level two hours post injection, then began to decline, and by the end of the 4th hr post injection, D percentage was still more than 200% above control levels. After the two middle doses of carbachol D percentage peaked during the 1st hr post injection and declined to contol levels during the 3rd and 4th hrs. The two lowest doses displayed time course profiles indistinguishable from saline controls.

These findings suggest that the phenomenon of carbachol-induced D sleep sign enhancement is mediated by acetylcholine receptors, and further support the cholinergic hypothesis of D sleep generation.

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245.15 CENTRAL ADMINISTRATION OF NEOSTIGMINE (NEO) ALTERS THE DISCHARGE FREQUENCY OF PONTO-GENICULO-OCCIPITAL (PGO) WAVES. R. Lydic, H.A. Baghdoyan, C.W. Callaway, and J.A. Hobson. Lab. of Neurophysiology, Harvard Medical School, Boston, Ma 02115

Microinjection of Neo into the pontine brain stem produces a behavioral state which is polygraphically and behaviorally similar to desynchronized (D) sleep. This pharmacological method for inducing a D sleep-like state (D-Neo) has been used to study the cholinergic hypothesis of D sleep generation and to draw inferences concerning sleep-dependent changes in autonomic function. Although such studies are predicated on the homology between D sleep and D-Neo, the similarities and differences between these two states remain incompletely understood. Since PGO waves occur during both D sleep and D-Neo, the present study is examining the cholinergic control of D-Neo and D sleep by quantitatively comparing PGO discharge frequency during each state.

Cats were implanted with guide tubes aimed for the pontine reticular formation into which 250 nl of saline or Neo was injected in doses of 0.2, 3., 6., or 20. micrograms. Following each microinjection, behavioral states and PGO wave frequency (waves/s) were recorded for 4 hrs.

PGO wave frequency (mean ± S.D.) averaged across all behavioral states was 0.13 ± 0.71 for saline and with increasing doses of Neo was: (0.06 ± 0.2), (0.31 ± 0.4), (0.64 ± 0.5), and (0.83 ± 0.6) waves/s. These differences were statistically significant by analysis of variance (Anova) ($p<0.0001$) and were attributable to behavioral state ($p<0.0001$), time post-injection ($p<0.0001$), and dose level of Neo ($p<0.002$). During D sleep after saline injections, PGO waves averaged 1.3 ± 2.1 waves/s. During the D sleep-like state evoked by increasing doses of Neo, PGO waves averaged: (0.87 ± .5), (0.81 ± .4), (0.88 ± .4), and (1.0 ± .5) waves/s. These differences were significant by Anova ($p<0.0001$). T-test comparisons revealed PGO wave frequency associated with all but the lowest dose of Neo to be significantly less than PGO wave frequency recorded during D sleep.

Therefore, across all behavioral states Neo produced a dose dependent increase in PGO wave frequency. During D-Neo, the PGO wave frequency was always less than the frequency of PGO waves during D sleep. Within D-Neo, however, increasing drug levels produced an increase in the frequency of PGO waves. Thus, D sleep and D-Neo are phenomenologically similar, but there are differences in PGO wave discharge frequency between the two states.

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245.16 FRONTAL DECORTICATION AND RESISTANCE OF CHOLINERGIC NEURONS IN RAT STRIATUM TO AGONISTS IN VIVO: RESTORATION STUDIES. S. Consolo*, G.L. Forloni*, G. Fisone* and H. Ladinsky* (SPON: A. Giachetti). Istituto di Ricerche Farmacologiche "Mario Negri", Milan 20157, Italy, and Istituto De Angeli, Milan 20139, Italy

We have recently reported that frontal cortical deafferentation of the striatum reduces the tone of the cholinergic neurons in this area and renders them resistant to the neuropharmacological action of a number of agonists of the opiate, dopaminomimetic, cholinomimetic, gabamimetic and purinergic classes capable of depressing cholinergic activity reflected as an increase in acetylcholine (ACh) content. The aim of this work was to restore the cholinergic action of one or more of the agonists by drugs or lesions. Thus, choline chloride, at the i.p. dose of 100 mg/kg, did not significantly affect striatal ACh in sham-operated rats or in decorticated animals but, when administered prior to R-apomorphine (APOM), methadone (METH) or 2-chloroadenosine (2-CADO) in the decorticated rats it allowed these drugs to express their full inhibitory effects on the cholinergic neuron. In addition, the refractoriness of the cholinergic neurons to APOM (but not to that of METH or 2-CADO) in the decorticated group was completely restored when we combined the cortical deafferentation with an acute (48 h) chemical lesion of the nigrostriatal dopaminergic input. Furthermore, the short-term chemical lesion of the serotonergic nuclei of the median and dorsal raphe reinstated the action of METH but not that of APOM and 2-CADO. The results support the idea that the deafferentated cholinergic neurons, already inhibited by the loss of the excitatory input, cannot be further depressed by drugs that normally slow down their activity and their ACh turnover rate but that these neurons are capable of a response (therefore potentially functional) when certain stimuli (choline chloride pretreatment or selctive lesions) are applied. Furthermore, these findings, providing insights into the cortical mechanisms regulating subcortical function, suggest that there are different populations of the cholinergic neurons in the striatum, perhaps all being under the excitatory control of the cortical (glutamatergic) neurons, but having different types of inhibitory control.

245.17 FURTHER EVIDENCE OF A CHOLINERGIC MECHANISM IN THE FUNCTION OF THE CORPORA CAVERNOSA PENIS. Dail, W. G., R. W. Hamill and N. Minorsky. Department of Anatomy, Univ. of New Mexico School of Medicine, Albuquerque, New Mexico and Monroe Community Hospital/Univ. Roch. Med. Ctr. Roch., NY.

The atropine resistance of nerve-induced penile erection and data that adrenergic blocking agents elicit erection have raised doubts that acetylcholine is the neurotransmitter released from postganglionic parasympathetic neurons in the penis. However, the recent demonstration of cholinergic muscarinic receptors in the corpora cavernosa penis strongly suggests the presence of cholinergic nerves in erectile tissue.

To determine if cholinergic nerves are present in the penis, choline acetyltransferase (CAT), a biochemical marker of cholinergic neurons, was measured in the erectile tissue of intact rats and in rats in which postganglionic fibers from the pelvic plexus were interrupted. In addition, the distribution of acetylcholinesterase positive (AChE+) nerve fibers in erectile tissue was assessed in control and axotomized rats. Seven days following axotomy of penile fibers from the pelvic plexus, total CAT activity in the erectile tissue fell from 5.47 ± 0.3 nmoles/hr to 1.621 ± 0.14 nmoles/hr. Total protein also fell from the control level of 373.5 µg ± 34.93 to 241.5 ± 10.96 µg in the denervated erectile tissue. When calculated as enzyme specific activity, CAT levels in the control tissue were 15.2 ± 1.5 pmoles/hr/microgram protein compared to 6.7 ± .37 pmoles/hr/microgram protein in the denervated tissue. Axotomy of nerve fibers from the pelvic plexus reduced the incidence of AChE+ nerve fibers around penile arteries and their branches in the erectile tissue. The density of AChE+ nerve fibers over the columns of intrinsic smooth muscle around cavernous spaces was also greatly decreased after axotomy. This study has shown that total CAT activity fell approximately 70% following axotomy of postganglionic penile neurons. When corrected for protein loss, enzyme specific activity fell by 56%. Remaining CAT activity and AChE+ fibers in the erectile tissue of denervated animals are probably due to penile neurons which lie distal to the site of lesion. Our results, which document a rich cholinergic innervation of penile erectile tissue, indicate that acetylcholine is an important neurotransmitter in the regulation of penile erection. Supported by NIH 1R011NS19839-03.

ACETYLCHOLINE: RECEPTOR ACTIONS

246.1 ELECTROLYTIC LESIONING OF THE HABENULA REDUCES THE PHOSPHORYLATION OF DISCRETE PROTEINS IN THE INTERPEDUNCULAR NUCLEUS. W.E. Heydorn, J. Patel*, G.J. Creed*, K.Q. Nguyen* and D.M. Jacobowitz, FDA, Rockville, MD. 20857, Lab. Clin. Sci., NIMH, and Psychobiology Branch, NIMH, Bethesda, MD 20892

Phosphorylation of individual proteins is an important step in the process of transforming extracellular signals into biologic responses. Thus, phosphoproteins are believed to play a major regulatory role in cell and neuronal functioning. Previous studies on phosphoproteins in brain have centered on identifying those proteins whose phosphorylation is regulated by specific second messengers (calcium, cyclic AMP, cyclic GMP). However, since intracellular second messengers are regulated by first messengers, such as neurotransmitters, it would seem appropriate to investigate the influence of specific neurotransmitters on the phosphorylation of individual proteins in brain. Thus, the current work was undertaken with the goal of identifying those proteins in the IP whose phosphorylation by cyclic AMP dependent protein kinase is altered by lesioning the habenula. The IP receives its major cholinergic input from the habenula. Therefore, by lesioning the habenula, we are able to study the influence of long-term cholinergic deprivation on the phosphorylation of individual proteins.

Male Sprague-Dawley rats received either a sham or a bilateral electrolytic lesion of the habenula. Five weeks later all animals were killed, their brains rapidly removed and frozen in dry ice. The IP was removed and the proteins within the IP were phosphorylated by using ^{32}P-ATP either in the presence or the absence of 8-bromocyclic AMP. Proteins were then resolved by two-dimensional gel electrophoresis. Gels were dried and exposed to film for 2-4 days. Phosphorylation was quantitated by scanning densitometry.

Twenty phosphoproteins in the IP were analyzed quantitatively over the pH range of 5-7 and Mr range of 100-10 kDa. Of these 20 proteins, 11 showed an increase in phosphorylation in sham lesioned animals as a result of cyclic AMP addition to the incubation bath. One protein was identified whose phosphorylation was inhibited by cyclic AMP. Lesioning the habenula resulted in a reduction in the phosphorylation of 3 proteins in the IP in the absence of cyclic AMP. The magnitude of this reduction was 55-83%. In the presence of cyclic AMP, a total of 6 proteins in the IP showed a reduction in phosphorylation following habenula lesioning. This reduction ranged from 21-64%. No proteins were identified in the IP whose phosphorylation was elevated by lesioning the habenula.

These results demonstrate that removal of the neuronal input from the habenula to the IP can significantly reduce the phosphorylation of individual proteins in the IP. The presence of a cholinergic input from the habenula to the IP suggests that acetylcholine may influence the phosphorylation of these proteins in vivo.

246.2 ACETYLCHOLINE INDUCES THE PHOSPHORYLATION OF AN 83,000 MOLECULAR WEIGHT PROTEIN IN SLICES OF RAT NEOCORTEX. P.L. Mobley. Dept. of Pharmacology., Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

A major mechanism by which various neurotransmitters in brain exert their influence on the function of target cells involves the activation of specific protein kinases and the subsequent phosphorylation of various intracellular proteins. The identification of these proteins and determination of their function is a key to understanding brain function. This study was conducted to identify proteins in the neocortex whose state of phosphorylation is regulated by cholinergic input.

Slices of the rat neocortex were preincubated for 20 min. at 37°C in oxygenated Kreb's ringer bicarbonate buffer, then washed and incubated in oxygenated phosphate-free Kreb's ringer bicarbonate buffer plus 0.5-1.0 mCi ^{32}P-H_3PO_4 for 40 min. at 37°C. After this incubation period either physostigmine (final concentration 100µM) or its saline vehicle was added to the incubation vials. Ten minutes later acetylcholine (final concentration 1mM) or its saline vehicle was added to each of the incubated vials. The incubation was terminated 2 min. following the addition of acetylcholine. Slices were homogenized in 1% sodium dodecyl sulfate and prepared for 2-dimension gel electrophoresis. From autoradiograms prepared from the 2-dimension polyacrylamide gels, the amount of ^{32}P incorporated into various phosphoproteins was estimated.

Using this technique, several proteins have been identified in the neocortex which appear to be phosphorylated by cholinergic dependent mechanisms. The most prominent of these is a protein with a mol. wt. of approximately 83,000 (Mr 83K) and an isoelectric point of approximately 4.0-4.5. In neocortex slices exposed to either 1mM acetylcholine for 2 min. or 100µM physostigmine for 12 min. the amount of ^{32}P incorporated into Mr 83K is enhanced by 50%. Additionally, the combination of acetylcholine plus physostigmine produces an even more dramatic increase in ^{32}P incorporation into this protein; approximately twice that observed in control.

These results suggest that one mechanism by which acetylcholine exerts its influence on target cell function in this brain region is by altering the state of phosphorylation of this Mr 83K protein. While it is not yet clear what protein kinase system is mediating the effect of acetylcholine, studies suggest that this protein is a substrate for a calcium and phospholipid-dependent protein kinase, protein kinase C.

246.3 STIMULATION OF PHOSPHOINOSITIDE BREAKDOWN BY ENDOGENOUS ACETYLCHOLINE IN RAT NEOSTRIATUM. J.L. Góngora* and J. Aceves : (SPON: Oscar C. Ramírez). Dept. of Physiology, Biophysics and Neurosciences. Centro de Investigación del IPN. 07000 México, D.F. México.

Binding studies have shown a high density of muscarinic receptors in neostriatum. Activation of this receptors stimulate breakdown of phosphatidylinositol 4,5-bisphosphate. Supposedly these receptors are normally activated by acetylcholine (Ach) released from cholinergic interneurons. Here we have explored this possibility measuring the accumulation of inositol 1-phosphate (Ins 1P) in striatal slices incubated with physostigmine.

Vibratome obtained slices of rat neostriatum were stabilized in Krebs solution containing 10 mM LiCl, and then incubated for 30 min. in 0.3 µM tritiated myo-inositol. Physostigmine at various concentrations was then added, continuing the incubation for an additional hour.

Extraction and elution of tritiated Ins 1P was done as described elswhere (M.J. Berridge et al., Biochem. J., 206: 587, 1982).

In striatal slices physostigmine stimulated dose-dependently the accumulation of Ins 1P (EC50: 0.9 µM). At 10 µM physostigmine, Ins 1P accumulated 17-fold above basal levels. N-methylatropine (2 µM) completely inhibited the effect of physostigmine, but TTX (1 µM) did not prevent the effect.

The results suggests that endogenously released Ach, presumably from cholinergic interneurons, stimulates phosphoinositide breakdown, the stimulation being mediated by muscarinic receptors. Failure of TTX to reduce the effect of physostigmine suggests spontaneous rather than action potential-evoked Ach release.

(Supported by a grant from CONACyT of Mexico).

246.4 MUSCARINIC RESPONSE IN RAT LACRYMAL GLAND CELLS DECREASES DUE TO LOSS OF A DIFFUSIBLE FACTOR ACTING BEFORE IP_3 PRODUCTION. J. Zimmerberg* and A. Marty*(SPON:S.Simon). PSL,DCRT and LCB, NIADDK, National Institutes of Health, Bethesda, MD and Laboratoire de Neurobiologie, Ecole Normale Superieure, Paris, France.

Often responses of cells studied with the tight-seal whole-cell recording technique differ from those of intact cells and change with time after initiation of whole-cell recording. To measure the liberation of intracellular calcium from internal stores, we monitered the calcium dependent K and Cl currents resulting from application of acetylcholine (Ach, 100-500 nM) to exocrine cells (Marty, A. Y.P. Tan, and A. Trautmann, J.Physiol. 357:293, 1984). The response to Ach diminishes over several minutes after initiation of whole-cell dialysis ("washout") (Trautmann,A., and A. Marty, PNAS, 81:611, 1984).

We applied Ach to cells with a variety of sizes while dialysing with patch pipettes of a variety of sizes. We found that the response was stable for some minutes, then decreased exponentially. The delay and time constant of the washout were directly proportional to the size of the pathway for diffusion (Gs) and inversely proportional to the cell volume ($Cslow^{3/2}$). In large cells with small pipette tips the response remained for more than 40 minutes. Thus washout was not due to receptor desensitisation. It appeared that some soluble intracellular factor, needed after receptor activation, was needed for liberation of intracellular calcium. Comparing the time constant of muscarinic washout to the time constant of the washout of potassium in a cell of similar size with a similar patch access resistance yields an estimate of the diffusion constant of this factor, from which an upper limit estimate of molecular weight of 1000-10000 daltons can be guessed.

Intracellular inclusion of IP_3 liberates calcium, of GTPγS potentiates the muscarinic response and slows washout (Evans, M.G. and A. Marty, PNAS, in press). Is polyphosphoinositide (PIP_2) hydrolysis a step in muscarinic activation? We think so, because adding a PIP binder, neomycin, blocked activation by Ach.

Is washout due to a failure to activate a phospholipase, a depletion of the substrate PIP_2 , or a depletion of calcium stores? Attempts to constantly replenish PIP_2 by inclusion of CTP, ATP, and inositol, while slightly increasing current amplitudes transiently in some cells, did not halt washout. Intracellular inclusion of ATP, GTP, cATP, or cAMP, together or alone, did not stop washout. We observed washout in one cell, gently removed the pipette after no further response to Ach was seen, and sealed to the same cell a new pipette containing IP_3 . A rapid and sustained response was seen upon initiation of the whole-cell mode. We conclude that the washout is due to the loss of a diffusible factor which acts after muscarinic receptor binding and before polyphosphoinositol release. We suspect the action of factor to involve a G protein.

246.5 AGONIST-STIMULATED HYDROLYSIS OF PHOSPHOINOSITIDES IN BRAIN SLICES AND MEMBRANES. Tamara L. Casebolt*, Gail V.W. Johnson and Richard S. Jope (SPON: J. Monti). Department of Pharmacology and Neuroscience Program, University of Alabama at Birmingham, Birmingham, AL 35294

Receptor-stimulated phosphoinositide hydrolysis is important for signal transduction in a wide variety of cell types. In brain, responses to agonists are relatively weak, and agonist-responsive cell free preparations have not been reported. This study outlines methods to maximize the agonist-induced release of inositol phosphates from rat cerebral cortex slices and membranes, investigates the regulation by calcium of this process, identifies the inositol phosphate products that are released upon receptor activation in both preparations, and identifies the dose-dependent effects of a phorbol ester (PMA) and of N-ethylmaleimide (NEM) on inositol phosphate release.

Cortical slices from male Sprague-Dawley rats were subjected to 3 consecutive incubations: a preincubation to restore energy charge, a labelling incubation in the presence of 0.53 µM [^{3}H]myo-inositol and a hydrolysis incubation after which the inositol lipids and aqueous products were extracted. Exclusion of calcium from the first two incubations produced a 250% increased labelling of inositol lipids. When calcium (1.3mM) was added back to the hydrolysis incubation in the presence of lithium chloride (10mM), carbachol (5mM) stimulated a 573% increase in aqueous product release over basal.

Cortical slice membranes were prepared from brain slices which were preincubated and labelled without calcium; the slices were thoroughly rinsed, vigorously homogenized, and centrifuged 10 minutes at 10,000 x g. The membranes were resuspended in buffer and the ability to respond to carbachol was investigated. An ED_{50} of 100 µM carbachol was observed for both membranes and slices. Calcium dose-dependently enhanced basal and carbachol stimulated hydrolysis in membranes; the greatest enhancement above basal occurred at the highest concentration of calcium (1.3mM). The major product of carbachol stimulation in slices was inositol-1-phosphate, while in membranes the production of inositol-1-phosphate, inositol bisphosphate and inositol triphosphate were stimulated approximately equally by carbachol. Lithium was required for detection of products in both slices and membranes. In brain slices, PMA and NEM significantly inhibited carbachol stimulated phosphoinositide hydrolysis in a dose-dependent manner. In membranes, GTP stimulated phosphoinositide hydrolysis. These results support recent suggestions that guanine nucleotide regulatory proteins are associated with receptor-coupled phosphoinositide hydrolysis. Supported by NIMH grant 38752 and NIA grant AG04719.

246.6 INHIBITION OF MUSCARINIC RECEPTOR-MEDIATED CYCLIC GMP FORMATION BY PHORBOL ESTERS IN MOUSE NEUROBLASTOMA CELLS. W.S. Lai* and E. E. El-Fakahany. (SPON: D.J. Pettibone). University of Maryland School of Pharmacy, Baltimore, MD. 21201.

Protein kinase C mediates the transduction of extracellular signals into intracellular events. Activators of protein kinase C include diacylglycerol produced from inositol phospholipids in response to extracellular stimuli, and tumor promoting phorbol diesters. These potent tumor promoters are known to affect agonist binding to various neurotransmitter receptors and to alter receptor mediated responses. Since cGMP formation is an established cellular response to muscarinic acetylcholine receptor activation in clone N1E-115 of mouse neuroblastoma cells, it was of interest to test the susceptibility of this response to modulation by phorbol diesters. Phorbol 12-myristate 13-acetate (PMA) treatment of cells at 37°C induced a reduction in carbamylcholine (CBC)-induced [^{3}H]cGMP formation with a $t_{1/2}$ of inhibition of 20.7 min and an IC_{50} of 50nM. Pretreatment of cells with 100nM of PMA for 60 min at 37 °C did not affect the basal level of [^{3}H]cGMP compared to control but resulted in decreasing [^{3}H]cGMP responses to 1mM CBC and 0.1mM histamine to 35% and 33%, respectively. However, [^{3}H]cGMP formation through direct activation of guanylate cyclase by sodium azide was not inhibited by PMA. In addition, PMA had no effect on [^{3}H]cGMP synthesis induced by calcium ionophore A23187, which activates guanylate cyclase bypassing neurotransmitter receptors. In the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, there was a small loss in the ability of PMA in inhibiting CBC-stimulated [^{3}H]cGMP response, suggesting that PMA has little, if any, effect on activation of phosphodiesterase. PMA did not affect [^{3}H]N-methylscopolamine binding in intact cells, indicating the absence of effects on muscarinic antagonist binding sites. Our data suggest that PMA exerts its effect on muscarinic receptor mediated cGMP formation either through changing the receptor conformation for agonists or by inactivating the coupling mechanism between the receptor and guanylate cyclase. (Supported by a contract from the U.S. Army Research Office #DAAG-29-85-K-0123).

246.7 AUTORADIOGRAPHIC STUDIES ON ^{3}H-QNB AND ^{125}I-SOMATOSTATIN BINDING SITES IN THE RAT FRONTAL CORTEX AFTER KAINIC ACID INJECTION INTO THE NUCLEUS BASALIS OF MEYNERT. S.Katayama, S.Kito, Y.Yamamura, S.Inagaki and H.Matsubayashi 3rd Dept. of Int. Med., Hiroshima Univ. Sch. of Med, Kasumi, Hiroshima, 734 Japan

Cell loss of the nucleus basalis of Meynert (nBM) is thought to be responsible for the cholinergic deficit seen in Alzheimer's disease. In addition, decreased somatostatin (SOM) content in the cerebral cortex has been reported. We attempted to evaluate some correlative aspects of muscarinic acetylcholine (mACh) and somatostatin binding sites in the cerebral cortex autoradiographically. Male Wistar strain rats (180-220 gm) were injected 2ul of kainic acid (1mg/ul) into the ipsilateral nBM. For mACh receptors ^{3}H-QNB (33.2 Ci/mmol, NEM) was used as ligand. Binding experiments were performed under the condition of 30 min incubation with ^{3}H-QNB (0.5 nM) in 50 mM Tris-HCl (pH 7.4) with or without 1 uM of atropine. The cryostat sections were then washed in the buffer for 5 min at 0 C. For somatostatin binding sites, ^{125}I-SOM-14(Tyr^{11}) was used as ligand. The frontal sections were preincubated at room temperature for 30 min in 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM of $MgCl_2$, 32 mM of sucrose and 1% BSA. Incubation was carried out in the same buffer plus bacitracin (0.5 ug/ml) at room temperature for 60 min in the presence of $5x10^{5}$ cpm of ^{125}I-SOM per ml with or without 10^{-6}M of unlabeled D-Trp8 -SOM. The sections were then washed for 5 min at 0 C. Obtained autoradiographic films were then computer-analyzed by IBAS II (Zeiss).

Through these experiments, we obtained the following results. Seven days after injection of kainic acid, ^{3}H-QNB binding sites were increased in the injection side in comparision with the control side. ^{3}H-QNB binding sites were decreased in the superficial layers (I and II) of the frontal cortex at the level of AP +0.2 mm to -0.6 mm, but didn't change in the deeper cortical layers, while AChE staining was reduced in the III-V layers in the lesioned side with serial sections in consistence with the previously reported distribution of acetylcholine and choline-acetyltransferase. This suggests that cholinergic degeneration in the III-V layers of the frontal cortex following the injection of kainic acid into the nBM causes postdenervation supersensitivity of mACh receptor in the I and II layers, likely dendritic areas of cortical neurons. On the other hand, ^{125}I-SOM binding sites, which were densely seen in the V-VI layers of the cortex, didn't change 40 days after the denervation of cortical cholinergic fibers. All these results showed that postdenervation supersensitivity caused the increase of ^{3}H-QNB binding sites on the 7th day after denervation. Besides, our observation on ^{125}I-SOM autoradiographs showed that on the 40th day there were no effects on ^{125}I-SOM binding sites by injection of kainic acid into the nBM.

246.8 SUPRESSIVE EFFECTS OF BOTH ACETYLCHOLINE AND SMALL CARDIOACTIVE PEPTIDE $_{B}$ ON THE EVOKED GWR OF THE ISOLATED APLYSIA GILL ARE BLOCKED BY CURARE. D.R.L. Cawthorpe*. W.F. Colmers and Ken Lukowiak (SPON: B. A. McVicar). Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1

The classical neurotransmitter, acetylcholine (Ach), induces both a gill contraction (at concentrations greater than 5 uM) and a reduction in baseline tension (at these and lower concentrations) when perfused through the isolated gill of Aplysia californica. Ach-induced contractions are blocked by the antagonist atropine; the reduction of baseline tension is mimicked by the agonist carbachol. Carbachol also inhibits p-chlorophenylthiocyclic AMP induced contractions of the isolated gill; this effect is blocked by the nicotinic antagonist curare. The endogenous peptide, small cardioactive peptide (SCP_B) is a potent inhibitor of the gill withdrawal response (GWR) evoked by mechanical stimulus to the isolated, perfused gill. That curare also blocks the supressive effects of SCP_B supports the hypothesis that SCP_B acts via a cholinergic mechanism. In this study, we examined the effects of Ach on the evoked GWR of the isolated, perfused gill. In addition, the effects of the co-perfusion of the nicotinic antagonist, curare were examined.

Preparations consisted of the siphon, mantle and gill of 150-300g Aplysia californica, with the abdominal ganglion and opaline and gametolytic glands removed. The preparation was positioned dorsal side down and pinned to the Sylgard base of a lucite dish, filled with artificial seawater (ASW; Instant Ocean) maintained at 15 ^{0}C. The perfusate, ASW, was cooled to the same temperature, and delivered to the afferent branchial gill vein via a glass cannula. Experimental solutions were prepared immediately prior to use. A suture thread, tied to a single gill pinnule, was attached to an isotonic force transducer (Grass FT03C), connected to a Grass polygraph. The GWR was evoked by a mechanical stimulus to the gill by a mechanical tapper.

The results show that perfusion of Ach at concentrations lower than 5 uM generally did not produce a gill contraction but did produce a 40% supression of the GWR at concentrations as low as 100 nM. Ach induced supression of the GWR were dose-dependent and reversible. After washout and recovery, Ach was co-perfused with curare 10 M which was found to block the supressive effects of Ach on the evoked GWR over the range of concentrations tested. That Ach and SCP_B both produce supression of the evoked GWR which is blocked by curare provides further evidence supporting the hypothesis that SCP_B acts via an inhibitory cholinergic mechanism. Supported by the MRC of Canada.

246.9 CHARACTERIZATION OF AN ENDOGENOUS MUSCARINIC AGONIST FROM BRAIN THAT IS NOT ACETYLCHOLINE. J. Baumgold. Clinical Neurogenetics Branch, N.I.M.H., Bethesda, MD 20817

Recent reports (J. Neurochem. 44 622, 1985 and 45 710, 1985) have described an endogenous substance from brain and from thymus which inhibits ^{3}H-QNB binding to muscarinic receptors. We have confirmed these findings, have characterised this factor further and show that it behaves like a muscarinic agonist in inhibiting adenylate cyclase.

Rat brain was homogenized in various solutions (1 gm of brain in 32 ml), the homogenate was centrifuged at 86,000 x g for 45 min. and the supernatants were used as the media for ^{3}H-QNB binding studies. We compared the amount of ^{3}H-QNB bound to washed membranes in these supernatants with the amount bound in media without supernatant. We found that when brain was homogenized in Krebs solution, the amount of binding to membranes in the presence of supernatant was 72 $\pm$ 6 % of the value in Krebs without supernatant, suggesting that the supernatant contained a factor that was inhibitory to the binding. Since this supernatant had not lost any inhibitory activity after having been incubated with membranes for over 24 hours, the factor can not be acetylcholine since acetylcholine would have been degraded by the cholinesterase. Divalent-ion-free Krebs failed to solubilize appreciable amounts of this factor since the binding in the divalent-ion-free supernatant was 95 $\pm$ 3 % of that in the same solution without supernatant. Of several other solutions examined 0.1 N HCl released the most factor. After neutralization, ^{3}H-QNB binding in HCl extract was 27 $\pm$ 13 % of that in neutralized HCl without supernatant.

Acid-extracts of brain were made and the acitvity of this extract in inhibiting prostaglandin E1 (PGE1) stimulated adenylate cyclase was studied. For these experiments, NG108-15 neuroblastomaglioma cells were grown in culture. These cells posses muscarinic receptors that are coupled in an inhibitory fashion to PGE1-stimulated adenylate cyclase. The activity of the cyclase was determined by assaying the amount of cAMP produced in 10 min. in response to PGE1 stimulation. We found that, like carbachol, the acid-extract of brain inhibited the cyclase in a dose-dependant manner and, thus, can be considered to be a muscarinic agonist.

These findings indicate that brain contains a factor other than acetylcholine that inhibits ^{3}H-QNB binding, and that behaves like a muscarinic agonist in inhibiting adenylate cyclase.

246.10 ACETYLCHOLINE MODULATES TWO TYPES OF POTASSIUM CONDUCTANCES IN AMPHIBIAN MOTOR NERVE TERMINALS. E.Hevron*, G.David* and Y.Yaari (SPON: A.K. Asbury). Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel.

In the vertebrate neuromuscular junction, the primary function of presynaptically-stored acetylcholine (ACh), is to excite the postsynaptic muscle fibre upon its release by a motor nerve impulse. It has been suggested previously that ACh may also modulate presynaptically the excitability of motor nerve terminals, but the mechanisms underlying this action have not been resolved. We have used the curarized frog (rana ridibunda) cutaneous pectoris nerve-muscle preparation to investigate how ACh affects delayed rectifier (gK,V) and calcium-activated (gK(Ca)) potassium conductances (gKs) in motor nerve terminals. External microelectrodes were used to record local circuit currents from the perineurium of preterminal motor axons during spike activation.

In normal Ringer solution the delayed rectifier potassium current can be identified. ACh, at relatively high concentrations (0.5-2 mM), suppressed it in a dose-dependent manner. The calcium-activated potassium current, which becomes prominent following gK,V blockade by aminopyridines, was similarly suppressed by ACh in comparable doses. Since these gKs are responsible for spike repolarization in motor nerve terminals, their blockade by ACh prolonged the duration of presynaptic depolarization. ACh effects were quickly reversible upon wash. They were insensitive to atropine and d-tubocurarine and could not be reproduced by muscarine or carbachol.

We conclude that ACh blocks gK,V and gK(Ca) in amphibian motor nerve terminals through cholinoceptive sites, which have different properties than classical cholinergic receptors. Possibly ACh exerts a quarternary ammonium-like blocking action on potassium channels, explaining the required high dose. As ACh injection into spinal motoneurons has been shown to block similar gKs in the soma-dendritic membrane, ACh may act from the cytosolic side of the presynaptic membrane. Its influx may be facilitated by the ACh transport system which normally mediates its nonquantal leakage. We suggest that the cholinergic sensitivity of presynaptic gKs provides ACh stores in motor nerve terminals a control over the number of transmitter quanta released by motor nerve impulses.

246.11 CHARACTERISTICS OF NICOTINE-INDUCED RELEASE OF ^{3}H-NOREPINEPHRINE FROM HYPOTHALAMIC SYNAPTOSOMES. C.J. Hillard. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Nicotine and other nicotinic receptor agonists have been shown to regulate monoamine neurotransmitter release from brain slices (Westfall, T.C. Neuropharmacol. 13: 693, 1974). In the present study, the effects of nicotine on ^{3}H-norepinephrine (^{3}H-NE) release from hypothalamic synaptosomes were determined and experiments were begun to assess the role of sodium in the release process.

Male Sprague-Dawley rats were decapitated and hypothalami were dissected. Synaptosomes were harvested using a slight modification of the discontinuous sucrose gradient method of Gray and Wittaker (J. Anat. 96: 79, 1962). Synaptosomes were gently suspended in Krebs Ringer HEPES buffer (KRH) containing (mM): NaCl (118.4), $MgSO_4$ (1.18), KCl (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), HEPES (10) and glucose (11.1) at pH 7.4 unless otherwise noted. Synaptosomes were incubated with ^{3}H-NE (approximately 50 nM) at 37°C for 30 min, then were collected by centrifugation at 3,000 rpm. Aliquots of the ^{3}H-NE loaded synaptosomes were incubated in KRH at 37°C for the desired length of time, then released ^{3}H-NE was separated by filtration through GF/B glass fiber filters.

Concentrations of nicotine between 10^{-7}M and 10^{-4}M produced a concentration-related increase in the release of ^{3}H-NE at 15 min after addition. At 10^{-4}M, ^{3}H-NE release was 112% of nonstimulated release. The increase in release was blocked by the addition of 10^{-6}M mecamylamine. Carbachol also increased ^{3}H-NE release although was about 10-fold less potent.

The time course of nicotine's effects was determined. Nicotine produced a significant increase in release over basal at 3,7,10,15 and 20 min after addition. The increase produced by nicotine (113% over basal at 20 min) was slightly less than that produced by high K^+ (126% over basal).

In order to determine whether nicotine-induced release was dependent upon extracellular sodium, sodium chloride in the incubation media was replaced with equimolar choline chloride. Nicotine had no effect on ^{3}H-NE release in the absence of Na^+. Therefore, these preliminary studies indicate that nicotine will release ^{3}H-NE from hypothalamic synaptosomes in a sodium-dependent manner. Furthermore, nicotine appears to exert this action through a cholinergic nicotinic receptor. (Supported by the PMAF and USPHS grants DA-03725 and 2 507-RR5434-22)

246.12 MECHANISMS OF ASCENDING CONTROL OF THALAMIC NEURONAL ACTIVITIES: ACETYLCHOLINE AND NOREPINEPHRINE. David A. McCormick and David A. Prince. Dept. Neurology Stanford University School of Medicine, Stanford, CA 94305

Ascending cholinergic and adrenergic projections from brainstem innervate the mammalian thalamus and may be involved in regulation of thalamo-cortical information processing. We investigated the actions of ACh and NE in thalamic neurons of rat and guinea pig brain slices maintained in vitro. In the guinea pig ACh induced a direct muscarinic hyperpolarization of all thalamic neurons located in the medial and lateral geniculate nuclei (MGN, LGNd), ventral lateral thalamus, and the nucleus reticularis (nRt) by activating a membrane K+ conductance (McCormick and Prince, Nature 319: 402-405.). In approximately 25% of guinea pig MGN and LGNd neurons, this response was followed by a slow depolarization which was largest near firing threshold and was associated with a decrease in input conductance (G_i) of 5 to 25 nS. In contrast, in rat LGNd, ACh caused predominately the direct slow depolarization and decrease in membrane conductance, with the hyperpolarization being either absent or only very small, indicating that significant species differences in responses to ACh exist in at least the LGNd.

Norepinephrine caused a slow depolarization associated with a decrease in G_i of 2 to 15 nS when applied to neurons in guinea pig MGN, LGNd, nRt, anteroventral and periventricular nuclei. The reversal potential of this response varied in a Nernstian manner when $[K+]_o$ was varied, indicating that it is due to block of a resting K+ conductance. Local electrical stimulation of the LGNd caused (in order) a fast epsp, two phases of ipsp, and a slow epsp. This slow epsp was associated with a decrease in membrane conductance and could last as long as 60 seconds. These characteristics and others indicated that this slow epsp may be mediated by NE, although this has not yet been tested pharmacologically. The early and late ipsps had reversal potentials of approximately -75 and -90 mV respectively in 5 mM $[K]_o$, suggesting an initial increase in $gCl-$ followed by an increase in $gK+$.

Thalamic neurons possess two basic firing modes: single spike firing at membrane potentials positive to approximately -55 mV and burst firing at membrane potentials negative to approximately -65 mV (Jahnsen and Llinas, J. Physiol. 349: 205-226). The hyperpolarizing action of ACh in guinea pig neurons resulted in the inhibition of single spike firing while promoting the occurrence of burst discharges. The slow depolarizing actions of ACh and norepinephrine had the opposite effect, inhibiting burst firing, while promoting single spike activity, and enhancing the cells' general responsiveness. These effects illustrate how the ascending cholinergic and noradrenergic systems may work to modulate the processing of sensory-motor information in the thalamus. Supported by NIH grants NS 12151, 06477 and 07331.

246.13 DESENSITIZATION TO THE STIMULATORY EFFECT OF PHYSOSTIGMINE ON RAT PLASMA ADRENOCORTICOTROPIN : LACK OF INTERACTION WITH NICOTINE B. Sharp*, H.S. Beyer* and K. McAllen* (SPON: J. Gottesman). Dept. of Medicine, Hennepin Cty. Med. Center and Univ. of Minnesota, Mpls, MN 55415.

In the rat, nicotine is a potent stimulus for the secretion of adrenocorticotropin (ACTH). However, we have recently reported that a single dose of nicotine rapidly desensitizes the ACTH response to the subsequent administration of nicotine. The present studies were performed to determine whether cholinergic synapse(s) (1) affect the secretion of ACTH and (2) mediate the secretion of ACTH by nicotine. Initially, 400-500g male Holtzmann rats received indwelling jugular cannulae 24-48h prior to sc injections of saline or physostigmine 0.4 mg/kg BW followed 2h later by physostigmine. Saline(S)/physostigmine (P) 0.2, 0.4 and 0.8 mg/kg BW significantly elevated plasma ACTH levels by +20 min and baselines were restored by +120 min. In contrast, the ACTH responses (expressed as integrated areas) to these doses given 2h after the first dose of physostigmine 0.4 were markedly diminished. ($S/P_{0.4}$ = 485±80 SE pg·min/ml vs $P_{0.4}/P_{0.4}$ = 138±71, p<.01; $S/P_{0.8}$ = 706±192 vs $P_{0.4}/P_{0.8}$ = 238±53 p = .036). Rats were decapitated to obtain trunk blood in further studies designed to evaluate cross-desensitization between physostigmine and nicotine. Physostigmine 0.4 or 0.8 sc, administered 2h prior to nicotine 0.25 mg/kg BW ip, had no effect on the ACTH responses to nicotine ($S/N_{0.25}$ = 529±96 SE pg/ml; $P_{0.4}/N_{0.25}$ = 498±65; $P_{0.8}/N_{0.25}$ = 425±58). Conversely, nicotine 0.5 ip, given 2h prior to physostigmine 0.15, 0.4 and 0.8 mg/kg BW, did not modify the ACTH responses to physostigmine. In two experiments using 26d old rats which showed mature responses to nicotine-induced ACTH secretion, mecamylamine (M) 2 mg/kg BW, a putative nicotinic-cholinergic channel antagonist, significantly inhibited the ACTH responses to nicotine (S/S = 82±7 SE pg/ml; M/S = 131 ±20; $S/N_{2.0}$ = 1838±137; $M/N_{2.0}$ = 473±44; $S/N_{2.0} > M/N_{2.0}$, P < .01 by ANOVA and S/S = 84±8; $S/N_{0.25}$ = 738±87; $M/N_{0.25}$ = 136±22; $S/N_{0.25} > M/N_{0.25}$ P < .01). In contrast, mecamylamine 2-10 mg/kg BW produced a partial nonsignificant inhibition of ACTH responses to physostigmine 0.4. Also, atropine sulfate 25 mg/kg did not modify the ACTH responses to physostigmine.

These studies show that physostigmine like nicotine is a potent stimulus for the secretion of ACTH in rats and that partial desensitization rapidly develops. However, cross-desensitization between nicotine and physostigmine does not occur and mecamylamine significantly inhibits the ACTH response to nicotine but not to physostigmine. Thus, nicotine and physostigmine may stimulate the secretion of ACTH by different mechanisms; whether nicotine stimulates ACTH secretion via a cholinergic synapse is open to question. (Supported by DA03977).

247.1 ANTEROGRADE AND RETROGRADE EVIDENCE FOR A CHOLINERGIC PROJECTION TO THE SUBSTANTIA NIGRA IN THE RAT. L.L. Butcher, E. Gould*, and N.J. Woolf. (SPON: M.L. Schlag-Rey) Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Cholinergic projections to the substantia nigra were studied using retrograde and anterograde tracers combined with immunohistochemical localization of choline acetyltransferase (ChAT; code 11/255; for characterization, see Eckenstein and Thoenen, EMBO J., 1, 363, 1982). Fluorogold, propidium iodide, and true blue were infused into the reticular part of the substantia nigra either iontophoretically (fluorogold) or by pressure injection (propidium iodide and true blue; 0.01-0.03 µl). Two surgical approaches were used for pressure injection. The first approach was made by introducing a microliter syringe perpendicularly into the reticular part of the substantia nigra. Because these injections traversed the compact part of the substantia nigra and the ventral tegmental area, control injections were made by approaching the reticular part of the substantia nigra at a 45 degree angle. In the latter case the cannulae traversed the hippocampus and largely avoided the compact part of the substantia nigra or the ventral tegmental area. Anterograde transport of phaseolus vulgaris leucoagglutinin (PHAL) was traced from ChAT-labeled cells that took up iontophoretically applied PHAL.

ChAT-immunoreactive cell bodies that also demonstrated retrogradely transported fluorescent label following tracer infusions into the reticular part of the substantia nigra were found primarily in the pedunculopontine tegmental nucleus. Numerous noncholinergic cells were also retrogradely labeled in the pedunculopontine tegmental nucleus. A few ChAT-positive somata in the dorsolateral tegmental nucleus, magnocellular preoptic area, and ventral pallidum also retrogradely transported tracers from the reticular part of the substantia nigra. Cholinergic projections from the pedunculopontine tegmental nucleus to the reticular part of the substantia nigra were confirmed with anterograde tracing. Fibers from ChAT-positive cells in the pedunculopontine tegmental nucleus could be traced to the substantia nigra, and PHAL boutons were observed in the reticular part of the substantia nigra.

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247.2 EVIDENCE FOR DESCENDING BASAL FOREBRAIN PROJECTIONS: ANATOMICAL AND PHYSIOLOGICAL STUDIES. K. Semba, P.B. Reiner, J.E. Disturnal, S. Atmadja*, E.G. McGeer and H.C. Fibiger. Division of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada V6T 1W5.

The basal forebrain (BF) has been identified as the source of a major projection system innervating a number of forebrain structures including the cerebral cortex, hippocampus, amygdala and olfactory bulb. While studying the effects of brainstem electrical stimulation upon the activity of BF neurons (Disturnal et al., Neurosci. Abstr.,'86), we encountered an unexpectedly large number of BF neurons which could be antidromically activated from the brainstem. In the present study, the anatomical and physiological properties of this descending projection were examined.

Forty-eight BF neurons in 13 rats were antidromically activated from the brainstem, 32 from the region of the pedunculopontine nucleus, 15 from the dorsal raphe and 3 from the adjacent laterodorsal tegmentum. Two cells were antidromically activated from 2 sites; the remaining cells could be activated from only one site. Latencies varied from 2-28 msec; over 50% were silent in these urethane anesthetized rats. The anatomical counterpart of this descending projection was confirmed by injections of true blue (TB) into the pontomesencephalic tegmentum which resulted in many retrogradely filled neurons in several regions of the BF. That at least some of these retrogradely labelled BF neurons had axons terminating within the brainstem tegmentum was suggested by the presence of anterogradely labelled varicose fibers in these same regions following BF injections of PHAL.

Double labelling studies using brainstem TB injections and cortical injections of propidium iodide demonstrated the existence of a small population of BF neurons which project both to the brainstem and the cerebral cortex. TB labelled neurons were generally medium sized, although the double labelled neurons were notably larger. Preliminary studies suggest that these double labelled neurons are also ChAT immunoreactive.

These data confirm and extend previous studies showing that there exists a significant descending projection from the BF to the brainstem, and, together with our data on brainstem afferents to the BF (Disturnal et al.,'86), suggest that reciprocal connections exist between the basal forebrain and the brainstem. Furthermore, these data suggest that this descending projection contains a minor, possibly cholinergic component which also projects to the cerebral cortex.

(Supported by the Medical Research Council of Canada.)

247.3 ANATOMICAL AND PHYSIOLOGICAL STUDIES OF BRAINSTEM AFFERENTS TO THE BASAL FOREBRAIN. J.E. Disturnal, P.B. Reiner, K. Semba, S. Atmadja*, E.G. McGeer and H.C. Fibiger. Division of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada V6T 1W5.

The basal forebrain contains a population of cholinergic neurons which project to the cerebral cortex. Although the major sources of afferent inputs to the basal forebrain have been identified, detailed information regarding their neurochemical and functional properties is limited. In the present report, anatomical, histochemical, and physiological techniques were used to study brainstem afferents to the basal forebrain.

WGA-HRP was injected into several areas of the basal forebrain. Retrogradely labelled cells were found in many regions of the brain; this study focuses upon those found in the pedunculopontine (PPN), laterodorsal tegmental (LDT), and dorsal raphe (DRN) nuclei. Retrogradely labelled neurons were concentrated ipsilaterally, although contralateral labelling was seen as well. That at least some of these retrogradely labelled brainstem neurons had axons terminating within the basal forebrain was suggested by the presence of anterogradely labelled varicose fibers in the basal forebrain following PHAL injections into the PPN.

Previous studies have shown that the PPN and LDT contain a large population of ChAT positive neurons, and that NADPH diaphorase is a reliable marker for these cholinergic neurons (Vincent et al.,'83). By combining retrograde HRP with NADPH diaphorase histochemistry, it was found that afferents to the basal forebrain arise from both presumptive cholinergic and non-cholinergic neurons in the PPN and LDT.

Since most cortically projecting basal forebrain (CPBF) neurons are cholinergic, extracellular recordings were made from antidromically identified CPBF neurons in anesthetized rats, and their orthodromic responses to brainstem stimulation determined using peri-stimulus time histograms. Thirteen CPBF neurons responded to PPN stimulation; 8 were excited and 5 were inhibited. Five CPBF neurons responded to DRN stimulation; 4 responses were excitatory and one inhibitory.

These data demonstrate the existence of brainstem afferents to the basal forebrain, at least some of which may be cholinergic. Activation of these inputs reveal both excitatory and inhibitory orthodromic influences on cortically projecting basal forebrain neurons.

(Supported by the Medical Research Council of Canada.)

247.4 FOREBRAIN PROJECTIONS OF CHOLINERGIC AND SUBSTANCE P-CONTAINING NEURONS IN THE LATERODORSAL TEGMENTAL NUCLEUS IN THE RAT. K. Satoh and H.C. Fibiger, Div. Neurological Sci., Dept. Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada

The laterodorsal tegmental nucleus (TLD) contains many cholinergic neurons, some of which also contain substance P. Recent retrograde tracing studies combined with immunohistochemistry revealed some forebrain projections from these cholinergic and/or substance P-containing neurons. In the present investigation, efferent fiber projections of this cell group to the medial prefrontal cortex (MPC), lateral septum (LS), and dorsal diencephalon were examined in rats using a variety of neuroanatomical tracing techniques.

Iontophoretic injections of Phaseolus vulgaris leucoagglutinin (PHA-L) into the TLD revealed a long, continuous fiber system that projects to the basal forebrain. The principal route passes through the ipsilateral dorsomedial tegmentum (dorsal tegmental pathway), caudal diencephalon (intermediate diencephalic bundle) and medial forebrain bundle (lateral hypothalamic bundle), and finally enters the diagonal band of Broca. The intermediate diencephalic bundle gives rise to fiber tracts to the parafascicular and lateral habenular nuclei (posterior thalamic path), and to the anterior thalamic nuclei, the lateral part of the mediodorsal nucleus and the intralaminar thalamic nuclei (anterior thalamic path). Rostral to the anterior commissure, PHA-L positive fibers in the diagonal band run dorsally towards the LS, while other fibers pass further rostrally to enter MPC.

Unilateral injection of WGA-HRP or fluorescent dyes (True Blue (TB) or primuline (Pr)) into MPC, LS, anterior thalamic region (AT) or mediodorsal thalamic region (MD) resulted in retrograde labeling of many neurons in TLD. In sections processed for combined retrograde tracing and choline acetyltransferase (ChAT) or substance P immunohistochemistry, ChAT positive neurons were retrogradely labeled by the tracer after injections into each of these sites. In contrast, retrograde labeling of substance P-containing cell bodies were observed after the injection of tracers into MPC or LS, but rarely after MD injections.

Injections of the two different fluorescent dyes into two different brain regions were performed in 10 rats. Tracer injections into two telencephalic structures (MPC vs LS) or into two diencephalic regions (AT vs MD) resulted in double labeling in a small population of TLD neurons. Double labeling was rarely seen after injections into MPC and MD.

These results indicate that the ascending cholinergic projection from TLD innervates diverse regions of the rat forebrain without a prominent divergent projection between telencephalic and diencephalic structures. Furthermore, the pontotelencephalic cholinergic system is characterized by the presence of substance P within the perikarya. (Supported by the Medical Research Council of Canada).

247.5 COMPARATIVE CHOLINERGIC HISTOCHEMISTRY OF THE MAGNOCELLULAR BASAL FOREBRAIN. K. Talbot*, N.J. Woolf, E. Gould*, and L.L. Butcher (SPON: J. Beatty). Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The organization and morphology of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE)-rich neurons of the magnocellular basal forebrain (MBF) were studied in albino rats, cats, and squirrel and bonnet monkeys. A battery of monoclonal and polyclonal antibodies against ChAT was tested immunohistochemcially on each species using the avidin-biotin peroxidase method. AChE material was prepared according to the pharmacohistochemical protocol of Butcher (in: Handbook of Chemical Neuroanatomy, vol. 1, 1983).

ChAT and AChE-rich cells in all three species were similarly distributed in the MBF, including the medial septal nucleus/diagonal band area, magnocellular preoptic area, and peripallidal nucleus basalis, as well as in the supratubercular zone filling the polymorphic olfactory tubercle and in the pericommissural area near the decussation of the anterior commissure. In each species, the ChAT and AChE cells had isodendritic forms with long dendritic processes embedded in a diffuse matrix of cholinergic neuropil. The geometry of dendritic fields was more complex in cats and monkeys.

A number of striking species differences occured in connectionally homologous parts of the MBF. Parts of the basal forebrain, such as the supratubercular zone and part of the diagonal band that have cholinergic projections to the olfactory bulb (see Woolf et al, Brain Res. Bull., 13, 751, 1984; Zaborszky et al., J. Comp. Neurol., 243, 488, 1986) were relatively more well developed in the rats and cats compared to monkeys. Conversely, basal forebrain regions such as the magnocellular preoptic area and peripallidal nucleus basalis which innervate isocortical fields (cf. Bigl et al., Brain Res. Bull., 8, 727, 1982; Mesulam et al., J. Comp. Neurol., 214, 170, 1983) were best developed in monkeys, and ChAT and AChE cells found in these regions of the primate were larger and more densely packed. The rostromedial magnocellular preoptic area (Ch4am) was similarly compact in monkeys, but diffuse in cats and especially rats. The pericommissural cholinergic cells were highly diffuse in rats, yet conspicuously dense (with thick, overlapping ChAT and AChE processes) in cats and monkeys.

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247.6 HISTOLOGICAL FIXATIVES AND CHOLINESTERASE ENZYME ACTIVITY. R.E.Foster,K.R.Olson*,J.H.Clark*,B.MacIver*,C.A.Broomfield*, D.E.Lenz*, and C.N.Lieske*. U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425

Over the past three decades histochemical studies of the localization and distribution of enzymes which hydrolyze alkyl esters, e.g., cholinesterases (ChEs), have utilized both fresh and fixed tissues, although fixatives were known to inhibit enzyme activity. Recent interest in the quantification of cholinesterase activity in preserved rather than fresh brain tissues has led us to examine parametrically the effects of glutaraldehyde (GA) and formaldehyde (FA) on acetylcholinesterase (AChE) and butrylcholinesterase (BChE). Solubilized AChE was obtained from eel electric organ or guinea pig brain homogenates and BChE was obtained from horse serum. FA was made from paraformaldehyde and GA was obtained in 50% aqueous form (Tousimis EM Grade). The activity of ChEs was determined by colorimetric assay using p-nitrophenyl acetate as the substrate. Except for stop-flow experiments, all enzyme reactions were carried out in 96-well microtiter plates (MTP). All experiments were replicated at least 4 times. The colorimetric reaction was measured by a Titertek MTP spectrophotometer. Typically, selected concentrations of FA and GA were mixed with the solubilized enzyme, one MTP column per concentration. After a given fixation period, the substrate was added to all wells, and the reaction was followed at regular intervals until all MTP columns had a quantifiable reaction. The control condition was always the enzyme-substrate reaction without fixative. All data were expressed as a percentage of control enzyme activity (Activity - Δ OD/min). At concentrations of 5% (W/V), both FA and GA produced maximal inhibition of AChE and BChE activity of 50 - 60%. At lower fixative concentrations, however, FA had a less inhibitory effect than did GA, but inhibitory effects of both were detected with concentrations as low as 0.05%. Time of incubation of the enzyme with fixative had little effect on the level of inhibition, suggesting that the fixative reaction reached completion very rapidly. Combinations of FA and GA were not additive but inhibited to the level of the most effective constituent. Experiments to determine whether the concentration of the enzyme was a factor indicated that over a dilution range of 3 - 20 there was no interaction with the fixative effects. Enzyme fixation experiments with stop-flow instrumentation indicate that the aldehyde-induced inhibition is probably caused by an allosteric machanism and not by direct binding at the active site.

247.7 ANALYSIS OF ENZYMES OF ACETYLCHOLINE METABOLISM IN RAT AMYGDALOID COMPLEX R.P. Hellendall*(1), D.A. Godfrey(2), C.D. Ross(2), D.M. Armstrong(3), J.L. Price(4)(SPON: P.Knott). Neurobio. Grad. Prgm., Mt. Sinai Sch. of Med., NY, NY 10029 (1); Dept. of Physio., Oral Roberts Sch. of Med., Tulsa, OK 74171 (2); University of California, San Diego, La Jolla, CA 92093 (3); Dept. of Anat. and Neurobio., Washington Univ. Sch. of Med., St. Louis, MO 63110 (4).

The distribution of choline acetyltransferase (ChAT) within the rat amygdaloid complex was studied to describe the pattern of cholinergic innervation believed to arise from the basal forebrain. Three methods were applied: 1)immunohistochemistry using a monoclonal antibody against ChAT in conjunction with the double peroxidase-antiperoxidase procedure; subsequent computer analysis produced high resolution densitometry images of the labeled sections; 2) quantitative histochemical mapping of ChAT activity from assays of contiguous samples microdissected from 20um thick freeze dried sections; 3) acetylcholinesterase (AChE) enzyme histochemistry of frozen sections from the same series.

All of the approaches indicate significant cholinergic activity in the basolateral amygdaloid nucleus (most prominent rostrally), the nucleus of the lateral olfactory tract (especially layer II), and the amygdalo-hippocampal area. Regions which showed reduced ChAT activity could not easily be distinguished from backround staining with the immunocytochemistry. AChE stain deposition appears to parallel ChAT activity though quantitative analysis was not conducted on the sections for AGhE. Lowest assayed ChAT activity is in the medial nucleus of the amygdala. Most other regions in the complex display activity at least as high as that found in homogenate of whole brain or in cortex. Gradients of activity are present in several regions including the basolateral and lateral amygdaloid nuclei and the nearby posterior piriform cortex. In the piriform cortex, high ChAT activity and dense AChE staining are present at the medial edge near the nucleus of the lateral olfactory tract. The immunocytochemical method shows a few intensely reactive somata within this area though less reactivity than in other areas containing high ChAT activity. A comparison of the results observed with the three techniques suggests an advantage in using them together since their respective strengths tend to complement each other.

The present study shows the amygdaloid complex of the rat contains a wide range of ChAT activity including a circumscribed area, corresponding to the basolateral nucleus, containing some of the highest activity found in the CNS. In light of evidence that the major source of cholinergic afferents to the basolateral nucleus is in proximity to an area in the basal forebrain projecting to cortical regions (Carlsen, J. et. al., J. Comp. Neurol., 234: 155,1985), our findings indicate the amygdala may serve as a discrete, useful model to study central cholinergic terminal fields.

247.8 IMMUNOCYTOCHEMICAL CHANGES IN MEDIAL SEPTAL-DIAGONAL BAND NEURONS FOLLOWING FIMBRIA-FORNIX TRANSECTION IN THE RAT. V.E. Koliatsos*, C.A. Kitt, J. Rosenfeld, L.C. Walker, N.H. Sternberger*†, L.A. Sternberger*†, B.H. Wainer§ and D.L. Price (SPON: M.D. Crutcher). The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2182; †University of Maryland, Baltimore, Maryland 21201; §University of Chicago, Chicago, Illinois 60637.

Fimbria-fornix (FF) lesions have been reported to cause retrograde cellular changes in neurons of the medial septal nucleus (MSN) and nucleus of the diagonal band of Broca (ndbB). Relatively little is known about the time course of these events; in addition, previous observations have been based exclusively on studies of Nissl, acetylcholinesterase (AChE), and choline acetyltransferase (ChAT) stained material. The present study was designed to examine the time course of changes in cytoskeletal elements (phosphorylated and nonphosphorylated neurofilament [NF] proteins) and in transmitter-related enzymes (AChE and ChAT) in individual neurons of the MSN and ndbB. At 1-2 days following FF transection, AChE histochemistry revealed some cell shrinkage with intensified perikaryal staining, whereas ChAT immunocytochemical staining of perikarya was decreased. Both AChE and ChAT stains demonstrated irregularly swollen fibers that were most prominent immediately rostral to the transection site. A few perikarya also contained abnormal accumulations of phosphorylated NFs. At 7-10 days following transection, many neurons did not stain for AChE and ChAT. The fiber reaction had spread to rostral septal levels, but AChE and ChAT staining intensity was diminished. Phosphorylated NFs were present in many perikarya. At this time, cell loss was apparent in Nissl-stained material. On day 25, the longest survival time studied, there was a dramatic reduction in AChE and ChAT staining and a significant increase in phosphorylated NF accumulations in cells in the MSN-ndbB; few cells appeared normal. There was no apparent change in the pattern of staining for nonphosphorylated NFs.

The results of this study suggest that FF transection leads to a series of changes in neurons of the MSN and ndbB including: perikaryal shrinkage with loss of ChAT immunoreactivity; increase in AChE staining; abnormal accumulation of phosphorylated NFs in perikarya; and the appearance of irregular fibers that stain for AChE and ChAT. Recent evidence indicates that phosphorylated NFs may be involved in the pathogenesis of neurofibrillary tangles in individuals with Alzheimer's disease. Investigations of cytoskeletal and transmitter-specific changes that follow transection of the FF pathway could provide insights into pathogenetic mechanisms of more complex diseases involving the cholinergic system.

247.9 A SET OF LIMBIC AND MOTOR NEURONS IN THE RAT BRAIN DEFINED BY Tor 23. D.T. Stephenson* and P.D. Kushner, The ALS Research Center, Pacific Medical Center, San Francisco, CA 94115.

We are asking whether functional networks in the mammalian brain can be identified on the basis of neuronal cell surface antigens, much the way the T-cell subsets of the immune system were first defined. A relationship between the motor and limbic systems is an old concept, well established in neurology, psychiatry, and psychology. Among discrete neurons within these two highly complex and widely dispersed central nervous system pathways, we have discovered a common cell surface epitope.

The monoclonal antibody Tor 23, which was made to cholinergic nerve terminals, immuno-stains the external plasma membrane of neurons which are 1) precisely distributed and 2) typically rare in any region, and 3) those regions are mostly either of the primary motor or the first order limbic system. Important to our analysis is the very obvious lack of Tor 23 binding throughout many areas of the brain, e.g. the thalamus and the corpus striatum. Also, in most regions Tor 23 positive neurons represent a small and frequently scattered cell population within a complex matrix of other, negative neurons.

Cell surface sets and subsets of neurons may or may not correspond to a single transmitter type. In our analysis we have found that Tor 23 positive neurons lie mostly in those very regions which have been shown to contain choline acetyltransferase (CHAT) positive neurons. Exceptions are as follows: regions CHAT positive and Tor 23 negative, the corpus striatum and some cranial motor nuclei; regions CHAT negative but Tor 23 positive, the deep cerebellar nuclei, the locus cereleus, and some cranial sensory nuclei.

BEHAVIORAL PHARMACOLOGY I

248.1 AN INVESTIGATION OF THE ACUTE, CHRONIC AND WITHDRAWAL EFFECTS OF CAFFEINE ON ANXIETY, EXPLORATION AND CONVULSIONS IN THE RAT. H.A.Baldwin*,S.E.File,A.L.Johnston* and L.J.Wilks*.(SPON: W.A. Friedman) Dept.Pharmacology,Sch.of Pharm.,Univ.London,London WC1N1AX(U.K.)

The effects of acute and chronic caffeine, and withdrawal of caffeine, were examined in male hooded Lister rats. Acutely treated rats received a single injection of caffeine (20 or 40 mg/kg IP). Chronically treated rats received 50 or 100 mg/kg/day caffeine in their drinking water for 21 days, and 20 or 40 mg/kg IP on the test day. To investigate the effects of caffeine withdrawal, after 21 days, the caffeine was removed and the animals tested 24 hours later.

The rats were tested in two animal models of anxiety, the social interaction test (File, S.E., J.Neurosci Methods 2:219, 1980) and the elevated plus-maze (Pellow, S. et al. J Neurosci Methods 14:149, 1985). In the social interaction test: acute caffeine produced a significant decrease in the time spent interacting, and in the number of rears but motor activity was unchanged. In contrast rats treated chronically showed no change in social interaction or rears whilst motor activity increased. Withdrawal rats showed no differences from controls. In the elevated plus-maze: Acute caffeine produced a decrease in the % number of entries on to the open arms whereas chronic and withdrawn caffeine had no effects.

In the holeboard acute caffeine produced a decrease in head dipping but had no significant effect on motor activity. Chronic and withdrawn rats showed no difference to controls. Finally the effect of caffeine treatment on PTZ induced seizures was investigated. Acute and chronic treatment produced a significant increase in the number of rats having full tonic-clonic seizures although the number of myoclonic jerks was unaltered. Rats withdrawn from caffeine showed no differences to controls.

In conclusion acute caffeine appears to have an anxiogenic action in both tests of anxiety, produces a decrease in exploratory behaviour in the holeboard and has proconvulsant activity with PTZ. 3 weeks of chronic treatment produces tolerance to the anxiogenic and exploratory effects but not to the proconvulsant properties of caffeine. There appear to be no effects of withdrawal from caffeine with respect to the behaviours investigated.

248.2 WITHDRAWAL FROM THREE DIFFERENT ANTICONVULSANTS: EFFECTS ON AGGRESSION, EXPLORATION AND SEIZURE THRESHOLD. L.J. Wilks* and S.E. File, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX

The effects of withdrawal from three anticonvulsant drugs, after 21 days of chronic drug treatment, were investigated in isolated male mice. Testing was carried out 24, 48 and 72 hours after a final dose of lorazepam (0.025 or 0.1 mg/kg/day during chronic treatment), sodium phenobarbitone (20 or 70 mg/kg) or phenytoin (10 or 40 mg/kg). Mice isolated for 28 days prior to the start of chronic treatment were tested at these time points for changes in aggressive behavior by introducing an untreated intruder into the home cage of the drug treated resident and recording the frequencies and times spent in spontaneous aggressive encounters in a 6 minute test. Parallel groups of mice were tested in the holeboard, to independently measure exploration, spontaneous motor activity and rearing and to detect any general stimulatory or sedative drug actions. Data for aggressive behavior is presented as percentage of total social interactions which were aggressive. Specific increases in aggression were observed 24 and 72 hours after the last dose of lorazepam (0.1 mg/kg) and a smaller increase 24 hours after the lower dose. No significant changes were observed in the holeboard at either dose. Specific increases in aggression were also observed 24 and 48 hours after the last dose of sodium phenobarbitone (70 mg/kg) despite reductions in holeboard behaviours at these times. Significant decreases were observed in rearing and motor activity 72 hours after the 20 mg/kg dose accompanied by decreases in aggression. A specific increase in aggression was observed at the 10 mg/kg dose of phenytoin after 24 hours. Significant decreases in all measures in the holeboard were observed 72 hours after both doses of phenytoin, and at the 40 mg/kg dose there was also a significant decrease in aggressive behaviour, which may therefore reflect a non-specific change. Seizure thresholds were measured at these time points by intravenous infusion of pentylenetetrazol. Significant decreases in seizure threshold were observed 48 and 72 hours after the last dose of lorazepam (0.1 mg/kg), 24 and 72 hours after phenobarbitone (20 mg/kg) and 24 hours after the 70 mg/kg dose and 72 hours after phenytoin (10 mg/kg).

248.3 THE EFFECTS OF SELECTIVE AGONISTS AND ANTAGONISTS FOR 5-HYDROXYTRYTAMINE RECEPTOR SUBTYPES IN A TEST OF ANXIETY IN THE RAT. A. L. Johnston*, S. Pellow and S. E. File. The School of Pharmacy, London WC1N 1AX.

Serotonergic pathways have long been implicated in the control of anxiety. The recent development of more specific 5-HT ligands has led to a resurgence of interest in the link between 5-HT and anxiety.

We have investigated the effects of some of these ligands (many reported to have possible anxiolytic activity) in a test of anxiety in the rat. The drugs studied were: 8-OH DPAT and TVX Q 7821 (putative agonists for 5-HT_{1A} receptor subtype); RU 24969 (putative agonist for 5-HT_{1B} receptor subtype); quipazine (non-selective 5-HT agonist); ritanserin (putative 5-HT_2 receptor antagonist); (-)-propranolol (putative 5-HT_1 receptor antagonist) and metergoline (non-selective 5-HT antagonist).

Our test of anxiety was the elevated plus-maze (Pellow et al, J. Neurosci. Meths.,14:149-167,1985); the apparatus consisted of four elevated arms in a plus formation, two of which were open and two of which were enclosed. The percentage of entries onto or time spent on the open arms compared to the enclosed arms is taken as a measure of anxiety; this is increased by anxiolytics and decreased by anxiogenics. The total number of arm entries is taken as a measure of overall activity.

8-OH DPAT (0.0625-0.25mg/kg) was without effect on either measure of anxiety. TVX Q 7821 (2.5-10mg/kg) reduced both measures, but this was due to a general depression in overall activity rather than anxiogenic activity. RU 24969 (0.1875-1.5mg/kg) displayed a partial anxiogenic profile by reducing the percentage of time spent in the open arms, but was without effect on percentage of entries made onto the open arms. Quipazine (2 & 4mg/kg) showed anxiogenic activity. Ritanserin (0.25-10mg/kg) also displayed an anxiogenic profile, while (-)-propranolol (5 & 10mg/kg) reduced only the measure of percentage of entries made into the open arms as compared to control values. Finally, at 4mg/kg metergoline increased both measures indicating anxiolytic activity.

The results from the non-specific ligands (quipazine and metergoline) are consistent with the theory that a reduction in 5-HT function reduces anxiety. If the more specific ligands prove clinically effective, this suggests a mechanism of anxiety to which the plus-maze is insensitive.

248.4 THE EFFECTS OF INTRACRANIAL ADMINISTRATION OF MESCALINE ON FR-40 OPERANT RESPONDING IN RATS. G.F. Heath*, Steven E. Lindley* and R.H. Rech. Dept. of Pharmacology and Toxicology, Michigan State University, E. Lansing, MI 48824.

The fixed-ratio-40 (FR-40) schedule of operant behavior in rats manifests a distorted response pattern after hallucinogenic drugs that is characterized by "pausing". That is, the lowest effective doses of indolealkylamine or phenalkylamine hallucinogens decrease response rates and increase inter-response times (IRTs) reciprocally. Most other classes of psychoactive agents reduce response rates without inducing pausing at threshold doses.

In previous studies intracranial LSD or 2,5-dimethoxy-4-methylamphetamine (DOM) at various brain sites has induced FR-40 pausing just as effectively as i.p. administration. LSD appeared to be somewhat more potent in prefrontal cortex (PC) and dorsal raphe than at other brain sites or by i.p. injection. Intracerebroventricular (ICV) LSD was less potent than dorsal raphe LSD to induce pausing, about the same potency as PC LSD, and slightly more potent than i.p. LSD. The 5-HT_2 receptor antagonist pirenperone was very effective in blocking the FR-40 disruption by i.p. hallucinogens, and PC is known to contain a high density of 5-HT_2 receptors.

The present study compares the effects of mescaline on FR-40 performance of rats when administered into the PC, ICV or by the i.p. route. Contrary to the case with LSD, where little dose differential was appreciated by the various routes, mescaline ICV was much more potent (30-fold or more) than by i.p. injection. However, PC mescaline was significantly less potent than ICV mescaline. This decreased potency was reflected as a lesser tendency to both decrease response rates and increase IRTs in comparable dose ranges, comparing PC and ICV dose-response patterns. Contrary to LSD, mescaline appears not to produce disruptive effects on FR-40 behavior by a primary action at the PC site. (Supported by NIDA Grant DA01836.)

248.5 EFFECTS OF CARBAMATE PRETREATMENT ON THE LATENCY TO ONSET OF SOMAN-INDUCED SEIZURES AND CONVULSIONS IN GUINEA PIGS: COMPARISON OF PYRIDOSTIGMINE AND PHYSOSTIGMINE. D.J. Hinman*,N.K.Marshall, D.L.Rickett and J.F. Glenn. Neurotoxicology Branch,U.S.Army Medical Research Institute of Chemical Defense, APG,MD 21010-5425

Pretreatment of animals with reversible inhibitors of cholinesterase such as the carbamates pyridostigmine (PYR) or physostigmine (PHY) reduces the lethality due to exposure to irreversible organophosphorous (OP) anticholinesterases. However, little is known about the effects of carbamate pretreatment on the occurrence or latency to onset of OP-induced seizures. In this study we tested the effects of carbamate pretreatment on behavioral convulsions and electroencephalographic (EEG) seizures induced by the OP soman (pinacolyl methylphosphonofluoridate, GD) in freely-moving guinea pigs. Furthermore, we compared the quaternary carbamate PYR, which has primarily peripheral actions, with the tertiary carbamate PHY, which has central actions as well.

The EEG was recorded from indwelling cortical electrodes (stainless steel screws). Following a 30 min. baseline recording, the subjects were given pretreatment IM, either saline, PYR (0.03 or 0.22 mg/kg) or PHY (0.03 or 0.26 mg/kg). The doses of PYR and PHY were selected to produce equivalent effects on whole blood AChE (cf. Lennox et. al. Life Sci 37:793, 1985), with target levels of 40 and 80% inhibition at the low and high doses, respectively. Thirty min. later the animals received 2LD_{50}GD (56 ug/kg, SC), followed in 30 sec. by atropine methylnitrate (AtM; 4mg/kg, IM) and pralidoxime (2PAM; 25mg/kg, IM). AtM was given to block peripheral muscarinic sites and 2PAM to reactivate GD-inhibited AChE peripherally. EEGs and behavior were monitored for 2 hrs. post-GD.

Results indicate that low doses of either PYR or PHY significantly increase the latency to onset of behavioral changes such as chewing, retching and hyperactivity (18-47% increase), behavioral convulsions (15-38% increase) and EEG seizures (14-27% increase), and significantly decrease the incidence of lethality (50, 0 and 8% incidence for saline, PYR and PHY, respectively). Little difference between the carbamates was noted at the lower doses, although several were noted at the higher doses. High doses of PYR either increase or do not change the latencies to onset, while high doses of PHY have no effect or actually decrease the latencies. With regard to effects on lethality, both PYR and PHY had equivalent protective effects with no indication of a dose-response relationship for either drug.

Supported in part by U.S. Army Contract DAAG29-81-D-0100 (Battelle; D.J.H.) and IPA agreement (Univ. Illinois; N.K.M.).

248.6 A COMPARISON OF THE ANTICONFLICT ACTIVITY OF SEROTONIN AGONISTS AND ANTAGONISTS IN RATS. C.L. AMRICK AND D.A. BENNETT. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, N.J. 07901.

Modulation of serotonin (5HT) neurotransmission has been reported to be effective in the clinical management of anxiety, with both antagonism of the 5HT-2 receptor (Ceulemans et al., Pharmacopsychiat. 18: 1985) and agonism of the 5HT-1A receptor (Rickels et al., J. Clin. Psychiatry 43: 1982) implicated. Preclinically, various animal models have been used to identify potential anxiolytics. Conflict models are considered to be especially effective in this regard (Davidson and Cook, Psychopharmacologia 15: 1969).

The following investigation was conducted to compare the profiles of anticonflict activity produced by representative 5HT-2 antagonists (ritanserin and ketanserin) and 5HT-1A agonists (8-OHDPAT and TVX Q 7821). In this procedure, male Wistar rats, previously trained to respond for milk reinforcement on two alternating schedules: a VI-30 sec/milk (unpunished) schedule and an FR-10/milk + footshock (punished) schedule, were used. A significant increase in punished responding above control responding was considered to reflect an anticonflict effect (i.e. anxiolytic activity). When the 5HT-2 antagonists were tested at various doses, only one intermediate dose produced a significant increase in punished responding. Responding was increased 171% above control by a 10 mg/kg p.o. dose of ketanserin and by 130% at 3.0 mg/kg p.o. dose of ritanserin. A significant decrease in nonconflict responding was noted with 30 mg/kg of ketanserin, while no significant response decrease was seen with ritanserin even at the 30 mg/kg dose.

By comparison, 5HT-1A agonists produced dose-dependent increases in FR-10 responding. 8-OHDPAT resulted in significant increases in responding of 120, 140 and 180% at 0.03, 0.054 and 0.1 mg/kg i.p., respectively. TVX Q 7821 showed an increase in conflict responding of 124 and 168% at the 3.0 and 5.4 mg/kg doses, respectively. Significant decreases in nonconflict responding were evident with the 0.1 and 10 mg/kg doses of each of these compounds, respectively. These results indicate a different anticonflict profile for 5HT-1A agonists and 5HT-2 antagonists. The relationship between preclinical conflict results with serotonin modulators and clinical efficacy requires further elucidation. It is possible, however, that 5HT-1A agonists may have a better anxiolytic profile with a potentially wider therapeutic window.

248.7 THE ROLE OF SEROTONIN$_1$ VERSUS SEROTONIN$_2$ RECEPTOR SUBTYPES IN PHENCYCLIDINE-INDUCED HYPERLOCOMOTION IN RATS. A. P. Leccese, A. Mattia* and J. E. Moreton. Dept. of Pharmacology and Toxicology, School of Pharmacy, University of Maryland at Baltimore, Baltimore, MD 21201.

Neurochemical investigations of the effects of phencyclidine (PCP) have suggested a role for serotonin (5-HT) in PCP-induced hyperlocomotion and stereotypy. The present experiment employed selective 5-HT agonists to differentiate the relative importance of 5-HT$_1$ versus 5-HT$_2$ receptors in PCP-induced hyperlocomotion measured with automated activity monitors. While 0 and 1.0 mg/kg ip PCP were ineffective, 2.0, 4.0, 8.0 and 16.0 mg/kg ip PCP caused a dose-dependent hyperlocomotion limited by ataxia at the largest dose. A dose-dependent influence on the time course of the hyperlocomotion during the three hr sessions was also observed; however, the 4.0 and 8.0 mg/kg doses induced relatively constant hyperlocomotion throughout the sessions. Therefore, dose response effects on locomotion were determined for differential 5-HT agonists in combination with 4.0 mg/kg PCP. The 2.5, 5.0 and 10.0 mg/kg ip doses of the 5-HT$_2$ agonist, quipazine, when given with 4.0 mg/kg PCP, elevated hyperlocomotion to levels greater than those induced by any dose of PCP alone. In contrast, the indirect 5-HT$_1$ agonists, L-tryptophan (25, 50 and 100 mg/kg) and fluoxetine (2.5, 5.0 and 10 mg/kg), failed to increase the hyperlocomotion induced by 4.0 mg/kg PCP. In addition, the largest dose of quipazine enhanced the effects of the otherwise ineffective 1.0 mg/kg dose of PCP, while none of the doses of quipazine influenced the ataxia-limited hyperlocomotion induced by 16.0 mg/kg PCP. These results suggest that the serotonergic component of PCP-induced hyperlocomotion is mediated by 5-HT$_2$, rather than 5-HT$_1$, receptor subtypes. Furthermore, these results indicate that the effect of quipazine was relatively specific to hyperlocomotion and that there was little enhancement of the ataxia-inducing properties of PCP. (Supported by UMAB Designated Research Initiative Funds.)

248.8 INTRACRANIAL ADMINISTRATION OF NANOLITER VOLUMES: AN ASSESSMENT OF MICROINJECTION RELIABILITY USING RADIOLABELED TRACERS. J.B. Mitchell and M.A. Bozarth. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, 1455 de Maisonneuve Blvd. West, Montreal, Quebec, Canada H3G 1M8

The techniques used for intracranial drug administration require discrete drug delivery and reliable injection of small volumes, ideally in the mid nanoliter range. These problems are exacerbated in studies using freely moving animals. The electrolytic microinfusion transducer (EMIT; Bozarth, M. A. and Wise, R. A., J. Neurosci. Methods, 2, 173, 1980) uses electrolysis to displace drug through the injection cannula. The entire unit, including drug reservoir, mounts directly onto the animal's head with only an electrical cable attached to the control apparatus. This eliminates components, such as the flexible infusion line, that may present problems for reliable, small volume injection.

The reliability of the EMIT method was assessed by injecting radiolabeled tracer (tritiated 2-deoxy-d-glucose) and determining the amount of radioactivity present in the brain tissue. The EMIT method reliably delivered volumes from 0.03 to 3.0 μl, with excellent linearity ($r > .98$). An injection volume of 0.1 μl, for example, showed repeatability within 5%. The reliability of the EMIT method was also compared to that of a microsyringe method of injection in freely moving animals. Although the microsyringe itself delivers reliable volumes, the microsyringe method of drug injection allowed significant leakage of radiolabeled tracer: in unrestrained animals a volume of approximately 0.4 μl leaked in 15 min. This unprogrammed delivery may be attributable to the elements between the microsyringe and the subject, such as the commercially available infusion line and the fluid swivel. The EMIT method, by eliminating these intervening components, virtually eliminated the problem of leakage.

Data will be presented on the EMIT method across a range of injection parameters, in vitro. Data will also be presented comparing the EMIT method with other microinjection techniques in current use. Both single and repeated drug injections will be examined in freely moving animals, and special attention will be given to the problem of drug leakage.

248.9 EVIDENCE FOR A CENTRAL MECHANISM IN THE BEHAVIORAL EFFECTS OF ASCORBATE. L.K. White, M. Block*, M. Carpenter* and G.V. Rebec, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Ascorbate (AA), which is found in high concentrations in the mammalian forebrain, appears to exert an important modulatory influence on neuronal function. In the neostriatum, for example, AA is released by certain drugs (Kamata et al., Brain Res., 362:331, 1986), and when applied iontophoretically, it accelerates the firing rate of neostriatal neurons, especially when administered in conjunction with glutamate (Gardiner et al., Brain Res., 344:181, 1985). Behavioral studies also suggest a neuromodulatory role for AA. Systemic injections of AA attenuate the behavioral response to amphetamine (Tolbert et al., Life Sci., 25:2189, 1979) and enhance the behavioral effects of haloperidol (Rebec et al., Science, 227:438, 1985). To determine if the behavioral actions of AA are mediated by a central rather than a peripheral mechanism, we monitored the behavioral response to amphetamine in animals that received a central infusion of AA.

Adult, male rats were implanted with a cannula in the left lateral ventricle. Following a recovery period of 5-10 days, the animals were connected to an infusion apparatus, which delivered saline or 300 µg/µl AA at a rate of 0.4 µl/min, and simultaneously received a subcutaneous injection of 1.0 mg/kg d-amphetamine. Individual items of the amphetamine behavioral response were recorded by observers who were unaware of the infusion conditions. Compared to saline, an intraventricular infusion of AA for 90 min attenuated the locomotion and rearing produced by amphetamine, especially during the first 30 min of the drug response. Data on AA-induced changes in the behavioral effects of haloperidol, which will be presented as well, also support a central mechanism in the behavioral alterations produced by AA.

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248.10 GENETIC ALTERATION OF CAFFEINE AND ADENOSINE BEHAVIORAL RESPONSIVENESS T.W. Seale, K. Abla*, O.M. Rennert* and J.M. Carney. Depts. of Pediatrics, Biochemistry and Pharmacology, Univ. Oklahoma Health Sci. Ctr., Oklahoma City, OK 73190.

We are interested in identifying and characterizing genetic determinants of brain function which specify altered behavioral responsiveness to CNS stimulants. Such data may have direct application to delineating the inherited and neurochemical bases for altered intrinsic behavior as well as to the better definition of genetic factors influencing stimulant abuse. The methylxanthine, caffeine, is the CNS stimulant most widely consumed by man. Considerable evidence supports the frequent occurrence of inter-individual variation in CNS and peripheral responsiveness to methylxanthines among human subjects. The mechanisms underlying such changes are unknown. Certain of the low dose effects of methylxanthines have been attributed to their specific interaction with adenosine receptors to antagonize the effects of endogenous adenosine on neural firing (Daly, J.W., Bruns, R.F., Synder, S.H., Life Sci. 28:2083, 1981). In our systematic study of inherited variation in methylxanthine responsiveness, we have identified inbred mouse strains with an array of altered behavioral responses to both low and high doses of caffeine and related methylxanthines (Seale, T.W., Johnson, P., Carney, J.M., Rennert, O.M., Pharmacol. Biochem. Behav. 20:567, 1984). CBA mice are significantly more sensitive to the stimulatory actions of caffeine and theophylline on locomotor activity than are mice of SWR strain. This efficacy limited effect is inherited in a polygenic manner as shown by analysis responsiveness to caffeine among progeny derived from conventional genetic crosses. Pharmokinetic changes in methylxanthine distribution do not explain these effects because blood and brain levels of caffeine are indistinguishable in the two strains. The two strains of mice also differ in their susceptibility to the inhibitory action of the adenosine agonist 5'-N-ethylcarboxamidoadenosine (NECA). SWR mice, which are hyposensitive to caffeine, are supersensitive to NECA compared to CBA mice (ED_{50} values respectively 11.6 and 31 n moles/kg i.p.). Brain levels of [^{3}H] NECA were identical in the two strains at dosages which maximized their differences in behavioral response. No differences in responsiveness to several other adenosine analogs including CHA, L-PIA and CADA were observed. These data indicate that inherent differences in methylxanthine responsiveness can be associated with intrinsic, inverse changes in adenosine agonist sensitivity. The specificity of the change in responsiveness to adenosine agonists suggests that a particular class of central adenosine receptors, perhaps a subclass of the A-2 type, is involved.

248.11 CONTROLLING OPERANT BEHAVIOR EXPERIMENTS WITH COMMODORE COMPUTERS AND PROMAL SOFTWARE: COST EFFICIENCY, "BOX BIAS," AND SPEED. D.R. Prim*, R.C. Michaelis, and F.A. Holloway. Univ. Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

This paper describes a unique combination of small computer hardware and software capable of programming and controlling operant behavior experiments, as well as collecting and manipulating large amounts of data. The use of Commodore computer equipment and programs written in Promal convey a number of advantages over many available small computer systems. The system is significantly more cost-efficient than many systems in use today. In addition, this system overcomes the major problem encountered when trying to interface computer equipment with behavioral testing apparatus, namely the inability to simultaneously sample all possible inputs. Most small computer systems employ input/output interfaces which sample inputs in serial fashion. Once any input is registered, the program executes the appropriate functions in response to that input before returning to resample the input port address. These systems work well when required to process a maximum of 10 total inputs per second from no greater than two behavioral testing chambers. However, when total input rates are greater than 10 per second, or when more than two behavioral testing chambers are sampled, this results in a "box bias," whereby the input from any chamber can upstage and mask out simultaneous input from any other chamber whose input is sampled later in the sampling sequence. The Commodore 64 user port is sampled in parallel, enabling the computer to register and respond appropriately to inputs from any or all behavioral testing chambers every time it samples the input port. This feature interacts with the fact that Promal software provides a significant increase in speed of execution when compared to the Microsoft Basic used in most small computers. Promal provides a significantly higher input port sampling frequency than Basic, especially when there are multiple chamber inputs to be processed. The combined advantages of high speed parallel input port sampling, inexpensive, locally obtainable equipment, and large amounts of data storage make this an excellent system for programming and controlling operant behavior experiments.
Acknowledgements: Purchase and maintenance of research subjects supported by USPHS grant 1 R01 AA06351. Software supplied by Dave Prim of the American Neuroscience Research Foundation.

248.12 EFFECTS OF THE PHOSPHODIESTERASE INHIBITOR ROLIPRAM ON THE ACOUSTIC STARTLE RESPONSE IN RATS. J.H. Kehne, M. Davis, and J.F. Tallman, Department of Psychiatry, Yale Univ. Sch. Med., 34 Park St., New Haven, CT 06508.

Previous work using intrathecal infusions of cAMP analogs or adenylate cyclase activators (J.H. Kehne et al., Neurosci. Abstr. 114.20, 1985) suggested that increasing cAMP levels in the lumbar spinal cord produces an increase in the amplitude of the acoustic startle response in rats. The present study used the selective phosphodiesterase inhibitor Rolipram (4-(3-cyclopentyloxy-4-methoxy-phenyl-2-pyrrolidone)) to further evaluate the relationship between spinal cord cAMP and acoustic startle.

Rats were implanted with chronic indwelling intrathecal catheters that terminated at the rostral margin of the lumbar enlargement. Rolipram (3.12 - 200 μg; dissolved in DMSO) infused intrathecally produced a marked excitatory effect on acoustic startle that began immediately after infusion and lasted for over an hour. The magnitude and time course of this excitation was similar to that seen following intrathecal infusion of cAMP analogs (Kehne et al., ibid).

Rolipram has been shown to be active following systemic administration, producing a behavioral syndrome which includes hypoactivity, forepaw shaking, grooming, head twitches, and hypothermia (H. Wachtel, Psychopharmacol., 77: 309, 1982). In the present study, IP administration of Rolipram (0.156 - 10 mg/kg, in DMSO) produced a dose-related elevation in startle amplitude. Rolipram was approximately 10-fold less potent in increasing startle amplitude following IP injection than following intrathecal injection. The dose range in which systemically injected Rolipram increased startle amplitude approximated the dose-range at which others report elevated cAMP levels in various CNS regions (H.H. Schneider, Biochem. Pharmacol. 33: 1690, 1984).

In summary, Rolipram markedly increased startle amplitude following either intrathecal or IP administration. Taken together with previous findings, these data are consistent with the hypothesis that increasing cAMP levels in the spinal cord causes an elevation of the acoustic startle response in rats. Furthermore, the efficacy of systemically administered Rolipram in increasing startle suggests that the startle response may be a useful behavior for evaluating compounds for Rolipram-like activity.
(The authors would like to thank Schering AG and Berlex Laboratories for supplying Rolipram).

248.13 DISCRIMINATIVE STIMULUS EFFECTS OF 2-AMINO-7-PHOSPHONOHEPTANOIC ACID (APH) IN PHENCYCLIDINE-TRAINED RATS. J. Willetts*, M.Y. Chapman* and R.L. Balster* (SPON: D.R. Compton), Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

APH protects against N-methyl-D-aspartate (NMDA)-induced seizures in mice (Czuckwar et al. Eur. J. Pharmacol. 108:273, 1985). Various electrophysiological studies have indicated that APH selectively antagonizes excitatory effects of NMDA (Harrison and Simmonds Br. J. Pharmacol. 84:381,1983; Harris et al. Brain Res. 323:132, 1984; Polc Eur. J. Pharmacol. 117:387, 1985). Phencyclidine (PCP) and PCP-like drugs also have anticonvulsant activity (Hayes and Balster, Eur. J. Pharmacol. 117:121, 1985) in addition to antagonizing excitatory NMDA effects on central neurons (Anis et al., Br. J. Pharmacol. 79:565, 1983). In view of the fact that APH and PCP share these pharmacological effects, we decided to investigate PCP-like discriminative properties of APH in rats.

Male Sprague-Dawley rats (N=6) were trained to discriminate between PCP (1.25 μg/kg i.p.) and saline on a two-lever, FR-32 schedule of food reinforcement as previously described (Slifer and Balster, Neuropharmacol. 24:1175, 1985). Stainless steel cannulae were stereotaxically implanted into the right lateral ventricle for icv drug adminstration. Generalization tests were conducted after icv administration of saline, 100-150 μg PCP and various doses of APH. During the last 10 min of a 30-min test session, saline (5 μl icv) produced 0% drug-lever responding (DLR), while PCP (100-150 μg icv) produced 97-100% DLR and no consistent effects on response rates. APH (dissolved in saline and neutralized with NaOH) at 3.75 μg produced 67-100% DLR in three rats, while 0-7% DLR was observed in another three rats. Of the three rats that failed to generalize from PCP at 3.75 μg APH, one produced 73% DLR at 5.0 μg APH, and the two remaining rats produced 62% and 74% DLR at 7.5 μg APH. Generalization to APH at these doses was often accompanied by substantial effects on response rates. At 15 μg APH, response rates in two of five rats were decreased below 0.05 per second, and the remaining rats showed 77-100% DLR (response rates 0.12-0.95 per second). Of the three rats tested at 30 μg APH, one rat had response rates of 0.01 per second, one rat showed 90% DLR (response rate 1.00 per sec) and the remaining rat 0% DLR (response rate 2.57 per second).

These data indicate that icv administration of APH shares some discriminative stimulus properties with systemic PCP in rats. On the other hand, PCP-lever selection was not as consistent after injections of APH as it was after icv administration of 100-150 μg PCP; thus, there is not a complete overlap in the effects of PCP and APH. The previously demonstrated interaction of PCP with excitatory amino acid transmission may play a role in its discriminative stimulus effects. (Supported by NIDA grant DA-01442)

248.14 EXPOSURE TO MAGNETIC RESONANCE IMAGING ATTENUATES FENTANYL-INDUCED ANALGESIA IN MICE. G.C. Teskey, K.-P. Ossenkopp, F.S. Prato*, and M. Kavaliers. Research Institute, St. Joseph's Hospital and Dept. of Psychology, University of Western Ontario, London, Canada N6A 5C2.

A substantial literature has documented the biological effects of exposure to a variety of magnetic field conditions. Nuclear Magnetic Resonance Imaging (MRI) is a powerful noninvasive diagnostic procedure that is growing in popularity and as a result interest in possible biological consequences of exposure to this procedure has also grown. We have previously shown that the magnetic and radio-frequency fields associated with MRI can alter murine morphine-induced analgesia (Ossenkopp et al., 1985, Life Sci., 37, 1507). We describe here the effects of exposure to MRI on analgesia induced by the opiate, fentanyl.

Adult male CF-1 mice were exposed (in groups of 5) to the time varying magnetic field (TVMF) component of the MRI procedure during the mid-dark period. Previous experiments had shown that the TVMF component had the strongest inhibitory effect on morphine-induced analgesia in mice (Prato et al., 1985, Radiology, 157(P), 219). The mice were placed in the imager and exposed to the TVMF for 23.2 minutes. They were then removed and given an intraperitoneal injection of 1.0 mg/kg fentanyl citrate and tested 5, 10, 15, or 30 minutes later for nociceptive threshold. This test consisted of placing the mice on a warm surface (50° C) and measuring the latency to show discomfort by exhibiting a paw-licking response. Sham imaged mice were treated in an identical fashion except that they were not exposed to the TVMF of the imager (these fields were shut off during the sham imaging procedure).

The maximum analgesic effect of fentanyl was observed 5 minutes after injection and returned to baseline by 30 minutes post-injection. The TVMF exposure significantly reduced the analgesic effects of fentanyl ($p < .01$) with the greatest inhibition occurring 5 minutes after injection. We conclude that magnetic field gradients attenuate fentanyl-induced analgesia in mice in a manner similar to morphine-induced analgesia.

(Supported by a National Health Research and Development Program grant with F.S. Prato principal investigator)

248.15 CHRONIC ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN (5-HTP) ATTENUATES 5-HTP INDUCED RESPONSE SUPPRESSION. A.R. Mayeda*, J.R. Simon, J.N. Hingtgen and M.H. Aprison. Depts. of Psychiatry and Biochemistry and Institute of Psychiatric Research, Indiana Univ. School of Medicine, IU Medical Center, Indianapolis, IN 46223.

Administration of a single dose of D,L-5-HTP results in a period of depressed food-reinforced operant behavior that has been correlated with serotonergic mechanisms in specific areas of the brain (Aprison et al., In: New Vistas in Depression, Pergamon, p. 171, 1982). This 5-HTP induced response suppression has been proposed as an animal model of depression and has led to the development of the hypersensitive postsynaptic serotonin receptor theory of depression (Aprison et al., In: Neuropharmacol. and Behav., Plenum, p. 23, 1978; Aprison and Hingtgen, In: Serotonin, Plenum, p. 627, 1981). This behavioral depression has been effectively blocked by several antidepressant drugs at clinically comparable doses on both acute and chronic schedules (Hingtgen et al., Pharmacol., Biochem. and Behav., 20: 425, 1984). The mechanism of action of these drugs as tested on our animal model appears to involve postsynaptic blockade. Tryptophan and 5-HTP have therapeutic effects on depressed patients (van Praag, In: Biol. Psychiat. Today, Elsevier, p. 485, 1979) which have been explained by the postsynaptic theory of depression. Thus, serotonin supersensitivity may be reversed in depressed patients by such treatment. Therefore, it is important to test the effect of chronic 5-HTP treatment in our animal behavioral paradigm. Rats (adult, male Wistar) were trained to press a lever for sweetened condensed milk on a VI 1 schedule of reinforcement. After response baselines were established, rats were injected with 25 mg/kg D,L-5-HTP i.p. every day for 9 days. Control rats were given the same dose of 5-HTP on days 1 and 9 but saline on intervening days. All injections were administered after the first 15 min. of the VI schedule and subsequent changes in response rates were measured. After an increase in the period of depression on day 2 in some rats, the chronic 5-HTP animals showed progressively less depression until day 8 when no depression was observed. Control rats showed the same amount of 5-HTP induced depression on day 1 as on day 9. Serotonin binding studies are now in progress to elucidate the receptor mechanisms involved in this psychopharmacological effect. The present data are consistent with the concept that repeated administration of 5-HTP results in the desensitization of serotonin receptors within 5 to 10 days, thus accounting for the therapeutic effect of 5-HTP as seen in depressed patients, and providing additional support for the hypersensitive serotonergic theory of depression. (Supported in part by Indiana Dept. of Mental Health Grant 178-679-005 and by training grant PHS MH 17107-03, NIMH).

248.16 SOME EFFECTS OF REPEATED ADMINISTRATIONS OF d-TUBOCURARINE IN RATS. S.E. Gramling. Dept. of Psych., Nicholls State Univ., Thibodaux, LA 70310

The across session decline in rate observed in animals treated with neuroleptics has frequently been attributed to the putative "anhedonic" (reward-reducing) effects of these drugs. Others have suggested that the gradual decline in rate may be due to a putative response contingent aversive effect of neuroleptics. The present experiment tested a drug with strong motor effects that is thought to be devoid of centrally-mediated reward effects (d-tubocurarine; CUR) in a procedure typically used to test neuroleptics. Comparison data regarding the effects of a "pure" motor effect drug such as CUR may be useful in separating the behavioral processes that mediate the rate reducing effects of neuroleptics.

Thirty-five water deprived rats leverpressed at stable rates for water reinforcement on a CRF schdeule and were then randomly assigned to one of five treatment condiditions. Rats in the reward (RWD) condition received vehicle injections and responded for water in the test situation. Rats in an extinction (EXT) condition received vehicle injections and responding did not result in water presentation. Two additional groups received injections of CUR (0.08 mg/kg or 0.12 mg/kg) and responded for water in the test situation. Response rate and duration data were collected via a laboratory computer system 15 min post injection.

Relative to rats in the RWD condition, rate was decreased in all conditions. Rats in the EXT condition exhibited a significant decrease in rate across the four treatment days. Rats in both drug groups, however, exhibited significant increases in rate across the four test days. In general, decreased rate was accompanied by an increase in response duration.

The across session pattern of responding produced by rats treated with CUR (monotonic increases in rate) do not resemble the across session pattern of responding typically produced by rats treated with a neuroleptic (monotonic decreases in rate) when tested over four test sessions. To the extent that CUR provides an adequate analog of the motor effects produced by neuroleptics, these results suggest that motor effects alone do not account for the across session decline in rate observed in rats treated with neuroleptics.

248.17 DSP4 BLOCKS MALE RATS' RESPONSES TO FEMALE ODORS, BUT NOT OTHER ODORS. C.A. CORNWELL-JONES. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Depleting forebrain norepinephrine (NE) neonatally with 6-hydroxydopa (6-OHDOPA) produces adult male rats that do not respond to female odors (Cornwell-Jones and Bollers, Brain Research, 268, 1983). However, neonatal 6-OHDOPA treatment results in high brainstem NE concentrations, in addition to forebrain NE reduction. In the present experiment, adult male Sprague-Dawley rats were injected systemically with 50mg/kg of the central noradrenergic neurotoxin DSP4, a procedure which depletes forebrain NE without affecting brainstem NE.

One week after injection, normally cycling female rats were placed in the males' cages and remained until males were tested four days later. Each male was given 4 emergence tests. The apparatus consisted of a start cage and a connecting tunnel which led into a bedding cage separated by a wire screen from a second tunnel. The bedding cage contained either 2 liters of shavings from a rat nest, or rat nest shavings scented with garlic solution. A screen covered the shavings. The second tunnel was either empty or contained an anesthesized female.

For each test, the animal was placed in the start box and allowed to move at will for 5 min. while latency to enter the bedding cage, number of entries and total time in the bedding cage were monitored. The frontal cortex and olfactory cortex from 8 aniamls in each treatment group were subsequently assayed for NE, dopamine (DA) and serotonin (5HT) using high pressure liquid chromatography with electrochemical detection.

The presence of an anesthesized female increased the mean number of entries into the bedding cage made by males treated with water, whether the bedding cage contained regular or garlic-scented nest shavings. In contrast, the presence of a female did not influence the number of entries made by DSP4-treated males. The lack of response to female odor did not reflect a general anosmia: both treatment groups made significantly fewer entries in the presence of garlic-scented than regular shavings, whether or not the anesthesized female was present. Drug treatment reduced NE concentrations by 68% and 66% in the frontal and olfactory cortex respectively. DA and 5HT concentrations were not significantly affected.

The results support previous evidence that forebrain NE may help modulate responses to conspecific odors. In the wild, NE may help channel a male's investigatory behavior towards potential mates.

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248.18 50 PERCENT CONVULSION DOSE OF LIDOCAINE AND VARIOUS AMINO ACIDS LEVEL IN NORMAL AND NIACIN DEFICIENCY ANIMALS, AND DURING CONVULSION STATE. KC Kim and SH Kim.Indiana University School of Medicine,Indianapolis, IN 46223 and Ball State University, Muncie, IN

Manifestation of CNS symptoms and finding of pathological abnormalities in the brain of niacin deficiency(N.D.)animals are well known.Study regarding amino acids(AA)level related to the functional activities on N.D.animals are lacking.The purpose of this preliminary study is to investigate the 50% convulsion dose(CD50)of lidocaine and AA level in blood during convulsion and nonconvulsion state in normal and niacin deficient mice.

Methods.White mice,CD-1 strain,were divided into two groups.Control and N.D.animals which was produced by feeding N.D. diet of 4 weeks.To determine the mean 50% convulsion dose(CD50),groups of ten mice were tested with various doses of lidocaine.AA analysis was done by using with Beckman AA analyzer.Latency time and duration of seizure was observed.CD50 was computed with the probit log dose regression line using the method of Litchfield and Wilcoxon. Two way analysis of variance was used for statistical analysis of AA level.Convulsion was determined by following three symptoms:1)loss of righting reflex,2)clonic and tonic seizure,and 3)opisthotonus.

Results.Behavioral change was not observed between the two groups. CD50 of lidocaine was 65.6 mg in control and 57 mg/kg in N.D. animals.This difference was not statistically significant.However,the duration of convulsion was 240% prolonged for N.D. animals.This was statistically significant.Three different groups of AA changes were observed in N.D. animals compared to control(number indicates the ratio of experimental to control) 1)Increased AA:(*statistically significant)thronine(2.24*),serine(1.68*),asparagine(1.53*),proline(2.00*),alanine(2.16*),citruline(1.30*),valine(1.31*),isoleucine(1.20) 2)Decreased AA:leucine(0.81),tyrosine(0.63*),lysine(0.41*) 3)AA not changed:glutamic acid,glutamine,glycine,phenylalamine,histidine arginine,taurine,tryptophan.During convulsion all AA which were increased significantly in N.D.animals were decreased(except isoleucine)in both control and N.D.animals.All AA were decreased significantly in N.D. animals were increased. AA that did not change in N.D. animals remained unchanged with convulsion.

Conclusion.AA related to GABA or putative inhibitory AA were unchanged in N.D. animals and along with normal animals did not change with convulsion.However,excitatory AA,aspargine and alanine were increased significantly and tyrosine and lysine were decreased in N.D.animals.These changes in excitatory AA level were reversed during convulsion in both N.D. animals and control animals.It could be postulated that increased excitatory AA may be responsible for CNS symptoms in N.D. animals and GABA or inhibitory AA may not be involved.

248.19 THE EFFECTS OF RU 24722, A NOVEL NOREPINEPHRINE RELEASING AGENT, ON LOCOMOTOR ACTIVITY AND GROOMING IN RATS. J. E. Johnston, R. L. Isaacson, N. Shube*, L. Sukienik*, and D. M. Warburton*. Dept. of Psychology and Cntr. for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901.

The experimental drug RU 24722 (Roussel) has been shown to decrease norepinephrine (NE) levels and increase levels of catecholamine metabolites in mouse brain, possibly through the enhanced release of NE (Takeda et al., Eur. J. Pharmacol. 106: 357, 1985). In the present study, the adrenergic specificity of RU 24722 was demonstrated in behavioral tests of juvenile and adult male rats. Twenty day old animals injected (i.p.) with 2.0 mg/kg RU 24722 performed better in a rotorod test of motor coordination than vehicle injected animals. However, at 25 days of age, the same dose of RU 24722 produced motor incoordination on the same test. Both of these age-dependent effects were counteracted by pretreatment with the α-adrenergic antagonist, phenoxybenzamine (Weiner, in Gilman et al. (Eds.), Pharmacol. Bases of Ther.: 1980). The motor effects of RU 24722 in young rats likely reflect ontogenetic changes in responsiveness to catecholaminergic drugs (Reinstein & Isaacson, Brain Res. 135: 378, 1977). In adult rats, doses of RU 24722 up to 8.0 mg/kg do not significantly alter locomotion or exploration in an open field. However, the number of grooming bouts increased in a dose-dependent manner while the duration of the bouts increased only at the 4.0 mg/kg dose. Phenoxybenzamine administration (5.0 mg/kg) blocked the RU 24722-induced increase in the frequency of grooming bouts, but, unexpectedly, enhanced the duration of the grooming episodes. A similar effect of phenoxybenzamine on the frequency of grooming bouts produced by low doses of morphine has been reported (Ayhan & Randrup, Psychopharm. 29: 317, 1973). However, because pretreatment with phenoxybenzamine increased the duration of grooming bouts, the total amount of grooming exhibited by the animals was greater than that of control treated animals.

248.20 SEROTONIN MODULATION OF HYPERKINESIA AND PHASIC NECK DYSTONIA INDUCED BY BETA, BETA-IMINODIPROPIONITRILE (IDPN) IN RATS. Bruce I. Diamond, Ph.D., Kapil Sethi, M.D.* and Richard L. Borison, M.D., Ph.D. Department of Psychiatry, Medical College of Georgia, Augusta, Ga.

It has been demonstrated that IDPN administration to a variety of animal species produces hyperkinesia associated with phasic neck dystonias. To elucidate the pathophysiology of this model, thirty male Sprague Dawley rats (250-300 g) were treated for seven days with IDPN (75 mg/kg;i.p.). On the eighth day irreversible circling, retropulsion and phasic retrocollic neck dystonia developed. Twelve rats received bilateral striatal Kainic acid (3 nmoles) lesions either before or after IDPN treatment. Kainic acid lesions either before or after IDPN treatment did not affect its behavior. IDPN treated animals demonstrated no striatal histological changes under light microscopy. Treatment with the serotonin precursor, 5-hydroxytryptophan (150 mg/kg;i.p.) and agonist TFMPP (10 mg/kg) dramatically decreased the neck dystonia and circling behavior. These drugs, however, exaccerbated retropulsion. The 5HT autoreceptor agonist, 8-DPAT, completely eliminated neck dystonia and retropulsion without affecting circling behavior. Cyproheptadine (10 mg/kg) eliminated retropulsion and had little affect on neck dystonia. These results suggest that IDPN induced hyperkinesia associated with phasic retrocollic neck dystonia is non-striatal in origin and mediated, in part, via the serotonergic system.

BEHAVIORAL PHARMACOLOGY II

249.1 THE EFFECTS OF ADENOSINE AGONISTS ON APOMORPHINE-INDUCED CLIMBING IN MICE. D. Luttinger and S. M. Patton*. Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

Adenosine agonists have been suggested to possess antipsychotic-like effects in animals (e.g. Heffner et al. The Pharmacologist 27:293, 1985). The present studies were conducted to test this hypothesis by assessing the ability of adenosine agonists to inhibit apomorphine-induced climbing (i.e., a procedure that detects antipsychotic compounds).

Male, Swiss-Webster mice weighing 17-24 g were treated orally with the test compound, fifty minutes later apomorphine (1 mg/kg) was administered subcutaneously. The mice were then placed into individual cylindrical cages 12 cm in diamter, 12 cm high with walls of vertical metal bars, 2 mm diameter and 1 cm apart. After a 10 min. habituation period, observations were made every 30 sec. for the next 10 min. by an observer that was blind to the treatments. The mice were scored at each time point for the number of paws on the vertical bars. These scores were summed over the 10 min. observation period. The effects of the compounds were compared to apomorphine/vehicle-treated controls by a Dunnett's test for multiple comparisons. The compounds tested included 5'-N-ethyl-carboxamideadenosine (NECA), N^6-cyclohexyladenosine (CHA), 2 chloroadenosine (CLA) and the R- and S- isomers of phenylisopropyladenosine (RPIA + SPIA). Like the antispychotic drugs, all the adenosine agonists inhibited apomorphine-induced climbing. The rank order of potency was: R-PIA=NECA(1 mg/kg)>CHA(3 mg/kg)>S-PIA=CLA(10 mg/kg).

In a second series of studies, theophylline (3 mg/kg) was administered intraperitoneally 30 min. after the adenosine agonist and 20 min. before apomorphine. This dose of theophylline had no effect on chlorpromazine or clozapine-induced inhibition of apomorphine-induced climbing. However, theophylline did inhibit the effect of RPIA, NECA and CHA on apomorphine-induced climbing, suggesting that the effect of the adenosine agonists was mediated by their interaction with adenosine receptors.

In separate studies, the active doses of the adenosine agonists were assessed for their effect on rotorod performance. Like the antipsychotic compounds tested (e.g. chlorpromazine, clozapine, thioridazine) most of the adenosine agonists impaired rotorod performance at doses active in the apomorphine-induced climbing procedure, R- and S-PIA were notable exceptions in that they did not impair rotorod performance at doses that inhibited apomorphine-induced climbing.

In summary, adenosine agonists produced an antipsychotic-like effect on apomorphine induced climbing. Unlike the antipsychotics tested, the effect of the adenosine agonists was reversed by the adenosine antagonist, theophylline. In addition, the data with the PIA isomers suggests that inhibition of apomorphine-induced climbing may be dissociable from ataxia (i.e. rotorod impairment).

249.2 STABILITY OF THE BEHAVIORAL AND NEUROPHARMACOLOGICAL EFFECTS OF LEARNED HELPLESSNESS: INESCAPABLE SHOCK DECREASES [^{35}S] TBPS BINDING IN RAT CEREBRAL CORTEX. Jacqueline N. Crawley[1], Stephen I. Deutsch[1], Steven M. Paul[1], Phil Skolnick[2], and Robert C. Drugan[1,2]. (Spon. F. Sulser) [1]Clinical Neuroscience Branch, NIMH and [2]Laboratory of Bioorganic Chemistry, NIADDK, NIH, Bethesda, MD 20892.

Recent work has shown that inescapable shock results in a facilitation of bicuculline-induced seizures 2 hours post-stress (Drugan et al., Br. Res. 342, 9-17, 1985). This finding suggests that there may be a perturbation in the benzodiazepine/GABA receptor complex relevant to the "Learned Helplessness" syndrome. We investigated the impact of escapable versus inescapable shock on [^{35}S] t-butylbicyclophosphorothionate (TBPS) binding to GABA-gated chloride channels, since the chloride channel is thought to be the effector for both $GABA_A$ and BDZ receptors. Rats exposed to inescapable shock had a 30% reduction in [^{35}S] TBPS binding in cerebral cortex in comparison to escapably-shocked and naive controls 2 hours post-stress.

A major problem in neurochemical studies of animal models of depression is that only about 50% of the rats exposed to inescapable shock develop the learned helplessness syndrome. This bimodal distribution results in considerable variability in biochemical analyses. We now report that subjects which showed learning deficits in their first experience in the learned helplessness paradigm also demonstrate escape failures 2 weeks or 4 weeks later in a second identical experience. Conversely, those rats that initially learned the escape task, also learned 2 weeks or 4 weeks later. This "stability" of learned helplessness might allow the initial performance of the subject to be a reliable predictor for separating the 2 populations (learn vs fail to learn). We are presently investigating [^{35}S]TBPS and [^{3}H]flunitrazepam two hours following the second session of inescapable shock, 2 weeks or 4 weeks after their first experience of learning or failing to learn the escape task. This "pretest" procedure may provide a useful screen which will create a homogenous treatment group for all future neurochemical studies of the learned helplessness phenomenon.

249.3 EFFECTS OF THE NUMBER OF PREEXPOSURES ON THE METHADONE-INDUCED PREEXPOSURE EFFECT IN TASTE AVERSION LEARNING IN THE RAT. R.J. Dacanay, J.P. Mastropaolo and A.L. Riley. Psychopharmacology Lab, The American University, Washington DC 20016.

Although the attenuating effects of drug preexposure on taste aversion learning are well documented, the basis for this attenuation remains unclear. Several explanations have been presented. For example, it has been suggested that environmental stimuli associated with the drug during preexposure block the subsequent learning of the relationship between the taste and toxicosis. This associative, environment-dependent effect has been reported with LiCl (Dacanay, R. J., & Riley, A. L., Anim. Learn. Behav., 10:91-96, 1982). It has also been suggested that pharmacological adaptation to the drug during preexposure reduces the subsequent ability of the drug to induce aversions. This nonassociative, environment-independent effect has been reported with morphine (Riley, A. L., Dacanay, R. J., & Mastropaolo, J. P., Anim. Learn. Behav., 12:157-162, 1984). Although from these earlier studies it appears that the mechanism underlying the preexposure effect in taste aversion learning is drug specific, i.e., associative for LiCl and nonassociative for morphine, in the present series of experiments we demonstrate that the mechanism underlying the effects of methadone preexposure on methadone-induced taste aversions varies with the specific parameters of preexposure, i.e., the mechanism is not drug specific, but parameter dependent.

Different groups of rats were given either three (Experiment 1), five (Experiment 2) or ten (Experiment 3) preexposures to methadone (12 mg/kg) in a distinctive environment. Following preexposure, subjects were given taste aversion conditioning with methadone in either the preexposure environment or home cage. Specifically, animals were given access to a novel saccharin solution in the preexposure or home cage and then injected with methadone. Subjects given three preexposures displayed an attenuation only when the preexposure and conditioning environments were the same. Subjects given five preexposures displayed an attenuation independent of the similarity of the preexposure and conditioning environments, although the attenuation was greater when the two environments were the same. Subjects given ten preexposures displayed equal attenuation independent of the similarity of the preexposure and conditioning environments.

Although it has been suggested that the mechanism underlying the US preexposure effect in taste aversion learning is drug specific, the present research demonstrating that the mechanism for a specific drug varies with the preexposure parameters argues against a drug-specific analysis. Instead of drug specificity, it is possible that the mechanism underlying the effects of drug preexposure is dependent upon the degree to which pharmacological adaptation occurs to the drug during preexposure. With greater adaptation, the preexposure effect becomes more nonassociative, i.e., pharmacological, in nature.

249.4 CONDITIONED TASTE AVERSIONS IN RATS AS A BEHAVIORAL BASELINE FOR THE ASSESSMENT OF THE STIMULUS PROPERTIES OF PHENCYCLIDINE. J.P. Mastropaolo, K.H. Moskowitz*, R.J. Dacanay and A.L. Riley. Psychopharmacology Lab, The American University, Washington, DC 20016.

Typically, drug discrimination is examined within the context of operant conditioning (cf. Stolerman, I.P. & Shine, P.J., Psychopharm., 86:1-11, 1985). In such designs, one behavior is reinforced during sessions preceded by an injection of a drug and a second behavior is reinforced during sessions preceded by an injection of the drug vehicle. The discrimination is evidenced by differential responding in the presence and absence of the drug stimulus. In the present series of experiments, we examined an alternative procedure for the assessment of drug discrimination learning, i.e., the conditioned taste aversion design (cf. Riley, A.L. & Tuck, D.L., Annals NY Acad. Sci., 443:381-437, 1985). In conditioned taste aversion learning, an animal is injected with a toxin following consumption of a novel taste. In such a design, animals avoid consumption of that taste on subsequent exposures. The question posed in the present series of experiments was whether an aversion to a taste would be acquired and displayed in one specific drug state, while the same taste would be consumed in another drug condition.

Prior to assessing drug discrimination learning within the taste aversion design, a dose of a drug had to be chosen which had no potential for supporting conditioning itself (Experiment 1), no unconditioned suppressant effects on consumption (Experiment 2) and was able to serve as a discriminative stimulus in an operant paradigm (Experiment 3). Based on the results of the first three experiments, a dose of 1.8 mg/kg of phencyclidine (PCP) was chosen to determine if the taste aversion design could be used to assay drug discrimination. In Experiment 4, different groups of rats were trained in a conditioned taste aversion paradigm with either the presence or absence of PCP as the signal for a saccharin/LiCl pairing. Specifically, for Group PL on days when PCP was injected 10 min prior to saccharin presentation, LiCl was injected immediately following saccharin access. On days when distilled water was injected 10 min prior to saccharin presentation, however, no LiCl was injected. For Group ML, these conditions were reversed. Differential consumption in the presence or absence of the drug stimulus emerged after three conditioning trials. In addition, substituting different doses of PCP produced a generalization gradient around the training dose. Following this, a range of doses of d-amphetamine (.18 - 1.8 mg/kg) and ketamine (3.2 - 18 mg/kg) were substituted for the training dose of PCP. Consistent with the findings from other procedures, there was no evidence of PCP-appropriate behavior following any dose of amphetamine, while ketamine produced PCP-appropriate behavior in a dose-related manner. The rapid control acquired by the drug stimulus in this procedure and the classification of compounds consistent with that found with other procedures suggest that the conditioned taste aversion design may be an efficient method for classifying drugs on the basis of their discriminative stimulus properties.

249.5 EFFECTS OF THE CONCURRENT INJECTION OF MORPHINE ON COCAINE SELF-ADMINISTRATION AND REINFORCING EFFICACY IN RATS. K.L. Marquis*, M.G. Webb* and J.E. Moreton. Dept. of Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharmacy, 20 N. Pine St., Baltimore, MD 21201.

The simultaneous self-administration by humans of a cocaine and heroin mixture ("speedballs") presumably has the effect of decreasing some of the side effects of cocaine and enhancing the pleasurable effects. This study examined the effects of combining morphine with cocaine on the pattern of cocaine self-administration and on reinforcing efficacy as assessed by the progressive ratio procedure. Six female Sprague-Dawley rats were trained to self-administer cocaine hydrochloride iv (1.0 mg/kg/inj) at FR10 in daily 90 min sessions. Rates of self-injection (inj/hr) were determined at 1.0, 0.5 and 0.25 mg/kg/inj cocaine in three rats. In the remaining three rats each cocaine dose was combined with morphine sulfate (0.5 mg/kg/inj) and rates were determined. Increases in the unit dose of cocaine produced dose-dependent decreases in injection rates. Injections occurred at relatively even intervals and were distributed across the entire session. The addition of morphine to the 1.0, 0.5 and 0.25 dose of cocaine reduced injection rates approximately 50%, 60% and 80%, respectively. The pattern of self-administration was also altered in that a large percentage of the total number of injections of the cocaine-morphine mixture occurred early in the sessions. Reinforcing efficacy, assessed in 6 hr progressive ratio test sessions, increased only slightly as the dose of cocaine increased. In these sessions, subjects responded rapidly for cocaine progressing through the ratio increments to a point where responding ceased for a 30 min interval. This break point was reached at each dose of cocaine prior to the end of the session. Break points determined for the cocaine-morphine mixtures failed to exceed that for cocaine alone; however, for the combination of 1.0 mg/kg/inj cocaine plus morphine all subjects continued to respond intermittently for the entire session. These data indicate that morphine will modulate the rate and pattern of self-administration of cocaine. This modulation may be the result of altered reinforcing efficacy or non-specific effects of morphine on responding for cocaine. (Supported by NIDA Grant DA 03173 and UMAB Designated Research Initiative Funds).

249.6 THE EFFECTS OF CHRONIC APOMORPHINE AND SULPIRIDE ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. D.M. Wood* and M.W. Emmett-Oglesby. Dept. of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107

Rats were trained to detect cocaine, 10.0 mg/kg, using a two-lever choice discrimination procedure. Following training, cocaine was generalized to, and apomorphine was substituted for the cocaine training stimulus, with approximate ED50's of 3.7 and 1.25 mg/kg, respectively. Subsequently, training was halted and cocaine, 20.0 mg/kg, was injected every 8 hours for 7 days. As compared to acute generalization and substitution testing, chronic administration of cocaine significantly shifted the generalization curve for cocaine two-fold to the right with an ED50 of 12.8 mg/kg, and also significantly shifted the substitution curve for apomorphine greater than four-fold to the right such that no dose of apomorphine produced cocaine-lever responding (doses higher than 5.0 mg/kg produced no lever selection during the 15 min. test session). In a second experiment, cocaine was dose-dependently generalized to the cocaine stimulus with an ED50 of 4.1 mg/kg. Subsequently, training was halted and apomorphine, 2.5 mg/kg, was injected every 8 hours for 7 days. Following chronic apomorphine administration, the dose-effect curve for cocaine was shifted significantly two-fold to the right with an ED50 of 14.2 mg/kg. In a third experiment, cocaine was dose-dependently generalized to the cocaine stimulus with an ED50 of 4.2 mg/kg. Sulpiride, 20.0 mg/kg, followed 15 min. later by cocaine, 20.0 mg/kg, was injected every 8 hours for 7 days. This treatment did not produce a significant shift of the cocaine dose-effect curve. The two-fold shift to the right of the cocaine generalization curve following chronic cocaine administration demonstrates tolerance to the discriminative stimulus properties of cocaine. The comparable magnitude of the shift of the cocaine dose-effect curve following chronic administration of apomorphine, and blockade of the development of tolerance by sulpiride, suggest that dopaminergic mechanisms may be involved in mediating tolerance to the discriminative stimulus properties of cocaine.

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249.7 PREVENTION OF LEARNED HELPLESSNESS AND ASSOCIATED DECREASES IN BRAIN NORADRENERGIC ACTIVITY BY INTRAHIPPOCAMPAL INJECTIONS OF NALOXONE. R.B. Hemingway, P.L. Prather*, A.C. Wright*, and T.G. Reigle. Dept. of Pharmacology and Toxicology, Univ. of Georgia, College of Pharmacy, Athens, GA 30602.

Previous studies in our laboratory have shown that the expression of learned helplessness (LH) is associated with a decreased or insufficiently activated noradrenergic activity in the brainstem and hippocampus (SN Abs. 11: 374.14, 1985). Since the major norepinephrine metabolites, 3,4-dihydroxyphenylethylene glycol (DOPEG) and 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG) are preferentially formed by pre- and postsynaptic enzymatic activity, respectively, these alterations in noradrenergic activity can be reflected by changes in the MOPEG/DOPEG concentration ratio. The present study investigated the interaction of hippocampal endogenous opiates with brainstem and hippocampal noradrenergic systems during the induction of LH in male Sprague-Dawley rats. Animals were exposed to an uncontrollable footshock stress 24 h before escape testing in a two-way shuttlebox task to demonstrate the development of LH. Paired animals previously exposed to an identical duration and frequency of escapable footshock served as controls. Rats were sacrificed immediately after shuttlebox testing, brains were removed and dissected and brainstem and hippocampal MOPEG and DOPEG were assayed by HPLC with electrochemical detection. Animals received bilateral 0.5 µg injections of naloxone in 0.5 µl isotonic NaCl or an equivalent volume of saline 15 min before the initial stress through previously implanted cannulae aimed at the anterior, dorsal hippocampus (AP + 4.7, L ± 1.0, V + 7.5; Paxinos and Watson, 1982). In agreement with previous results, escape deficits indicative of LH were exhibited only by animals that received prior inescapable footshock and this was associated with decreased MOPEG/DOPEG ratios in the brainstem and hippocampus. However, both the development of LH and the observed decreases in MOPEG/DOPEG ratios were prevented by intrahippocampal injections of naloxone. Thus, the hippocampal release of endogenous opiates during uncontrollable stress appears to be responsible for the subsequent escape deficits and reduced brainstem and hippocampal noradrenergic activity associated with the expression of LH.

249.8 RECOVERY OF LOCOMOTOR ACTIVITY FOLLOWING FENTANYL, ALFENTANIL AND SUFENTANIL. M.J.Benvenga, J.F.Capacchione* and T.P.Jerussi. Pharmacology Group, Anaquest, Murray Hill, NJ 07974.

Fentanyl, alfentanil, and sufentanil are opioids that resemble morphine qualitatively in effects on the central nervous system. In conjunction with skeletal muscle relaxants, they can be used to produce general anesthesia. In animals, this anesthesia can be correlated with the loss of righting (LOR) reflex whereby an animal placed on its back fails to spontaneously turn over. LOR for opioids usually coincides with general central nervous system depression and recovery can be assessed by measuring locomotor activity (LMA). The recovery from LOR was determined for fentanyl, alfentanil, and sufentanil.

Using male Sprague-Dawley rats (N=6/dose), dose response relationships for LOR were determined for each of the three opioids. Other rats (N=6/dose/drug) were administered i.v. the ED0, ED50, ED100, or ED150 doses for the LOR of each drug and placed in automated activity monitors immediately after regaining their righting. Controls, injected with distilled water, were treated similarly. The times at which the drugged animals reached control values for various parameters of LMA were automatically recorded. Mean recovery times for each LMA parameter at each dose and a "general recovery index" (GRI) consisting of a single mean of all parameters across all four doses was then calculated.

Differences in recoveries were evident between the three opioids. At the ED100, in which all animals displayed LOR, the mean recovery of alfentanil (9.5 min.) was shorter than either sufentanil (13.7 min.) or fentanyl (17.6 min.). In fact, at all doses tested, alfentanil consistently had the shortest and fentanyl the longest recovery times. Moreover, the respective GRI's for alfentanil, sufentanil and fentanyl were 6.8, 12.5, and 17.3 minutes. The results also indicated that there was no apparent relationship between potency, duration of LOR, and recovery. The results of this study compare favorably with clinical observations that alfentanil has a quicker recovery from anesthesia than either fentanyl or sufentanil.

249.9 EFFECTS OF MORPHINE, ETHYLKETOCYCLAZOCINE, N-ALLYLNORMETAZOCINE AND NALOXONE ON LOCOMOTOR ACTIVITY IN THE RABBIT. C.W. Schindler and M.F. White.* NIDA Addiction Research Center, Baltimore, MD 21224.

Based on studies in the chronic spinal dog, Gilbert and Martin (J. Pharmacol. Exp. Ther. 197:517, 1976) proposed a three receptor model for the action of morphine and morphine-like drugs. Morphine, ethylketocyclazocine (EKC), and N-allylnormetazocine (NANM) were proposed to interact with the mu, kappa and sigma opiate receptors respectively. Naloxone is thought to be an antagonist at all three opiate receptors. The concentration and distribution of these opiate receptors has been shown to vary considerably across species of animals (Robson et al., Eur. J. Pharmacol. 112:65, 1985). For example, in both rats and mice the concentration of mu receptors in the brain is over twice that for the kappa receptor, while for the rabbit there are approximately equal numbers of both mu and kappa receptors. The purpose of the present experiment was to determine the effects of opiates in rabbits on a behavioral measure used frequently in the study of opiates in rats and mice--open-field locomotor activity.

Rabbit motor activity was measured in a one meter square activity monitor. Three lights on each of two perpendicular walls created a grid of light beams which were detected by photocells on the opposite wall. All light beam interruptions were counted and were collected in 30 second intervals for a period of 90 minutes. Rabbits (2.0-2.5 kg) were tested twice per week on either Monday and Thursday or Tuesday and Friday. A pilot group of rabbits was tested without injection to determine a baseline level of activity. Activity scores for these animals were highest on day 1, then decreased and stabilized over the subsequent 5 days of testing. Therefore, for opiate studies, doses were tested only on days 2-6.

Doses of .3, 1.0, 3.0, and 10.0 mg/kg were tested for morphine, NANM, and naloxone; EKC was tested at .03, .01, .3, and 1.0 mg/kg. Rabbits received all doses of a single drug, including saline control, over the 5 test sessions. Rabbits receiving morphine showed only a dose-dependant decrease in motor activity. In rabbits tested with EKC and NANM, the lowest dose produced a slight increase in activity over saline control, but activity scores were decreased at higher doses, although not to as great a degree as for morphine. Naloxone also decreased activity at the highest dose tested. Analysis based on 5-min. components of the 90-min. session revealed a similar pattern of results.

These results indicate that the opiates in general produce decreases in motor activity in the rabbit. The small increases in activity observed with EKC and NANM may indicate that these drugs can increase activity at lower doses.

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249.10 REPEATED PRE-EXPOSURE TO AMPHETAMINE SENSITIZES RATS TO THE LOCOMOTION INDUCED BY MORPHINE ADMINISTERED EITHER SYSTEMATICALLY OR INTRACRANIALLY INTO THE VENTRAL TEGMENTAL AREA (VTA). J. Stewart and P. Vezina. Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, Montreal, Canada H3G 1M8.

Groups of 10 rats each were administered d-amphetamine sulfate (1.0 or 3.0 mg/kg), i.p., on five occasions, once every third day, prior to being placed in an activity box for two hours (Conditioning Groups). Saline injections were given in the home cages on the second day. Pseudoconditioning Groups received saline in the activity boxes and amphetamine in the home cage. A Control Group received saline injections in both environments. No injections or tests were given on the third day. Three days following the last amphetamine injection, all animals were tested in the activity boxes after a 1.0 mg/kg, i.p. injection of morphine sulfate and activity was recorded for two hours. Conditioning Groups, that had received either 1.0 or 3.0 mg/kg amphetamine in the activity boxes, showed significantly higher levels of activity with this low dose of morphine than did either the Pseudoconditioning or the Control Groups. These data demonstrate cross-sensitization between the activity effects of morphine and amphetamine and led us to explore its neural basis.

In a second experiment, three groups of 10 rats (Conditioning, Pseudoconditioning, and Control Groups) were prepared with bilateral injection cannulae aimed at the VTA and were then given pre-treatments of 1.0 mg/kg amphetamine sulfate or saline, i.p., under the same regimen as in the first experiment. This was followed by a series of tests during which all animals were given bilateral injections of 5.0 µg morphine sulfate in 0.5 µl saline volume into the VTA prior to being placed in the activity box for two hours. Once again, animals in the Conditioning Group were more active than animals in either of the other two groups. These data suggest that common changes in the mesolimbic dopamine system may underlie the sensitization of activity seen following repeated amphetamine and morphine exposure. As we have reported previously for the sensitization seen with repeated administration of morphine to the VTA, this cross-sensitization between the effects of amphetamine and morphine on activity appears to be expressed only in the environment where the animals have previously experienced the drug.

249.11 SENSITIZATION TO THE LOCOMOTOR EFFECTS OF MORPHINE ADMINISTERED REPEATEDLY TO THE VENTRAL TEGMENTAL AREA BUT NOT TO THE NUCLEUS ACCUMBENS OF THE RAT BRAIN. P. Vezina and J. Stewart. Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, Montreal, Canada H3G 1M8.

Several recent reports have demonstrated that morphine action in both the ventral tegmental area (VTA) and the nucleus accumbens (N.Acc) produces an increase in locomotor activity. Since it has been suggested that the former action of morphine involves the mesolimbic dopamine (DA) system and the latter does not (see Kalivas et al., J. Pharmacol. Exp. Ther., 1983), the present experiment was conducted to determine whether, as has been found with the VTA, repeated morphine administrations to the N.Acc result in a progressive increase in or sensitization of the acute locomotor effect elicited by morphine at this site.

Rats were prepared with bilateral injection cannulae aimed at the VTA or the N.Acc. After a recovery period, animals were given four injections of 5.0 µg morphine sulfate in 0.4 µl saline volume/side or the equivalent volume of saline and placed in an activity box for 60 min. At least two days separated each injection. As expected, both VTA and N.Acc animals injected with morphine showed increased locomotion relative to saline injected animals. Further, VTA animals showed a progressive increase in locomotion with repeated injections. However, while morphine continued to elicit locomotion in N.Acc animals, repeated injections did not lead to sensitization. These findings are in agreement with the idea that sensitization of the locomotor effects of opiates involves the mesolimbic DA system. Such a consequence of repeated activity may be a special property of this system. The lack of sensitization with N.Acc morphine, however, does not necessarily imply a lack of synergism between DA and morphine action at this site. Rats in a separate group were administered both morphine to the N.Acc and 1.0 mg/kg amphetamine sulfate, i.p., and placed in the activity boxes. These animals showed a high degree of sensitization with repeated injections, greater than that seen in animals administered amphetamine alone. Thus, while morphine in the N.Acc elicits increased locomotion which does not show sensitization with repeated injections, it may act to enhance mesolimbic DA system mediated sensitization.

249.12 ISOLATION HOUSING ENHANCES ETHANOL AND COCAINE SELF-ADMINISTRATION IN RATS. S. Schenk*, K. Gorman*, G. Lacelle* and Z. Amit (SPON: G. Fouriezos), CSBN, Concordia Univ., Montreal, Quebec

The present experiment was designed to investigate the influence of the early environment on self-administration of ethanol and cocaine. Rats were obtained at weaning (21 days of age) and housed for 6 (cocaine) or 12 (ethanol) weeks in either a socially isolated (1 per single cage) or aggregated (4 per double cage) condition. Following this housing manipulation, the "ethanol" rats were placed in individual cages and presented with 2 tubes, 1 containing tap water and the other ethanol. The ethanol concentration was initially varied from 2-10% over a period of 18 days. Thereafter, it was maintained at a concentration of 10% for an additional 20 days. Ethanol consumption (g/kg) and preference (percentage of fluid intake) was measured on a daily basis. The "cocaine" rats were prepared with indwelling jugular catheters. They were then exposed to an operant chamber for 3 hrs daily in which a lever press delivered a 0.1 ml infusion of cocaine (0.1-2.0 mg/kg/infusion) over 12 seconds. Response rates for each dose of cocaine were compared to those of rats that received infusions of the saline vehicle solution with each lever press. The minimum dose that would support responding at twice the operant rate was determined for each rat. Isolated rats consumed greater quantities of ethanol and had a higher preference ratio than did their group-housed counterparts. All of the isolated rats self-administered cocaine at rates substantially above the operant level whereas none of the group housed rats did. These data suggest that a large proportion of the variability in drug-taking behavior can be accounted for by environmental factors and point to the necessity for consideration of these factors in studies of drug abuse.

249.13 CONDITIONING IN COCAINE-INDUCED BEHAVIORAL SENSITIZATION. S.R.B. Weiss*, D. Murman*, R.M. Post and A. Pert. Biological Psychiatry Branch, NIMH, 9000 Rockville Pike, Bethesda, MD 20892

In many species of animals, psychomotor stimulants can produce progressively increasing behavioral effects with repeated administration (behavioral sensitization). These effects are long lasting and have been shown to be related to dose, gender, and inter-administration interval. We and others have demonstrated that cocaine-induced behavioral sensitization is also heavily dependent upon conditioning. For example, cocaine (10 mg/kg) administered for ten days produces a marked behavioral sensitization in rats that are pretreated and tested in the same environment, but not in rats pretreated with equal doses of cocaine in the home cage. In order to further examine this phenomenon we 1) pretreated with a single injection of cocaine at a higher dose (40 mg/kg) and 2) inhibited this behavior with various pharmacological agents.

Cocaine (40 mg/kg) administered in the test cage but not in the home cage produced marked behavioral sensitization to a lower dose of cocaine (10 mg/kg) the following day. Systematic variation of the similarity of pretreatment and test environments (injections in Plexiglas test cage versus wire home cage; and in the test room versus housing room) further indicate that the degree of similarity of the two environments is a determinant of the degree of sensitization. Haloperidol (0.5 mg/kg), diazepam (5 mg/kg), and muscimol (1.5 mg/kg) all inhibited cocaihe-induced hyperactivity (40 mg/kg) and significantly blocked sensitization to cocaine (10 mg/kg) the next day compared to vehicle controls.

Interestingly, rats treated solely with diazepam or muscimol (but not haloperidol) showed an enhanced response to cocaine the next day, suggesting a role of GABA systems in context-independent cocaine responsiveness. Taken together, these data demonstrate that environmental similarity and expression of cocaine-induced behavior (i.e., hyperactivity in the same environment unblocked by specific or nonspecific antagonists) are critical variables in the development of increased responsiveness to cocaine following prior exposure.

249.14 EFFECTS OF CHRONIC CHLORDIAZEPOXIDE AND MORPHINE ON SEPARATION DISTRESS IN DOMESTIC CHICKS. L. Crepeau*, L. Normansell*, & J. Panksepp (SPON: F. DeEskinazi) Dept of Psychology, Bowling Green State University, Bowling Green, OH 43403.

For several years we have been investigating the neurochemical control of separation distress in young chicks by measuring the frequency with which they emit distress vocalizations (DVs) when isolated. In order to evaluate benzodiazepine/GABA influences on this behavior, 2 day old chicks were injected i.p. twice daily for a week with 20mg/kg chlordiazepoxide (CDP), one injection 20 minutes before each daily 5 minute test trial. Drugged animals' vocalizations were reduced an average of 60% for that week. However, a follow-up 14 day experiment demonstrated reliable DV suppression across days only during the first of 3 five minute test trials (conducted 20, 30, and 40 minutes post injection). CDP modulation of DVs therefore appears to reflect sedation rather than an anxiety reduction. Other evidence suggests that benzodiazepine class drugs do not directly affect DV production. Animals treated for either 7 or 14 days and then abruptly taken off the drug failed to manifest any behavioral signs of withdrawal. DV rates among these animals increased to, but failed to exceed, vehicle group rates. Additionally, no group differences were found among animals injected with either the GABA antagonist picrotoxin (1mg/Kg), CDP (10mg/Kg), a combination of both drugs, or vehicle.

The effects of chronic opioid manipulations on DVs were also investigated. Groups of chicks were centrally injected with 2.5 ug morphine (in 3 ul H2O) daily for 5-6 days beginning at either 1, 5, 10, 15, or 21 days of age. DVs were initially suppressed to less than 20% of controls in the younger birds, and this suppression showed tolerance. By the end of testing, treated animals vocalized to the same extent as controls. Chicks injected i.p. with 2.5mg/kg morphine for 7 days beginning at 2, 8, or 14 days of age also exhibited DV attenuation. Unlike centrally injected birds, however, the peripherally treated animals did not show tolerance. No sedation was apparent in either of the morphine treated groups. Clearly morphine and CDP have differential effects on separation-induced DVs, with morphine having the much greater effect. This suggests that DVs do not represent simple anxiety, but some other form of trepidation.

249.15 RATE AND FORCE OF OPERANT RESPONSE ARE DIFFERENTIALLY SENSITIVE TO THE MULTIPLE PHARMACOLOGICAL EFFECTS OF HALOPERIDOL. M. M. La Cerra, S. C. Fowler and A. Ettenberg. Dept. of Psychology, Univ. of California, Santa Barbara, CA 93106 and Depts. of Psychology and Pharmacology, Univ. of Mississippi, University, MS 38677.

Twenty-four rats were reinforced with sweetened condensed milk for reaching through an aperture in the operant chamber to exert pressure on a force-sensing disc. The effects of two doses of haloperidol (0.04, 0.08 mg/kg) on operant responding were evaluated under two different behavioral conditions. One treatment consisted of drug administration plus 8 minutes of reinforced operant responding on 5 separate days (the "anhedonia" group) while the other treatment consisted of identical drug experience but operant plus drug treatment only on the 5th day (the "test day"). For this latter group the remaining 4 drug days were spent in the operant chamber, but in these 8-minute sessions access to both the reinforcement dipper and the operandum were prevented ("test-chamber control"). On the test day the anhedonia group exhibited significantly lower response rates than the test-chamber controls, but the dose effect on rate was not significant. Peak force was not affected by the differential behavioral treatment, but the higher dose produced significantly higher force. During subsequent extinction responding (neither group was drugged) the anhedonia rats showed significantly higher rates than the test chamber controls. However, there were no carry over effects of behavioral treatment on peak force: an extinction-related rise in peak force was observed equally for both groups, despite the between-group differences in rate, and prior dosage had no effect on peak force in extinction. The dissociation between force and rate on both the test day and the extinction day suggests that peak force may reflect the motor effects associated with neuroleptic challenge, whereas rate is a metric for the motivational or associational effects of these drugs.

249.16 CHRONIC NEUROLEPTIC TREATMENT: EFFECTS ON VACUOUS CHEWING MOVEMENTS AND BRAIN GABA FUNCTION. S. Mithani*, S. Atmadja* and H.C. Fibiger (SPON: J.H. Quastel). Div. Neurol. Sci. Dept. Psychiatry, Univ. of British Columbia., Vancouver, B.C., V6T 1W5.

Nigral glutamic acid decarboxylase (GAD) has been reported to be reduced in primates exhibiting haloperidol (HAL)-induced tardive dyskinesia (Gunne et al., Nature 309: 347, 1984) and in rats with HAL-induced vacuous chewing movements (VCM's) (Gunne and Haggstrom, Psychopharm., 81:191, 1983; Gunne et al., Psychopharm., 77:134, 1982). The present study was designed to determine if neuroleptic-induced VCM's could be correlated with regional changes in GAD activity.

Male Long-Evans rats received i.m. injections of flupenthixol (FLU) decanoate (4 mg/kg, once a week), or HAL decanoate (28.5 mg/kg, once every 3 weeks), or vehicle. VCM's were assessed weekly by the method of Gunne et al (Psychopharm., 77:134, 1982). The animals were killed after 16 or 40 weeks of treatment, and the brain was dissected into striatum, globus pallidus, entopeduncular nucleus and subtantia nigra. The striatum was further divided into dorsolateral, ventrolateral, dorsomedial and ventromedial sections. GAD activity was measured by a modification of the method of McGeer et al., (Brain Res., 32:425, 1971).

Neuroleptic-treated animals showed increases in the rate of VCM's after 10-12 weeks, and this remained fairly stable over the duration of the experiment. Significant decreases in nigral GAD activity in neuroleptic treated rats were observed after 40, but not 16 weeks of treatment. Other brain regions examined did not show any consistent changes in GAD activity.

The decreases in nigral GAD activity following chronic neuroleptic treatment are consistent with those reported by Gunne and Haggstrom (Psychopharm., 81:191, 1983). However, the present results do not indicate that VCM's are causally associated with reductions in nigral GAD activity since no decreases in nigral GAD activity were observed at 16 weeks by which time the neuroleptic induced VCM's had already reached near maximal rates.

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249.17 HALOPERIDOL AND EXTINCTION HAVE DIFFERENT EFFECTS ON RATS' OPERANT FORCE-TIME WAVESHAPES. S. C. Fowler, M. M. LaCerra and A. Ettenberg. Depts. of Psychology and Pharmacology, Univ. of Mississippi, University, MS 38677 and Dept. of Psychology, Univ. of California, Santa Barbara, CA 93106.

Food-deprived rats (N=9) were reinforced with sweetened condensed milk for pressing a force-sensing operandum on a continuous reinforcement (CRF) basis and were then exposed to extinction. Force-time waveforms were recorded (at a sampling rate of 512 hz) for all responses above 10g. Three quantitative indices were computed from these waveshapes: time from onset of response to peak force attainment (rise time), time from peak to response termination (fall time), and maximum force reached during a response (peak force). During CRF, haloperidol (0.04, 0.08, 0.16 mg/kg) increased the fall, but not the rise time of the response force waveforms. In extinction 5 rats received 0.08 mg/kg of haloperidol and 4 rats got the lactic acid vehicle. Unlike the haloperidol treatment during CRF, extinction was characterized by an increase in both the rise and the fall times for the force-time waveforms; all 9 rats (both drug and vehicle) displayed increased rise times in extinction ($p<0.01$, Wilcoxon signed rank test). These results suggest that the force-time waveshape provides a distinctive marker for neuroleptic versus extinction treatment: in extinction compared to CRF plus haloperidol, the force maxima occurred later in the response. Additional analyses based on peak force of response showed that the peak force rise frequently observed as a consequence of extinction was not attenuated by haloperidol treatment. Haloperidol's failure to mask the effects of extinction (i.e., rise times and peak force were increased in the extinction-drug condition) suggests that this drug does not reduce the rats' responsiveness to nonreinforcement.

249.18 THE EFFECTS OF 6OHDA LESIONS OF THE NUCLEUS ACCUMBENS ON COCAINE DISCRIMINATION IN RATS. S.I. Dworkin, C. Bimle* and N.E. Goeders. Psychiatry Research Unit, Louisiana State University School of Medicine, Shreveport, LA 71130

Drugs having high abuse liability are both self administered and have distinctive discriminative stimulus properties. Cocaine is considered to have high abuse liability, is readily self administered and produces distinctive interoceptive cues at relatively low doses. These effects along with many of the other behavioral and pharmacological effects of cocaine have been linked to dopaminergic systems. Lesions of the nucleus accumbens, a major dopaminergic site, attenuated cocaine self-administration indicating the involvement of the dopaminergic innervations in the reinforcing effects of the drug. However, similar lesions failed to alter cocaine-induced place preference which is most likely the result of both the reinforcing and stimulus properties of the drug. Therefore, this study was initiated to evaluate the effects of 6-hydroxydopamine (6OHDA) lesions of the nucleus accumbens on the discriminative cue produced by cocaine using a drug discrimination procedure.

The discriminative stimulus properties of cocaine were assessed in nine rats both before and after 6OHDA lesions of the nucleus accumbens. The subjects were trained on a two-lever, food-reinforced drug discrimination paradigm. The rats were trained to discriminate 10.0 mg/kg cocaine from saline. After determination of the generalization gradients (7-0.82 mg/kg cocaine), the rats were lesioned with the neurotoxin followed by a redetermination of the generalization gradients. Before the lesion both the 7.0 and 4.9 mg/kg dose resulted in over 90% drug appropriate responding and produced a slight decrease in overall response rates. The two lowest doses (1.18 and 0.82) produced over 90% responding on the saline lever. The lesion produced a slight decrease in control rates of responding and eliminated the drug related decreases in responding seen with the higher doses before the lesion. Furthermore, the lesion flattened the drug generalization gradient such that the two highest doses resulted in 75% and 57% respectively, drug appropriate responding and the two lowest doses resulted in 67% saline appropriate responding. Dopaminergic innervations of the nucleus accumbens are involved in the stimulus properties of cocaine.

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249.19 RESPONDING MAINTAINED BY CONCURRENT ACCESS TO FOOD, WATER AND INTRAVENOUS COCAINE. J.E. Smith, K. Schultz*, S.M. Dworkin* and S.I. Dworkin. Psychiatry Research Unit, Louisiana State University School of Medicine, Shreveport, LA 71130.

In most non-laboratory situations several different reinforcers may be simultaneously available to an organism. This situation can be evaluated in the laboratory by the use of concurrent schedule procedures. One type of concurrent arrangement involves presenting different reinforcers contingent on responses emitted on levers located in different areas of a conditioning chamber. Several studies have evaluated the complex neurobiological and behavioral interactions that can occur when several reinforcers are concurrently available. This experiment was initiated to determine the effects of adding cocaine as a reinforcer for rats previously trained on a concurrent food and water schedule.

Six adult male Fisher 344 strain rats were initially given continuous access to two levers with responses on one resulting in presentation of a food pellet (45 mg) and responses on the other resulting in 10 seconds of access to a 0.10 ml water dipper. The response requirements were increased over several days to a fixed ratio 10. After five days of stable responding the rats were implanted with chronic jugular catheters and allowed to recover. The rats were returned to the conditioning chambers and a third lever was introduced. Responding on this lever resulted in an intravenous infusion of cocaine (0.5 mg/kg per infusion delivered over 4.9 seconds). The effects of cocaine self-administration on food and water reinforced responding were evaluated. Contrary to results from an earlier study of morphine self-administration which showed large changes in dose to not affect food or water intake, this single dose of cocaine resulted in a 34% decrease in food and a 54% increase in water deliveries per day. While morphine was self-administered during both the light and dark cycle, cocaine intake occured primarily in the dark cycle. Several studies have evaluated the effects of stimulants on food intake. Indeed, anoretic effects have been well documented to occur during initial administration with tolerance developing rapidly to this effect. Such was not observed in this study. If access to cocaine was not limited the animals continued to decrease food intake and eventually died, during this time responding on the drug lever was still maintained. (Supported in Part By USPHS Grant # DA-03628).

BEHAVIORAL PHARMACOLOGY III

250.1 DURATION OF PENTOBARBITAL ANESTHESIA AND SEPTAL-HIPPOCAMPAL CHOLINERGIC ACTIVITY. J.R. Zucker*, J. Zabawska*, H. Lai*, and A. Horita*(SPON:R. Haschke). Depts. of Anesthesiology, Pharmacology, and Psychiatry, University of Washington, Seattle, WA 98195

Pentobarbital depresses the activity of the septal-hippocampal cholinergic pathway (SHCP). In a previous paper (Zucker et al., J. Pharmacol. Exp. Ther. 235:398, 1985) we have hypothesized that the duration of pentobarbital anesthesia is inversely related to hippocampal cholinergic activity. Intraseptal drug microinjection that enhances activity of the SHCP, shortens the pentobarbital-induced loss of righting reflex, a measure of anesthesia, in the rat and vice versa. To test this hypothesis we microinjected an alpha agonist (phenylephrine), a serotonin agonist (MK-212), and an acetylcholine agonist (arecoline) into the septum of pentobarbital-anesthetized rats and measured the duration of loss of righting reflex. Both phenylephrine and arecoline significantly shortened, whereas MK-212 prolonged the duration of anesthesia. These data are in agreement with out hypothesis since activation of septal noradrenergic (Robinson et al., J. Pharmacol. Exp. Ther. 208:476, 1979) and cholinergic (our unpublished data) innervations enhance SHCP activity, and septal serotonergic innervation (Robinson, Life Sci. 32:345,.1983) decreases activity.

The following table summarizes data (from the literature) showing an inverse relationship between duration of anesthesia and septal-hippocampal cholinergic activity in pentobarbital-anesthetized rats injected intraseptally with drugs:

Intraseptal Drug	Duration of Anesthesia	SHCP Activity
TRH	↓	↑
ACTH	↓	↑
Morphine	↑	↓
Substance P	↑	↓
Arecoline	↓	↑
Phenylephrine	↓	↑
MK-212	↑	↓

250.2 NICOTINE EXERTS A COMPLEX EFFECT ON VARIOUS INDICES OF LOCOMOTOR BEHAVIOR. A. Jerome*, S.H. Hagenmeyer-Houser, M.D. Bunsey, and P.R. Sanberg (SPON: G.E. Cordingley). Dept. of Psychology, Ohio University, Athens, OH 45701 and Div. of Psychobiology, Dept. of Psychiatry, University of Cincinnati, Cincinnati, OH 45267.

Previous studies assessing the effects of nicotine on locomotion in non-tolerant rats have yielded mixed results. Both increases and decreases in rearing, as well as increases, decreases, and no effect on ambulation have been reported by studies utilizing comparable doses. Ambulation may be conceptualized as being a multidimensional concept, comprised of a number of distinguishable components. Some portion of the inconsistency of reported results may be due to methodological differences in the assessment of activity. The present study examined a multivariate "activity-print" profile of nicotine in order to help clarify the discrepant results.

Rats were injected s.c. with nicotine (0.2, 0.4, 0.8 mg/kg) or vehicle and then placed into Digiscan-16 Animal Activity Monitors (Omnitech, Inc.) for 60 minutes during the nighttime. Measures of ambulation, vertical activity (rearing), rotational and stereotypic behaviors were analyzed by repeated measures ANOVA techniques utilizing orthogonal polynomials (trend analysis). The results indicated that 5 of 7 measures of ambulation were significantly affected by dose: movement time and number of movements exhibited positive linear trends, speed of movement and rest time showed negative linear trends, average distance exhibited a negative quadratic trend, while horizontal activity and total distance were not affected by dose. Significant decreases were apparent on all three measures of vertical activity: vertical time and vertical movement displayed negative linear trends, while vertical activity showed a negative cubic trend. Rotational movement and stereotypy were not significantly affected by dose.

These findings support previous research that has shown nicotine to have a dose related depressant effect on rearing. However, these results indicated that nicotine exerted a complex effect on ambulation that could not be accurately portrayed by reference to a single parameter. Various aspects of ambulation were either increased, decreased, or unaffected by nicotine. These results are not consistent with a general locomotor stimulant interpretation of nicotine's effects, and suggest the presence of complex neuropharmacological mechanisms. Supported by Pratt Family and Friends, HDF, HDFA, MH40127, and Omnitech Electronics.

250.3 COMPARISON OF ATROPINE SULFATE AND ATROPINE METHYLNITRATE, WITH OR WITHOUT PRALIDOXIME, AS THERAPY FOR SOMAN-INDUCED SEIZURES IN GUINEA PIGS. N.K. Marshall, D.J. Hinman* and J.F. Glenn. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425

Atropine sulfate (AtS) and oxime reactivators such as pralidoxime chloride (2PAM) are recommended therapy for poisoning by organophosphorous (OP) anti-cholinesterases such as soman (pinacolyl methylophosphonofluoridate, GD). Behavioral convulsions and electroencephalographic (EEG) seizures are major signs of GD poisoning. Most previous studies of effects of AtS on OP-induced seizures used anesthetized animals. In this study we have recorded GD-induced seizures in unanesthetized guinea pigs. Since no prior study specifically tested interactions between AtS and 2PAM as therapy for Op-induced seizures, we studied effects of each therapeutic drug alone and in combination. Finally, we compared tertiary (AtS) and quaternary (Atropine Methylnitrate, AtM) amines to test the effects of central and peripheral muscarinic blockade, respectively, on GD-induced seizures.

EEGs were recorded from indwelling cortical electrodes from 30 min. before until 2 hours after administration of $2LD_{50}$ GD (56ug/kg,SC), and the following therapies 30 seconds after GD: I-Saline; II - AtS (4mg/kg, IM); III - AtM (4mg/kg, IM); IV - 2PAM (25mg/kg, IM); V - AtS + 2PAM; VI - AtM + 2PAM. The results were:

		------ Seizure ------			---- Lethality ----	
Grp. #	n	Incidence (%)	Onset (min)	Duration (min)	Incidence (%)	Time (min)
I	8	75#	7.0±0.9	6.8± 2.9	100	13.5± 2.2
II	8	100	8.1±1.0	25.8± 5.1*	100	33.9± 5.6**
III	8	100	7.8±0.7	19.9± 2.6*	100	27.8± 2.7**
IV	9	89	5.9±0.9	25.4± 3.9*	89	31.3± 3.8**
V	9	100	9.4±1.3	86.4±11.9**	44*	65.8±13.3*
V	9	100	7.3±0.7	74.9±15.0**	44*	35.1± 4.9*

* - P<.05; ** - P<.01, Compared to Control (Group I); #-2 animals died prior to expected time of seizure onset.

Each treatment alone increased the duration of seizures and delayed the time of death, but did not decrease the incidence of lethality; there were no differences between AtS and AtM. With the combination therapies (Grp. V & VI) decreases in lethality were also observed; in addition, the time to death was greater for the AtS + 2PAM group than for the AtM + 2PAM group ($p<0.05$). ATM + 2PAM appear to be additive, whereas ATS + 2PAM appear to be supperadditive.

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250.4 EFFECTS OF INTRACEREBRAL NICOTINIC AGONISTS ON LOCOMOTOR ACTIVITY: INVOLVEMENT OF MESOLIMBIC DOPAMINE. A. Pert, and C.C. Chiueh. (Spon: C.T. Bennett). Biological Psychiatry Branch and Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20892

Nicotine has potent effects on locomotor behavior. At high doses it produces an initial depression of locomotor output which is usually followed by slight excitation. Little in known regarding the precise mechanisms underlying these actions. The purposes of these studies were to define the neuroanatomical foci through which nicotinic agonists produce their locomotor effects and to elucidate some of the underlying neuropharmacological mechanisms. Rats were implanted with stainless steel cannulae guides aimed for the lateral ventricle or a number of defined intracerebral sites. Intraventricular injections of nicotine (1,10 or 100 n moles) produced a dose dependent depression of locomotor activity which was followed by slight excitation 15 minutes later at the highest dose. Cytisine (1,10 or 100 n moles), a potent nicotinic agonist, also produced an initial depressant effect that was followed by a prolonged dose dependent excitation. Pretreatment with mecamylamine antagonized both the depressant as well as excitatory effects produced by intraventricular cytisine. Injections of cytisine (5 n moles) bilaterally into the ventral tegmental area (VTA) produced increases in locomotor activity while injections into the anterior ventral and ventral lateral thalamus as well as the nucleus accumbens produced an initial depression. Injections into the ventral posterior thalamus, reticular formation, globus pallidus, caudate nucleus, substantia nigra an cerebellum had no effect. Enhanced locomotor activity induced by injections of cytisine into the ventricles or VTA were antagonized by 6-OHDA lesions of the n. accumbens. Injections of cytisine into the VTA were also accompanied by increases in dopamine as well as DOPAC in the n. accumbens while injections of cytisine into the n. accumbens had no effect on dopamine or dopamine metabolites in this structure.

It seems that the enhanced locomotor activity induced by nicotinic agonists is mediated predominantly through activation of dopamine neurons in the VTA which project to mesolimbic structures while the depressant effects are mediated through a number of other structures including certain thalamic nuclei as well as the n. accumbens.

250.5 NIGRAL MUSCIMOL EFFECTS ON STRIATAL DOPAMINE ACTIVITY AS A FUNCTION OF AGE. E.F. Sperber, N.S. Sharpless, J.N.D. Wurpel*, S.L. Moshé, Depts. of Neurology and Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461

The substantia nigra (SN) is an important site for the mediation of seizures by GABA. Microinfusion of the GABA agonists, muscimol or GVG, in the adult rat SN suppresses seizures induced by several experimental procedures. In contrast, similar infusion of muscimol in the immature rat SN increases seizure susceptibility. It therefore appears that the GABA-nigral system has different effects on seizures as a function of age. The nigrostriatal dopamine (DA) pathway is one of the main nigral efferents. If the effects of nigral muscimol on seizures are mediated via the nigrostriatal DA pathway, then infusion of muscimol in the SN should affect striatal levels of DA and/or its metabolites.

Twenty-one 14 day old rat pups and 10 adult rats were implanted bilaterally with cannulae into the pars reticulata of the SN (SNr). Following a 2 day recovery period, animals were injected with either 100 ng of muscimol (12 pups and 5 adults) per site or an equivalent volume (0.25 ul) of saline (9 pups and 5 adults). Thirty minutes later the rats were killed and the striata were analyzed for DA and its metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) by high performance liquid chromatography (HPLC) with electrochemical detection. Standard histological techniques were used to confirm the cannulae placements in the SNr.

Microinfusion of muscimol in the adult SNr significantly increased striatal concentrations of DOPAC ($p<.02$) and HVA ($p<.05$) while DA concentrations did not differ from those of saline-treated rats. The ratios of DOPAC/DA (+147%) and HVA/DA (+122%) reflected increases in DA utilization. In contrast, in the 16 day old pup, microinfusion of muscimol had no effect on DOPAC and HVA levels while DA concentrations were increased ($p<.001$). The ratio of DOPAC/DA (-65%) and HVA/DA (-52%) reflected a decrease in DA utilization in pups.

These results suggest that muscimol infusion in the SN, has opposite effects on striatal DA and its metabolites in adult and immature rats; increased turnover in adults and decreased in pups. Previous research has shown that nigral muscimol has anticonvulsant effects in adult rats and proconvulsant effects in rat pups. Our findings suggest that the nigrostriatal pathway may underlie these age-related differences in seizure susceptibility.

250.6 BEHAVIORAL EFFECTS OF CHRONIC EXPOSURE TO SELECTIVE D-1 and D-2 DOPAMINE RECEPTOR AGONISTS. A.R. Braun* and T.N. Chase. (SPON: J.R. Walters) Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20892.

Behavioral supersensitivity following chronic administration of dopamine agonists is a well recognized phenomenon. The mechanism which underlies this process, however, is unclear. We have utilized agents which are selective for the D-1 and D-2 dopamine receptor subtypes in order to examine the role played by either receptor in the process of agonist induced supersensitivity.

Forty male Sprague-Dawley rats (280-300 gms) were pretested for behavioral response to .5 mg/kg apomorphine S.C. A behavioral checklist was employed which allowed quantification of individual motor behaviors; stereotypic behavior was scored using a modified Ernst Scale. Scores were ranked and the animals divided into 4 groups, each having equivalent apomorphine sensitivity. All animals were injected intraperitoneally for 18 days with either the selective D-2 agonist LY 171555 (3 mg/kg given once daily), the selective D-1 agonist SKF 38393 (8 mg/kg given twice daily), both drugs at the same doses, or normal saline. Following a 72 hour drug-free interval, animals were again challenged with apomorphine. Animals treated with SKF 38393 alone or in combination with LY 171555 appeared behaviorally supersensitive when compared with control animals ($P < .005$). Animals chronically treated with LY 171555 appeared behaviorally subsensitive ($P < .005$).

In a second phase of the study the effect of chronic treatment on the behavioral response to the selective agonists themselves was examined. Following a one week drug free interval all animals were scored for behavioral response (intervals of intense grooming behavior) to 8 mg/kg SKF 38393. Animals chronically treated with SKF 38393 displayed an augmented behavioral response to this drug when compared with all other groups ($p < .01$). There were no remarkable differences between the groups when behavioral response to 3 mg/kg of LY 171555 was analyzed following a second one week drug free interval. Animals were again challenged with apomorphine following a third week-long drug free interval; the same patterns of behavioral response seen in the initial post-test were again seen and remained statistically significant.

Our results thus suggest that the behavioral supersensitivity associated with chronic exposure to mixed dopamine agonists may depend on chronic D-1 receptor stimulation. Chronic administration of the selective D-1 agonist alone induces behavioral supersensitivity in response to both apomorphine and to the D-1 agonist itself. On the other hand, chronic treatment with a D-2 agonist alone induces behavioral subsensitivity which may be mediated by down-regulation of the D-2 receptor.

250.7 COMPARISONS OF SOME BEHAVIORAL AND NEUROPHARMACOLOGICAL EFFECTS OF CAFFEINE IN MICE, RATS, AND RHESUS MONKEYS. (SPON: J. Barrett). P. Scott* and J.R. Glowa. Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205

The effects of caffeine were compared on suppressed and non-suppressed responding in three species. Responding was maintained under multiple fixed-interval (FI) 3-min (rhesus monkeys), 5-min (mice), or variable-interval (VI) 45-sec (rats) schedules in which responding under one component produced a mild electric shock and suppressed responding. Suppressed responding was decreased to between 6-35% of the rate of response in the component that did not produce shock. Caffeine (0.3-100 mg/kg) increased both suppressed and non-suppressed responding in each species at intermediate doses, with suppressed responding being increased to a greater extent. Pentobarbital (in mice) and midazolam (in rats and rhesus monkeys) also increased both suppressed and non-suppressed responding, but the increases in suppressed responding with these drugs were typically greater, and the increases in non-suppressed responding were typically less, than the increases seen with caffeine. In the rat, Ro 15-1788 (1-100 mg/kg) slightly increased both suppressed and non-suppressed responding at intermediate doses, with the increases in non-suppressed responding being greater. Over the range of doses studies, Ro 15-1788 did not alter the rate-increasing effects of an intermediate dose (10 mg/kg) of caffeine. In the rat, 1-PIA (0.01-0.3 mg/kg) only decreased both suppressed and non-suppressed responding. However, intermediate doses of 1-PIA potenitated the rate-increasing effects of caffeine on suppressed responding. The results are interpretted as caffeine having a moderate, adenosine-mediated, psychomotor stimulant effect which appears to differ from that of other "classical" psychomotor stimulants.

250.8 PHENCYCLIDINE INDUCED ROTATIONAL BEHAVIOR IS NOT DOPAMINE DEPENDENT. H.D. Everist, C.C. Chiueh, and A. Pert. Biological Psychiatry Branch and Lab. of Cerebral Metabolism, NIMH, Bethesda, Maryland 20892

Phencyclidine (PCP) produces rotational behavior ipsilateral to a unilateral 6-OHDA lesion of the nigrostriatal dopamine pathway. In addition, unilateral injections of PCP into the substantia nigra results in rotational behavior contralateral to the injection. The assumption has been that PCP induced rotational behavior is mediated through enhanced dopaminergic activity. We have found little evidence to support this notion. In the first series of studies, we evaluated the ability of reserpine and alpha-methyl-paratyrosine (AMPT) pretreatment to antagonize PCP, amphetamine or methylphenidate induced rotational behavior in 6-OHDA lesioned rats. Reserpine antagonized methylphenidate and AMPT antagonized amphetamine while neither compound was effective against PCP. Findings from additional studies also make it unlikely that PCP is acting through dopaminergic mechanisms in the caudate nucleus or nucleus accumbens. While 6-OHDA lesions of the n. accumbens attenuated amphetamine induced rotational behavior in nigrostriatal lesioned rats, similar lesions were ineffective against PCP. Furthermore, systemically administered amphetamine failed to produce any asymetry in animals that had been injected unilaterally in the striatum with PCP. In addition, while amphetamine induced rotational behavior was accompanied by significant alterations in dopamine and DOPAC contralateral to the lesion, PCP had little effect on these neurochemicals.

Unilateral injections of PCP into the substantia nigra produced profound rotational behavior contralateral to the injection yet had little effect on dopamine or dopamine metabolites in either the contralateral or ipsilateral caudate. Knife cuts rostral to the substantia nigra were ineffective in altering rotational behavior induced by injections of PCP into this structure while knife cuts caudal to the substantia nigra decreased such rotational behavior significantly.

These findings suggest that PCP induces rotational behavior in lesioned rats through a dopamine independent mechanism. PCP induced rotational behavior followings unilateral injections into the substantia nigra appears to involve pathways that project caudally and is also independent of the ascending nigrostriatal system.

250.9 MICRODIALYSIS OF THE NUCLEUS ACCUMBENS: EFFECTS OF AMPHETAMINE AND PHENYLPROPANOLAMINE ON DOPAMINE AND ITS METABOLITES IN AWAKE RATS. L. Hernandez and B. G. Hoebel. Dept. Psychology, Princeton Univ., Princeton, NJ 08544.

The internal stimulus properties of systemic AMPH (d-amphetamine 1.0 mg/kg) can generalize to PPA (dl-phenylpropanolamine, 10 and 20 mg/kg in rats at 80% of normal body weight (I. Stafford & B. G. Hoebel, Soc. Neurosci. Abstr. 10:1072, 1984). To correlate brain neurochemistry with the stimulus effects of AMPH and PPA, very small microdialysis probes (Brain Res. Instr. Co.) were used to sample monoamines in the nucleus accumbens (NAC) before and after drug administration.

In 6 male rats prepared with a guide shaft implanted above the NAC, a moveable microdialysis probe was placed with its 0.2 mm diameter cellulose tip lodged in the NAC. AMPH (2 mg/kg), PPA (20 mg/kg) or saline (0.3 ml) were given in counterbalanced order in daytime tests. In 20 min samples of dialsate, extracellular DA increased more after AMPH then after PPA, but both drugs had significant effects on DA release at these doses. AMPH decreased DOPAC and HVA; whereas PPA had the opposite effect, suggesting that AMPH blocked DA metabolism. PPA increased 5-HIAA; AMPH did not.

The increase in extracellular DA in the NAC after both drugs may be partly responsible for the similar discriminative stimulus properties observed with high doses of these drugs in underweight animals. Increased 5HT turnover after PPA may be partly responsible for PPA anorexia. Both drugs may also generate a catecholaminergic anorexic cue in other parts of the brain. Observed differences in effects of AMPH and PPA on DA and 5HT metabolites may help explain why AMPH is readily abused and PPA is not.

250.10 EFFECTS OF CHOLINOLYTES ON MAZE PERFORMANCE IN THE RAT: ATTENUATION AND ADDITIVE EFFECTS OF PYRIDOSTIGMINE. Grauer, E.*, Y. Kapon* & G. Porath*. (SPON: A. Levy). Dept.of Pharmacology, Israel Institute for Biological Research, P.O.Box 19, Ness-Ziona 70450, Israel.

The scope of behavioral deficits expected after subcutaneous administration of either atropine or benactyzine was evaluated in rats trained to perform various maze tasks. The animals were required to choose one of two levers, press it and return to the choice point in order to obtain the reward and begin a new trial. The correct choice, depending on the task, was based on one of the following: a. Visual discrimination in a light+/dark+ procedure, b. Spatial discrimination in an unsignaled single alternation procedure, c. Complex visual/spatial discrimination in a successive discrimination procedure. An increase in the number of errors was regarded as indication for central effect of the drugs, while a decrease in rate of performance was an indication for peripheral effects. The lowest doses found to affect both general response rate and error score at specific maze tasks, were 4 mg/kg of atropine and 1 mg/kg of benactyzine. These behavioral effects were only slightly increased when the dose of the cholinolytes was increased up to four fold. Behavioral effects induced by the "threshold" doses were found to be attenuated by a pretreatment with pyridostigmine (130 μg/kg, i.m.). Pyridostigmine, at this low dose, was found to attenuate only the increase in the number of errors but had no effect on the decrease in the rate of responding. However, when tested with the high doses of the cholinolytes, pyridostigmine's attenuation effect on the number of errors disappeared. In addition, an additive effect was noted on the decrease in the response rate. Results are discussed in terms of possible central effects, either direct or indirect, of quatenary compounds.

250.11 MDMA ("ECSTASY"): DRUG DISCRIMINATION AND BRAIN BINDING PROPERTIES. R.A.Glennon,*1 M.Titeler,*2 R.A.Lyon *2 & M.Youssif,*1 (SPON: L.S.Harris) 1 Dept of Medicinal Chem, Sch of Pharmacy, MCV/VCU, Richmond, VA 23298, and 2 Dept of Pharmacol, Albany Med College, Albany, NY.

N-Methyl 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA; "Ecstasy") has recently attracted attention because of its psychoactive action and abuse potential. In humans, S(+)-MDMA is at least twice as potent as its racemate or R(-)-enantiomer (Anderson et al, NIDA Res Monograph 22:8, 1978). Structurally-related agents include its N-desmethyl analog (MDA), its N,alpha-didesmethyl analog (HPA), and 1-(2-methoxy-4,5-methylenedioxyphenyl)-2-aminopropane (MMDA-2.)

Sprague-Dawley rats (n=5) were trained to discriminate 1.0 mg/kg of racemic MDMA HCl from 1.0 ml/kg saline (VI 15-sec schedule of reinforcement for food reward) using a standard 2-lever operant procedure. The MDMA-stimulus generalized (2.5-min extinction session) to S(+)-MDMA HCl with the latter being more potent (ED50= 0.23 mg/kg) than racemic MDMA (ED50=0.52 mg/kg).

Radioligand binding studies were conducted using rat frontal cortex [tritiated 5-HT (5-HT1), ketanserin (5-HT2), DOB (5-HT2H; 5-HT2 high affinity state)], and striatal [N-methylspiperone (D2)] homogenates.

	Ki (nM)		
	5-HT1	5-HT2(KET)	5-HT2H
MDMA	6,850	8,300	-
R(-)MDMA	4,200	3,300	310
S(+)MDMA	11,300	15,800	1,860
MDA	7,150	5,300	-
R(-)MDA	4,800	3,400	-
S(+)MDA	14,000	13,100	-
HPA	750	4,500	-
MMDA-2	2,300	3,700	-

None of the agents displayed an appreciable affinity for D2 sites (i.e. Ki values were > 25,000 nM). In the case of MDMA isomers, Ki values are R(-)-MDMA: 25,400 nM, and S(+)-MDMA: > 100,000 nM.

If the effects (human and discriminative stimulus) of MDMA are due primarily to the actions of the S(+)-isomer, it is unlikely to be related to a direct interaction of these agents with 5-HT1, 5-HT2, or D2 receptors. (Supported in part by NIDA grant DA-01642.)

250.12 ACUTE TOLERANCE TO THE LOCOMOTOR STIMULANT EFFECTS OF NICOTINE: AN INDICATOR OF RECEPTOR DESENSITIZATION? R.L. Hakan and C. Ksir. Dept. of Psychology, University of Wyoming, Laramie, WY 82071.

Rats given daily low doses of nicotine show an increased locomotor response to nicotine. This is coincident with an increase in the number of CNS nicotinic cholinergic receptors. It is possible that a secondary blockade, such as occurs at peripheral nicotinic receptors, might also occur in the CNS. Such a blockade could then explain the upregulation of CNS receptors as a homeostatic adaptation

Two groups of rats were given nicotine (0.2 mg/kg) each day for five days so they would show a sensitized response to nicotine. On the sixth day the rats were placed in photocell activity cages for a one-hour adaptation period, after which five injections were given at 20-min intervals. One group received 0.2 mg/kg nicotine for each injection, the other group received saline. After the first injection the nicotine-treated rats showed significantly more activity than the saline-treated rats. With each subsequent injection at 20-min intervals, the response to nicotine diminished. This demonstrates a rapid tolerance to this behavioral effect of nicotine, as would be expected if nicotine produces a secondary antagonism of its receptor.

In a second experiment rats were given two injections each day of 0.2 mg/kg nicotine, with varying time intervals between the two injections. Second injections which followed the first by 30, 45 or 60 min produced significantly reduced responses compared to the first injection on that day. This secondary antagonism disappears by 90 min.

In the next experiment rats were given three injections on each test day. After the first injection of 0.2 mg/kg nicotine, activity was recorded for 10 min to establish an initial response level. Then an additional 1.6 mg/kg was given. On each test day a third injection, 0.2 mg/kg, was given at a different time after the large injection. If this third injection followed the large injection by 30, 60, 120, 180 or 360 min, the response to it was significantly lower than that day's response to the first 0.2 mg/kg injection. By 550 min after the large injection the response to 0.2 mg/kg was not different from that day's initial response. Assuming a 90-min half-life for nicotine in rat brain, and comparing the time course of the apparent recovery of receptor function after 0.2 and 1.6 mg/kg, it is not possible to reject the hypothesis that the inactivation of the receptor reflects continued presence of nicotine at the site.

The following model is proposed: nicotine first stimulates and then antagonizes its receptor. Repeated antagonism results in an increased number of nicotinic receptors. The increased numbers of receptors then produce an increase in the initial agonistic action of nicotine.

250.13 NICOTINE RESPONSE AND NICOTINIC RECEPTORS IN MICE SELECTED FOR DIFFERENTIAL SENSITIVITY TO ETHANOL. C.M. de Fiebre*, L.J. Medhurst*, L.L. Miner*, and A.C. Collins* (SPON:A.P. Jones). Inst. for Behav. Genetics, University of Colorado, Boulder, CO 80309.

Nicotine and ethanol, two of the most commonly used drugs by man, are often used simultaneously. In an attempt to ascertain whether common genes influence response to ethanol and nicotine, we have examined nicotine response and nicotinic receptor binding in the long-sleep (LS) and short-sleep (SS) mice which have been bred for differential sensitivity to ethanol anesthesia. Using a test battery for measuring nicotine effects in mice we have found that the LS are more sensitive than the SS to nicotine's depressant effects as measured by decline in y-maze activity, heart rate and body temperature following nicotine administration. Also, the LS mice are more sensitive than the SS mice to the seizure-producing action of nicotine. Similar to our findings with C3H and DBA mice (Miner, L.L., et al., J. Pharmacol. Exper. Ther., 231:545, 1984), nicotine-induced seizure sensitivity segregated into F1, F2, and backcross (F1 X LS and F1 X SS) generations in a manner which could be explained by nicotine-induced seizures being regulated by a single autosomal gene. The LS and SS mice did not differ in brain ^{3}H-nicotine binding but, the more seizure-sensitive LS mice have a higher number of hippocampal α-bungarotoxin (BTX) binding sites than SS mice. This difference is not as great as that seen in C3H and DBA mice. Therefore, it seems that differences in hippocampal BTX binding cannot totally account for differences in seizure sensitivity. The preinjection of a subseizure dose of nicotine resulted in a shift to the right of the seizure dose-response curves in both lines of mice with the LS curve showing a greater shift. Such a shift might result from the desensitization of nicotinic receptors. Therefore, LS nicotinic receptors might desensitize to a greater degree than do SS receptors. Biochemical studies have demonstrated that alcohols stabilize the nicotinic receptors from the electric organ of Torpedo in its desensitized state, presumably as a result of altering lipid-protein interactions (Boyd, N.D. & Cohen, J.B., Biochem., 23:4023, 1984). It may be that selection for differences in ethanol-induced sleep-time resulted in differences in membrane lipids surrounding the nicotinic receptor. These differences may contribute to the differences in nicotine-induced seizure sensitivity.
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250.14 PATTERN OF SEIZURES INDUCED BY PICROTOXIN: A DOSE-RESPONSE STUDY. J. Thomas & R. L. Isaacson, Center for Neurobehavioral Science and Dept. of Psychology, University Center at Binghamton, Binghamton, New York 13901.

As a preliminary step to the investigation of interventions that might antagonize epileptiform seizures, this study was undertaken to define the characteristics of picrotoxin-induced seizures. Male rats were injected subcutaneously with various doses of picrotoxin (3, 4, 5, 6, 8, or 10 mg/kg). The animals were placed in a plexiglas chamber and observed over the next hour. Four distinct behavioral patterns of seizures were identifiable: (a) myoclonic episodes, characterized by quick whole body twitches and jerks; (b) "partial" seizure episodes characterized by extension and forward arching of the head, neck, and fore part of the body while maintaining body posture in the hind limb area; and asymmetric tonic contraction or extension that shifted from one hind limb to the other, terminating with the re-establishment of posture along with clonic movements of the forepaws; (c) "atonic" episodes characterized by a fixed, "frozen" posture, staring, loss of muscle tone and an inability of the animal to right itself; (d) generalized seizures characterized by loss of postural control, whole body movements, muscular ridgidity with the head arched upward and back, and bilaterally symmetrical limb extension.

The occurrence of the seizures was predictable with myoclonic, "partial", and "atonic" seizures occurring, in that sequence, during the first half hour after injection. Generalized seizures did not occur in any animal at the 3 mg/kg dose. At the 4 mg/kg dose, generalized seizures occurred in 25% of the animals. At that dose additional episodes of myoclonic or "partial" seizures sometimes preceded or occurred instead of the episode of generalized seizures. At doses of 5 mg/kg and above, the generalized seizures always occurred and there was no deviation in the sequence from the pattern described. The generalized seizures always followed the "atonic" episode. The latency to myoclonus was not significantly influenced by the doses tested while the latency to generalized seizure decreased with increasing doses of picrotoxin. The morphology of the seizures was not influenced by any dose of picrotoxin.

These results indicate that picrotoxin-induced seizures are made up of several distinct types of seizures and the occurrence of generalized seizures is determined by the dose of picrotoxin and is independent of the occurrence of other types of seizures. This suggests that the different patterns of seizures are independent to some degree and it may be possible to manipulate the occurrence of each of them through different pharmacological methods.

250.15 CHANGES IN COCAINE POTENCY WITH MAGNESIUM EXCESS AND DEFICIENCY. K.M. Kantak. Laboratory of Behavforal Neuroscience, Dept. Psychology, Boston University, Boston, MA 02215.

Several lines of evidence suggest that the dopaminergic synapse is the site of the rewarding actions of the psychomotor stimulants. Magnesium (Mg^{2+}) is capable of activating tyrosine hydroxylase and potentiating dopamine receptor binding. We have previously established that behaviorally in mice, Mg^{2+} produces an inverted-U shape function toward offensive aggression, produces tolerance at behaviorally activating doses, and activates catecholamine systems. These effects are highly suggestive that Mg^{2+} has stimulant-like properties and should therefore interact with drugs of abuse such as cocaine and alter its potency. In the present studies potency changes were assesed using aggressive behavior in mice.

The acute administration of 7 doses of cocaine HCl (0.125 to 20 mg/kg) produced inverted-U shape functions in attacks. Cocaine action was potentiated in the excess Mg^{2+} group by almost a factor of 10 in that the average dose of cocaine that produced the maximum facilitation in attacks was 3.7 mg/kg in the control group, and was 0.53 mg/kg in the excess Mg^{2+} group. Conversely, a 15% required-Mg^{2+} diet reduced the potency of cocaine. No facilitation in attacks could be observed up to a dose of 40 mg/kg cocaine. When 0.5 mg/kg cocaine was administered chronically for 15 days, an increase in cocaine potency was detected with excess Mg^{2+}, and a decrease in cocaine potency was detected with low Mg^{2+}. The behavioral reducing properties of chronic cocaine on aggression were the greatest in the excess Mg^{2+} group (50% of baseline) and the least in the low Mg^{2+} group (10% of baseline) as compared to the control group (20% of baseline). These studies indicate that Mg^{2+} status can alter the potency of cocaine on behavior, possibly through a common influence on brain dopamine. This has implications for the abuse liability of cocaine in that the status for this mineral varies considerably in the human population and can therefore contribute toward the understanding of predisposing factors in cocaine abuse. These data also suggest a potential for using Mg^{2+} to help treat cocaine addiction because of its ability to activate the dopamine system.

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250.16 **BILATERAL KAINIC ACID LESIONS OF THE LATERAL HABENULA ALTER DOPAMINERGIC MEDIATED BEHAVIORS.** Paul M. Carvey, LiChiung Kao and Harold L. Klawans. Depts. of Pharmacology and Neurological Sciences. Rush-Presbyterian St. Lukes Medical Center,Chicago, IL 60612.

2-deoxyglucose autoradiography has demonstrated that neuroleptic agents increase and dopamine (DA) agonists decrease glucose utilization in the lateral habenula (LH). The recent discovery of the mesohabenular dopaminergic pathway and an understanding of the influence the LH has on the activity of the raphe complex also suggests that the LH participates in the behavioral expression of DA mediated behaviors.

A total of 102 male, albino rats were studied. The LH was lesioned bilaterally with kainic acid (0.075 mmg in 0.1 mml) in 1/3 of the animals (L-LH), 1/3 of the animals were sham-operated (S-LH) and the remaining animals acted as unoperated controls (U-LH). L-LH animals were hyperactive initially and normoactive but aggressive in the weeks following surgery. L-LH animals exhibited a permanently (5 weeks) increased stereotypic responsivness to 0.35 and 0.75 mg/kg apomorphine and to 4.0 mg/kg amphetamine. When 1.25 and 2.00 mg/kg apomorphine was administered, the L-LH animals were normosensitive relative to S-LH and U-LH animals. Animals normosensitive to the higher doses were found to be hypersensitive when studied at lower doses. The hypoactivity and yawning induced by 0.03 mg/kg apomorphine was unaffected by LH lesions. In order to test whether LH lesions effected neuroleptic induced alterations in behavior, L-LH animals were treated with haloperidol (0.75 mg/kg) for 3 weeks. These animals were subsensitive to apomorphine (0.75 mg/kg) relative to S-LH haloperidol treated animals and normosensitive relative to untreated controls (U-LH).

These results suggest that the LH participates in the expression of DA mediated behaviors in a dose specific fashion. Since the LH is the primary afferent fiber source to the dorsal raphe, and LH lesions have been shown to decrease raphe activity, the potentiated stereotypic response observed here may result from disinhibition release of striatal DA neurones. This hypothesis is consistant with the dose specific modulation we have shown to exist between the DA and serotonin systems.

250.17 AMPHETAMINE INDUCED CONDITIONED PLACE AVERSION IN FEMALE LONG-EVANS RATS. N.L. Costello*, J.N. Carlson*, S.D. Glick, (SPON: C.L. Bowman). Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208

In recent years the place preference paradigm has been utilized as an animal model of drug reinforcement (eg. Mucha, van der Kooy, O'Shaughnessy, and Bucenieks, Brain Res. 243:91-105, 1982; Spyraki, Fibiger, and Phillips, Brain Res. 253:195-203, 1982; van der Kooy, Swerdlow, Koob, Brain Res. 259:111-118). Previous place preference studies have primarily used male rats. Since sex differences in the sensitivity to a number of pharmacological agents have been noted, the present experiment investigated the ability of this paradigm to reflect such differences. Unexpectedly, female Long-Evans rats exhibited a place "aversion" to a dose of d-amphetamine (1.25 mg/kg) which is within the range of doses shown to produce place preferences in male rats. (Spyraki, Fibiger, and Phillips, Brain Res. 253:185-193, 1982). Although female rats treated with d-amphetamine did exhibit an increase in the total time spent in the drug associated environment, this increase was significantly less than that shown by saline treated control animals. Further studies will explore the dose-dependency of this finding and the extent to which such sex differences are quantitative or qualitative in nature. (Supported by NIDA grant DA03817).

250.18 QUANTIFICATION OF DA SUPERSENSITIVITY AFTER BILATERAL 6-OHDA DENERVATION IN MICE P.K. Randall, R.J. Mandel, E.G. Mudie, and R.E. Wilcox Depts. of Physiology and Biophysics and Psychology, USC, Andrus Gerontology Ctr., L.A., CA 90089 and College of Pharmacy, U. Texas, Austin, Texas 78712

Denervation-induced supersensitivity in the dopamine-containing nigrostriatal pathway of the mouse on the order of 30 fold has been reported utilizing rotational behavior in a dual lesion preparation (Mandel and Randall, Br. Res. 330:358, 1985). Supersensitization in the same system induced by chronic neuroleptics, on the other hand, has been reported to be on the order of 2 fold using shifts in dose response curves for stereotypic behavior (Randall, Life Sci. 37:1419, 1985). In this experiment we examined alterations in the dose response curve for apomorphine-induced stereotypic behavior resulting from bilateral 6-OHDA lesion of the nigro-striatal pathway.

Sixty C57BL/6J mice were divided into 3 treatment groups: bilateral 6-OHDA lesion, bilateral sham surgery, and untreated controls. After a 3 week recovery period the dose response-curve for apomorphine-induced behavior was determined in each group using an incomplete block design (APO doses=0.125, 0.25, 0.5, 1, 2, and 4 mg/kg, IP). Each mouse was rated for stereotypic behavior (Severson et al. Br. Res. 210:201,1981) for 1 hr. after receiving the appropriate dose of APO. After the behavioral portion of the experiment, the mice were sacrificed and their striata dissected for determination of 3H-spiperone binding.

Mice with bilateral 6OHDA lesions were significantly more sensitive to APO as determined by nonlinear curve fitting ($F(14,9)=5.2, p<.01$) than both the sham and control groups which did not differ in their response ($F(13,9)=1.13, p>.05$). Examination of quantal dose-response curves for individual rating levels (Randall and Randall, Beh. Neurosci. 100:85,1986) indicated that different components of the APO-induced response showed differential shifts in sensitivity. Lower rated behaviors shifted more (4-5 fold) than more highly rated behaviors (no significant shift). Spiperone binding was significantly elevated in striatum in the bilateral lesion group versus control and sham ($F(2,40)=3.68, p<.05$). No self-mutilatory grooming behavior was observed in the lesioned mice.

High ratings in the rating scale employed are heavily weighted for oral behavior (biting, licking, etc) which appear to be related to action at the D-1 receptor subtype (Yurek and Randall, Life Sci. 37:1665,1985). These data are therefore consistent with differential supersensitization of the D-2 receptor by denervation.

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250.19 EFFECTS OF SULPIRIDE PRETREATMENT ON APOMORPHINE-INDUCED BEHAVIOR IN MICE. D.M.Yurek and P.K. Randall. Dept. of Physiology & Biophysics, USC Sch. of Med., Andrus Gerontology Ctr., Los Angeles, CA 90089.

Previous studies have demonstrated that pretreatment with a selective D-2 receptor antagonist will enhance the expression of Apomorphine-induced stereotypic behavior while simultaneously eliciting catalepsy in mice. To further characterize this phenomenon we performed a series of studies which combined various doses of the selective D-2 receptor antagonist, Sulpiride, with a standard dose of Apomorphine (2.0 mg/kg) and various doses of Apomorphine (0.56, 1.0, 1.8 & 3.2 mg/kg) with doses of Sulpiride ranging from 1.0 ng - 2.0 ug. Sulpiride was administered i.c.v. and Apomorphine was administered i.p. Doses of Sulpiride ranging from 30 ng - 0.25 ug produced modest catalepsy accompanied by enhanced Apomorhine-induced oral behavior, especially licking. Apomorphine-induced licking is rarely if ever observed in mice without Sulpiride pretreatment. At doses greater than 2.0 ug strong catalepsy is observed with a concomitant decrease in climbing and an apparent blockade of oral behavior. However, even at 10.0 ug of Sulpiride, sensory stimulation releases the inhibition of stereotypic behavioral expression. With Sulpiride pretreatment the apomorphine threshold for oral behavior is reduced below that for climbing so that climbing unaccompanied by continuous oral behavior is rarely observed. Sulpiride has little effect on climbing at doses below 2.0 ug.

These data are consistent with a release of D-1 mediated behaviors by D-2 blockade. In addition, blockade of D-1 related behaviors by high doses of Sulpiride may result from the antagonist acting on an independent system resulting in an overall reduction in motor output.

250.20 RESPONSES OF ANTERIOR HYPOTHALAMIC/PREOPTIC NEURONS TO INTRACEREBRAL INJECTIONS OF CARBACHOL. S.M. Brudzynski*, R.S. McLachlan, J.P. Girvin and F. Bihari* (SPON: A.J. Hudson). Epilepsy Unit Research Lab., Departments of Clinical Neurological Sciences and Physiology, University of Western Ontario, London, Ontario N6A 5C1.

Intracerebral injection of carbachol (CCh) into the anterior hypothalamic/preoptic area (AH/POA) is known to decrease spontaneous locomotor behaviour and rearing activity in freely behaving rats and in high doses to induce seizures. The neuronal correlate of these behavioural changes is, however, not known. The aim of the present study was to determine the electrophysiological response of spontaneously firing neurons in AH/POA to direct pressure injections of CCh.

Extracellular single unit recordings from the medial AH/POA of 25 urethane anesthetized rats were carried out 500 s before and 1000 s after ipsilateral injection of CCh (0.1-0.2 µL, 0.5-1.5 µg) from a glass pipette (50-100 µm outer diameter) 0.5-1 mm away from the recording glass microelectrode. The selected dose range is comparable to that used in chronic behavioural experiments. The EEG was recorded from the cortical surface bilaterally and from the microelectrode.

CCh decreased the mean firing rate (m.f.r.) in 19 of 24 neurons located in AH/POA and neighbouring structures (79% of all units). Using the criterion of greater than 30% change in m.f.r. compared to preinjection level, firing decreased in 10 neurons (42%), increased in 1 (4%) and did not change in 13 (54%) during the first 500 s post injection. Some neurons in which m.f.r. decreased had a subsequent increase in firing rate. In a control series of 22 neurons in AH/POA, sham treatment (filled pipette in situ but no injection, n=14) or injection of isotonic saline (n=8) did not markedly change m.f.r. Using the same criterion of at least 30% change, firing decreased in 2 (9%), increased in 2 (9%) and did not change in 18 neurons (82%). On the other hand, similar pressure injections of a 1M solution of L-glutamate dramatically increased m.f.r. of all tested cells (n=7) in AH/POA. Higher doses of CCh (2-4 µg) induced EEG spikes or electrographic seizures at times associated with transient increases of m.f.r. of AH/POA units which had initially shown a reduction in firing rate. The results indicate that the initial cholinergic effect on medial AH/POA neurons by pressure injections of CCh is largely inhibitory. However, with time excitation may predominate possibly relating to the eventual development of seizure activity.

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BEHAVIORAL PHARMACOLOGY IV

251.1 PHARMACOLOGICAL AND GENETIC STUDIES OF ANXIETY USING MICE IN A PLUS-MAZE. Richard G. Lister. Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

The plus-maze is a new test of anxiety that has recently been validated in rats (Pellow et al., J. Neurosci Meths. 14:149, 1985). The test is rapid, and sensitive to the effects of both anxiolytic and anxiogenic agents. This study examines whether the test can be adapted for use in mice, since it would then provide a potentially powerful tool for examining genetic factors underlying individual differences in anxiety and responses to anxiolytics.

The plus-maze consisted of two open arms, and two closed arms with plexiglass sidewalls raised 39 cm above the floor. Mice were tested for 5 min in a holeboard apparatus immediately before a 5 min test in the plus-maze. The behavioral measures from the plus-maze are: 1) the percentage of arm entries made into open arms 2) the time spent on the open arms as a percentage of the total time spent on both open and closed arms and 3) the total number of arm entries. The first and second measures which are used as indices of anxiety are highly correlated (r=0.86).

In NIH Swiss mice the anxiolytics chlordiazepoxide (5-10 mg/kg), ethanol (0.8-1.6g/kg), sodium pentobarbital (7.5-30 mg/kg), and alprazolam (0.25-0.5 mg/kg) increased the percentage of time spent in open arms. The anxiogenic agents picrotoxin (1-2 mg/kg), FG 7142 (5-10 mg/kg), and caffeine (15-60 mg/kg) decreased the percentage of time spent in the open arms. D-amphetamine (1-4 mg/kg) and imipramine (7.5-15 mg/kg) failed to alter the percentage of time in the open arms. Although there was no change in the percentage of entries made into open arms with repeated testing, the effect of chlordiazepoxide on this measure was reduced in animals tested once before in the apparatus in an undrugged state. This suggests that caution should be exercised in retesting animals.

The percentage of entries into open arms was lower in DBA/2 and C57B1/6 mice than in the NIH Swiss mice. Ethanol (0.8-2.4 g/kg) produced an anxiolytic effect in DBA/2 mice by causing a dose-related increase in the percentage of open arm entries. However, no such effect was found in C57B1/6 mice. This provides another example of a difference between these two strains in their behavioral response to alcohol.

These studies suggest that the plus-maze test can be modified for use in mice for detecting anxiolytic and anxiogenic drug effects and that it may be a valuable tool with which to study the pharmacology and genetics of anxiety.

251.2 FURTHER EVIDENCE OF A GABA MECHANISM DISTINGUISHING LONG-SLEEP AND SHORT-SLEEP MICE. T.D. McIntyre and H.P. Alpern. Behavioral Neuroscience Program, Dept. of Psychology, University of Colorado, Boulder, CO 80309.

We have argued that mechanisms responsible for sleep time differences in mouse lines selectively-bred for long and short ethanol-induced hypnosis (LS/SS) are not alcohol specific. LS are more sensitive to the soporific effects of alcohols, enflurane, isoflurane, nitrous oxide, paraldehyde, chloral hydrate, trichloroethanol, methyprylon, γ-butyrolactone, adenosine, chlordiazepoxide, thiopental, pentobarbital, phenobarbital and barbital (McIntyre & Alpern, 1985 for review). Evidence implicates the transmitter GABA in the central actions of many hypnotic-depressants. The GABA agonists THIP and baclofen more effectively inhibit bar-holding in LS, while the indirect GABA agonist AOAA enhances ethanol and barbiturate-induced sleep time in both lines. Finally, it has been shown that SS are more sensitive to bicuculline and flurothyl-induced convulsions, as well as being more prone to ethanol and morphine withdrawal-induced convulsions. The evidence indicates, therefore, that these lines were selected for a more general CNS process than alcohol sensitivity, such as GABA-mediated brain excitability. These experiments further assess this hypothesis by examining the effects of convulsive doses of flurothyl, pentylenetetrazol, bicuculline, picrotoxin, caffeine, methyl-ß-carboline, and strychnine, as well as the effect of the anticonvulsant phenobarbital and the benzodiazepine antagonist RO15-1788 on pentylenetetrazol, bicuculline and caffeine induced convulsions. When the agent induced both myoclonus and clonus (flurothyl, pentylenetetrazol, methyl-ß-carboline and bicuculline), SS had a shorter latency to myoclonus, while LS had a lower latency to clonus. The exception is for bicuculline, where SS had a lower latency than LS for both myoclonus and clonus. For agents producing only clonus (picrotoxin, caffeine, strychnine), LS had a longer latency than SS for clonus. Both phenobarbital and RO15-1788 dose-dependently increased convulsive thresholds for pentylenetetrazol, bicuculline and caffeine-induced convulsions. These results substantiate a GABA hypothesis for the differences between LS and SS, and suggest that the mechanisms mediating myoclonus and clonus are independent.

251.3 SINGLE GENE MEDIATION OF MYOCLONUS IN LONG-SLEEP AND SHORT-SLEEP SELECTIVELY-BRED MICE. H.P. Alpern and T.D. McIntyre. Behavioral Neuroscience Program, Dept. of Psychology, University of Colorado, Boulder, 80309.

We have suggested elsewhere that the Long-Sleep and Short-Sleep lines (LS/SS) of mice were not selectively-bred for specific alcohol sensitivity, but rather a more general CNS process such as GABA-mediated differences in brain excitability. For instance, LS are more sensitive than SS to the soporific effects of alcohols, barbiturates, and benzodiazepines, as well as miscellaneous hypnotics such as adenosine (McIntyre and Alpern, 1985, for review). Additionally, when several convulsants (flurothyl, pentylenetetrazol, methyl-β-carboline, or bicuculline) which antagonize different aspects of the benzodiazepine-GABA receptor-chloride ionophore were examined, it was shown that flurothyl, pentylenetetrazol and methyl-β-carboline induced myoclonus more rapidly in SS than in LS, but clonus more rapidly in LS than in SS. Bicuculline, however, induced both myoclonus and clonus more rapidly in SS than in LS mice (this session). In view of this, we speculated that hybrids (F1's), produced by crossing LS with SS, would display patterns of sensitivity that reflect differences in patterns of inheritance. To test this, F1's were administered either flurothyl, pentylenetetrazol, bicuculline, or methyl-β-carboline. The results indicate that F1's have mean myoclonic latencies for flurothyl, methyl-β-carboline and pentylenetetrazol that are statistically identical to LS. This dominance pattern suggests that a single gene is responsible for myoclonus induced by these agents. Bicuculline, however, exhibits myoclonic and clonic latencies that are intermediate in comparison to the parental lines. This may also be due to a single-gene effect but with no dominance, although more complex genetic models could also account for the bicuculline data. In summary, these results mirror previous findings: (1) flurothyl, pentylenetetrazol and methyl-β-carboline exhibit identical reverse convulsive susceptibility patterns in LS and SS, as well as identical patterns of inheritance in F1 offspring; and (2) bicuculline, which induces both myoclonus and clonus more rapidly in SS than in LS, also displays a fundamentally different F1 inheritance pattern.

251.4 GABAERGIC DRUGS CAN ENHANCE OR ATTENUATE CHLORDIAZEPOXIDE-INDUCED HYPNOSIS IN A HETEROGENOUS STRAIN OF MICE. B. Throop*, S. Levy*, T.D. McIntyre and H.P. Alpern (*Spon: J. Werner). Behavioral Neuroscience Program, Dept. of Psychology, University of Colorado, Boulder, 80309.

We have suggested elsewhere that the mechanism(s) responsible for differences in mouse lines selectively-bred for long and short ethanol-induced hypnosis (LS/SS) are not alcohol-specific. LS are more sensitive to the soporific effects of alcohols, barbiturates, benzodiazepines and miscellaneous hypnotics, and GABA transmission has been implicated as the mechanism of action for most of these depressants (McIntyre and Alpern, 1985, for review). Further, we have reported (this session) that when analeptics produce myoclonus and clonus (flurothyl, pentylenetetrazol, methyl-β-carboline, bicuculline), SS had a shorter latency to myoclonus, while LS had a shorter latency to clonus. The exception to this is for bicuculline, where SS had a lower latency to both myoclonus and clonus. To ascertain the generality of the hypothesis that GABA plays a major role in the expression of brain excitability, the non-selected heterogenous strain (HS) from which LS and SS were derived were administered chlordiazepoxide in conjunction with several drugs that have major actions on the GABA system.

Initially, 50, 75, 100, 125 and 150 mg/kg of chlordiazepoxide was administered to independent groups, to construct a dose-response function, and a dose-dependent increase in hypnosis was found. Then the indirect GABA agonist aminooxy-acetic acid (10 and 20 mg/kg), administered to separate groups in conjunction with 100 mg/kg of chlordiazepoxide, increased hypnosis in a dose-dependent manner. Finally, the GABA antagonists (+)-bicuculline (1.0 and 2.0 mg/kg), picrotoxin (7.0 mg/kg) and pentylenetetrazol (50.0 mg/kg), administered to separate groups in conjunction with 100 mg/kg of chlordiazepoxide, dramatically decreased sleep time. These results show that the HS strain of mice displays drug responses similar in nature to the LS and SS and substantiate the claim that GABA plays a major role in the expression of depressant and analeptic drug effects.

251.5 DIAZEPAM PRETREATMENT INCREASES THE DIAZEPAM-INDUCED ANXIOLYTIC RESPONSE BY DECREASING MUSCLE RELAXATION. D.A. BENNETT AND C.L. AMRICK, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, N.J. 07901.

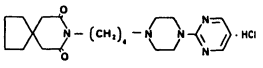
Anxiolytics produce their maximal anticonflict effects in "drug sophisticated" animals (i.e. animals that have received repeated or homecage (HC) pretreatment with a benzodiazepine - BZ) (Margules and Stein, Psychopharmacologia 13: 1968; Corradi et al., Pharmacologist 27: 1985). The present investigation was conducted to determine whether the increase in anticonflict activity produced by HC pretreatment with a BZ is related to either: 1) Tolerance of an initial muscle relaxant effect or 2) An increase in anxiolytic efficacy of the drug per se.

Drug-naive rats, trained in a chronic conflict paradigm, were pretreated in their HC with either diazepam (DZ) (100 mg/kg po), CGS 9896 (100 mg/kg po) (a BZ receptor agonist without muscle relaxant properties - Bennett and Petrack, Drug Dev. Res. 4: 1984) or vehicle (veh). One week later, rats were administered either DZ (10 mg/kg po) or CGS 9896 (30 mg/kg po) and tested in the conflict situation. Rats pretreated with DZ in the HC showed a 417% increase in conflict response rates when tested with DZ, while rats pretreated with either veh or CGS 9896 showed only a modest increase in conflict responding. Therefore, stimulation of the BZ receptor alone (HC pretreatment with CGS 9896) is not sufficient to induce maximal anxiolytic activity when acutely challenged with DZ. Rats pretreated with veh, DZ or CGS 9896 in the HC and subsequently tested with CGS 9896 showed robust increases in conflict responding of 348, 354 and 353%, respectively, in the test situation. These results suggest that a non-muscle relaxant BZ agonist, upon initial administration, produces maximal anxiolytic activity.

Another group of naive rats were pretreated in their HC with either DZ (100 mg/kg po), CGS 9896 (100 mg/kg po) or veh, one week prior to being tested for muscle relaxation on the rotorod following various doses of DZ. In veh or CGS 9896 pretreated rats, DZ produced muscle impairment with ED50 values of 12.6 and 13.9 mg/kg po, respectively; while the ED50 value for muscle relaxation in rats pretreated with DZ was significantly higher (ED50= 44.6 mg/kg po). These results suggest that HC pretreatment with DZ produces tolerance to the muscle relaxant effect of DZ as measured in the rotorod procedure. Again, stimulation of the BZ receptor (in the HC) by CGS 9896 did not produce tolerance to the muscle relaxant effects of DZ.

In conclusion, the maximal DZ anxiolytic response noted from animals that received HC pretreatment with DZ ("drug sophisticated" animals) is due to a tolerance of the initial muscle relaxant effect of the BZ and is not due to an increase in anxiolytic efficacy of the compound per se.

251.6 DISCRIMINATIVE STIMULUS PROPERTIES OF BUSPIRONE HCL (MJ 9022-1). W. J. Millard, K. Moynihan*, A. Murzyn*, and J. Szostak*, Laboratory of Psychobiology, Mount Holyoke College, South Hadley, MA 01075-1462.



The discriminative stimulus properties of an atypical anxiolytic, buspirone HCl, were studied in the rat. A choice procedure was used in which reinforcement for each of two response alternatives was differentially associated with the administration of either buspirone or vehicle. A dose of 2.00 and 2.50 mg kg^{-1} bwt, but not 1.00, resulted in the acquisition and maintenance of differential responding.

Stimulus control of responding by buspirone was dose-dependent (0.75-5.00 mg kg^{-1}), and specific as indicated by substitution tests with sodium pentobarbital (3.16-31.60 mg kg^{-1}) and chlordiazepoxide (1.58-15.80 mg kg^{-1}).

The mechanism of action for buspirone was further examined in tests with MJ-13805-1, an analog of buspirone.

251.7 INTRAVENOUS SELF-ADMINISTRATION OF MIDAZOLAM IN RATS.
J.M. Finlay*, C. Szostak* and H.C. Fibiger (SPON: A. Jakubovic). Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5.

Intravenous self-administration (SA) has been used to provide an indication of the abuse potential of a drug. Laboratory animals typically show low levels of responding when given restricted access to intravenously administered benzodiazepines. However, in these instances low levels of responding may be a function of a long duration of drug action coupled with a short duration of drug access. Thus, we have assessed the potential for benzodiazepine SA in rats using the short acting benzodiazepine midazolam. In order to assess the pattern of responding, rats were given 24 hour access to the drug.

Following implantation of chronic intrajugular cannulae, rats were given 24 hour access to a single lever. During the acquisition phase, responses on the lever resulted in an infusion of midazolam (.05 mg/.18 ml/infusion) coincident with the illumination of a cue light. Over the course of the acquisition phase, each subject (n=6) established a preferred level of baseline responding that remained stable over subsequent 24 hour blocks. Evaluation of responding within the 24 hour period revealed that maximal levels of responding coincided with hours 6-10 of the dark portion of a 12 hour light:dark cycle. Upon attainment of stable levels of responding rats were tested under extinction conditions. During this phase, the experimental contingencies of acquisition were maintained with the exception that saline was substituted for midazolam. With repeated extinction sessions, levels of responding decreased below drug baseline performance for all rats. Rats that remained patent following extinction were tested for reacquisition of midazolam SA. In all cases, rates of responding increased to at least the level obtained during the initial acquisition phase.

We have observed that rats, given continuous access to midazolam, exhibit reliable and stable levels of responding across sessions and that within a session responding is maximal during the dark phase. Responding for SA of midazolam is subject to both extinction and reacquisition. These results suggest that short acting benzodiazepines may have significant abuse potential.

J.M.F. is an MRC student and C.S. is an NSERC scholar.
Supported by the Medical Research Council of Canada.

251.8 BEHAVIORAL TOLERANCE TO DIAZEPAM AS A CONSEQUENCE OF THE DOSE ADMINISTERED CHRONICALLY.
J. Tizzano*, A. Bannon *, R. Liberto*, J. Anderson*, D. Roberts*, D. Muchow* and M. Kallman. Departments of Psychology and Pharmacology, University of Mississippi, University, Mississippi 38677.

Tolerance to the sedative effects of benzodiazepines has been attributed to both physiological and behavioral variables. The present study used the presession (PS) and postsession (PO) injection strategy first developed by Chen (1968) to evaluate the importance of behavioral and physiological factors in the development of tolerance to various doses of diazepam (DZ). Forty-eight male rats were trained to lever press for water reinforcement on a fixed ratio (FR)-30 schedule. After response rates on the FR schedule stabilized, an initial dose effect determination was conducted for DZ (1.0 - 17.5 mg/kg; IP). Animals were then randomly assigned to one of four chronic dosing conditions (0.0, 1.0, 3.0 or 10.0 mg/kg; N = 12 rats/condition). Each group was further divided into two squads (N = 6) with one squad in each group receiving the chronic injections of drug PS (15 min) and the other receiving injections PO (30 min). Following the period of repeated drug administration, dose effect functions were redetermined for DZ. Response rate and duration of response were collected by a laboratory computer system as measures of the effects of DZ. The acute effects of DZ produced a dose dependent decrease in response rate and a dose dependent increase in response duration. When dose effect functions for DZ were redetermined after 30 days of chronic drug administration, a non-parallel shift to the right of the dose response curve was observed indicating the development of tolerance. The response rate data indicated that tolerance develops regardless of whether rats received PS or PO dosing. However, the duration measure indicated a greater degree of tolerance for rats injected PS and the degree of tolerance observed was dependent on the chronic dose of DZ administered. These findings suggest that behavioral factors are more important in the development of tolerance to DZ effects on duration of responding on the FR-30 schedule and that the level of behavioral tolerance which develops depends on the dose of drug administered chronically. Both physiological and behavioral variables are important in the development of tolerance to DZ.

(This research was supported in part by a Faculty Research Award to M.K. from the University of Mississippi, University, MS.)

251.9 BEHAVORIAL EFFECTS OF VASOPRESSIN AND RELATED PEPTIDES AFTER ADMINISTRATION TO CEREBRAL CORTEX AND BASAL FOREBRAIN OF THE RAT.
G. Meisenberg and M. Uddin* (SPON: W.H. Simmons). Ross University Medical School P.O.Box 266 Roseau, Dominica, W.I.

Little is known about the possible roles of vasopressin, oxytocin and related peptides on forebrain structures. Therefore, we evaluated behavioral effects of 8-arginine vasopressin (AVP), oxytocin (Oxy) and the vasopressin V-1 antagonist 1-(, -cyclopentamethylene, -mercapto propionic acid- 2-(O-methyl)tyrosine, 8-arginine vasopressin (P-AVP) after administration to surface structures of the forebrain in rats. Male Sprague-Dawley rats, weighing 275-325g, were implanted with guide cannulae on the medial dorsal cerebral cortex (+2.7, lat 1.3, dv 3.2mm, =22°) and the ventromedial surface of the olfactory tubercles (+4.7, lat 1.5, dv 8.0mm, =10°, incisor bar 3.2mm above interaural line). Peptides were injected in 5.0 l artificial cerebrospinal fluid. Spontaneous behavior was determined in a rectangular open-field, 100 x 100cm, whose floor was divided in 25 squares. Open-field testing was done 7-12 minutes after intracerebral injection of peptide or vehicle. Positively and negatively reinforcing effects were evaluated in an "unbiased" place-preference paradigm. After administration on the dorsal cortex, both AVP and P-AVP (2.5 g each) caused profound reductions of spontaneous motor activity with increased periods spent immobile. Doses of 500ng of either peptide induced effects of lesser magnitude, while 100ng were inactive. 2.5 g of P-AVP, but not AVP showed "cataleptogenic" properties, as evidenced by an increased latency to leave the central square at the beginning of the open-field test. After injection on the olfactory tubercles, P-AVP (2.5 g, 500ng) caused behavioral alterations qualitatively and quantitatively similar to those after administration to the dorsal cerebral cortex, while AVP (100ng) induced "barrel rotation" (3/9) or limbic seizures (1/9) in addition to variable behavioral abnormalities. Oxy (100ng, 500ng, 2.5 g) was inactive at either site. P-AVP showed no significant rewarding or aversive properties at either site (2.5 g) in spite of its marked effects on spontaneous behavior. These results suggest that the presence of vasopressin or vasopressin-related peptides and their possible physiological roles in basal forebrain structures and the cerebral cortex should be re-evaluated.

251.10 ANXIOLYTIC SCREENING IN MICE: STUDIES OF PARAMETRIC VARIATIONS AND COMPOUND SELECTIVITY IN A DRINKING CONFLICT PROCEDURE.
B.R. Cooper, M.E. Judge*, J.L. Howard. Pharmacology Department, Wellcome Research Laboratories, 3030 Cornwallis Rd., Research Triangle Park, NC 27709.

A rapid, simple drinking conflict test was studied in mice as a potential anxiolytic screening procedure. The test consisted of a modification of two procedures, a) the Vogel et al. (1971) conflict procedure which is routinely used as an anxiolytic screen in rats, and b) the acquisition session of a one trial approach-avoidance procedure used in mice (Judge and Quartermain, 1982).

Male CD-1 mice (15-25 gms.) were water deprived before testing. The test chamber was a 6 x 6 x 6 inch white plexiglas cube with a clear hinged lid, solid aluminum floor and a stainless steel drinking tube protruding through a 0.5 inch hole above the floor. The test session consisted of placing individual mice, previously injected with vehicle or a test drug, into the chamber and allowing them to drink for 5 seconds. After this initial drinking period, an approach-avoidance conflict was established by administering brief electric shocks through the water tube for subsequent drinking. The test was terminated when the mouse suppressed contact with the water tube for 60 seconds, and the test measure consisted of the number of shocks received up to this point. Anxiolytic drugs produced a dose related antagonism of lick suppression. Three variables were found to significantly influence the ability of the test to detect a 4 mg/kg i.p. dose of chlordiazepoxide (CDZ):

1) A 48 hour period with no access to water resulted in significantly less variability than 24 hours of water deprivation, and eliminated the need for any pre-test adaptation to the apparatus.
2) Varying shock level from 0.2 to 1.0 mA resulted in the optimum separation of CDZ from saline at 0.3 mA, with little separation at 0.2 or 1.0 mA.
3) Varying the cumulative contact duration necessary to produce a shock resulted in a separation of CDZ from saline at long intervals (around 1.0 second) at which mice make about 10 licks per shock as in the Vogel procedure, however, the most rapid and reliable determinations of the presence of CDZ were achieved when the contact duration was restricted to 0.1 seconds.

Judge, M.E., and Quartermain, D., Physiology & Behavior, 1982, 28:585-590.
Vogel, J.R., Beer, B. and Clody, D.E. Psychopharmacologia, 1971, 21:1-7.

251.11 EFFECTS OF IMIPRAMINE ON CARDIODYNAMIC ACTIVITY DURING STRESS. Steiner, S., Schoenheimer, J.*, Hamberger, M.*, Klutchko, B. & Friedhoff, A. Clinical Technologies Associates, Inc., Mt. Kisco, New York 10549.

Tricyclic antidepressants are the most widely prescribed compounds for the treatment of clinical depression, and imipramine is the benchmark antidepressant. Documentable cardiotoxic effects of imipramine have been obtained from patients with pre-existing cardiovascular disorders, particularly conduction defects. Overdose data also clearly demonstrate imipramine's cardiotoxicity at high doses. However, little data has been presented thus far to support the presumed danger of lesser cardiotoxic effects at therapeutic doses with individuals free of cardiovascular disease (Glassman, 1984).

This study employed the technique of electrical impedance cardiography to monitor the cardiac performance of subjects following the administration of imipramine. This technique allows the noninvasive monitoring of the ejection of blood from the left ventricle and its waveform represents the velocity of the blood as it is ejected during systole (Miller & Horvath, 1978).

The subjects were nine patients, diagnosed as clinically depressed according to Research Diagnostic Criteria, and for whom imipramine had been prescribed. Impedance cardiograph records were obtained on three occasions, each involving both resting and stress conditions. The first recording was obtained immediately prior to receiving the first dose of imipramine (50 mg). The second recording was one hour after the initial dose, and the third record was obtained after taking a therapeutic dosage of imipramine (100-150 mg) for three weeks. The study in no way dictated or modified the treatment these patients received.

Most subjects demonstrated a pertubation in the waveform early in systole, representing a transient interruption in the development of maximal ejection velocity. This change in the waveform is obvious to the eye and its dimensions are measurable. The phenomenon was observed just one hour after administration of imipramine in many subjects and three weeks later it was even more pronounced. It was observed that the remaining subjects, who did not demonstrate the phenomenon after the initial dose, typically showed the effect after three weeks of administration. This observed change in the impedance cardiogram is similar to the waveform seen in patients with conduction defects (Mohapatra, 1981). The authors believe this to be an early precursor to more serious conduction defects.

These results indicate that the effects of imipramine on myocardial performance can be observed after the first dose and at therapeutic doses in individuals with no cardiovascular disease. Therefore this protocol may be useful in early clinical trials.

251.12 CIRCLING BEHAVIOR FOLLOWING UNILATERAL INTRACEREBROVENTRICULAR MICROINJECTIONS OF CHOLECYSTOKININ$_8$ (CCK$_8$) IN RATS. M.A. Morency, G.M. Ross and R.K. Mishra. Depts. of Psychiatry and Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Intracerebroventricular (ICV) microinjections of CCK$_7$ ($3x10^{-8}$ mol) have been reported to elicit barrel rotations accompanied by characteristic limb flexion and extension and contralateral postural asymmetry (Mann et al., 1980, Pharmacol. Biochem. Behav. 13: 125-127). With this high dose, however, there was no spontaneous locomotor activity other than barrel rolling. The present study examined the effects of lower doses of CCK$_8$ on circling behavior.

Male Sprague-Dawley rats were implanted with a chronic indwelling cannula (20 gauge) aimed at the right or left lateral ventricle. Each rat was tested 5 times on alternate days. Prior to the first and fifth sessions, rats were injected with the vehicle (10 µl); doses of 5 µg ($4x10^{-9}$ mol), 500 ng ($4x10^{-10}$ mol) and 50 ng ($4x10^{-11}$ mol) CCK$_8$ in 10 µl were administered in a counterbalanced order prior to the second, third and fourth sessions. Test sessions began with the central injection and placement in a Lafayette multi-activity recording box. During 5 observation periods (0-3, 6-9, 15-18, 21-24 and 27-30 min) all complete turns ipsiversive and contraversive to the side of the cannula were recorded. An activity count was obtained for the 30-min session.

The 3 doses of CCK$_8$ produced a marked increase in contraversive turning ($p < .01$). Furthermore, animals exhibited postural asymmetry, ipsilateral limb flexions and contralateral limb extensions. The two higher doses of CCK$_8$ induced barrel rotations in some rats. The highest dose of CCK$_8$ (5 ng) significantly reduced spontaneous locomotor activity; this was reflected in significantly lower numbers of total turns and lower activity counts ($p < .01$).

Preliminary evidence indicates that contraversive circling and a similar behavioral sequela is induced by desulfated CCK$_8$. In addition, proglumide, a CCK antagonist, does not appear to attenuate the circling behavior induced by CCK$_8$. Thus, this behavioral syndrome appears to be a non-specific, possibly toxic, effect. (Supported by the Natural Sciences and Engineering Research Council of Canada.)

251.13 EFFECT OF ARGININE VASOPRESSIN ON ARTERIAL PRESSURE, HEART RATE, AND AUDITORY EVOKED POTENTIALS IN THE RAT. M.A. Nathan, N.K. Squires*, and E. K. O'Malley*. Dept. of Pharmacology, Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX, 78284, and Dept. of Psychology, State Univ. of N.Y., Stony Brook, NY, 11794.

Arginine vasopressin (AVP) has been claimed to affect memory consolidation and/or retrieval (de Wied et al., 1984). However, the issue is controversial and some have argued that AVP only indirectly affects memory perhaps by raising mean arterial pressure (MAP) or by increasing arousal (Koob et al., 1985; Sahgal, 1984). The present study was conducted to examine the possibilities that AVP increases arousal, and that the arousal may be associated with an elevation of MAP. Male Long-Evans rats (n = 4, weight 325-365 g) were instrumented with an arterial cannula for recording MAP and heart rate (HR) and a subcutaneous cannula for administration of AVP or vehicle. Screws were implanted in the skull for recording auditory evoked potentials (EPs). All measurements were made 2-4 days after instrumentation. AVP (1-15 µg/kg) or vehicle were given immediately prior to each measurement period. EPs were recorded during presentation of free field tones (8 kHz, 30 msec duration, rate of 1 per 2 secs, 75-85 dB). ANOVA and correlation analyses were performed (significance point, $p<0.05$). There was a significant dose-related increase in MAP (vehicle = 116 ± 3 mmHg; 15 µg/kg = 158 ± 10 mmHg) and a significant decrease in heart rate (vehicle = 318 ± 16 bpm; 15 µg/kg = 214 ± 27 bpm). EPs (amplitude from N20 to P30) also increased significantly as a function of dose (vehicle = 60.7 ± 6.94 µV; 15 µg/kg = 97.42 ± 12.18 µV). However, the increased amplitude was observed only when the animals were resting quietly or appeared to be sleeping. Moreover, AVP appeared to depress somatomotor behavior, especially at the higher doses. MAP and EPs were positively correlated (+0.48) and a larger negative correlation existed between HR and EPs (-0.66). Response latency also differed (HR = 12 ± 4 min; EPs = 17 ± 4 min; MAP = 21 ± 6 min). We conclude that: (1) the increases in EP amplitude were not dependent upon an elevation of MAP because EP changes were not consistently preceded or accompanied by increases in MAP, (2) AVP acted centrally because HR decreases were frequently seen in advance of an increase in MAP and the initial decrease in HR after peripheral administration of AVP has been shown to be of central origin (Undesser et al., 1985), and (3) AVP affected EPs but the changes were associated with decreased, rather than increased, arousal.

251.14 EFFECTS OF BENZODIAZEPINE AGONIST AND ANTAGONISTS ON FINE MOTOR DISCRIMINATION IN THE RAT. C.E. Lau*, J.L. Falk and M. Tang*. Dept. of Psychology, Rutgers University, New Brunswick, NJ 08903.

The effects of midazolam, a short acting benzodiazepine, on fine motor discrimination was assessed in the rat. Animals were trained to hold down a manipulandum within a 15-27g forceband for 1.5 sec to obtain a 45-mg food pellet. A total of 50 pellets could be obtained in each session. Acute doses of midazolam maleate (0.75, 1.5 and 3.0 mg/kg s.c.) given 30 min pre-session produced dose-related increase in dyskinesia and decreases in work rate, tonic accuracy and efficiency. The benzodiazepine antagonist Ro 15-1788 (5 and 10 mg/kg s.c.) given 15 min pre-session, however, did not have any effect on the parameters under study. Low doses (0.5 and 2.0 mg/kg p.o. 1-hr pre-session) of another antagonist CGS 8216 were also ineffective in affecting any behavioral changes while a higher dose (10 mg/kg) produced behavioral disruptions similar to that observed after a low dose of midazolam. Both RO 15-1788 and CGS 8216, however, were effective in reversing the disruptive effects of midazolam in a dose-related fashion.

251.15 A CRITICAL EXAMINATION OF THE CONDITIONED DEFENSIVE BURYING PARADIGM AS A MODEL FOR IDENTIFYING ANXIOLYTICS. R.M. Craft*, G.T. Pollard, and J.L. Howard. Department of Pharmacology, Burroughs Wellcome Company, 3030 Cornwallis Rd., Research Triangle Park, NC 27709.

Treit et al. (Pharmacology, Biochemistry & Behavior 15:619, 1981) showed that conditioned defensive burying (CDB) provoked by a shock delivered from a novel object in an environment to which a rat has become habituated is suppressed by several conventional anxiolytics in a dose-dependent manner. They have suggested CDB as an anxiolytic screen free from the problems inherent in non-ethological models. The purpose of this experiment was to determine whether suppression of burying is drug-class specific and whether the drugs that suppress burying do so only at doses that do not disrupt other behaviors as well.

Thirty minutes before the test session, each rat was injected orally with a conventional anxiolytic (chlordiazepoxide 4, 8, 16, 32 mg/kg), a novel anxiolytic (buspirone 8, 16, 32 mg/kg), a neuroleptic (chlorpromazine 1, 2, 4, 8 mg/kg), an antidepressant (imipramine 4, 8, 16 mg/kg), an opiate analgesic (morphine 2, 4, 8 mg/kg), or vehicle. Subjects were observed for 10 minutes, and the duration of all behaviors (categorized as one of six types) was recorded: burying, other behaviors that caused bedding to be displaced (primarily digging), locomotor behaviors (walking and rearing), gnawing (on corn-cob bedding), grooming, and resting (completely still, or sniffing or teeth chattering while stationary).

Burying was significantly suppressed by the typical anxiolytic chlordiazepoxide but not by the atypical anxiolytic buspirone. Imipramine and morphine also suppressed burying in a dose-dependent manner. Chlordiazepoxide and morphine significantly suppressed burying only at doses that also suppressed locomotor behaviors, while imipramine suppressed burying without affecting locomotion. Chlorpromazine caused a nonsignificant decrease in burying. All drugs that suppressed burying also suppressed digging and grooming. The two anxiolytics significantly increased gnawing; other drugs did not affect this behavior. We conclude that CDB is not superior to existing models of "anxiety" in terms of drug class specificity.

251.16 A SUBJECTIVE EFFECT OF DIAZEPAM WITHDRAWAL IS BLOCKED BY PHENOBARBITAL. Diane A. Mathis*, Cynthia M. Harris, Smart O. Idemudia*, Michael W. Emmett-Oglesby, and Harbans Lal. (SPON: Edward Orr). Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas, 76107.

The subjective effect of diazepam withdrawal has been measured in an animal model for anxiety using a two-lever choice, free operant paradigm (Lal and Emmett-Oglesby, Neuropharmacology 22:1422, 1983). Rats trained to discriminate an anxiogenic drug, pentylenetetrazol (20 mg/kg) from saline, were given generalization tests with drugs which substitute for or block the pentylenetetrazol (PTZ) stimulus. The stimulus effect of diazepam withdrawal precipitated by RO15-1788 substituted in a dose-dependent manner for the pentylenetetrazol stimulus and was blocked by pentobarbital (Emmett-Oglesby, et al, Eur. J. Pharmacol. 92:127, 1983). The present study tested phenobarbital blockade of diazepam withdrawal. Rats were injected ip for 16 days with diazepam (80 mg/kg, t.i.d). RO15-1788 (10mg/kg) produced PTZ-lever selection by 88% of the rats. Pretreatment with phenobarbital, 15 min prior to the RO15-1788 injection, blocked PTZ-lever selection in a dose-dependent manner, with full blockade at 80mg/kg. Phenobarbital also blocked PTZ-lever selection in non-dependent subjects. These results suggest that the drugs of the barbiturate class are effective in blocking a subjective effect of diazepam withdrawal and that the pharmacological profile of the discriminative stimulus from precipitated diazepam withdrawal is similar to that of PTZ. Since the site of action of phenobarbital may be the supramolecular complex containing the diazepam receptors and GABA-modulated chloride ionophores, alterations in the receptor/ionophore complex may mediate the subjective effects of diazepam withdrawal. (Diazepam and RO15-1788 were generously provided by the Hoffman-LaRoche Company. Supported by NIDA Grant RO1 DA03521A).

251.17 MAUDSLEY REACTIVE AND NON-REACTIVE RAT STRAINS: DIAZEPAM AND PENTOBARBITAL EFFECTS ON CONFLICT BEHAVIOR. R.L. Commissaris*, G.M. Harrington*, A.M. Ortiz* and H.J. Altman (SPON: M.A. Marrazzi). College of Pharmacy and School of Medicine, Wayne State University, Detroit, MI 48202 and Dept. Psychology, Univ. Northern Iowa, Cedar Falls, IA 50614.

Based on open field defecation rates, two distinct strains of Maudsley rats have been identified. The Maudsley Reactive (MR) strain exhibits high levels of open field defecation, while the Maudsley Non-Reactive (MNRA) strain exhibits low levels of open field defecation. This difference in open field behavior has been interpretted as an indicator of "emotionality". These Maudsley MR and MNRA rat strains have also been shown to differ in their basal behavior in the conditioned suppression of drinking (CSD) procedure, a second "model" behavior for the study of anxiety and/or emotionality in rats. The present studies were designed to compare these MR and MNRA rat strains in terms of their response to typical anti-conflict agents in the CSD paradigm. In daily 10-minute sessions, water-deprived rats were trained to drink from a tube that was occasionally electrified (0.5 mA), electrification being signalled by a tone. Consistent with previous reports, MNRA rats accepted significantly more shocks than did MR rats during control (non-drug) sessions. Further, rats of the MNRA strain were more responsive to the anti-conflict effects of both diazepam and pentobarbital. This strain difference was observed in both male and female rats. Thus, the Maudsley MR and MNRA rat strains, bred originally for their differences in open field behavior, also differ markedly in their responsiveness to anti-anxiety agents in the CSD paradigm. These findings further the hypothesis that the Maudsley rat strains may represent a genetically-based "animal model" for the study of emotionality and/or anxiety.

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251.18 AN OCTADECANEUROPEPTIDE (ODN) DERIVED FROM DIAZEPAM BINDING INHIBITOR INCREASES AGGRESSIVE BEHAVIOR IN MICE. M. Kavaliers and M. Hirst, Department of Psychology, University of Alberta, Edmonton, Alberta, Canada, T6G 2E9 and Department of Pharmacology & Toxicology, University of Western Ontario, London, Ontario, Canada, N6A 5B7.

Diazepam binding inhibitor (DBI) is a naturally occurring neuropeptide that is capable of displacing benzodiazepines and beta-carboline derivatives from their specific binding sites. It has been proposed that rat and human brain DBI functions as a precursor for the octadecaneuropeptide (ODN), Gln-Ala-THr-Gly-Asp-Val-Asn--Thr-Asp-Arg-Pro-Gly-Leu-Leu-Asp-Leu-Lys, and possibly other neuropeptides which function as endogenous ligands for benzodiazepine recognition sites. These observations, along with the results of initial behavioral and pharmacological investigations, have led to the proposal that DBI and ODN may have physiological roles in the regulation of behavioral patterns associated with conflict situations, anxiety and aggression.

Social conflict and aggression are major features of natural animal behavior. Intraspecific aggressive encounters consist of a number of components: threats, attacks, fighting and subsequent defeat and submission. Thus, removal of a small mouse from its group-housed environment and placement in the home-cage of a larger, singly-housed mouse causes the defeat and submission of the intruder animals. Accordingly, it was considered of interest to examine the effects of ODN on the offensive and defensive aggression displayed by mice.

Intracerebroventricular (i.c.v.) administrations of ODN increased, in a dose-dependent manner (0.75-5.0 nmoles), the propensity for and intensity of offensive aggression in dominant resident mice. ODN also increased the number of bites required to obtain defeat in i.c.v. treated subordinate mice during aggressive interactions, as well as reducing the subsequent defeat-induced opiate analgesia. In a parallel manner, ODN initiated and increased the display of aggressive behavior in minimally aggressive BALBc male mice. The facilitatory effects on aggression occurred without any evident effects on the basal activity levels or nociceptive responses of the animals. These results indicate that ODN can function as pro-conflict peptide and both induce and increase the display of aggressive behavior.

251.19 EFFECTS OF GABAERGIC DRUGS ON PENILE REFLEXES AND COPULATION IN RATS. R. E. Leipheimer* and B. D. Sachs (SPON: W. A. Wilson). Dept. of Psychology, U-20, Univ. of Connecticut, Storrs, CT 06268.

Several components of penile reflexes in rats are dependent on actions of the striated penile muscles. In view of evidence that GABA terminals synapse in the area of motor neurons which innervate the perineal muscles, we studied the effects of GABA agonists and antagonists on penile responses "ex copula" and during copulation.

Males known to respond in ex copula tests (restrained in supine position with penile sheath retracted) were assigned to control (saline injected) or one of the following treatment groups: baclofen (1 or 2 mg/kg), a GABA-B receptor agonist; THIP (5 mg/kg), a GABA-A receptor agonist; or bicuculline (0.25 mg/kg), a GABA-A receptor antagonist. Tests began 20 min after sc injection.

Baclofen significantly reduced penile erection in ex copula tests: 3/7 males responded after 1 mg/kg and 1/7 after 2 mg/kg. The effects were stronger on the somatic than on the autonomic components of penile reflexes. Baclofen-treated males that responded also had significantly longer latencies to first erection than males in the other groups, all of which displayed penile reflexes. THIP did not inhibit reflexes; Indeed, it tended to potentiate them. Bicuculline had no apparent effect on penile reflexes.

Because baclofen inhibited penile reflexes in ex copula tests, we assessed the effects of this drug (same dosages) on copulatory behavior. There were no significant differences between saline-injected and baclofen-treated males in any parameter of copulatory behavior. In addition, baclofen did not appear to inhibit penile responses during mating: All males attained intravaginal intromissions and ejaculations, as determined by the presence of spermatozoa in vaginal smears taken at the end of each test. This pattern of behavioral effects of baclofen (absence of reflexes ex copula but not in copula) is analogous to the effects of systemic estradiol treatment in castrated rats, and suggests that higher centers may nullify the inhibitory actions of baclofen on penile reflexes during copulation.

We tentatively conclude that central (probably spinal) GABA-B receptors play an inhibitory role in the complex synaptic mechanisms which regulate penile reflexes. However, pending the outcome of ongoing studies on the effects of intrathecal application of GABAergic agents, we can not rule out the possibility of effects of these drugs outside the CNS.

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251.20 ROTATIONAL BEHAVIOR IS INDUCED BY SEROTONIN AGONISTS IN RATS WITH UNILATERAL 6-HYDROXYDOPAMINE-INDUCED SUBSTANTIA NIGRA LESIONS. R. Gerber, C. A. Altar and J. M. Liebman. Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Rotational behaviors are induced by 5HT-1 agonists, including 8-OH-DPAT and RU 24969, in rats in which dorsal raphe (DR) 5HT-containing neurons have been unilaterally destroyed by 5,7-dihydroxytryptamine (5,7-DHT) (Blackburn et al, Psychopharmacology 83: 163-165, 1984). As a further test of this model for 5HT agonism, it was hypothesized that 5HT-1 agonists should show a high degree of selectivity for rotational behavior in 5,7-DHT-lesioned rats, as opposed to 6-OH-DA-lesioned rats. Conversely, DA agonists should fail to induce rotational behavior in 5,7-DHT-lesioned rats.

Neurotoxins were microinjected through 25 gauge needles stereotaxically aimed at target brain regions. One group of anesthetized rats received 6-OH-DA HBr (11 ug/4ul) in the right SN. Another group was administered 5,7 DHT creatinine sulphate (1.6 ug/ 2 ul) in the right side of the DR one hr after pargyline (50 mg/kg i.p.) pretreatment. Two weeks post-surgery, 6-OH-DA-lesioned rats were challenged with SKF 38393 (3.0 mg/kg s.c.) and 5,7-DHT-lesioned rats with 8-OH-DPAT (3.0 mg/kg i.p.), and rotational behavior was immediately assessed in an Omnitech Digiscan analyzer. Rats were used for subsequent experimentation only if they exhibited more than 30 net contralateral turns within 60 min after SKF 38393 or within 90 min after 8-OH-DPAT.

Confirming previous reports, 8-OH-DPAT (0.3-3.0 mg/kg s.c.) and RU-24969 (3.0-10 mg/kg s.c.) induced contralateral rotational behavior in 5,7-DHT-lesioned rats. Neither apomorphine nor 3-PPP were active in this model. Surprisingly, however, 8-OH-DPAT (1.0-3.0 mg/kg) and RU-24969 (10 mg/kg) also induced contralateral rotational behavior in unilaterally 6-OH-DA lesioned rats. The potency of these compounds and the magnitude of their effects were comparable in the 5,7-DHT and 6-OH-DA models.

It seemed possible that the 6-OH-DA treatments might have inadvertently depleted striatal 5HT levels along with DA levels, causing a denervation of striatal 5HT receptors. Therefore, striatal 5HT and DA content was assessed in eight representative 6-OH-DA lesioned rats. Striatal DA was depleted by 99% while 5HT levels were unchanged.

Alternatively, it has been hypothesized that denervated DA receptors may lose some of their selectivity for DA agonists (Fenton et al., Eur. J. Pharmacol. 106: 363-372, 1984). This hypothesis was proposed to account for the ability of SKF 38393 and 3-PPP to induce rotational behavior in some (but not all) 6-OH-DA-denervated rats. We suggest that highly denervated (>99% DA depletion) DA receptors may also be responsive to some agents nominally identified as 5HT agonists.

INVERTEBRATE NEURODEVELOPMENT I

252.1 LEECH DEVELOPMENT IN TWO DIMENSIONS: AN ORGAN CULTURE PREPARATION. Margaret I. Law, Ph.D. Dept. of Molecular Biology, Univ. of California, Berkeley, CA 94720

Embryos of the glossiphoniid leech, Haementeria ghilianii, were grown in organ culture after removal of their yolk and yolk membranes. Yolkless embryos were pinned out flat in sterile dissecting dishes filled with a modified L-15 culture medium and maintained at 24°C for 3-8 days. Two aspects of the development of these cultured embryos were compared with the normal developmental pattern: expression of the serotonin neurotransmittor phenotype and axonal growth.

Twelve embryos were explanted between stages 9(0/4) to 9(1/4), one to two days before immunocytochemical labeling with serotonin antibodies normally detects any serotonin containing neurons (D.K.Stuart and J.C. Glover, in prep.). Upon culture of the embryos, serotonin containing neurons became sequentially detectible in the explanted embryos in an order comparable to that in which particular serotonin containing neurons appear in intact embryos. However, the serotonin phenotype was expressed in the explants more slowly than in intact embryos.

Immunocytochemical labeling also revealed that an identified serotonin containing neuron in explanted embryos developed its normal axonal projections, both within the nerve cord and into the periphery. Immunocytochemical labeling with monoclonal tubulin antibodies (Amersham) showed that the explanted embryos continue to form commissural, connective and segmental nerves, as well as muscles, in culture.

Growth of peripheral axons of an identified sensory neuron (P_V) was followed in embryos explanted at stage 10(1/5). Intracellular injections of lucifer yellow dye into P_V neurons in some embryos immediately before explantation indicated the initial extent of their peripheral axons. Dye injections of P_V neurons in other, sibling embryos after 3-8 days in culture revealed a substantial additional growth and arborization of P_V axons. Moreover, in several of these cultured embryos normal resting and action potentials could be recorded.

The ability to grow dissected leech embryos in organ culture opens up many potential experimental manipulations which were hitherto impossible in the intact embryos.

252.2 EARLY DIFFERENCES BETWEEN ALTERNATE N BLAST CELLS IN THE LEECH EMBRYO. S.T. Bissen* and D.A. Weisblat (SPON: B. Holton). Dept. of Zoology, University of California, Berkeley, CA 94720.

The early embryo of leech contains five bilateral pairs of stem cells, the M, N, O/P, O/P and Q teloblasts. Each teloblast undergoes iterated, highly unequal cleavages to produce a coherent column of cells, called blast cells. In each column, or bandlet, blast cells are born at the rate of one per hr at 23°C, and the older cells lie distal to the younger ones. Thus in each bandlet, the age of each blast cell corresponds to its position in the bandlet. The proliferation of the blast cells ultimately gives rise to the segmental tissues of the leech. In the n bandlet, which is produced by the N teloblast, two adjacent blast cells give rise to each segmental complement of cells; each blast cell generates a distinct set of progeny. These two alternating classes of blast cells in the n bandlet can also be distinguished by the timing and symmetry of their first divisions; one divides unequally about 22 hrs after its birth and the other divides equally about 28 hrs after its birth. Furthermore, ablation and other studies provide indirect evidence that the alternate n blast cells may be determined to be different by events occuring at the time of their births.

To characterize the differences between the newly born n blast cells, DNA synthesis and RNA transcription were examined in these cells in Helobdella triserialis embryos using autoradiography. N teloblasts were injected with a fluorescent lineage tracer and allowed to produce several blast cells. The embryos were then injected with [^{3}H] thymidine triphosphate (TTP) or [^{3}H] uridine triphosphate (UTP), both of which diffuse rapidly throughout the embryos. In embryos injected with [^{3}H] TTP, silver grains were only present over the nuclei, even after long incubation periods, which suggests that the [^{3}H] TTP was incorporated into DNA. The n blast cells began synthesizing DNA very soon after birth and continued for several hrs. These data suggest that the two classes of n blast cells have G_1 and S phases of similar lengths, but differ in the length of their G_2 phases. In embryos injected with [^{3}H] UTP, grains were localized over the nuclei after short incubation periods, however, after longer periods of incubation, grains were present throughout the cytoplasm as well as over the nuclei, which suggests that the [^{3}H] UTP was incorporated into RNA. The n blast cells started accumulating RNA within the first hour or two after birth and continued for at least 10 hrs. These results provide more direct evidence that the alternate n blast cells may become different at birth or immediately thereafter.

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252.3 GROWTH AND DECREMENTAL CONDUCTION IN THE PLEXUS OF THE HORSESHOE CRAB, LIMULUS POLYPHEMUS. W. H. Fahrenbach., Laboratory of Electron Microscopy, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Lateral inhibition in the lateral eye of Limulus is mediated by passive conduction (Hartline et al., Cold Spring Harbor Symp. Quant. Biol. 17: 125, 1952) via collaterals of eccentric cells, secondary receptor neurons of the ommatidia. During growth spacing between ommatidia increases in direct relationship to body size, i.e., prosomal width. Collaterals, on the other hand, which have to span one half of the inhibitory field, increase only logarithmically in diameter during growth. For example, average collateral diameters increase from 110 nm in a 12 mm animal with an inhibitory field diameter of about 250 µm to 0.69 µm in an adult with a prosomal width of 200 mm and an inhibitory field of 6 mm. If the known membrane resistivity of the eccentric cell axons (9000 Ωcm^2) is applied to calculation of the length constant for collaterals, the result is congruent with decremental conduction in very small animals, but lags far behind the spatial requirements for the adult. Even an assumption of orthograde spiking does not constitute a solution to the inconsistency, since postsynaptic hyperpolarization is conducted over the same collaterals in the retrograde direction.

Conversely, the presumptive membrane resistivity can be calculated from the diameter of the collaterals and their requisite conduction distances, i.e., one half of the inhibitory field. This approach yields values ranging from 4.6 $k\Omega cm^2$ for neurites in the smallest animal to 142 $k\Omega cm^2$ for the largest diameter collaterals in the adult. The smallest collaterals in a large eye, provided they stretch over the full extent of the inhibitory field, would require a membrane resistivity of about 400 $k\Omega cm^2$. To check whether these values are within reason for Limulus, length constants for various decrementally conducting visual fibers (Behrens and Fahy, J. Comp. Physiol. 141: 239, 1981) were matched with their respective diameters, extracted from my own preparations. The most extreme case consists of axons of the lateral rudimentary eye, which have length constants of 8-21 mm, diameters of 10-25 µm, and corresponding membrane resitivities of 190-530 $k\Omega cm^2$. Thus, a precedent exists for very large membrane resistivities in Limulus, which are not beyond those found in other invertebrates.

The structural gradients that have been described in the plexus (Fahrenbach, Proc. Roy. Soc. B, 225: 219, 1985) appear to act synergistically with the electrotonic fields to concentrate inhibitory input on the immediate proximity of the target receptor. Despite its seeming functional simplicity, lateral inhibition in the Limulus compound eye is a complex, analog stage of visual processing, generated in a stochastically interconnected neuropil, whose subtly graded output serves to modulate the digitally encoded primary visual signal in the eccentric cell axons.

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252.4 CRITICAL TIMING IN THE NEURONAL REGULATION OF MUSCLE FIBER DEATH DURING CLAW TRANSFORMATION. DeF. Mellon and M.M. Quigley*. Department of Biology, University of Virginia, Charlottesville, VA 22903. The transformation of claw type in Alpheus is accompanied by biochemical and structural modifications of the claw closer muscle. These changes include complete degeneration of a central band of muscle fibers, and a switch in isoform expression of contractile and regulatory proteins in the flanking regions of the muscle. In untransformed pincer claws the flanking regions of the closer muscle comprise intermediate speed fibers, while the central band consists of fast type fibers. During transformation a major biochemical modification that occurs in the flanking region fibers is a switch in the troponin T isoform: pincer closer expresses one isoform, and snapper closer another (Quigley & Mellon, 1984). Furthermore, we have shown that transection of the second nerve root within the transforming pincer claw allows morphological changes in the claw to proceed, but it prevents the TnT modification (Quigley, Cox, & Mellon, 1985). We now report that transection of the second root, or some of its distal branches, also protects the central band of fast muscle fibers from degeneration in an otherwise transforming claw. Details of the nervous pathway involved in the degeneration signal have yet to be worked out, but afferent as well as efferent axons appear to be involved in the elaboration or transmission of the message. We have now determined that the central band of muscle fibers is sensitized by the nervous system within 120 hours after pincer claw transformation is triggered. Nerve section prior to snapper removal results in protection of the fast fibers; nerve section at least 120 hours following snapper removal does not, and the central fibers die following the first molt. The window of sensitivity may be much narrower, and its width also depends upon ambient temperature. Our studies support the hypothesis that muscle fibers in the transforming claw are marked for death or modification by temporally restricted information elaborated over nervous pathways in the affected limb. Since observations by other workers and by ourselves implicate an ecdysteroid-like factor in regulating the molt cycle of shrimps, cooperative interaction between nervous and hormonal signals may control the events in muscle restructuring.

252.5 DISCOVERY AND IDENTIFICATION OF THE STEROIDOTROPIC NEUROPEPTIDE, PROTHORACICOTROPIC HORMONE (PTTH), REGULATING DEVELOPMENT IN THE GYPSY MOTH, LYMANTRIA DISPAR. E. P. Masler, T. J. Kelly*, B. S. Thyagaraja*+, C. W. Woods*, R. A. Bell* and A. B. Borkovec*. Insect Reproduction Laboratory, Agricultural Research Service, U. S. Dept. of Agriculture, Beltsville, MD 20705 and +RSRS, Central Silk Board, Kollegal, Karnataka, India.

Benchmark experiments by Kopec, studying metamorphosis and development in L. dispar over sixty years ago, resulted in some of the earliest reports of an association between the nervous and endocrine systems (Biol. Bull., Wood's Hole 42: 322-342, 1922). Subsequent studies in a number of species have identified the primary metamorphic neurohormone involved as PTTH which stimulates the prothoracic glands to produce ecdysteroids necessary for development and metamorphosis. However, very little work has been done with Lymantria. We report here the discovery of PTTH in the gypsy moth and analysis of the hormonal activities.

A two-phase in vitro bioassay is used for the detection of PTTH activity. Prothoracic glands from fifth-instar larvae are incubated with test extracts, ecdysteroid in post-incubation control and test media is quantified by radioimmunoassay and ecdysteroid levels are used as a measure of PTTH activity.

Activity is specifically associated with the brain and retrocerebral complex. Dose response kinetics show a 10-fold activation range with a half maximal dose near $4x10^{-3}$ tissue equivalents per microliter. Two forms of the hormone are identified by high performance size exclusion chromatography as PTTH-I (20± 5 kiloDaltons) and PTTH-II (5 ±1 kiloDaltons). The developmental physiology of prothoracic gland activation, characterization of the neurohormones and their purification are discussed.

252.6 METAMORPHOSIS OF IDENTIFIED VISUAL INTERNEURONS WHICH STEER FLIGHT IN THE DRAGONFLY. R.M. Olberg. Dept. of Biol. Sci., Union College, Schenectady, NY 12308.

In an effort to understand the metamorphosis of behaviorally relevant interneurons, I have identified eight large "target interneurons" in the adult dragonfly and studied the structure and response properties of the same interneurons in the larva. Since the dragonfly is hemimetabolous, it metamorphoses directly from an aquatic larval stage to its aerial adult stage, accomplishing a total switch in its behavioral repertoire within a 2 hour period.

Intracellular injection of Lucifer yellow revealed that the target interneurons descend from the brain to the thoracic ganglia. Each passes consistently through one of three longitudinal tracts in the prothoracic ganglion, the Dorsal Median Tract (MDT), Dorsal Intermediate Tract (DIT), or Dorsal Median Tract (DMT). They are named according to the tract through which they pass. All eight target interneurons are strictly visual, and respond only to movements of small patterns, such as black squares, moving on a white background. The receptive fields of the cells are large, encompassing about a hemisphere. Three neurons (DIT1, MDT2, MDT4) respond only to very small (2° - 8°, visual angle) targets; four (DIT2, DIT3, MDT1, DMT1) show consistent responses over a wide range of target sizes; and one (MDT3) responds only to large (16°-32°) targets. None responds to movement of a large checkerboard pattern which covers a hemisphere of the visual field. Five of the interneurons show pronounced directional selectivity. Target movement in the antipreferred direction elicits hyperpolarizing responses in most of these. A large pattern moving in any direction inhibits the response to target movement.

These neurons mediate, in part, the visual control of flight orientation. High frequency (150 Hz) stimulation of individual target interneurons elicits pronounced asymmetrical wing movements. This finding, coupled with their highly selective visual responses indicates that the target interneurons convey turning signals to the flight system in response to objects moving relative to the animal.

At least one of the target interneurons (DMT1) is also active in the larva, even though the behavior of the larva is radically different from that of the adult. The persistence of this neuron from larva to adult requires a shift in the behavioral consequences of its activity. Other target interneurons (MDT's) are inactive during larval life, only to be switched on upon adult eclosion. A correlation exists between the region of compound eye added during the final molt and the receptive fields of the interneurons which are first active during the adult stage.

252.7 HORMONAL CONTROL OF NEURONAL REORGANIZATION DURING INSECT METAMORPHOSIS. R. B. Levine, Dept. of Biology, Rice University, Houston, TX 77005.

During the metamorphosis of the hawkmoth, Manduca sexta, many larval neurons persist to serve new behavioral roles in later stages of life. These neurons undergo dramatic structural and functional changes as part of this process. The detailed information available regarding the titers of developmentally-relevant hormones in this insect makes it possible to use them as tools in the effort to understand how metamorphic changes are induced and regulated. In general, preparation for metamorphosis is initiated by the "commitment" pulse of the steroid 20-hydroxyecdysone (20-HE) when it appears at the end of the last larval instar for the first time in the absence of the juvenile hormone (JH). Cells begin to express their commitment in response to a second "prepupal" surge of ecdysteriods. The relevance of these cues to the nervous system was demonstrated in the following study.

One set of larval sensory neurons (SNs) persists into the pupal stage to participate in a new pupal reflex (the gin trap reflex). These SNs expand their arbors within the CNS during the final 4 days of larval life, and begin to evoke a very different motor response in the pupa. We have shown previously that when JH is applied to the peripheral somata of these SNs in the prepupa during the commitment pulse of ecdysteroid, they retain their larval branching pattern within the pupal CNS and fail to evoke the gin trap response (Levine et al., J. Neurosci. 6:293, 1986). Thus, as with other cells in the animal, the decline in JH is crucial for the pupal development of these nerve cells. Two lines of evidence indicate that 20-HE is also important. First, JH-treated SNs begin to grow pupal arbors and evoke the gin trap reflex 4-5 days after pupation, coincident with the normal ecdysteroid surge that causes adult development of the rest of the animal. This delayed pupal development could be prevented by blocking the rise in 20-HE, and reinstated by infusing the steroid. Second, animals deprived of the prepupal ecdysteroid pulse retained their larval form. The SNs failed to expand their processes and could not evoke the gin trap response. Topical application of 20-HE dissolved in undecane to the peripheral cell bodies of the SNs, however, caused them to expand their processes within the larval CNS. Adjacent, untreated SNs retained their larval form. Thus, the steroid acts upon the somata of these neurons to control the growth of their distant axonal processes. This growth is independent of steriod-dependent cues within the CNS. Interestingly these SNs did not gain the ability to evoke the gin trap response, suggesting that 20-HE may also be required within the CNS for the pupal development of other neurons in the pathway. (Supported by NSF #BNS 8308907).

252.8 POSTEMBRYONIC DEVELOPMENT OF LEG MOTOR CIRCUITS IN THE MOTH MANDUCA SEXTA. K.S. Kent, F.G. Rothenberg*, R.B. Levine, Department of Biology, Rice University, Houston, TX 77001.

During the metamorphosis of insects, motor circuits undergo structural and functional changes that permit the expression of new behaviors. As a prelude to studying the signals that induce these changes, we are defining elements of the prothoracic-leg motor circuits of the moth, Manduca sexta, and examining the morphological and physiological alterations that take place in these motor circuits as the insect metamorphoses from larva to adult.

Using cobalt staining methods,we have traced the projections of sensory neurons arising from individual hair-like sensilla on the larval leg. The sensory axons project into the prothoracic ganglion and have arborizations in two distinct regions of ventral neuropil, one near the lateral edge of the ganglion and another near the midline. Axons from sensory cells on the posterior surface of proximal leg segments also have arborizations in the mesothoracic ganglion. Cobalt staining of sensory neurons of the adult leg reveals a pattern of arborizations in the prothoracic ganglion that is different from that in the larva. Fibers project to a large area of ventral neuropil occupying most of the ipsilateral anterior quadrant. Some fibers also project posteriorly to the pterothoracic ganglion and others project anteriorly into the brain.

Backfilling the main larval leg nerve with cobalt reveals approximately 14 somata in the prothoracic ganglion. While the majority of motorneuronal somata are ipsilateral to the cobalt-filled leg nerve, one soma lies on the contralateral side of the ganglion. A midline cell in the posterior half of the ganglion sends a process into both ipsilateral and contralateral leg nerves. Using intracellular recording techniques, we have been able to individually identify and characterize some of these motorneurons. Tactile stimulation of the hair-like sensilla on the leg indicates that the motorneurons receive robust sensory input. Dye-injection reveals that these motorneurons have dense dendritic arborizations in the ventral lateral region of neuropil in which the sensory neurons also have arborizations. Some motorneurons also have arborizations in a dorsal region of neuropil near the ipsilateral midline, a region that does not contain arborizations from leg sensory neurons.

Backfilling the main leg nerve of pupae and adults suggests that many or all larval leg motorneurons, including the midline and contralateral cells, are present throughout metamorphosis. The dendritic arborizations of these motorneurons in the adult appear to be confined to a lateral region of neuropil. The possibility that some of the motorneurons present during pupal and adult stages are persistent larval motorneurons has been confirmed by the use of retrograde tracers with long survival times. Supported by NIH grant #NS 07781 and NSF grant #BNS 8308907.

252.9 DEVELOPMENTAL CHANGES IN MECHANOSENSORY CIRCUITS DURING METAMORPHOSIS OF THE HAWKMOTH. D. M. Linn* and R.B. Levine. (SPON: D. Johnston). Dept. of Biology, Rice University, Houston, TX 77005.

During insect metamorphosis many larval neurons persist to serve new roles in the pupa and adult, often undergoing striking functional and structural changes. To better understand the scope and behavioral relevance of these changes we have followed elements of the simple neural circuits responsible for mechanosensory reflex behavior in different stages of the hawkmoth, Manduca sexta. The mechanoreceptive hairs that cover the surface of the larval abdomen and thorax are innervated by sensory neurons (SNs) which project somatotopically into the CNS (Levine et al. J. Comp. Physiol. 157:1, 1985). They evoke weak position-specific responses in motorneurons (MNs) innervating the body wall muscles of several segments. In general, the SNs excite ipsilateral and inhibit contralateral MNs, but this depends upon the segment being stimulated and the animal's posture. These SNs do not synapse directly upon the MNs. A small group of intersegmental interneurons has been identified which receive sensory input from well-defined regions of the body surface, and which excite MNs. During the final two days of larval life the animals enter a hyperexcitable state. Responses to SN stimulation cease to be position-specific, consisting instead of rapid abdominal flexion and rotation. During this period the SNs cause large depolarizations and short bursts of action potentials in the MNs. In the hours prior to pupal ecdysis MN responses decrease markedly, but specific SNs evoke new reflexes immediately after ecdysis.

A localized small subset of the SNs persist into the pupal stage to innervate hairs within cuticular pits known as the gin traps. Stimulation of hairs within these structures evokes a strong defensive reflex, in which the SNs cause an excitatory response in specific MNs that is more robust than that in the larva. (Bate, J.E.B. 59:121, 1973). One change necessary for the new pupal "gin trap" reflex is the expansion of SN arbors within the CNS (Levine et al., J. Neurosci. 6:293, 1986). We are now exploring other aspects of this circuit. Although some of the larval interneurons are retained into the pupal stage, it is not yet clear whether they are involved in the new reflex. The MNs which are retained to participate in the pupal behavior undergo a striking functional change near the end of larval life. Depolarizing current injected into the somata or dendrites of larval MNs evokes only small, passively-invading action potentials. In the pupa, current injected into the same MNs evokes a large, non-linear depolarization and delayed rectification. These changes may be related to the increased effectiveness of specific excitatory inputs necessary for the new pupal reflex.

Another localized small group of SNs persist, and reorganize their central projections to initiate the pupal "cocking" reflex. These SNs excite contralateral and inhibit ipsilateral MNs such that the gin traps open in preparation for the defensive reflex. (Supported by NSF #BNS8308907).

252.10 **ANTENNAL-NERVE AFFERENTS INFLUENCE THE DISTRIBUTION OF NON-ANTENNAL SYNAPSES IN THE ANTENNAL LOBE OF MANDUCA SEXTA.** Leslie P. Tolbert. Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

The antennal lobes of the brain of the moth Manduca sexta depend upon the presence of afferent axons from the antennae for their normal histological development (Hildebrand et al., PNAS 76:499; Kent, Ph.D. dissertation, Harvard Univ., 1985; Oland & Tolbert, ms. submitted). The normally afferented mature lobe comprises a central area of coarse neuropil surrounded by fine-textured, spheroidal glomeruli, where virtually all the synaptic interactions among afferent axons and antennal-lobe neurons occur. An antennal lobe that has been deprived of antennal afferent axons throughout development is smaller and lacks histologically conspicuous glomeruli. Oland and Tolbert (submitted) have shown that the histological development of normal and unafferented antennal lobes diverge as soon as antennal-afferent axons begin to reach the lobe in the normal case. While coarse and fine-textured regions of neuropil segregate in both cases, glial invasion of the neuropil and subsequent glial delineation of glomeruli does not occur in the absence of antennal afferent axons.

In the present study, I have compared the distribution of synapses in normal and in chronically unafferented antennal lobes throughout their development. Synapses between antennal-lobe neurons are present from the earliest stages examined (stage 2 of the 18 stages of metamorphic adult development). Through stage 4, the stage at which antennal afferent axons normally begin to reach the lobe, the synapses are distributed homogeneously through the neuropil of the antennal lobe at a packing density of about 2-3.5 synapses/100 um^2. From stage 5 through stage 9 in normal development, as antennal axons continue to grow in and begin to synapse, there is a loss of synapses from the central area; by stage 9, almost all synapses are found in the newly formed glomeruli (at a packing density at least 30 times that in the coarse neuropil). In the unafferented lobe, in contrast, synapses persist in the central, coarse neuropil at about 2/100 um^2 through stage 9, and by maturity (emergence of the adult moth) are found at 6/100 um^2 there, compared with only a 4-fold higher packing density in the fine-textured neuropil.

These data indicate that antennal afferent axons play a role in sculpting the synaptic neuropil not just at a histological level, but also at the ultrastructural level. Their presence causes the elimination of synapses in the center of the lobe's neuropil (at least some of which were present before the arrival of the afferent axons and therefore do not involve those axons). Current work is aimed at discovering the cellular basis for this effect. (Supported by NIH grant #NS 20040.)

252.11 REDUCTION OF GLIAL POPULATION BY γ-IRRADIATION DISRUPTS DEVELOPMENT OF GLOMERULI IN MANDUCA SEXTA ANTENNAL LOBE. Lynne A. Oland and Leslie P. Tolbert, Dept. Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

The synaptic neuropil of the mature antennal lobe of the moth Manduca sexta is organized into spheroidal glomeruli which contain the highly branched arbors of antennal sensory axons and antennal-lobe neurons and whose borders are defined by glial cells. Glomerular organization, which is characteristic of primary olfactory centers in vertebrates and invertebrates alike, arises during metamorphic adult development from a neuropil that is homogeneous in texture and surrounded by a thin rim of glial cells. We (manuscript submitted) have shown that the first signs of division of the neuropil into glomeruli occur in the glial population. Coincident with the arrival of the earliest antennal afferent axons (during stage 4 of the 18 stages of *adult development*), glial cells begin a burst of mitosis, extend processes *into the neuropil*, and, during stage 5, begin to migrate inward in patterns reminiscent of glomerular borders. In contrast, if antennal axons are prevented from ever reaching the antennal lobe, glomeruli do not develop and glial cells remain restricted to a thick layer outside the neuropil. Our results suggest that the glial cells may be in a position to act as intermediaries in developmental interactions between afferent axons and antennal-lobe neurons. We have begun to test this hypothesis by examining the neuropil in antennal lobes that have a normal complement of neuronal elements but in which we have severely reduced the glial population.

In order to reduce the number of glial cells, we have exposed developing animals to γ-radiation at times when glial cells but not neurons are dividing. Autoradiography of antennal lobes from animals injected with ^{3}H-thymidine at stages 2 - 12 revealed a burst of glial proliferation extending from stage 4 through stage 9; neurons have essentially completed division before stage 2. Animals were irradiated during stages 4 and 5, and were sacrificed at stage 12, when the lobe is *normally* histologically mature. Doses of 5,000 - 20,000 rads had no apparent effect. At 40,000 rads, the number of glial cells in the antennal lobe was reduced by at least half, and at 80,000 - 160,000 rads, glial cells were even more severely reduced. In all cases, neurons appeared healthy and antennal afferent axons grew into the lobe. While at 40,000 rads, a few possibly glomerular profiles could be distinguished, at the higher doses, no glomeruli were evident, and the organization of the neuropil resembled that of an unafferented lobe.

These results support the hypothesis that glial cells are necessary elements in the construction of antennal-lobe glomeruli.

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252.12 LECTINS AS PROBES FOR GLYCOCONJUGATE EXPRESSION DURING DROSOPHILA NEUROGENESIS. J.R. Fredieu* and A.P. Mahowald* (SPON: L. Hershey) Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106

A variety of plant lectins, which bind carbohydrate moeities on glycoproteins and glycolipids, were used to examine cell surface glycoconjugate expression during neurogenesis in Drosophila melanogaster (Oregon R). Staged whole embryos were dechorionated in 50% chlorox, the vitelline membrane removed in heptane, fixed in 10% formalin, and incubated overnight in 5-10 g/ml of FITC-conjugated lectin.

Peanut agglutinin (PNA) binds cells in the ventral midline of the embryo at 10 hours of development. By 12 hours, the complete ventral neuropil binds PNA, followed by ganglion cells at later stages. Pre-incubation with a 0.2M solution of either D-galactose or GalNAc has no effect on binding.

Bauhinia purpurea agglutinin (BPA) and ricinus communis (RCA II) mimic the binding observed with PNA; i.e. cells of the ventral midline and the ventral neuropil bind both BPA and RCA II at the same developmental stages as PNA. In contrast, BPA and RCA II bind ventral ganglion cells at earlier embryonic stages than does PNA. Also, BPA recognizes a component of the peripheral nervous system. The binding of BPA and RCA II is inhibited by pre-incubation with D-galactose or GalNAc.

Dissociated cells from gastrula stage embryos plated on glass coverslips in the appropriate media will differentiate into a variety of cell types including neurons. As predicted from the whole embryo studies, PNA, BPA, and RCA II bind to cultured neurons. In addition, lectins from griffonia simplicifolia, maclura pomifera, and wheatgerm bind to cultured neurons, although undetected in the whole embryos. This discrepency may be due to a decrease in the cell-type specificity of lectin binding in the intact embryo. Soybean agglutinin recognizesthe surface of culturd neurons, but fails to bind a comparable component in the embryo.

These results indicate: 1. glycoconjugates are selectively expressed in the Drosophila nervous system at critical periods during neurogenesis; 2. although cultured Drosophila neurons bind specific lectins, some lectin binding does not correlate with that observed in the whole embryo; 3. some lectins bind subpopulations of neurons both in culture and in the whole embryos indicating differential expression of surface glycoconjugates between developing Drosophila neurons.

252.13 INDUCTION OF NEUROMA FORMATION AND SPECIFIC TARGET MUSCLE DELETION IN THE DROSOPHILA TS MUTANT SHIBIRE. M.R. Hummon and W.J. Costello. Dept. Zool. and Biomed. Sci./College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

All muscles of the Drosophila melanogaster escape response, an adult behavior, develop during the pupal period. Use of the ts mutation shibire permits selective disruption of development by heat pulse (HP)(30C for 6h), resulting in lethality (HP onset at 0-12h of development @22,~0-7h @25C), in adults with damage to the escape response (~12-60h @25C), or in adults with normal behavior and phenotype (~70-96h @25C). Effects on the escape response depend on time of HP, and include muscle fusion (Costello and Salkoff, 1983, Neurosci. Abs. 9:832) and disruption of an interneuron and of latency of response of muscles (Hummon & Costello, 1984, 1985, Neurosci. Abs. 10:1032;11:918). We now report that a particular HP reliably generates adults with partial deletion/fusion of certain target muscles and with bilateral neuromas at the terminus of some of the identifiable motoneurons that should innervate these muscles.

All shibire pupae (n=8) exposed to HP with onset at 30h @22C (~20h @25C) developed, but none emerged without aid. All showed similar cuticular anomalies, including a virtual absence of head and thoracic chaetae (contra Poodry et al., 1973, Dev. Biol. 32: 373). After Epon embedment, flies (n=3) were serially sectioned (5 µm) in transverse and then frontal section, to permit tracing of the identified neurons and muscles of the escape response. Selected thick sections were re-embedded and sectioned for TEM. The indirect fibrillar flight muscles are represented by fewer muscles, fewer fibers, or both. For DLM (wing depressors), instead of 6 fibers per side, there are 1 or 2. For DVM I, II, III (wing elevators), there is usually only a single fiber per muscle, or no muscle, as opposed to the normal 3, 2, 2 fibers respectively. The normal 5 motoneurons for DLM are present near the thoracic ganglion, but some of their axons terminate in a conspicuous neuroma (approx. 150 x 50 µm), while others continue towards the remaining fibers. The neuroma is a tangle of glia and of large and very small axons with numerous axonal hemisynapses facing glia, axons, or space. Thus there is complete correspondance between presence of the neuroma and the deletion or fusion of target fibers.

The primary defect of shibire flies involves disruption of membrane recycling and of the pinch-off mechanism of endocytosis (Kosaka & Ikeda, 1983, J. Cell Biol.97:499; Costello & Hummon, 1985, Neurosci. Abs. 11:918). Anomalies in shibire adults exposed to pupal HP should reflect processes sensitive to abnormal membrane *behavior*, as well as indirect effects of primary anomalies. We will use the time course of neuroma and target muscle development in shibire pupae following HP at 30h (22C) to clarify which anomaly, neuron or muscle, is the primary defect. Supported by Muscular Dystrophy Association.

252.14 *DROSOPHILA* STOCKS USED FOR STUDYING GIANT-FIBER-PATHWAY MUTATIONS MAY DIFFER NOT ONLY IN STRUCTURE AND FUNCTION OF CENTRAL NEURONS BUT ALSO IN GLYCOGEN STORAGE AND SECRETION BY GUT EPITHELIUM. D. G. King, Department of Anatomy and Department of Zoology, Southern Illinois University, Carbondale, IL 62901.

Certain behavioral mutations cause specific physiological and anatomical abnormalities in identified neurons of the *Drosophila* giant fiber pathway. Current investigations seek to understand the genetic and cellular mechanisms which underly these neuronal changes (reviewed by Wyman, Thomas, Salkoff and Costello in: A. Selverston, ed., Model Neural Networks and Behavior, Plenum 1985).

Microscopic examination (LM and EM) of cardia epithelium in three *Drosophila* stocks--*Pas*, *ben* and *nj75*, each bearing one mutation affecting the startle response--has disclosed several differences between these stocks and the wild-type strain Hochi. *Pas* flies display increased periodic acid-Schiff (PAS) stain reaction in the head of the stomadeal valve, in granules within tall cells of the cardia outer wall, and in the peritrophic membrane. EM of *Pas* flies demonstrates greater concentrations of glycogen in stomadeal valve cells; LM appearance suggests similar concentrations occur in *ben* and *nj75*. EM of *Pas* and *ben* reveals an increase in the staining density of apical secretory vesicles in cardia outer wall tall cells, when compared with Hochi. All three mutant stocks display longer secretory pegs on these tall cells and some shape differences in the zone of short secretory cells along the neck of the stomadeal valve. The peritrophic membrane, which appears to arise largely from the short cells, is thicker, less uniform, and more deeply stained in the mutant stocks. The correspondence of PAS staining variation with cell morphology variation in each of several cell types suggests that a common theme in all these differences may be variation in the storage and secretion of polysaccharides.

Whether the gut variation described above is caused by, or only coincidental with, the genetic variation which causes behavioral lesions is not yet known. If both gut and neural variation were to share a common genetic basis, then the similarity among three mutant stocks would suggest that they all reflect modifications in one underlying cellular process. However, the possibility that similar epithelial changes could be induced by each of three distinct behavioral mutations seems highly unlikely. A more probable explanation is that similar gut variants in each of the three mutant stocks arise from the common genetic background shared by these flies but not by Hochi. Future plans call for comparisons which can distinguish between these two alternatives.

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252.15 FORMATION OF ADULT MOTOR SYSTEMS IN DROSOPHILA: SCANNING ELECTRON MICROSCOPE STUDY. W.J. Costello. Dept. Zool. and Biomed. Sci./ Col. Osteo. Med., Athens, OH 45701

As a holometabolous flying insect, Drosophila has two life stages: larva and adult. The transition stage (pupa) consists of a dramatic reprogramming from a crawling form to a flying form. Major changes must occur in the motor systems controlling such movement: larval components degenerate, de novo adult components form, and some larval components can be modified into adult structures. To investigate these changes and establish a baseline for comparison with mutants affecting the nervous system, I conducted a scanning electron microscope study of the developing pupal nervous system in Canton-S flies.

Particular attention was paid to the motor system controlling the escape response of adult flies. This motor system consists of jump muscles (TTM), wing elevators (DVM), and wing depressors (DLM). Motor axons to the TTM and DLM exit the thoracic ganglion via an adult nerve, the PDMN. Another adult nerve, the ADMN, exits anteriorly and contains motor axons to a DVM set. Together, the PDMN and ADMN form a major part of the escape response system.

In the larva, one nerve exiting the mesothoracic part of the ganglion can be identified during differentiation throughout the pupal stage. During early pupation, the nerve maintains connection to sites of larval muscles which have degenerated. These sites are collecting points of adult myocytes which will become TTM, DVM, and DLM. This nerve begins forming distinct branches to these sites by gradually separating itself into three major portions. By late pupal stage, this nerve has been modified to consist of distinct parts: ADMN innervating a set of DVM; PDMN innervating DLM; TTM nerve usually branching from PDMN. Thus one larval nerve eventually gives rise to three major adult nerves which are part of the escape response.

Knowing this larval origin of adult nerves serves to explain several anomalous phenomena seen in development and in phylogeny. Although normally the TTM nerve branches from the PDMN a short distance from the ganglion, often the TTM nerve exits the ganglion as a separate trunk. Occasionally, DLM motor axons will travel a short distance with the ADMN before separating to join the PDMN just before ADMN and PDMN exit the ganglion. Also, in some related species the PDMN and ADMN will exit the ganglion via a common trunk.

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252.16 FUNCTIONAL PLASTICITY IN THE FLY'S VISUAL SYSTEM. K. Kral* and I.A. Meinertzhagen. Department of Psychology, Dalhousie University, Life Sciences Centre, Halifax, Nova Scotia, Canada B3H 4J1.

The regulation of the strength of synaptic connection between groups of neurons by the functional experience to which the neurons are exposed during development or early adulthood has been the target of particular attention in the visual system. Here we report that the number of feedback synapses formed upon photoreceptors in the fly's eye depends on visual experience. Since the high-gain afferent synapse of photoreceptors transforms light-induced receptor depolarisations to transient hyperpolarisations of second-order monopolar cells L1 and L2 so as to encode temporal contrasts, we have chosen such contrasts (flicker) as an optimal rearing stimulus.

Flies (newly enclosed females) and pupae (>67% development stage) of Musca domestica were subjected to a 2-day period of stimulation in an integrating sphere illuminated transmurally by an LED array (λ565mm) with 25Hz sine-wave flicker. Experimental animals had one eye occluded with black latex paint and comparisons were made between flicker-reared (FR) and occluded (OC) eyes. The resultant adults were quickly dissected under white light, prepared for electron microscopy and sections of their first optic neuropile, or lamina, examined by quantitative methods to ascertain the frequency of contained synaptic profiles in the proximal region, close to the chiasma. The frequencies per cartridge of two classes of synaptic contact were scored: afferent tetrad synapses of photoreceptor terminals, at which input is provided repeatedly upon clusters of four postsynaptic elements including L1 and L2 (one each) and, usually, α processes of an amacrine cell; and feedback synapses formed by L2 back upon R1-6. For flicker-reared 4-day adults the mean ratio of FR:OC lamina synaptic frequencies was: tetrads 1:0.84, feedback synapses 1:1.60. Thus the mean frequency of tetrad synapses is somewhat greater in flicker rearing, while that of feedback synapses L2→R1-6 is smaller by about 60%; the feedback ratio thus decreasing to about 53%. If individual synaptic contacts have unaltered electrophysiological gains following differential rearing, the decreased feedback ratio should cause a longer time-course of recovery of L2 transients. Newly emerged flies showed comparable FR:OC L2→R1-6 synaptic ratios after two days but had higher absolute synaptic frequencies; L2 synapses are thus, additionally, lost with age.

Laughlin (1984)[1] has suggested that L1 and L2 optimally encode the range of temporal contrasts to which they are exposed in normal vision. This range of optimal encoding could be modifiable by altered contrasts to which the cells are exposed, if synaptic strength shows functional plasticity during development. This study provides anatomical evidence for such functional plasticity.

Supported by grants EY-03592 from NIH and A0065 from NSERC.

[1]Laughlin, S.B., NATO A.S.I., vol. 74. Plenum: New York, 1984.

MOTIVATION AND EMOTION I

253.1 CARBACHOL ANTAGONIZES THE EFFECTS OF MUSCARINIC ANTAGONISTS ON FREQUENCY THRESHOLDS FOR MEDIAL FOREBRAIN BUNDLE SELF-STIMULATION. O. Kofman, Dept. of Psychology, University of Toronto, Toronto, Ontario, Canada, M5S 1A1.

Previous studies have shown that microinjections of carbachol in the ventral tegmentum induced a conditioned place preference in rats. When the muscarinic antagonists atropine sulfate and scopolamine hydrobromide were injected in the ventral tegmentum, frequency thresholds for intracranical self stimulation in the lateral hypothalamus and dorsal tegmentum were elevated (Yeomans, et al., 1985; Kofman and Yeomans, 1985). These effects were dose dependent and robust over two or more sessions. Since atropine is known to have local anesthetic and calcium channel blocking properties as well, the present study undertook to antagonize the reward blocking effects of atropine by pretreating animals with the cholinergic agonist carbachol.

Rats were trained to bar press for a train of cathodal square wave pulses delivered via electrodes implanted in the lateral hypothalamus. After at least five stable frequency thresholds were obtained for half-maximal rates of bar pressing, animals were injected with 0.5 ul of carbachol (1 or 2 ug) or CSF via a cannula in the ventral tegmentum. Fifteen minutes later, animals were re-injected with either CSF or atropine sulfate (60 ug) and replaced in the self-stimulation apparatus. Frequency thresholds were measured until they had returned to baseline. The order of injections was CSF-CSF, CSF-Atropine, and Carbachol-Atropine; sessions were at least 48 hr. apart.

When injections of atropine sulfate were preceded by CSF, frequency thresholds were increased up to 360% from baseline. Animals pretreated with 1 ug carbachol 15 minutes before atropine injections had frequency thresholds elevations to only 160% of baseline; 2 ug carbachol reduced the atropine effect to less than 145%. This supports the suggestion that the reward blocking effects of atropine act via muscarinic receptors. Similarly, we have found preliminary evidence that atropine pre-treatment can block carbachol-induced conditioned place preference.

253.2 FACILITATION OF LATERAL HYPOTHALAMIC SELF-STIMULATION BY AMPHETAMINE MICROINJECTIONS INTO NUCLEUS ACCUMBENS L. M. Colle* and R. A. Wise. (Spon: E. Miliaressis) Ctr. for Studies in Behav. Neurobiol. Concordia Univ., Mtl, Quebec, H3G 1M8.

Systemically administered amphetamine increases the rate of responding and decreases the threshold for self-stimulation (SS) presumably by enhancing the rewarding impact of the stimulation. Intracranially administered amphetamine (into either the nucleus accumbens or the striatum) also increases the rate of responding for SS, however, it is not clear that this increase in responding reflects an enhancement of the rewarding impact of the stimulation, or an increase in the performance capacity of the rats. In the present study, the effects of intracranial d- and l-amphetamine were assessed in a paradigm which has been shown to distinguish performance from reward effects: the curve-shift paradigm. Rats were implanted with monopolar stimulating electrodes aimed at the lateral hypothalamus, and 22 gauge guide cannulae aimed at the anterior part of the striatum. The animals' rates of lever-pressing were determined at several stimulation frequencies (rate-frequency function) under drug and no-drug conditions. D- and l-amphetamine were each applied to the ventral striatum and the nucleus accumbens in doses of 0, 2.5 ug, 5.0 ug, and 10.0 ug in .5 ul of saline in each animal. When applied to the nucleus accumbens ipsilateral to the stimulating electrode d-amphetamine caused a dose-dependent shift to the left in the rate-frequency function; when applied to the nucleus accumbens contralateral to the stimulating electrode only the highest dose of d-amphetamine caused a shift to the left. There was no change in the animals' asymptotic response rate following either drug. Following either intra-striatal d-amphetamine or intra-accumbens or intra-striatal l-amphetamine, there was no change in the rate-frequency function. The shift to the left following intra-accumbens d-amphetamine is thought to reflect an enhancement of the rewarding impact of the stimulation. These data compliment those which have show that intra-accumbens neuroleptics decrease the rewarding impact of SS suggesting that the dopamine terminals of the nucleus accumbens are involved in mediating the rewarding impact of lateral hypothalamic SS.

253.3 RELATIVE EFFECTS OF NALTREXONE ON NUCLEUS ACCUMBENS, LATERAL HYPOTHALAMIC AND VENTRAL TEGMENTAL SELF-STIMULATION IN THE RAT. T.E.G.West* and R.A.Wise. Centre for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada. H3G 1M8.

The effects of opioid blockers on brain stimulation reward have not been consistent from one report to the next. Differences in stimulation site and period of testing have been suggested as explanations of the inconsistencies. In the present study we compared early with late testing periods and three stimulation sites along the medial forebrain bundle in the same animals. The curve-shift paradigm was used to assess the effect of naltrexone (2.5, 5, 10, 20 mg/kg) on self-stimulation. Naltrexone generally shifted the rate-frequency function to the right, increasing stimulation frequency needed for normal rates of responding. Maximal (asymptotic) response rates were not altered by naltrexone, though the stimulation frequency required to produce responding at maximal rates was increased. Naltrexone affected responding for nucleus accumbens stimulation the most and responding for ventral tegmental stimulation the least. There was a greater attenuation of responding during the second test period, 50 minutes after injection, than during the first test period, 20 minutes after injection. If animals were not tested during the first period, responding was immediately attenuated at the start of the second period; thus stimulation experience was not necessary for response decrements. A time course of the effect of naltrexone on response rates was determined using a single stimulation frequency; peak effects were observed 45 to 85 minutes after injection. It is not clear why lateral hypothalamic and ventral tegmental self-stimulation were differentially affected by naltrexone. It is thought that the rewarding effects of lateral hypothalamic and ventral tegmental stimulation involve activation of the same medial forebrain bundle fibers, and these fibers are thought to synapse between these two sites. It may be that stimulation at different anterior-posterior levels of the system spreads to different numbers of reward-relevant fibers, or that stimulation near the axon terminals has different potency than stimulation closer to the soma.

253.4 LOCALIZATION OF BRAIN STIMULATION REWARD SITES IN THE PONTINE TEGMENTUM: A MOVEABLE ELECTRODE MAPPING STUDY. Pierre-Paul Rompré and Sandra Boye*. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Canada H3G 1M8.

It was recently reported that the midline region of the metencephalon contains a large band of self-stimulation sites which extend dorso-ventrally from the ventral central grey to the pontine nuclei (Rompré and Miliaressis, Brain Res. 359:246-259, 1985). Moving from caudal to rostral, the concentration of the reward-relevant neurons increases significantly and reaches a peak in the vicinity of the dorsal raphé, at the frontal level of the decussation of the superior cerebellar peduncle. The present study was a detailed investigation of the localization of brain stimulation reward sites in caudal and lateral pontine tegmentum regions. Eleven male, adult hooded rats were implanted with a single moveable electrode and tested for self-stimulation a week after surgery. The electrode was first implanted at 6 mm below the surface of the skull and subsequently moved down by steps of 0.16 or 0.32 mm. Stimulation consisted of 400 ms train of cathodal, rectangular pulses of fixed intensity (200 uA) and duration (0.1 ms). Self-stimulation was recorded from zero to maximum performance by varying the frequency of stimulation (frequency/response function). An estimate of the threshold was obtained from the average of six replications of the frequency/response function determined at each site. Out of 212 sites sampled, only 64 supported self-stimulation. Ninety-two percent of the rewarding sites are localized in the medial area, between the frontal levels passing through the median raphé and raphé pontis nuclei. In the region comprised between the frontal levels passing through the raphé magnus and the raphé pontis, only nine out of the 110 tested sites supported self-stimulation. These positive sites are localized in the vicinity of the locus coeruleus and parabrachial nuclei. These results suggest that neurons originating from caudal regions of the pontine tegmentum are less likely to constitute a major portion of the large band of reward-relevant neurons located in the medial and rostral metencephalon.

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253.5 DISRUPTION OF THE CORTICOCORTICAL CONNECTIONS BETWEEN THE MEDIODORSAL AND SULCAL PREFRONTAL CORTICES ALTERS THE ASSOCIABILITY OF REWARDING CORTICAL STIMULATION TO PLACE *STIMULI* AND TO TASTE STIMULI IN RATS A. Robertson and A. Laferriere*. Dept. of Psychology, Univ. of Alberta, Edmonton, Alberta T6G 2E9.

The present experiments examined the effect of transecting the direct corticocortical connections between the mediodorsal and sulcal divisions of the rat prefrontal cortex on the ability of rewarding stimulation of the mediodorsal (prelimbic) area to impart secondary reinforcing effects to different classes of stimuli.

In the first experiment, rats showing mediodorsal self-stimulation (SS) were tested in a conditioned taste preference (CTP) paradigm, in which the rewarding stimulation was paired with a novel flavor, and preference for that flavor later assessed in a two bottle test. Nonlesioned (control) rats readily displayed a one trial CTP to a solution of decaffeinated coffee but subjects with bilateral knife cuts also showing mediodorsal SS did not. This failure to establish a CTP could not be attributed to a loss of the ability to discriminate the paired flavor because lesioned animals displayed normal neophobic responses to the taste. The lack of a CTP also could not result from gross learning deficits since sulcal SS could support a CTP in animals given similar knife cuts. In the second experiment, rats displaying mediodorsal SS were tested for a conditioned place preference (CPP) after mediodorsal SS had been repeatedly paired with a distinctive environment in a counterbalanced design. Nonlesioned subjects showed no CPP but rats having received bilateral knife cuts reliably did so. These effects were not due to changes in activity or exploratory patterns.

The disruption of the mediodorsal-sulcal connections thus had the effect of preventing the formation of a CTP derived from mediodorsal stimulation while facilitating, or unmasking the induction of a CPP obtained with the same type of reinforcing stimulation. The cuts thus appear to change the associability of distinct classes of stimuli to the reinforcing effects produced by mediodorsal cortical stimulation. The results suggest the presence of prepotent relations between the rewarding consequences of prefrontal stimulation and distinct stimulus or processing domains. Furthermore, intrinsic prefrontal connections appear to be important in establishing such stimulus-reinforcer associations. The results can be viewed in the context of known functional dissociations of prefrontal divisions and suggest that prefrontal reward-related processes might be multiple and hierarchially organized.

253.6 COMPARISON OF THE EFFECTS OF CHOLINERGIC AGONISTS ON THE CUE PROPERTIES AND REWARDING EFFECTS OF BRAIN STIMULATION IN THE VENTRAL TEGMENTAL AREA. J.P. Druhan, H.C. Fibiger and A.G. Phillips. Dept. Psychol.; Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Recent pharmacological studies suggest a role for cholinergic (ACH) receptors in reward processes produced by activation of neurons in the ventral tegmental area (VTA). The present experiment examined this hypothesis further by assessing the effects of peripherally injected cholinergic agonists on brain stimulation reward (BSR) from sites in the VTA. In the first experiment, rats were trained to self-stimulate for a range of current intensities, varied randomly within each session. When baseline response rates had stabilized, the rats were tested on every third day following separate i.p. injections of physostigmine (.20, .35 & .50 mg/kg) plus methyl-scopolamine (.50 mg/kg), pilocarpine (1.25, 2.5 & 5.0 mg/kg) plus methyl-scopolamine (.50 mg/kg), nicotine (.20, .40 & .80 mg/kg), saline plus methyl-scopolamine (.50 mg/kg) and saline alone. Both physostigmine (.50 mg/kg) and pilocarpine (5.0 mg/kg), in conjunction with methyl-scopolamine (MS), produced an attenuation of ICSS rates relative to tests with lower doses of these drugs or with saline. All doses of nicotine facilitated ICSS rates relative to saline tests. The MS treatment had no effects relative to injections of saline alone.

The second experiment assessed the effects of ACH drugs on the cue properties of VTA stimulation to determine whether their influence on BSR might be associated with comparable changes in the perceived intensity of the stimulation. Rats were trained to discriminate between discrete trains of high and low intensity VTA stimulation according to a procedure described elsewhere (see Druhan et al, Neuroscience Abstracts, 344.12, 1985). Stimulus generalization gradients were obtained for each rat after injections of physostigmine, pilocarpine, nicotine, methyl-scopolamine and saline. All doses and injection procedures were the same as for experiment 1. The higher doses of both physostigmine (.35 & .50 mg/kg + MS) and pilocarpine (2.5 & 5.0 mg/kg + MS) enhanced the perceived intensity of the stimulation. By contrast, the perceived intensity of the stimulation was not affected by either nicotine or MS plus saline. These experiments demonstrate that ACH drugs may have differential effects on ICSS and stimulus generalization gradients obtained with VTA stimulation. This may represent a pharmacological dissociation of the cue properties from the rewarding effects of VTA stimulation. However, acceptance of this conclusion requires confirmation that the effects of ACH compounds are related to actions on VTA reward processes rather than on motor systems.

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253.7 CYTOCHROME OXIDASE MAPPING OF REWARD PATHWAYS.
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Wong-Riley and her colleagues have demonstrated the relationship between the amount of activity and cytochrome oxidase levels in developing and mature CNS neurons associated with visual pathways. Sensory deprivaton, deafferentiation, and impulse blockage by tetrodotoxin all yield similar results, progressive decreases in cytochrome oxidase activity in the geniculate and striate cortex, suggesting that this enzyme, a key factor in oxidative metabolism, can serve as a useful marker for labelling functionally relevant CNS trajectories. In view of these findings, we were interested in the possibility that a sustained increase in neural activity might be accompanied by an increase in cytochrome oxidase levels in relevant structures. In this study, the energy demands of reward-related neurons were altered by having rats bar-press for unilateral lateral hypothalamic stimulation. Each lever depression resulted in a 0.5 sec train of 0.1 ms cathodal rectangular pulses with the current fixed at 0.3-0.4 mA. The 15-20 min test sessions were held daily for 5-47 days. On the day following the final test, animals were perfused and the brains prepared for cytochrome oxidase histochemistry using the procedure described by Wong-Riley (Brain Res., 1979, 171, 11-28). Generally, we observed increases in cytochrome oxidase staining in a number of brain regions ipsilateral and in a few regions contralateral to the stimulating electrode; in addition, the differences appeared to be greater in rats that were allowed to self-stimulate for more days. In the stimulated hemisphere, darker reaction products were noted in the caudate, preoptic region, various thalamic nuclei, different portions of the medial forebrain bundle, lateral habenula, red nucleus, and substantia nigra reticulata, while in the unstimulated hemisphere, the nucleus accumbens was consistently darker. Ipsilateral darkening probably reflects the increased metabolic activity from the direct activation of neurons near the electrode tip; contralateral darkening may reflect one of two possibilities - either there is an increased activity in contralateral structures that are directly innervated by fibers that cross over or there is an inhibition of structures ipsilateral to the stimulating electrode. There is reasonable agreement between the above list of structures and the list compiled by Gallistel and his group (J. Neurosci., 1985, 5, 1246-1261) for 2-DG marking of neurons directly driven by rewarding brain stimulation. Cytochrome oxidase thus promises to be a useful tool for delineating the neural pathways mediating reward and could be easily extended to other stimulation-produced behaviors.
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253.8 BRAIN STIMULATION REWARD SITES IN THE STRIA MEDULLARIS.
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The anterior medial forebrain bundle was mapped for brain stimulation reward, with particular attention to sites in the stria medullaris. Male Long Evans rats were implanted with moveable electrodes that allowed testing at 0.25 mm intervals over a 2.5 mm dorsal-ventral range. Rate-intensity curves were determined at each stimulation site tested. Reward thresholds and maximal rates of bar pressing were calculated for each stimulation site. A continuous progression of positive sites for self-stimulation was found at the anterior planes tested. Of these positive sites, at least 25 sites were found within the anatomical boundaries of the stria medullaris. The majority of these positive sites in the stria medullaris were characterized by high maximal rates of bar pressing ranging from 80-120 presses per minute. These high rates in the condensed region of the stria medullaris were in contrast to the low-medium rates across the other anterior levels tested in the medial forebrain bundle. The reward thresholds in the stria medullaris ranged from 21-54uA. Several negative sites for self-stimulation were found immediately dorsal, ventral and lateral to the stria medullaris. Thus it appears that the myelinated fibers of the stria medullaris play a special role in brain stimulation reward. Whether activation of these fibers contribute to the rewarding effects of lateral hypothalamic area stimulation remains to be determined.

253.9 FOOD DEPRIVATION DOES NOT SIGNIFICANTLY AFFECT MAXIMUM RESPONSE OR THRESHOLDS FOR LATERAL HYPOTHALAMIC OR POSTERIOR HYPOTHALAMIC BRAIN STIMULATION REWARD.
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It has long been believed that food deprivation facilitates responding for brain stimulation reward when feeding-reward sites in the lateral hypothalamus (LH) are stimulated but not when copulation-reward sites in the posterior hypothalamus (PH) are stimulated (Olds, 1958; Caggiula, 1970). It is difficult to reconcile this belief with recent data suggesting that the directly activated substrate of medial forebrain bundle reward passes without synapse between the lateral hypothalamic and ventral tegmental areas (Gallistel et al., 1980). In addition, it is not known whether food deprivation increases the reward value of the stimulation or the the performance capacity of the animal. For these reasons, the effects of food deprivation on lateral and posterior hypothalamic self-stimulation were reexamined.

In the present experiment, the effects of food deprivation on LH and PH brain stimulation reward were tested in a lever-press variant of the curve-shift paradigm. Eight adult male Long-Evans rats were each stereotaxically implanted with one electrode in the LH and another in the ipsilateral PH. The animals were trained to press a lever on a continous reinforcement schedule for 0.5 sec trains of 0.1 msec pulses. Stimulation intensity was adjusted for each animal so that stimulation frequencies between 28 to 33 Hz were sufficient to maintain minimal (threshold) levels of responding. A descending range of frequencies, from 69 to 25 Hz were tested daily. This range included low values that did not sustain responding and extended to high values that maintained asymptotic response rates. When frequency thresholds were between 28 and 33 Hz for three consecutive days, the effects of 24, 48, and 72 hrs of food deprivation were tested. Rate-frequency functions for each level of food deprivation were obtained from the mean of three trials for each frequency tested. No level of food deprivation altered responding for either LH or PH stimulation at any frequency level tested. Neither frequency threshold nor maximal responding for LH or PH stimulation was influenced by deprivation.

The present data are inconsistent with the view that there is an LH-specific reward system uniquely associated with feeding reward. Our data are consistent with the more recent view that the same fibers of passage link the reward sites in the LH, PH, and ventral tegmental area. Our data suggest that the responsiveness of these fibers to stimulation, as well as the "appetite for self-stimulation", is not increased by hunger as previously suggested. Earlier reports appear to have resulted from selection of individual cases from data reflecting large individual differences.

253.10 DOPAMINE RECEPTOR BLOCKADE OF NUCLEUS ACCUMBENS: ATTENUATION OF SELF-STIMULATION FROM THE VENTRAL TEGMENTAL AREA BUT NOT FROM THE MEDIAL PREFRONTAL CORTEX.
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The mesolimbic dopamine projection to the nucleus accumbens is thought to underlie the rewarding properties of certain drugs of abuse as well as intracranial self-stimulation (ICSS). These and other findings have led to the view that the mesolimbic dopamine system is a critical link or a final common path in the reward system.

While many studies have shown dopamine to be importantly involved in ICSS derived from the medial forebrain bundle (MFB), little is known about dopamine involvement at ICSS sites distal to the MFB. The present experiments explored the contribution of the mesolimbic dopamine projection to the nucleus accumbens in ICSS derived from the medial prefrontal cortex.

Two groups of rats were implanted with electrodes aimed at either the medial prefrontal cortex or the ventral tegmental area. In addition, all animals were implanted with bilateral cannulae aimed at the nucleus accumbens. The animals were tested using the reward summation paradigm in which quantitative shifts in reward value can be assessed independently of impairments in performance capability (Stellar, Kelley, and Corbett, Pharmacol. Biochem. Behav. 18: 433-442, 1983). The dopamine blocker cis-flupenthixol was injected (0.5 µg/µl or 1.0 µg/µl) bilaterally into the nucleus accumbens and the effects were examined in the above paradigm.

Dopamine blockade of the nucleus accumbens produced consistent decrements in the rewarding effects of ventral tegmental ICSS without evidence of impaired performance capacity. The same injections failed to alter ICSS from the medial prefrontal cortex.

These results do not support the idea that the mesolimbic dopamine projection to the accumbens is a critical link in the reward system. Other mesolimbic dopamine projections (e.g. amygdala) may contribute to prefrontal ICSS or perhaps ICSS from this region is independent of any important dopamine influence.

253.11 THE EFFECTS OF OPERANT ON SELF-STIMULATION TRAIN DURATION THRESHOLDS ARE HEMISHERE-SPECIFIC. A. Markou and R.A. Frank. Department of Psychology, University of Cincinnati, Cincinnati, Ohio 45221.

Behavioral paradigms for the investigation of drug effects on brain stimulation reward should provide quantitative measures which separate effects on reward and performance. In order to assess the influence of drugs on the performance capability of the animal, multiple operant studies have been conducted. Interpretation of the results of these studies are based on the assumptions that reward thresholds for electrical brain stimulation are independent of the operant and that an operant manipulation simply changes response effort. The present study was designed to test the validity of the above assumptions. Bipolar electrodes were implanted bilaterally in the ventral tegmental area of 8 male Sprague-Dawley rats. Animals were trained to barpress, nosepoke and move an omnidirectional lever to obtain brain stimulation from the two electrodes. Train duration thresholds and maximal response rats were assessed for all three operants, on both brain sides for each animal. A repeated-measures ANOVA on thresholds indicated that there was a significant side x operant interaction ($p < .05$). Barpressing and omnidirectional lever thresholds were significantly different from nosepoking thresholds on the right side ($p < .05$). In addition, thresholds for barpressing were lower on the left side versus the right side ($p < .05$). Histological analyses indicate that this pattern of results cannot be attributed to differences in electrode placement. Furthermore, there was a differential effect of operant on maximal response rates. Nosepoking maximal response rates were significantly different from barpressing and omnidirectional lever rates on the right side ($P < .05$) but not on the left side ($p > .05$). The results of this study indicate that the effect of an operant on self-stimulation thresholds is hemisphere-specific. The side x operant interaction also suggests that unlike a performance manipulation (e.g., weight on the lever) which induces small shifts in thresholds, changing the operant can produce larger threshold shifts. Thus, altering the operant is not simply an effort manipulation.

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253.12 THE EFFECT OF COCAINE ON BRAIN STIMULATION REWARD DOES NOT TOLERATE DURING CHRONIC DRUG ADMINISTRATION. R. A. Frank, S. Martz* and T. Pommering*. Dept. of Psychology, Univ. of Cincinnati, Cincinnati, OH 45221.

The present research explored the influence of chronic cocaine HCl on self-stimulation train duration thresholds. Bipolar stimulating electrodes were implanted in the ventral tegmental area of 16 male Sprague-Dawley rats under pentobarbital anesthesia. Following appropriate training, the animals were divided into three groups that were matched for self-stimulation thresholds. Subsequently, the rats were tested for three days using saline injections. The subjects' self-stimulation response rates at 14 train durations ranging from 0 to 150 msec were assessed during 90 sec stimulation test periods separated by 30 sec time-outs. Once stimulation train duration response functions had been collected for the predrug baseline period, the subjects received either saline, 10 mg/kg or 15 mg/kg cocaine HCl 15 min before testing on 18 consecutive days. Next, a three day, postdrug baseline was run using saline injections. Cocaine induced a significant decrease in self-stimulation thresholds at both 10 and 15 mg/kg which was evident during the first day of drug administration and continued through the eighteeth day of testing. The two drug groups returned to baseline threshold levels during the postdrug test period. In a final attempt to induce either tolerance or sensitization of cocaine's effects on train duration thresholds, the rats in the two drug groups received three daily injections of 25 mg/kg cocaine HCl for three days. Control animals received saline during this period. The animals were then tested for three days at their original dosage levels. During this final testing phase, cocaine induced threshold shifts that were comparable to those observed during the 18 day drug administration period. Thus, the facilitative effect of cocaine on brain stimulation reward showed little evidence of tolerance or sensitization in the present study. This result suggests that cocaine-induced euphoria persists despite repeated drug injections, a finding that is undoubtedly important for understanding cocaine self-administration in both animals and humans.

253.13 A COMBINED BEHAVIORAL AND ELECTROPHYSIOLOGICAL APPROACH TO IDENTIFYING NEURONS RESPONSIBLE FOR THE REWARDING EFFECTS OF MEDIAL FOREBRAIN BUNDLE STIMULATION. P. Shizgal, D. Schindler*, and P-P. Rompré. Center for Studies in Behavioral Neurobiology, Concordia University, Montréal, QUE H3G 1M8.

The identification of neurons subserving brain stimulation reward is complicated by the fact that many cells in addition to those responsible for the rewarding effect are fired by the stimulation. To address this problem, we first characterized reward fibers by means of behavioral techniques and then used these results to interpret single unit recordings. Of the neurons driven by the self-stimulation electrode(s), those with characteristics that matched the behaviorally-derived values were deemed the most likely to play a role in the rewarding effect.

In the first experiment, Yeomans' behavioral method (Physiol. Behav., 1975, 15, 593) was used to assess the refractory periods of the directly stimulated cells responsible for the rewarding effect of medial forebrain bundle (MFB) stimulation. Single unit recordings were then obtained using the same subjects, stimulation electrodes, and stimulation fields. In 10 rats, the activity of 191 cells was recorded, of which 90 were antidromically driven from the MFB reward sites. Refractory period estimates, obtained using Swadlow's method (Exp. Neurol., 1982, 65, 514) when possible, were collected from 50 of these cells; 27 fell within the range of the behaviorally-derived values (roughly 0.4 - 1.5 msec). The recording targets lay in the septum, diagonal band of Broca, lateral preoptic area, and neighboring basal forebrain structures.

A much tighter set of constraints on the unit data can be obtained by carrying out the behavioral version of the collision test (Shizgal et al., J.c.p.P., 1980, 94, 227) before the electrophysiological recording. The collision curves rise extremely steeply and provide estimates of both anatomical and physiological properties of the reward substrate. For a neuron to be compatible with these constraints, its axon must project through both stimulation fields, be fired from both sites at the currents used in the behavioral test and conduct at a rate compatible with the well-defined estimate of the collision interval obtained behaviorally. When the current is reduced to levels that failed to yield collision-like effects in the behavioral test, one or both of the stimulating electrodes should fail to fire the axon. To date, the sample of cells studied in this manner is small, but it is clear that the constraints imposed by the combination of the refractory period and collision tests are far more selective than those imposed by the refractory period data alone.

253.14 BEHAVIORAL INDICES OF DECREASED BRAIN STIMULATION REWARD DURING AMPHETAMINE WITHDRAWAL. P.R. Hartley, D.B. Neill*, S.A. Murray*, G.K. Mumford*, and G.W. Vogel*. Depts. of Psychology and Psychiatry, Emory University, Atlanta, GA 30322.

Fragmentary reports from humans indicate that withdrawal from heavy amphetamine use is accompanied by mood depression. Research with rats performing intracranial self-stimulation (ICSS) has consistently shown reduced ICSS responding during amphetamine withdrawal. This reduced responding is hypothesized to result from a decrease in the reward value of the brain stimulation. However, the change could be due to impaired motoric ability, fatigue, or subnormal arousal.

We prepared rats with bipolar electrodes chronically implanted in the lateral hypothalamus (AP 5.4, L 1.8, H -2.3; atlas of Pellegrino, Pellegrino and Cushman) and trained them to bar-press for 150 msec stimulation trains (0.5 msec biphasic pulses, 100 pps, 50-150 uA). These rats were then placed in one of three ICSS procedures: FR-1, FR-20, or autotitration. After behavioral stability was attained, they were injected twice daily with 10 mg/kg of d-amphetamine sulfate for 10 days. No testing was carried out during this time. Testing resumed on the day following the last dose.

Rats performing on FR-1 (fixed ratio-1; every bar-press delivered stimulation) showed a 50% decrease in response output on the first day of withdrawal; responding then recovered to pre-drug levels over the succeeding 5-10 days. This was consistent with previous reports.

Rats performing on FR-20 (every 20 bar-presses delivered stimulation) would not maintain responding for 5-10 days into withdrawal. Recovery to pre-drug response levels did not occur in most animals during 4 weeks of post-drug testing. Both run time and post-reinforcement pause were abnormal during this time. These results suggested that the FR-20 procedure was much more sensitive to the withdrawal effects than the FR-1 procedure.

In the autotitration procedure, stimulation intensity decreased 3 uA every 5 responses; the rats could reset the intensity to its starting level by depressing a reset lever. During withdrawal, the rats reset earlier than pre-drug. The rats acted as if the reward value of the stimulation was decreased. Measures of time to reset, total responses, and response rate also changed in a manner similar to the changes seen following decreased starting intensity in undrugged rats.

The results of these studies support the hypothesis that the reward value of brain stimulation is decreased during amphetamine withdrawal. The FR-20 and autotitration procedures may be particularly useful for examining this effect.

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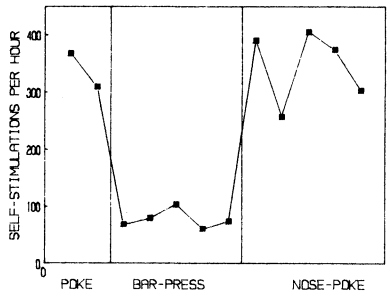
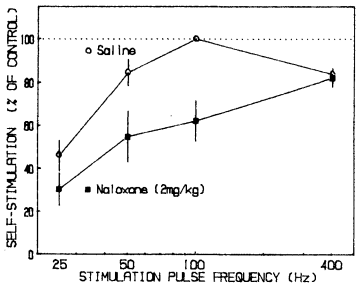
253.15 HIPPOCAMPAL SELF-STIMULATION: DEPENDENCE ON EASE OF RESPONSE AND PHARMACOLOGY. V.R. Bautista*, K.E. Stevens, J.D. Belluzzi and L. Stein. Department of Pharmacology, University of California, Irvine, CA 92717. (SPON: Sue P. Duckles)

The hippocampus has been implicated in memory and learning processes, but suggestions that it also has a role in behavioral reinforcement are not well supported. While there are published reports that rats will perform a lever-press response for electrical stimulation of the dentate gyrus or other hippocampal sites, the rates of such hippocampal self-stimulation (SS) are very low. We were unable in a pilot experiment to train most rats to bar-press for hippocampal self-stimulation, even after extensive shaping; however, the same rats learned spontaneously to perform a nose-poke response for the hippocampal reward. In the experiment shown in the Figure, naive rats with electrodes in the CA1, CA3, or dentate gyrus areas of hippocampus initially were trained to SS in the nose-poke test, were switched to a bar-press test for five sessions, and then were returned to the nose-poke test. SS rates in the bar-press test fell to 20% of the nose-poke rate, but immediately recovered when rats were returned to the nose-poke task. All hippocampal placements supported SS: mean 1-hour nose-poke scores rates were CA1 = 403, CA3 = 420 and dentate gyrus = 539. Amphetamine (1 mg/kg) dramatically increased nose-poke SS rates at all 3 sites; in some cases SS rates were increased 10-fold, strongly implicating a catecholamine in hippocampal reward. This catecholamine neurotransmitter is unlikely to be dopamine (and therefore could be norepinephrine), since haloperidol (0.05 mg/kg) failed to decrease SS at any of the sites tested. Morphine (1 mg/kg) produced no significant effect on hippocampal SS, but naloxone (2 mg/kg) selectively decreased SS at the CA3 site. Possibly, SS of the CA3 region is regulated by endogenous opioids. (Supported by AFOSR Grant 84-0325 and NIH Fellowship DA-05288-01.)



253.16 NALOXONE SUPPRESSION OF NUCLEUS ACCUMBENS SELF-STIMULATION: DEPENDENCE ON STIMULATION PULSE FREQUENCY. J. McCollum*, J. Black, J.D. Belluzzi, and L. Stein, (SPON: C.N. Christian). Department of Pharmacology, University of California, Irvine CA, 92717.

It is well-established that the opiate antagonist naloxone suppresses self-stimulation (SS) of the nucleus accumbens and other reward areas rich in endorphins. This and other evidence has been interpreted to suggest that nucleus accumbens self-stimulation depends upon the activation of endorphin neurons and the consequent release of endogenous opioids which function as reward transmitters. This endorphin-reward hypothesis predicts that enhancement of the release of endorphins will decrease the behavioral efficacy of naloxone due to increased competition for reward receptors. In the present experiment, endorphin release was varied by systematic manipulation of the pulse frequency of the rewarding electrical stimulus. Animals with nucleus accumbens electrodes were trained in one-hour daily sessions to nose-poke for electrical brain stimulation (150 msec train of 0.2 msec monophasic square pulses, 100 Hz, 375 μA). After SS rates had stabilized, baseline pulse frequency-response curves were established for each animal in the range, 25-400 Hz. Such pulse frequency-response curves were then established following injections of naloxone (2 mg/kg, s.c.) and saline (1 ml/kg, s.c.). The open circles in the Figure show saline self-stimulation rates at each pulse frequency as a percent of the saline rate at 100 Hz (the standard pulse frequency used throughout training). Black squares represent naloxone self-stimulation scores as a percent of saline self-stimulation scores at each of the indicated pulse frequencies. The principal finding is that naloxone suppression of SS decreased substantially with increasing pulse frequency. If endorphin release increases as pulse frequency increases, these results support the idea that, at higher frequencies, a given dose of naloxone is less able to compete for endorphin reward receptor sites. (Supported by AFOSR grant 84-0325)



253.17 A COMPARISON OF THE POTENCY OF HEROIN AND ITS ACTIVE METABOLITES, 6-ACETYLMORPHINE AND MORPHINE, ON REWARDING AND AVERSIVE BRAIN STIMULATION. C.B. Hubner and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

The enhanced euphorigenic and analgesic potency of heroin as compared to morphine has raised questions concerning the mechanism responsible for these effects. The present study addresses this question by examining the relative potency of heroin and its active metabolites, 6-acetylmorphine and morphine, on two brain-stimulation models which measure the rewarding and analgesic properties of drugs. Increased sensitivity for rewarding brain stimulation (lowering of the reward threshold) is used as an animal model of drug-induced euphoria and is thought to be predictive of abuse liability in man. An attenuation of the aversive quality of the stimulation in the brain-stimulation escape paradigm (elevation of the escape threshold) represents a means by which to assess a drug's analgesic activity.

Electrodes were implanted in male rats in either the medial forebrain bundle (MFB), an area for which stimulation is positively reinforcing, or the mesencephalic reticular formation (MRF), a site for which stimulation is aversive. Animals were trained to turn a wheel manipulandum to obtain rewarding electrical stimulation to the MFB or to escape from aversive electrical stimualtion to the MRF. Brain-stimulation reward and escape thresholds were determined by using a modification of the psychophysical method of limits. The results indicate that the minimally effective dose of morphine which produced a significant reduction of the reward threshold value was approximately thirty-three times larger than the smallest dose of heroin needed to produce an effect. Based on an examination of the lowest dose required to produce a significant raising of the escape threshold, heroin was found to be approximately five times more potent than morphine. In terms of the minimally effective dose, the monoacetyl derivative of heroin, 6-acetylmorphine, was found to be equipotent to heroin on both the brain-stimulation reward and escape paradigms, suggesting that 6-acetylmorphine makes a major contribution to the rewarding and analgesic effects of heroin.

The enhanced reinforcing effects of 6-acetylmorphine as compared to its analgesic effects may be attributed to its binding to opiate receptors preferentially associated with the mediation of reward. (Supported in part by NIDA grant DA 02326 and NIDA Research Scientist Award (CK) DA 00099).

254.1 A PARTIAL REINFORCEMENT EXTINCTION EFFECT INDUCED BY INTERMITTENT HALOPERIDOL TREATMENTS IN WATER REINFORCED RATS. A. Ettenberg and C.H. Camp. Department of Psychology, University of California, Santa Barbara, California 93106

Dopamine antagonist neuroleptic drugs have been reported to produce dose-dependent reductions in water-reinforced operant behaviors. This finding is consistent with the view that such drugs can attenuate the rewarding properties of water reinforcement. However, animals treated with neuroleptics exhibit deficits in a wide variety of behaviors of which operant responding is only one. In addition, interpretations about the significance of these results are complicated by the fact that the conclusions drawn in the literature have been based almost exclusively upon observations of animals that were drugged at the time of testing. Clearly a more optimal test procedure would be one in which the investigation of putative reward deficits was conducted some time after the direct pharmacological effects of the drug had subsided. In this way the behavioral measures would not be confounded by drug-induced motoric or sedative properties.

Animals trained on schedules of partial reinforcement subsequently make many more responses during extinction than animals trained on a schedule of continuous reinforcement (CRF). Thus a reward manipulation during training (i.e. periodic reward omission) alters behavior of animals some time later, during extinction. This phenomenon is called the "partial reinforcement extinction effect" (PREE) and is well established in the animal learning literature. If neuroleptics greatly attenuate reward, then one might predict that periodic drug treatment in CRF animals would later produce an increased resistance to extinction comparable to that produced in animals who experienced periodic reward omission. In the present study, thirsty rats were trained to traverse a straight alley once a day for 30 s access to water reinforcement. Once trained, the animals experienced a 30-day reinforcement period immediately followed by a 21-day extinction period. Rats that found no water in the goal box on 33% of the reinforcement trials subsequently demonstrated an increased resistance to extinction compared to CRF rats. A weaker, but statistically reliable, PREE was also observed in CRF rats pretreated with 0.15mg/kg of haloperidol (i.p.) on 33% of reinforcement trials. Animals pretreated with a smaller 0.075mg/kg dose of haloperidol behaved no differently from vehicle-treated CRF animals. Such results cannot easily be accounted for by some general drug-induced performance deficit since the rats were not drugged during the critical extinction trials. Although the present results were not as strong as those previously observed for food reward (Camp & Ettenberg, Soc. Neurosci. Abstr.,1984, 10, 265), the data do suggest that neuroleptic agents can attenuate the positive reinforcing properties of water.

Supported by a Regents Junior Faculty Fellowship and by NSF grant BNS 85-10387.

254.2 EFFECTS OF ANXIOLYTIC AGENTS ON FIRING OF LATERAL SEPTAL UNITS IN ACUTE AND CHRONIC PREPARATIONS. E. Yadin, E. Thomas, and M. P. Vaughan*. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010.

We have found that single-unit activity in the lateral septal nucleus of Sprague-Dawley rats consistently increases upon presentation of a conditioned inhibitor of fear and decreases during the presentation of a conditioned excitor, in a Pavlovian differential conditioning paradigm. This pattern of activity, together with a large body of behavioral evidence, has led us to the proposition that one of the functions of the lateral septum is the inhibition of aversive states. Since anxiolytic drugs also inhibit aversive emotional states, it seemed reasonable to expect that unit activity in the septum would reflect the action of anxiolytics.

In well-conditioned animals, the effects of intraperitoneal injections of benzodiazepine anxiolytics and the non-benzodiazepine anxiolytic, buspirone, on the patterns of septal firing were tested. In general, the results for both agents in these freely-moving animals were consistent. First, there was an increase in the spontaneous activity of the cells in the absence of the conditioned stimuli. Secondly, the suppressive effects of a conditioned fear stimulus were completely blocked. Thirdly, the increase in the rate of firing to a conditioned inhibitor of fear (itself a possible index of an anxiolytic function for the septum) was enhanced.

In the acute preparation, the results were quite different. In anesthetized animals, the rate of single-unit firing in the lateral nucleus of the septum was decreased after administration of chlodiazepoxide, a benzodiazepine anxiolytic drug. The effects of buspirone on firing patterns in this area were more variable. Some cells responded by decreased rates of firing while others increased their spontaneous firing rates.

Discrepancies between patterns of single-unit firing in the acute and chronic preparations have been documented for other structures (Rasmussen, K. & Jacobs, B. L., Brain Res, 344:240, 1985). One possibility is that while the effects seen in the acute preparation may be a reflection of the direct action of the drug on the cells, in the chronically implanted, freely-moving animal, the firing patterns may be influenced by the conditioned emotional state of the animal in the apparatus.

254.3 BEHAVIORAL ABNORMALITIES INDUCED BY RIGHT CORTICAL AND ELECTROLYTICAL ACCUMBAL LESIONS. S. E. Starkstein*; T H. Moran; K.L. Kubos, R.G. Robinson, Johns Hopkins Univ Sch Med. Balt., MD

The nucleus accumbens, by receiving main projections from limbic forebrain areas (amygdala and hippocampus) and sending efferents to structures involved in locomotion (ventral globus pallidus) is strategically located for limbic-motor integration. Hyperactivity has been reported following both bilateral and right accumbal electrolytic lesions (Kubos et al, in press) and bilateral and right cortical suction lesions (Pearlson et al, Brain Res 218:233,1981).

Since the accumbens also receives projections from the frontal cortex, we carried out the present study to investigate if both the cortical and accumbal lesions were inducing hyperactivity in a similar fashion by interrupting the same pathways at different levels.

Male Sprague-Dawley rats housed in running wheel cates were divided into four groups following acquisition of baseline activity levels. G1 received both right cortical suction (RCS) and accumbal electrolytic lesions (RAE), G2 received a RCS lesion and a RAE sham lesion, G3 received a RAE lesion and a sham RCS lesion, while G4 received both RCS and RAE sham lesions. Daily spontaneous running wheel activity was measured for 30 postoperative days. A significant increase in mean activity during the period from 12 to 21 days postoperative was observed for G1 (cortical suction and accumbal lesions) of 150%, G2 (cortical suction lesion) of 180%, and G3 (accumbal lesion) of 130% as compared G4 controls.

These findings indicate that the hyperactivity induced by cortical and accumbens lesions were not additive, and in fact cortically elicited hyperactivity was reduced by the accumbal lesion. While cortically elicited mechanisms may not act directly through the accumbens, there appears to be an important interaction between cortex and accumbens in eliciting lateralized hyperactivity following unilateral lesions.

254.4 SINGLE-UNIT RESPONSES IN THE LATERAL AND MEDIAL SEPTAL AREA TO CONDITIONED EMOTIONAL STIMULI. E. THOMAS AND E. YADIN. Dept. of Psychology, Bryn Mawr College, Bryn Mawr PA 19010.

We report here on single-unit activity recorded from the lateral and medial septum of the albino rat during Pavlovian aversive conditioning. We employed a differential conditioning paradigm in which one stimulus (either a light or a tone) was paired with the US and the other was presented unpaired with the US. The CS-US interval was 10 sec. The US was a 1 ma square wave shock lasting 1 sec.

The single-unit data reported here are consistent with our previous multiple-unit recording data, which have suggested that activity in the lateral septum is inhibited by fear-eliciting stimuli and activated by conditioned inhibitors of fear. In the present study we have found single units in the lateral septum which both fire in the presence of a conditioned inhibitor of fear (CS- in our Pavlovian differential conditioning paradigm) and which are inhibited by a conditioned excitor of fear. In addition, we have observed a number of units which show only one of these characteristics i.e., units which fire to a conditioned inhibitor of fear but which do not respond to a conditioned excitor of fear or units which inhibit to a conditioned excitor but which do not respond to a conditioned inhibitor. In general however, there is great consistency of response to the CSs in the lateral septum. Of the considerable number of units assessed to date, none has shown a response inconsistent with the notion that lateral septal activity is enhanced by stimuli that reduce fear and is suppressed by stimuli that signal fear.

The picture in the medial septum is somewhat more complicated, with cells showing a variety of response patterns including: 1)"non-plastic cells" which show no response to any CS during conditioning. 2) cells which show theta bursting in the presence of a conditioned inhibitor of fear and suppression of theta in the presence of a conditioned fear stimulus. These changes in theta bursting are highly correlated with locomotor activity during the CSs. 3) cells which respond to conditioned stimuli in a manner directly opposite to those in the lateral septum. The existence of this last category of cell has suggested to us that there may be, at least in part, a reciprocal relationship between the lateral and medial septum in the modulation of emotion.

254.5 LOW-DOSE PENTYLENETETRAZOL DISCRIMINATION ESTABLISHED BY A STIMULUS-FADING TECHNIQUE. Cynthia M. Harris and Harbans Lal, Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107

Pentylenetetrazol (PTZ) discrimination, using PTZ at 20 mg/kg, has served as an animal model for investigating the neurobiology of anxiety and as an assay for anxiogenic or anxiolytic actions of drugs (Lal and Emmett-Oglesby, Neuropharmacology 22:1423, 1983). In this paradigm anxiogenic treatments substitute for PTZ; however, some test treatments substitute only partially for the stimulus produced by PTZ given at 20 mg/kg. Partial substitutions may be produced by treatments which 1) are similar to, but of weaker intensity than the training stimulus, 2) are qualitatively different from the training stimulus, or 3) disrupt stimulus control. One approach to assessing the presence of a "weak" anxiogenic stimulus is to test these treatments against a "weak" PTZ stimulus. Previous attempts to train rats to discriminate low doses of PTZ directly were unsuccessful (Shearman and Lal, Fed. Proc. 38:256, 1979). We therefore employed stimulus fading as a training technique. Rats were first trained to detect a high dose and then were given successively lower doses until the previous ED50 was consistently detected. The criterion for learning the discrimination was correct lever selection in 8 consecutive sessions in which either drug or saline were tested in an irregular sequence. A group of 32 rats were trained to discriminate PTZ, 20 mg/kg. Acquisition at this dose required 31.7 (+/- 2.5) sets of saline/drug sessions, and 94% of subjects were trained within 60 sessions. Twenty-four of these rats were then retrained with successively lower PTZ doses (18, 16, 14, 12, and 10 mg/kg). The dose for each rat was adjusted according to his performance. Doses were reduced after each block of training sessions in which the rat selected the correct lever at least 4 of 5 times. Acquisition of the discrimination at 10 mg/kg required 17.6 (+/- 3.4) additional sets of drug and saline sessions. The dose-response curve for detection of PTZ by these rats was shifted to the left, and the ED50 was reduced to half. These results indicate that a low-dose PTZ discrimination can be established by the stimulus-fading technique. (Supported by NIAAA Grant RO1AA06890)

254.6 FOREBRAIN INCORPORATION OF ^{3}H-2-DEOXYGLUCOSE (^{3}H-2-DG) DURING ELECTRICAL STIMULATION OF MEDIODORSAL (MD) NUCLEUS OF THE THALAMUS AND INSULAR CORTEX. Shirley L. Buchanan and D. A. Powell. Neuroscience Lab., WJB Dorn VA Hospital and Univ. of South Carolina, Columbia SC.

We have previously established that electrical stimulation of both insular cortex and the mediodorsal (MD) thalamus produces cardiovascular adjustments in the rabbit. Although these structures are reciprocally connected, stimulation of insular cortex produces essentially parasympathetic-like inhibitory changes consisting of heart rate (HR) decelerations and depressor responses, whereas stimulation of MD produces what appears to be sympathetic activation, consisting of pressor responses and cardiac decelerations. In the present experiment rabbits were stimulated either in MD or insular cortex to elicit these responses, subsequent to a bolus injection of ^{3}H-2-deoxyglucose (1.67 mCi/kg). Forty-five min later the animal was sacrificed and perfused with 10% formalin. The brain was removed and sectioned (40 µm) on a cryostat; after drying the sections were placed on film for 2-DG autoradiography. ^{3}H-2-DG activity was quantified in selected CNS structures by densitometry.

Stimulation of the anterior insular cortex elicited increased ipsilateral 2-DG activity along the entire rostral-caudal extent of the insular cortex, as well as the regions dorsal and ventral to the posterior rhinal fissure. Contralateral insular cortex also showed increased 2-DG activity compared to nonstimulated control animals. In all animals with stimulating electrodes in insular cortex ipsilateral mediodorsal nucleus of the thalamus and the amygdala also showed increased 2-DG activity. However, there was no consistent pattern of unilateral activation in some structures known to receive efferents from insular cortex (e.g., parabrachial N. and the N. tractus solitarius). Stimulation of MD resulted in increased 2-DG activity in prefrontal and insular cortex. Contralateral MD as well as thalamic midline nuclei also showed increased activity in some animals. Both insular and MD stimulation resulted in increased 2-DG uptake in the caudate-putamen complex.

These data suggest that the connections that have been previously established through traditional neuroanatomical retrograde and anterograde tracing techniques to exist between MD and frontal cortex are active during electrical stimulation of these structures that produces autonomic changes.

Supported by VA Institutional Research Funds

254.7 LESION OF A BRAINSTEM OUTPUT OF THE LIMBIC SYSTEM ABOLISHES THE REWARDING EFFECTS OF PSYCHOACTIVE DRUGS. A. Bechara* and D. van der Kooy (spon.: Y. Israel). Dept. of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Canada M5S 1A8.

The medial forebrain bundle (MFB) has been identified as a prime substrate for reward effects. The MFB projects down to a wide swath of midbrain and rostral pons referred to as the "limbic" midbrain". We now report that a selective removal of neuronal cell bodies in the nucleus tegmenti pedunculopontinus (TPP) region of the limbic midbrain abolish the rewarding effects of systemically administered morphine and amphetamine. Employing a place conditioning paradigm separate groups of drug naive rats, lesioned with 0.2 ul of 4% ibotenic acid (IA) or vehicle injections into TPP bilaterally, were administered 1 mg/kg doses of amphetamine or morphine (s.c.). Significant place preferences for distinctive environments paired with morphine or amphetamine were observed in sham but not in the IA lesioned rats. IA lesions of the PAG did not block morphine place preferences. The lesions had no effect on the aversive aspects of amphetamine (1 mg/kg i.p.) or morphine (15 mg/kg i.p.) as shown in the conditioned taste aversion paradigm. Rats with sham and IA lesions in the TPP equally showed the aversions for a distinctive taste paired with morphine or amphetamine. In addition, TPP lesions did not disrupt the aversive effects of other drugs (lithium chloride 75 mg/kg i.p. or naloxone 10 mg/kg s.c.) as shown in the place conditioning paradigm. The lesions did not disrupt the analgesic effects of morphine. Significant increases in tail flick latencies after morphine injection were seen in both TPP and sham lesioned rats when compared to saline injected rats. Furthermore, these TPP lesions did not affect the rewarding impact of food or the preference for sweet tastes. Finally, because the TPP region has been implicated in the control of locomotion, we tested open field activity in the TPP and sham lesioned rats. The IA lesions of TPP blocked both the locomotor excitation produced by amphetamine (1 mg/kg s.c.) and the conditioned increase in locomotion produced by morphine (16 mg/kg s.c.), but did not block the catalepsy produced by morphine (16 mg/kg s.c.). We conclude that lesions of the TPP block the rewarding and locomotor effects of opiates and stimulants, without affecting the aversive or analgesic effects of the drugs. We suggest that the neural circuits mediating the rewarding effects of various stimuli acting at forebrain sites may have an obligatory exit point from the limbic system in the TPP region of the brainstem.

254.8 PARAPHERNALIA-INDUCED COCAINE CRAVING. M.S. Gold, J.P. Byron*, C.A. Dackis, and D.R. Sweeney. Research Facilities, Fair Oaks Hospital, Summit, NJ 07901 and Hampton Hospital, Mt. Holly, NJ 08060.

Drug craving is a major reinforcer of addiction that must be addressed with any viable treatment approach. In order to assess whether exposure to cocaine paraphernalia induces cocaine craving, we studied 12 hospitalized cocaine addicts (11 males, 1 female, mean age = 28.3, S.D.=8.1). After signing informed consent, patients were asked to rate their cocaine craving on two consecutive days by marking a 100 mm. line anywhere between the two poles, labelled "not at all" and "extremely." Baseline ratings were made at 11:00 and 13:00, and again at 14:00 hours, after exposure to the provocative stimulus (PS).

The PS involved a 20 minute period (13:30 to 13:50) during which patients reinacted their specific rituals of cocaine administration by handling cocaine paraphernalia and a powder (lactose) that resembles cocaine sulfate. Paraphernalia were available to accomodate intravenous, free-base, and intranasal routes of administration. After completion of the ritual, patients sniffed cocaine scent twice to conclude the PS intervention. Baseline rating means were calculated and compared to the post-PS rating with a Student's t-test. On day 1, mean craving ratings increased significantly ($t=3.1$, $p < 0.005$) from 14.2 to 59.8 (Difference=45.6, S.D.=18.8). On day 2, mean craving ratings also increased significantly ($t=2.5$, $p < 0.01$) from 12.5 to 46.7 (Difference=34.2, S.D.=21.2). There was no significant difference in provoked craving between days 1-2.

These data indicate that exposure to cocaine paraphernalia is a potent stimulus of cocaine craving. Cocaine intoxication is such a potent reinforcer that the sight, smell and feel of cocaine and articles of paraphernalia become conditioned cues, capable of inducing intense craving. Other unknown conditioned cues may also exist and be clinically significant. Furthermore, provoked craving by paraphernalia was not significantly extinguished. Since craving usually preceeds relapse in cocaine addicts, active avoidance of paraphernalia and perhaps other environmental cues is an important aspect of recovery from the addiction.

254.9 HALOPERIDOL DIFFERENTIALLY ANTAGONIZES AMPHETAMINE-INDUCED SHIFTS IN THRESHOLD FOR BRAIN STIMULATION AND LOCOMOTOR ACTIVITY. M.R. Lynch and R.J. Carey (SPON:V.Moser). Dept. Psychiatry, S.U.N.Y. Health Sci. Ctr. and V.A. Med. Ctr., Syracuse, NY 13210

Pharmacological treatments affect BSR rate-intensity functions by shifting the curve horizontally, thereby affecting asymptotic response rates, or displacing it laterally indicating a shift in the threshold for reward. DA agonists and antagonists affect this latter measure in an opposite manner: amphetamine decreases and neuroleptics increase thresholds. Likewise, DA agonists stimulate and the neuroleptics depress spontaneous motor activity. While haloperidol antagonizes the locomotor stimulation produced by 1.0 mg/kg d-amphetamine, it does not block the shift in BSR threshold observed following 2.0 mg/kg d-amphetamine: Eight rats were implanted with platinum electrodes in the VTA (A-4.0,L+2.5,D-8.1). After 10 days, they were shaped to bar press for biphasic square wave stimulation (100 hz, .1 msec interpulse interval, .2 sec train duration). Rate-intensity data were collected by stepping-down the current intensity from that which produced maximum response rate, by 50 µA very 7 min (descending sequence). The first 2 min of each stimulation period served as a warm-up for the new (lower) intensity. Stimulation periods were 5-min in duration, separated by 2-min time-outs when house lights and white noise were turned off and stimulation was not available. Animals were also tested for baseline in an open field. When measures were stable each rat was tested with 0.1 mg/kg haloperidol and the lactic acid vehicle (administered 1 hr before testing). One-half were also tested with 1.0 mg/kg d-amphetamine and the other 4 rats with 2.0 mg/kg (1/2 hr before testing). Lastly, each rat was tested with two injections - the haloperidol followed 1/2 hr later by the respective dose of amphetamine. Drug tests were counterbalanced and spaced 48 hr apart, with return to baselines conducted in between. Animals were always observed for 10 min in the observation box first, then tested in the rate-intensity procedure. While both doses of amphetamine stimulated crossings in the open field, they did not increase asymptotic performance in the BSR paradigm (a measure believed to reflect changes in motor performance). The higher dose did however, lower the mean BSR threshold by greater than one 50 µA step. The interesting finding was that pretreatment with 0.1 mg/kg haloperidol blocked the amphetamine-induced threshold shift, but was without effect on rate increases in locomotor behavior. Therefore, the interaction between these two drugs may be due to more than merely a pharmacological antagonism, as the combination produces vehicle-like performance on measures of BSR, but does not block the stimulation of locomotor activity.

254.10 MEDIAL PREFRONTAL CORTEX STIMULATION INCREASES NUCLEUS GIGANTOCELLULARIS STIMULATION-INDUCED ESCAPE. M.J. Delp* and E.E. Coons* (Spon: D.Quartermain). Psychology Dept. New York Univ., New York, NY 10003

Stimulation of pure reward and reward-escape sites in the lateral hypothalamus respectively ameliorates and exacerbates aversion produced by nucleus gigantocellularis (NGC) stimulation (Carr and Coons, Science, 215:1516, 1982; Simson and Coons, Neurosci. Abstr., 9: 908, 1983). The present study examines the effects of medial prefrontal cortex (MPFC) stimulation on NGC aversion.

Female rats were chronically implanted bilaterally in NGC and MPFC. In Experiment 1, blocks of NGC stimulation trials were given alternately with blocks of (NGC+MPFC) stimulation presented concurrently. In both NGC and (NGC+MPFC) conditions, a bar press (BP)response terminated brain stimulation for 3 seconds. In all animals, the addition of either contralateral or ipsilateral MPFC stimulation to NGC stimulation produced increased escape responding compared to NGC stimulation alone (t_{21}=7.96, p<.001). This was true for both MPFC pure reward and reward-escape animals.

Subsequent work addressing possible explanations of this finding showed that increased NGC escape in the presence of MPFC stimulation is not due to some aversive aspect of the MPFC stimulation summating with NGC aversion, even in the reward-escape animals. Similarly, MPFC activity does not, through heightened arousal, increase the perceived aversiveness of NGC stimulation. Further, a lateralized effect of MPFC on NGC escape suggests that MPFC does not increase NGC escape through a non-specific facilitation of behavior.

Other explanations of the effect of MPFC activity on NGC escape are possible; among them, that MPFC stimulation increases the salience of associations in the NGC escape paradigm. The frontal lobes have been implicated in the organization of stimulus-response contingencies. Results of Experiment 1 are interpreted in this context as showing that MPFC activity facilitates a particular class of motivated behavior, i.e. escape behavior, by increasing the salience of the NGC escape-BP response-negative reinforcement contingency.

254.11 INTRAAMYGDALA AND INTRACEREBROVENTRICULAR (ICV) MIDAZOLAM AND RO5-3663: EFFECTS ON THE INTEROCEPTIVE DISCRIMINATIVE STIMULUS PRODUCED BY PENTYLENETETRAZOL. Dan Benjamin,* Michael W. Emmett-Oglesby, and Harbans Lal. (SPON. I.M. Korr) Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Tx. 76107.

Pentylenetetrazol (PTZ) is an anxiogenic drug which produces an interoceptive discriminative stimulus (IDS) in animals that is mimicked by anxiogenic stimuli and antagonized by anxiolytic drugs in a dose-dependent manner (Lal and Emmett-Oglesby, Neuropharmacol. 22:1423, 1983). PTZ discrimination is a useful animal model of generalized anxiety syndrome. Although there is ample evidence for an anxiogenic effect of PTZ, a CNS site for the production of the PTZ IDS is an untested assumption. In the present experiments, effects of central application of PTZ, RO5-3663, (an anxiogenic benzodiazepine, Lal et al., Fed. Proc. 40:310, 1981), and midazolam, an anxiolytic benzodiazepine were tested. Rats were trained to discriminate the stimulus properties of PTZ in a food-reward (FR10), two-lever operant task: responses on one lever were reinforced after PTZ 20 mg/kg injection, responses on the other lever were reinforced after saline. Trained animals were implanted bilaterally with indwelling guide cannulae in the lateral ventricles and amygdala. After PTZ was given ip., the proportion of rats selecting the PTZ-appropriate lever was dose dependent. The PTZ stimulus was reliably blocked by midazolam given ip. When PTZ was injetted ICV at subconvulsant doses, a maximum of 50% of rats selected the PTZ-lever. All rats selected the saline lever after artificial CSF. Given ip., RO5-3663 generalized to PTZ at a dose of 10 mg/kg, when applied ICV, 154 nmol resulted in full generalization to PTZ. This represents about a 100-fold increase in potency over ip. administration. When injected ICV 1 min before PTZ 20 mg/kg, midazolam blocked PTZ-lever selection in a dose-dependent manner at doses from 18 to 144 nmol When injected into the amygdala, midazolam blocked PTZ-lever selection dose-dependently, at doses from 4.5 to 72 nmol. When injected into the amygdala, an area dense in benzodiazepine receptors (Young and Kuhar,JPET 212:337, 1980), midazolam produced maximal blockade of PTZ-lever selection at one half the ICV dose necessary, and at one seventh the ip. dose. These results suggest that: 1) The PTZ IDS can be mimicked and antagonized by central application of small quantities of drug; 2) The effects seen after central administration are not due to transport of drug into the periphery, as active doses were too low to produce effects after peripheral administration; 3) The site of the PTZ IDS is central because it can be produced by central injections of PTZ or RO5-3663 and blocked by ip. injections of anxiolytics; conversely, the PTZ IDS can be produced by ip. injections of PTZ or RO5-3663 and blocked by central injections of midazolam.

254.12 EVIDENCE THAT VENTRAL PALLIDAL PROJECTIONS TO PEDUNCULOPONTINE NUCLEUS BUT NOT MEDIODORSAL THALAMUS MEDIATE LOCOMOTOR ACTIVITY. G.J. Mogenson and M. Wu*. Dept. of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

Neural projections from the ventral striatum to the ventral pallidal area have been implicated in locomotor activity initiated from limbic integrative activities (Mogenson & Nielsen, Brain Res. Bull., 11:309-314, 1983). Two major outputs from the ventral pallidal area that might mediate this hyperkinetic response are projections to the pedunculopontine nucleus (PPN) of the midbrain and to the mediodorsal nucleus of the thalamus (MD). Earlier we reported that locomotor activity initiated by unilateral injections of picrotoxin into the ventral pallidal area was reduced significantly when procaine was administered to the ipsilateral zona incerta to block VP-PPN projections (Mogenson, Swanson & Wu, Brain Res., 334:65-76, 1985). The present study investigated the effects of administering procaine to the mediodorsal thalamus on locomotor activity elicited by injection of picrotoxin into the VP. Chronic bilateral cannulae were placed into the VP and MD of male Wistar rats. One week later a series of tests was made in an open field chamber equipped with lights, photocells and counters to record the number of photo-beam interruptions as an index of locomotion. Following unilateral injections of picrotoxin (0.15 µg/0.2 µl) into the VP the locomotor index was 274.4 ± 28.6 in 20 min compared to 46.8 ± 7.6 after unilateral injections of isotonic saline (n=21, t=7.65, p<0.001). In subsequent tests a comparison was made of injecting procaine into the MD either ipsilateral or contralateral to the picrotoxin injection into the VP. Following the ipsilateral MD injection the locomotor index was 292.2 ± 24.1 and following the contralateral MD injection the locomotor index was 274.7 ± 30.0 (n=21, t=1.08, p>0.2 and t=0.01, p>0.5 respectively). It is concluded from these experiments that signals from ventral striatum and ventral pallidum associated with locomotor activity utilize ventral pallidal output projections to PPN but not ventral pallidal output projections to MD. Since the PPN is part of the mesencephalic locomotor region it appears that ventral striatal-ventral pallidal-PPN projections may have a role in limbic-motor integration and mediate the locomotor component of certain adaptive behaviors.

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254.13 **DOPAMINE AND REWARD: CONDITIONED PLACE PREFERENCE INDUCED BY DOPAMINE D_2 RECEPTOR AGONIST.** D.B. Gilbert*, J.E. Dembski*, L. Stein and J.D. Belluzzi. Dept. Pharmacol., Coll. Med., University of California, Irvine, Irvine, CA 92717

Dopamine-containing brain systems have been linked to reward function in self-stimulation, self-administration, and conditioned place preference experiments. The purpose of the present research was to study the role of dopamine receptors in reward using the conditioned place preference paradigm. Dopamine D_1 and D_2 receptor agonists and antagonists, alone and in combination with d-amphetamine, were administered to rats in the nonpreferred compartment of a 2-chambered apparatus. N-0437 (3 mg/kg), a dopamine D_2 receptor agonist, induced a significant place-preference, whereas SKF 38393 (20 mg/kg), a specific D_1 receptor agonist, induced no such preference. d-Amphetamine, an indirect catecholamine agonist, has previously been shown to induce place preference, but the receptors involved have not been identified. In a second experiment, we combined a low dose (0.3 mg/kg) of d-amphetamine, which did not induce significant place preference, with sulpiride, a dopamine D_2 antagonist, at a dose (30 mg/kg) which blocks presynaptic auto-receptors leading to increased release of dopamine. Sulpiride potentiated the effect of amphetamine to produce a significant place-preference. Sulpiride alone (30 mg/kg) induced no place-preference and blockade of D_1 receptors with SCH 22390 (0.1 mg/kg) did not potentiate d-amphetamine effects in place-preference. These results suggest that 1) postsynaptic dopamine D_2 receptors mediate reward since a D_2 agonist induced conditioned place preference, 2) amphetamine's rewarding action depends on dopamine release since blockade of presynaptic D_2 receptors facilitates amphetamine-induced conditioned place preference, and 3) dopamine D_1 receptors are not involved in reward function since neither D_1 agonists nor antagonists had any effect in conditioned place preference.

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254.14 EVENT-RELATED POTENTIALS DIFFERENTIATING POSITIVE FROM NEGATIVE AFFECTIVE STIMULI. S.J. Sims, H. Begleiter. S.U.N.Y. Health Sciences Center at Brooklyn. Brooklyn, New York 11203

Affective reactions are important components of the causes of both normal and diseased behavior (affect is here defined as the subjectively-felt reaction to a stimulus). However, affect has eluded objective study because of a need to rely on necessarily indirect verbal reports and emotional behaviors. Biological studies have pursued the goal of quantifying the physiological mechanisms of affect, thereby making affect an objective phenomenon amenable to scientific experimentation.

However, event-related potential (ERP) studies have, with rare exception, used stimuli supposedly devoid of affect. This practice raises two major questions: 1. How much of the brain activity attributed to "non-affective" information processing is in fact activity of the affective brain systems? and 2. What ERP activity would result from the presentation of affectively-weighted stimuli?

Here we report the first findings of ERP's differentiating stimuli consisting of pictures of nudes and war wounds. Right-handed males (n=9) mean age 24, viewed slides subtending 8° of visual arc and projected for 400ms, one every 3 seconds. The 25 positive (female nudes), 25 neutral (household objects) and 25 negative affect slides (war wounds) were presented in random sequence. The ERP's were sorted according to the subject's button presses indicating "like", "neutral" or "dislike".

The results at the PZ electrode show a large positive component at 500ms (here called P500) which is present in the "like" ERP but is either reduced or absent (see below) in the "neutral" and "dislike" ERP's ($P < .01$ by peak amplitude measurement). This is the largest component elicited by affectively-weighted stimuli to date. The P500 has a different latency than the P3 elicited by the auditory or visual oddball paradigms in the same subject, although there is some overlap.

In order to better determine the component structure of the waveforms, and in particular to identify components whose latency changes, the Fast Fourier Transform (FFT) was applied to the ERP's. The FFT magnitude spectral density results show clear differences between the "like", "neutral" and "dislike" waveforms, at various frequencies. Topographical maps constructed from the 21 electrodes used, show localized foci of activity at these frequencies, with a single focus of peak activity per frequency, per condition (e.g. "like") in nearly all instances.

In summary, the use of affective stimuli and data analysis which included the FFT has resulted in findings with implications for the study of affective brain systems and for systems physiology in general.

MOTIVATION AND EMOTION III

255.1 THE EFFECTS OF N-METHYL-DL-ASPARTIC ACID LESIONS OF THE LATERAL PREOPTIC AREA ON THE MATERNAL BEHAVIOR OF POSTPARTUM RATS. M. Numan, K.P. Corodimas*, M.J. Numan* and W.D. Piers*. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

Previous research has shown that medial preoptic area (MPOA) neurons are essential for maternal behavior in rats and that a neural projection route which travels between the MPOA and the ventral tegmental area (VTA) of the midbrain is equally important. The present study is the first in a series examining the structure of this projection route. MPOA efferents project directly to the VTA via the lateral hypothalamus (LH). Indirect MPOA projections to the VTA also exist in that the MPOA projects to the lateral preoptic area (LPOA) which, in turn, projects to the VTA via the LH. The present work examines the importance of the LPOA for maternal behavior.

Fully maternal postpartum rats received one of the following treatments: (1) Bilateral radiofrequency (RF) lesions of the LPOA; (2) Bilateral excitotoxic amino acid lesions of the LPOA employing N-methyl-DL-aspartic acid (NMA)(9.3 µg in .7 µl of phosphate buffer (PB)). NMA is known to selectively destroy neuronal cell bodies, while sparing fibers of passage. (3) Bilateral injections of .7 µl of PB into the LPOA; (4) Bilateral NMA lesions of the LH. The maternal behavior of the rats was studied daily for one week postoperatively. The results showed that NMA and RF lesions of the LPOA severely depressed retrieving, nest building and nursing behavior. In contrast, NMA lesions of the LH were without effect on maternal behavior.

In order to show that NMA lesions, as performed in our laboratory, spare fibers of passage, a second experiment was undertaken. Rats received either RF or NMA lesions of the LH, or injections of PB into the LH. One week postoperatively, horseradish peroxidase (HRP) was injected into the VTA (50% solution; .05 µl), and 48 hrs later the rats were perfused. HRP histochemistry was subsequently performed on the brains and the number of HRP labeled cells at the level of the LPOA was charted. Preliminary analysis indicates that while RF lesions of the LH block the retrograde transport of HRP from the VTA to the LPOA, NMA lesions of the LH do not. Therefore, NMA does not appear to destroy LPOA axons at the level of the LH.

In conclusion, these results indicate the importance of LPOA neurons for maternal behavior, lending support to the view that an MPOA-to-LPOA-to-VTA circuit underlies maternal behavior.

255.2 **EFFECTS OF HANDLING AND AMPHETAMINE ON THE ACQUISITION OF LEVER PRESSING FOR BRAIN SELF-STIMULATION IN RATS.** **Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, Georgia Mental Health Institute, 1256 Briarcliff Rd, Atlanta, GA 30306.**

Changes in dopamine (DA) neurotransmission can influence the acquisition of operant responses and the performance of intracranial self-stimulation (ICSS). An increase in DA utilization in the mesocortical system has been reported in rats following the application of a stressor. Work in our laboratory has demonstrated that acquisition of lever pressing for ICSS in medial prefrontal cortex is facilitated by the DA agonist d-amphetamine (AMPH). We report here on the effects of handling and of AMPH on the acquisition of lever pressing for ICSS in the ventral tegmental area-posterior hypothalamic region (VTA-PH), the origin of the ascending DA fibers.

Adult male rats were implanted in VTA-PH with bipolar stimulating electrodes. In the first experiment, one group of animals (H) (n=6) was handled daily during the week following surgery; whereas, the other group (N) (n=5) was left undisturbed in the home cages. During subsequent acquisition testing, all animals were allowed to learn on their own to lever press for ICSS in daily 15 min sessions. Handled animals required significantly ($p<0.05$) less time (min) to reach a criterion of 10 presses/min (H = 6.5±1.3; N = 21.2±6.7). The time to the first lever press was similar for the two groups. In tests with other, non-implanted animals, the number of lever presses and the amount of locomotor activity in a novel environment was similar in the handled and non-handled groups; suggesting that changes in motor activity were not solely responsible for the effect on acquisition.

In the second experiment, VTA-PH implanted rats were allowed to self-learn lever pressing for ICSS in daily sessions following injection of either saline (n=7) or AMPH (0.25 mg/kg, n=8). No significant differences were found between saline and AMPH treated rats for time to attain criterion or time to the first lever press; although the time to criterion tended to be longer for animals receiving injections than in non-injected animals.

These data show that rats could quickly acquire a lever pressing task for ICSS in the VTA-PH. The speed of acquisition was not affected by this dose of AMPH but was enhanced by prior handling. This may have reduced their level of stress during the initial acquisition procedure. The trend toward slower acquisition following injection stress supports this view.

(Supported by the Georgia Department of Human Resources.)

255.3 EFFECT OF VENTRAL TEGMENTAL AREA LESIONS ON AUTOSHAPED LEVER PRESS RESPONSES IN THE RAT. W. J. Wilson and V. L. Tannehill*. Department of Psychological Sciences, Indiana University - Purdue University at Fort Wayne, 2101 Coliseum Blvd. East, Fort Wayne, IN 46805, USA.

Lesions of the ventral tegmental area (VTA) in the rat facilitate responses motivated by the pairing of a neutral conditioned stimulus (CS) and an aversive unconditioned stimulus (US). This results in enhanced performance in signalled two-way avoidance tasks (Wilson & Baeske, 1985, submitted). We believe that this effect is mediated by damage to the dopaminergic projection from VTA to nucleus accumbens, because manipulations that increase dopamine in the accumbens attenuate aversively classically conditioned motor responses (Wilson & Soltysik, 1985). These studies collectively implicate the mesolimbic dopamine system in the inhibition of motor conditioned responses that arise through aversive classical conditioning. The present study examines the role of the VTA in motor responses that arise in an appetitive classical conditioning task, the autoshaping of lever press responses when presentations of the lever predict food delivery.

Rats received either electrolytic lesions of the VTA, or sham lesions (under 42 mg/kg sodium pentobarbitol anesthesia), and then were reduced to 80% of their pre-surgery free-feeding weight for the remainder of the experiment. Rats were exposed to one of two experimental procedures in 10 daily sessions. The Autoshaped rats received 50 pairings of a retractable lighted lever and a 45 mg food pellet in each session. The Random Control rats received 50 independent presentations of the lever and food in each session. The number of trials in which a lever press occurred in each session was recorded for each rat. Pressing the lever had no effect on food delivery in this stage of the experiment.

After 10 daily sessions, all rats were switched to an Omission procedure, in which food was not delivered on those trials in which a lever press response occurred. Omission training continued for 10 daily sessions.

Data collection is ongoing. If the VTA-lesioned rats acquire the autoshaped lever press response more rapidly than the sham-lesioned rats, it will indicate that the VTA lesion facilitates appetitively motivated responses, just as it is known to facilitate aversively motivated responses. If VTA and sham rats are equivalent, it will suggest a specific role for the VTA in aversively motivated behaviors.

Wilson, W. J., & Baeske, E. G. (1985). Society for Neuroscience Abstracts.

Wilson, W. J., & Soltysik, S. S. (1985). Acta Neurobiologiae Experimentalis, 45, 91-105.

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255.4 AN AUTORADIOGRAPHIC ANALYSIS OF THE PATHWAYS MEDIATING AFFECTIVE DEFENSE AND QUIET BITING ATTACK FROM THE MIDBRAIN PERIAQUEDUCTAL GRAY IN THE CAT. M.B. Shaikh and A. Siegel. Dept. of Neuroscience, N.J. Medical School, Newark, N.J. 07103.

It is now well established that affective defense (AD) as well as quiet biting "predatory" (QBA) aggressive reactions can be elicited by stimulation of the midbrain periaqueductal gray (PAG) of the cat. The purpose of this study was to identify the ascending and descending pathways from the PAG subserving each of these forms of aggressive behavior.

Initially, an AD or QBA response was identified by electrical stimulation through a cannula electrode situated in the PAG. The attack responses were subsequently elicited following injections of homocysteic acid through the same cannula electrode in order to demonstrate that these responses were the result of direct stimulation of cell bodies within the PAG. Then, one of the following autoradiographic tracing procedures was utilized: (1) ^{3}H leucine was injected through a cannula electrode and the animal was sacrificed after 4-14 days survival period; or (2) a C^{14} 2-deoxyglucose (2DG) solution was systemically injected and electrical stimulation was applied through the cannula electrode in order to metabolically activate the pathways associated with each of these responses.

In general, observations resulting from tritiated amino acid injections paralleled those obtained utilizing 2DG. The principal ascending pathway associated with AD was traced to the anteromedial hypothalamus and medial thalamus. Concerning descending projections, label was traced into the central tegmental fields of the midbrain and pons, locus coeruleus, and motor and main sensory nuclei of the trigeminal complex. With regard to QBA, a different projection pattern appears to be present. Label was followed rostrally for a short distance only to the level of the posterior hypothalamus. Caudally directed fibers associated with QBA appeared to be more restricted than those linked to AD. These axons were traced primarily to the superior colliculus, central tegmental fields and raphe complex of the midbrain and pons.

The results suggest that: (1) the PAG serves as a critical structure for the expression of at least the vocalization component of AD since axons associated with this behavior supply nuclei of NV; and (2) the expression of QBA from the PAG may involve ascending fiber bundles innervating the hypothalamus rather than any of its descending components.

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255.5 ENDURING CHANGES IN "ANXIETY LEVEL" RESULT FROM EARLY POSTNATAL HANDLING OF RAT PUPS. S.K. Goldsmith and R.E. Ruth Neuroteratology Lab., Committee on Neuroscience, Univ. Ill. Chicago, 60608.

Early handling produces alterations in adult behavior. Recently an elegant behavioral test was developed to measure "anxiety (Pellow, et.al, 1985)." We studied the effects of early post-natal handling on this measure and the permanence of any changes.

Male rat neonates were randomly assigned to handled (H) or unhandled (U) foster litters. On postnatal day 1 (PD0=birth) pups from H litters were removed from the home cage and gently handled for 30 minutes/day through PD22. To control for maternal *separation we* removed dams which nurtured the U litters for an equivalent period of time. Beginning at PD60 each rat was observed for 5 minutes during the dark cycle on a plus-maze designed to measure anxiety (Pellow et.al, 1985). Each rat was tested on 3 separate occassions separated by a 48h interval between tests. The dependent variable was percent-time spent on the open arms. Half of the animals were tested a fouth time at PD240. A second behavior, neophobia, was tested in a two-bottle paradigm. Following a 45 minute deprivation at the start of the dark cycle, two water bottles (a novel solution of 0.1% saccharin in tap water or tap water only) were presented to the rats for 30 minutes; the novel solution was placed in the spatially non-preferred position. After 48h the test was repeated with a different novel solution (25.0% apple juice). We measured the percent of total intake represented by the novel fluid. A third behavior, open-field activity, was observed during the dark cycle in a 5 x 3 foot grid (3 replications 48h apart). Squares traversed/5 minutes was recorded.

As reported (Tucker and Johnson, 1983) the H rats were significantly more active in the open-field, crossing 50% more squares than the U rats. The H rats were less neophobic, consuming a higher percentage of both novel fluids (+30%, +23%). The greatest difference was observed on the anxiety test; the U rats spent 1/3 of the 5 minute test period, but the H rats spent 2/3 of the period, on the open arms. This relationship remained in the same proportions across the 48h replications and again on PD240.

The increased activity (Semenova et.al, 1981) and decreased neophobia (Mason, et.al, 1978) associated with early handling are consistent with a role for norepinephrine in the mediation of early handling effects (O'Shea, et.al, 1983). However the results indicate that other systems also participate in this process, since scores on the plus-maze are influenced primarily by anxiolytic but not by antidepressant nor antipsychotic drugs (Pellow et.al, 1985).

255.6 OPPOSITE EFFECTS OF MEDIAL THALAMIC MU AND KAPPA OPIOID ACTIVITY ON MOTIVATIONAL-AFFECTIVE RESPONSES. K.D. Carr and E.J. Simon. Department of Psychiatry, New York University School of Medicine, N.Y., N.Y. 10016.

Medial thalamus contains a high density of opioid receptors and seems particularly enriched in the mu receptor type. The present study evaluates the effect of medial thalamic mu opioid activity on motivational-affective responses in the rat. Rats were chronically implanted with microinjection cannulae (22 ga. guide) aimed 2 mm above the target site in the lateral segment of the mediodorsal thalamic nucleus. Bilateral infusion of DAGO, the mu-selective agonist, at a dose of 5.0 ug (in a 0.5 ul volume, infused over 1 minute via 28 ga. injector extending 2 mm beyond guide) consistently produced catalepsy that was immediately naloxone-reversible (1.0 mg/kg, i.p., n=4). DAGO in doses of 0.25 ug and 1.0 ug did not produce visible signs of sedation or motor impairment yet had a substantial effect in tests of motivational-affective behavioral responses. Lateral hypothalamic electrical stimulation frequency thresholds for eliciting feeding behavior and self-stimulation reward were determined in separate groups of rats (n=3 & n=4, respectively). Tail-shock voltage threshold for eliciting a pain vocalization response was also determined (n=4). DAGO (0.25 ug) produced a mean elevation of +35% in feeding threshold, +12% in self-stimulation threshold, and +40% in pain vocalization threshold. DAGO (1.0 ug) produced a mean elevation of +120% in feeding threshold, +35% in self-stimulation threshold, and +165% in pain vocalization threshold. U50 488, the kappa-selective opioid agonist, in doses up to 25.0 ug, showed no sign of elevating threshold for any of these behavioral responses. On the contrary, rats displayed modest reductions in threshold for self-stimulation reward and pain vocalization. Higher doses of U50 488 are currently being tested.

These results suggest that mu opioid activity within medial thalamus diminishes motivational-affective responsiveness of the organism. Preliminary data suggest that kappa opioid activity may enhance such responsiveness. (Supported by DA 03956 to K.D.C. and DA 00017 to E.J.S.)

255.7 EFFECTS OF NALOXONE UPON HYPOTHALAMICALLY ELICITED AFFECTIVE DEFENSE BEHAVIOR AND THE MODULATION OF THIS RESPONSE BY THE AMYGDALA IN THE CAT. M. Brutus and A. Siegel. Dept. of Neuroscience, N.J. Medical School, Newark, N.J. 07103.

The amygdala, medial hypothalamus (MH) and the periaqueductal gray (PAG) form reciprocally interconnected neural substrates for both elicitation and modulation of aggressive reactions. These sites are also known to contain high concentrations of enkephalin-containing perikarya and nerve terminals. Our laboratory has recently shown that stimulation induced PAG inhibition of hypothalamically elicited affective defense behavior (AD) can be blocked by intracerebral injections of naloxone into the same PAG sites which modulate this response (Pott et al., 1984). We have also observed that systemic administration of naloxone can facilitate AD behavior elicited from the PAG (Shaikh et al., 1986). These findings suggest that endogenous opioids may be involved in the regulation of AD. Therefore, the major goal of the present study was to examine what role endogenous opioids may play in the regulation of hypothalamically elicited AD and in the modulation of this response by the amygdala.

Moveable stimulating electrodes were stereotaxically placed into the MH sites from which AD could be elicited and into amygdaloid sites from which significant modulation of this response could be obtained. Stable baseline threshold values for AD were determined over a 1-2 week period. The experimental design also employed paired trials of single stimulation of the attack site in the hypothalamus and dual stimulation of the hypothalamus and modulating site in the amygdala. The order of presentation of these paired trials followed an A-B-B-A sequence. This procedure was followed on alternate days by this same paradigm except that naloxone hydrochloride (10 mg/kg, I.P.) or normal saline (0.9%, I.P.) was injected 5 min. prior to experimental testing.

Our results indicate that, in 8 cases, peripheral administration of naloxone significantly reduced AD latencies, while similar changes were not observed for control conditions. These results are consistent with prior studies which indicate that endogenous opioids inhibit aggression. Of those sites in the amygdala which modulated AD, naloxone partially blocked the facilitation effect resulting from dual stimulation in 3 cases. This preliminary data further indicates that endogenous opioids may also play a role in the overall amygdaloid mechanism which facilitates AD. Experiments currently in progress are also attempting to determine if endogenous opioids play any role in amygdaloid inhibition of AD.

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255.8 NEONATAL CAPSAICIN INCREASES PLAY DEFICIT PRODUCED BY XYLOCAINE. D.S. Sacks* and J. Panksepp.(SPON: J. Rossi III) Dept. of Psych., Bowling Green State University, Bowling Green, OH 43403

Social play in rats has been shown to be dependent upon the sensory feedback occurring during play (Soc. Neurosci. Abst. 11:288, 1985) The disruption of play due to cutaneous anesthesia of the anterior dorsal surface of an animal's body is indicative of the importance of specific sensory input in the modulation of play. However, the role of specific sensory information from these sites is unclear. Capsaicin has been shown to selectively damage only C-fiber sensory afferents while leaving unmyelinated efferent fibers of SP-I neurons intact (Peptides 4:769-774, 1983). In our study, C-fiber de-afferentation occurring after the neonatal administration of subcutaneous Capsaicin was used to study the role of these fibers in the transmission of this sensory feedback.

Pinning behavior as well as play solicitations as measured by dorsal contacts, was examined in 10 pairs of juvenile Long-Evans rats after neonatal treatment with subcutaneous administration of either Capsaicin (50 mg/kg) or vehicle within 48 hours post partum. At 21 days of age, pups were individually housed and tested for play during 5 min. observation periods every other day until 45 days of age. During the last 2 sessions, each animal of the play pair was administered 4 bilateral subcutaneous injections (.4 ml. per injection) of 2% Xylocaine or vehicle in the scapular region, 10 minutes prior to play testing.

Neonatal treatment with Capsaicin did not reliably alter play. As expected, a reliable decrease in pinning as well as an increase in play solicitation gestures was found following somatosensory desensitization by the Xylocaine injections. However, the disruptive effects of this temporary desensitization was reliably intensified in animals receiving the neonatal Capsaicin treatment. Whereas Xylocaine reduced pinning by 26% in controls, it yielded a 45% decrease in the Capsaicin treated animals. Play solicitations increased over 3 fold in controls following Xylocaine but a 4 fold increase was found in the Capsaicin treated animals.

These results are indicative of the importance of C-fibers in the communication of alternative sources of sensory information in play. While destruction of C-fibers did not reduce play by itself, indicating that C-fiber degeneration does not eliminate the important sensory feedback cues from the anterior dorsal areas required in modulating play, these fibers do communicate sensory information that can be used secondarily as an alternate source of sensory feedback if the primary areas are unavailable for the animal.

255.9 PHENOMENOLOGIC LINK BETWEEN SUBSTANCE ABUSE AND EATING DISORDERS. A.L.C. Pottash, J.M. Jonas*, M.S. Gold, J.A. Cocores*. Research Facilities, Fair Oaks Hospital, Summit, NJ 07901 and Delray Beach, FL 33445.

Studies of individuals with eating disorders have demonstrated an unusually high prevalence of substance abuse among these populations (Hudson, J.I., Pope, H.G., Jonas, J.M. et al, Br J Psychiatry, 142:133-138, 1983). The nature of this linkage remains unclear, however, and may be attributable to the presence of a common third disorder (such as depression) or to a shared "addictive" diathesis. To investigate this latter possibility, we have studied the prevalence of eating disorders among patients with addictive disorders (cocaine abusers) and members of families of alcoholics (adult children of alcoholics), using a semi-structured interview designed to generate lifetime DSM-III diagnoses of bulimia and anorexia nervosa (ANN). A biological link between addictive disorders and eating disorders has been suggested by a report of naltrexone reversal of bulimia (Jonas, J.M., Gold M.S., Lancet, 1(8484):807, 1986).

Among a group of 259 cocaine addicts, we found that 2% (6) had a lifetime diagnosis of ANN, 22% (57) had a lifetime diagnosis of bulimia, and 7% (19) had suffered from both disorders. Among children of alcoholics, fully 34% of 106 people surveyed met DSM-III criteria for either bulimia (29%), ANN (3%), or both disorders (2%). While the incidence of ANN is comparable to that seen in other surveys, the incidence of binge-eating and bulimia were elevated in both of these populations. These preliminary data support a link between substance abuse and eating disorders, and suggest that eating disorders and substance abuse may be mediated by endogenous and exogenous opioids.

255.10 Antidepressant Effects on Behavior and Cerebral Glucose Metabolism in Rats. S. Caldecott-Hazard, P.R. Emmanuel, J. Mazziotta, M. Phelps. Nuclear Medicine and Biophysics, UCLA, L.A., CA 90024

We previously reported that open field behavior is reduced and cerebral glucose metabolism is changed in each of 3 rat models of depressed behavior as compared to controls (Neurosci Abstr. 1985). The changes in metabolism include increased metabolism in the lateral habenula (LHAB), and decreased metabolism in the prefrontal cortex (PCX), anterior ventral nucleus of the thalamus (AVT), and inferior colliculus (ICOL). We now report the effects of 2 antidepressant drugs, tranylcypromine (a MAO inhibitor) and desipramine (a tricyclic) on behavior and metabolism in our depression models. The models were induced by injections of alpha-methyl-para-tyrosine (AMPT), withdrawal from chronic amphetamine (Amp WD), or 3 weeks of stressors. ^{14}C-2-deoxyglucose (2DG) was used to study cerebral metabolism. Tranylcypromine (TRANYL) was injected once (10 mg/kg ip) at 30 hours prior to open field testing or 2DG administration in the AMPT model, or immediately following removal of the amphetamine-containing pellets (20 hours prior to testing) in this model. TRANYL was injected once/day for 3 weeks (10 mg/kg ip) prior to testing in the stress model. Control rats given this dose of TRANYL by either protocol did not differ significantly from each other or from undrugged controls in behavior. With 2DG, controls given 1 injection of TRANYL had increased metabolism in the ICOL as compared to 3-week-injected or undrugged rats. Each of the models given TRANYL showed a significant reversal of the reduced behavior seen with the models alone. In each model, TRANYL also significantly reversed the depression-correlated increase of metabolism in the LHAB. Furthermore, TRANYL reversed the decreased metabolism in the ICOL (AMPT and Amp WD models) and the PCX (Amp WD model).

Desipramine (DESIP) was injected twice/day (10 mg/kg ip) for 3 weeks in all models. Open field behavior was significantly reversed from its reduced state in the models alone. Other DESIP injection protocols (3 injections total, or 7 days of twice/day injections), administered in the AMPT and Amp WD models, did not affect behavior. 2DG studies in the 3 models given DESIP are still in progress. In all of these cases, the drug is injected for 3 weeks. Results from the stress model indicate that glucose metabolism in the PCX, but not the LHAB, is significantly reversed from its decreased state in the model alone. While TRANYL and DESIP both reverse depressed behavior, they may predominantly affect different areas of the brain and hence work via different mechanisms. Supported by grant #NS00979.

255.11 AVERSIVE EFFECTS OF NALOXONE IN NAIVE AND MORPHINE-TREATED MICE: A DEMONSTRATION OF SPECIES GENERALITY. M.J.K. Walker*, M.D. Gritti* and R.F. Mucha. Addiction Research Foundation of Ontario, Toronto, Canada M5S 2S1 and Department of Pharmacology, University of Toronto, Ontario, Canada M5S 1A8.

A recent report that naloxone-induced taste aversion is not seen in mice is inconsistent with findings in rats of both taste and place aversions produced by naloxone (Psychopharmacology, 1984, 82, 322). In view of the limitations that this has for generalizing any motivational effect of naloxone seen in laboratory animals to humans, and for use of mouse in the study of opiate-related motivation, the present study examined the hypothesis that, in mice, naloxone is not aversive. This involved confirmation of previous findings in rats, modification of the procedures for use in mice, and testing of this species for naloxone conditioning.

Subjects were sexually mature males obtained from Charles River Canada Inc. (Quebec). Conditioning involved a sensitive two-flavor taste procedure, used previously in rats (Psychopharmacology, 1985, 86, 274). The two flavors were adjusted so that non-trained subjects showed no overall preference for one flavor. In rats, Flavor 1 comprised 9.3 mM MSG and 96 mM NaCl, and Flavor 2, 0.4 mM saccharine and 1.1 mM citric acid (mixed with tap water). For the mice, the solutions were similar except for the saccharine concentration of 0.26 mM. The subjects were water-deprived overnight and trained 3 times with each flavor given by itself at intervals of no less than 4 h and no longer than 24 h; consumption of one flavor was followed by an injection (SC) of naloxone and the other flavor by placebo (saline). Testing was carried out in water-satiated animals given access to the two flavors for 1 day.

As seen previously, rats (Sprague-Dawley) showed an aversion to the flavor paired with naloxone at doses as low as 0.1 mg/kg. Mice (CD_1) also showed aversive effects, but the lowest dose producing significant conditioning (1 mg/kg) was higher than that for the rats. Doubling the number of naloxone pairings in mouse failed to produce any conditioning at 0.1 mg/kg. However, this dose was aversive in mice implanted with a 37 mg morphine pellet for 3 days prior to the start of conditioning, indicating that as for rats naloxone aversive effects are potentiated by morphine treatment.

It was concluded that motivational changes produced by naloxone do occur in mouse. They may reflect processes similar to those in the rat, although the mouse does appear less sensitive than the rat. In addition, it appears that the economical laboratory mouse may be exploited for the study of drug-related motivation.

(Supported by MRC grant 9552; naloxone kindly supplied by duPont.)

255.12 EVIDENCE FOR DOPAMINE INVOLVEMENT IN REWARD USING A LATENT EXTINCTION PARADIGM. K.E. Asin and D. Wirtshafter. University of Illinois at Chicago, Dept. of Psychology, P.O. Box 4348, Chicago, IL 60680.

One of the most hotly debated topics in physiological psychology is the role of dopamine (DA) in reinforcement. Evidence for the involvement of DA in reward has come from studies on the behavioral effects of neuroleptic drugs. Especially problematic in resolving the dispute is the difficulty in dissociating the motoric demands placed on the animal from the attainment of reward in neuroleptic treated animals. Additionally, the behavior of neuroleptic treated rats is typically compared to the behavior of untreated animals and this may lead to interpretive difficulties due to possible state dependency. In an effort to circumvent these difficulties, we examined the effects of haloperidol (H) on the running response of animals trained on a straight alley task using a latent extinction-like (LE) paradigm. Our results support the notion that dopamine is involved in reward mechanisms.

In the first experiment we examined the conditions under which LE could be obtained in our lab. Rats were given 60 acquisition trials on a straight alley task for food reward. After this time, half of the animals (GB) were given 10 placements directly into the now unbaited goal box, while the other rats (NB) were given 10 placements in an empty, neutral box. When, immediately thereafter, animals were given 6 conventional extinction trials, GB rats showed more rapid extinction compared to NB rats, demonstrating that LE had occurred.

In the next experiment we used a similar paradigm but substituted haloperidol for the no-reward condition. Thus, rats were given 60 training trials on a food-rewarded straight alley task across 10 days. On the following day all animals were injected with .15 mg/kg haloperidol (ip); 45 min. later half of the rats (GBH) were given 10 goal box placements with food present, while the others (NBH) were given placements in a neutral box, also with food present. Rats were then given 6 trials to run down the alley for food reward. A MANOVA indicated that although both groups showed declines in run speeds across the 6 trials, group GBH ran significantly slower than group NBH, even on the first test trial. We do not feel that this difference reflects an influence of the drug on motoric systems since both groups had had similar motoric outputs (i.e. they both had been allowed to consume rewards immediately prior to testing). Since H treated rats given direct goal box placements showed slower run speeds compared to rats placed in a neutral box, we feel that our study suggests that injections of haloperidol can interact with reward systems in a manner at least somewhat similar to extinction and that this interaction can occur even **before** the performance of the conditioned response.

255.13 DIFFERENTIAL CONTROL OF THERMOREGULATORY BEHAVIORS AGAINST HYPERTHERMIA BY SUPERFICIAL AND DEEP THERMORECEPTORS IN RATS. W.W. Roberts. Dept. of Psychology, Univ. of Minnesota, Minneapolis, MN 55455.

The principal behavioral responses of rats to hyperthermia induced by ambient heat are grooming, which cools by evaporation of saliva spread over the fur; locomotion, which leads to escape from the warm area; and relaxed body extension, which reduces heat production by muscle tonus and active behaviors. During the early stage of exposure to ambient heat, when the signal of hyperthermia is derived solely or mainly from superficial heat receptors directly exposed to the heat, grooming and locomotion are predominant, and postural extension is weak or absent. During the later stage, as deep body tissues become hyperthermic, postural extension emerges and becomes progressively stronger and more frequent. This temporal sequence suggests that superficial heat receptors preferentially activate grooming and locomotion, while deep receptors preferentially activate relaxed postural extension.

Predominant (though not pure) deep hyperthermia was induced by three methods: a) exposure to ambient heat followed by escape to a thermally neutral environment, b) intermittent exposure to weak microwave irradiation in a thermally neutral environment, and c) injection of isoproterenol in cold-acclimated rats in a thermally neutral environment. In all three cases, postural extension was markedly increased above the small amount present when deep tissues were normothermic in a thermally neutral environment, and even rose about 40-50% above the amount present during the later stage of exposure to ambient heat. The amounts of grooming and locomotion, however, were about the same or less than during exposure to thermally neutral conditions.

These findings confirm the predominant control of grooming and locomotion by superficial receptors and postural extension by deep receptors. As a consequence, initial exposure to ambient heat elicits saliva spreading and escape activity. If they do not prevent deep tissues from becoming hyperthermic, postural extension emerges to suppress tonus and other heat-producing activities except for continuation of some intermittent grooming and locomotion. If escape to a thermally neutral environment then occurs, extension predominates and locomotion and grooming are minimized.

256 SPECIAL LECTURE. INOSITOL LIPIDS AND NEURAL FUNCTION. Michael J. Berridge*, A.F.R.C. Unit of Insect Neurophysiology and Pharmacology, Dept. of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, U.K.

Many transmitters in the brain act by stimulating the hydrolysis of an inositol lipid (PIP_2) to give diacylglycerol (DG) and inositol 1,4,5-trisphosphate ($Ins1,4,5P_3$). These two second messengers mark the beginning of a highly versatile signalling system which may have a unique role to play in modulating neural activity. Special emphasis will be given to the way in which this bifurcating signalling system functions in both pre- and post-synaptic events. By mobilizing calcium from the endoplasmic reticulum, $Ins1,4,5P_3$ regulates the intracellular concentration of calcium thereby effecting both excitability and facilitation. There are two separate pathways for metabolizing $Ins1,4,5P_3$. In one pathway it is sequentially dephosphorylated to free inositol. Inhibition of some of these dephosphorylation reactions by lithium might explain the therapeutic action of this ion in controlling manic-depressive illness. The alternative pathway begins with an inositol trisphosphate kinase which phosphorylates $Ins1,4,5P_3$ to inositol tetrakisphosphate ($Ins1,3,4,5P_4$) which is then dephosphorylated to form the trisphosphate isomer $Ins1,3,4P_3$. These new inositol polyphosphates ($Ins1,3,4,5P_4$ and $Ins1,3,4P_3$) could conceivably have additional second messenger functions.

The DG/C-kinase limb of the signal pathway may regulate both transmitter release and excitability by modulating a variety of physiological processes many of which depend upon changes in potassium permeability. Receptor-stimulated inositol lipid hydrolysis may thus play a crucial role in the nervous system by modulating transmitter release through subtle alterations in excitability.

257 SYMPOSIUM. BRAIN SPECTRIN: STRUCTURE, LOCATION, AND FUNCTION. S. R. Goodman*, M. S. Hershey Med. Center (Chairperson); I. S. Zagon, M. S. Hershey Med. Center (Co-Chairperson); V. Bennett*, John Hopkins Univ.; G. Lynch, Univ. of Cal.-Irvine; M. Willard, Washington Univ.

The discovery of nonerythroid spectrin in 1981 has led to an explosive amount of research activity designed to understand the functions of this unbiquitous cytoskeletal family of proteins. In this symposium, we will bring the neuroscience community to the cutting edge of research performed over the past five years, which has led to our initial understanding of the structure, location, and function of brain spectrin (fodrin). Dr. V. Bennett will discuss the structure of brain spectrin, and the identification of proteins (ankyrin, synapsin I) associated with spectrin in brain. A brief presentation will be given by Dr. Keith Krebs, a postdoctoral fellow in Dr. Goodman's laboratory, on the discovery of a new spectrin binding protein in brain named amelin. Amelin is a 93 kD basic protein which is antigenically and structurally related to erythrocyte protein 4.1, but is distinct from synapsin I. Dr. S. R. Goodman will discuss his recent discovery of two distinct mammalian brain spectrin isoforms, brain spectrin(240/235) which is located in axons and presynaptic terminals; and brain spectrin(240/235E) which is located in neuronal cell bodies, dendrites, and postsynaptic terminals, as well as in certain glial cells. The structure and associations of these isoforms will be compared. Dr. I. S. Zagon will discuss the different expression of these two brain spectrin isoforms during mammalian brain development. Dr. M. Willard will discuss the movement of spectrin (fodrin) in the axon and its relationship to the movements of other proteins (e.g. synapsin I), as well as its relationship to the movement of proteins in other systems (e.g. lymphocyte capping). Dr. G. Lynch will discuss the hypothesis that the degradation of brain spectrin by calpain, triggered by physiological events, results in long-lasting changes in synaptic structure and function.

258 SYMPOSIUM. PHYSIOLOGICAL CONTROL OF MAMMALIAN VOCALIZATION. J.D. Newman, NICHD, NIH (Chairperson); J. Panksepp, Bowling Green State University; P. MacLean, NIMH; J. Buchwald, UCLA Medical Center; W. O'Neill, University of Rochester Medical Center.

Study of the physiological control of mammalian vocalization has shifted recently from the traditional emphasis on brainstem mechanisms of phonation in general to the study of forebrain mechanisms involved in the expression of specific vocalizations given in specific behavioral contexts. This symposium presents an overview of research reflecting this contemporary trend in the study of mammalian vocal physiology. Two topics addressed by the speakers are the evolution of mechanisms mediating expression of the isolation call, one of the most basic mammalian vocalizations, and the role in phonation of the anterior cingulate gyrus, a forebrain structure for which there is no counterpart in submammalian vertebrates. Upon maternal separation, mammalian infants emit species-typical vocalizations commonly referred to as "isolation calls." Recent studies have identified some of the key neural circuits and related neurochemical systems involved in isolation call production. An important part of this circuitry is the mediofrontal limbic cortex (cingulate gyrus), which has been implicated in sound production in a range of mammals, including humans.
The topics to be covered by the syposium speakers include: a comparison of neural and neurochemical control of separation-induced vocalizations in rodents and in chicks (Jaak Panksepp); the evolution of the thalamo-cingulate division of the limbic system, and its relevance to the elaboration of mammalian audiovocal communication from ancestral therapsid reptiles (Paul MacLean); the neural and hormonal control of separation-induced vocalization in kittens (Jennifer Buchwald); the role of anterior cingulate gyrus and periaqueductal gray in the vocalizations of bats (William O'Neill).

259.1 LAMINAR AND REGIONAL SPECIFICITY OF TANGLE AND PLAQUE DISTRIBUTION IN ALZHEIMER'S DISEASE (AD): A QUANTITATIVE STUDY OF VISUAL AND AUDITORY CORTICES. D.A. Lewis, M.J. Campbell, G.W. Huntley,* D.L. Benson,* R.D. Terry* and J.H. Morrison. Scripps Clinic and Res. Fnd., La Jolla, CA 92037 and UCSD, La Jolla, CA.

We quantified the regional and laminar distribution of neurofibrillary tangles (T) and senile plaques (P) in the following cortical regions from brains of patients with AD: Brodmann's areas 17 (primary visual), 18 (visual association), 20 (higher-order visual association), 41 (primary auditory) and 22 (auditory association). Sections 40 μm thick were stained with Thioflavin S. For each area, the number of T in a 250 μm cortical traverse and the number of P in a 500 μm cortical traverse were counted in 10 and 8 sections, respectively. The mean number of T in each cortical layer is shown below:

Area	Cases	I	II	III	IV	V	VI	Total
17	6	0	0	0.6	0.1	0.1	0	0.9
18	6	1.0	1.7	13.5	0.3	3.5	0.7	19.7
20	6	1.0	0.6	12.5	3.1	18.0	1.3	35.5
41	4	0	0.1	0.7	0	0.4	0	1.2
22	4	0	0.2	4.3	0.5	11.3	0.3	16.5

The laminar and regional distribution of T in human cortex is directly correlated with the distribution of pyramidal neurons furnishing long corticocortical (CC) projections in monkey cortex. For example, both T and long CC projection neurons are sparse in area 17, have a substantially greater representation in area 18, predominantly in layer III, and are present in even greater numbers in area 20, in both layers III and V. A similar association is evident in the auditory system. In contrast, mean P number is similar in visual areas 17 (18.0), 18 (18.2) and 20 (21.3). However, over 70% of P in areas 18 and 20, but only 22% in area 17, are of the immature type. P are present in all layers but are most numerous in III and IV. Immature P are more common in supragranular layers and mature P in deep layers.

In summary, the precise correlation between the distribution of T and the origin of long CC projections suggests that T reside in the pyramidal cells which furnish these projections. In contrast, P have different and less specific regional and laminar distributions. These findings, in concert with the different regional and laminar distributions of immature and mature P, suggest that multiple neuronal systems (e.g. long CC projections, subcortical afferents, and intrinsic cortical neurons) may contribute to P formation. Supported by MH-00519, AG05131, and the MacArthur Foundation.

259.2 SMI 32; A POTENTIAL IMMUNOHISTOCHEMICAL MARKER FOR THE CORTICO-CORTICALLY PROJECTING CELLS COMPROMISED IN ALZHEIMER'S DISEASE (AD). J.H. Morrison, D.A. Lewis, M.J. Campbell, G.W. Huntley*, D.L. Benson*, and C. Bouras*†. Scripps Clinic and Res. Fdn., La Jolla, CA 92037 and †Inst. Univ. de Psychiatrie, Geneva, Switz.

Quantitative analyses of the distribution of plaques and tangles (Lewis et al, this volume) suggest the following: AD results in part from the breakdown of corticocortical connections and the pyramidal cells which furnish these projections. In this view, the dementia of AD may reflect a neocortical disconnection syndrome. This hypothesis requires the direct demonstration that corticocortically projecting neurons degenerate in AD, and that their degeneration is correlated with plaque and tangle formation. The Sternbergers (PNAS;80,1983) have developed a series of monoclonal antibodies to non-phosphorylated neurofilament protein (N-PNFP). Neurofilament protein and other cytoskeletal components have been implicated in the pathology of AD. In immunohistochemical studies in monkey and human neocortex, SMI 32 (anti-N-PNFP) labels predominantly pyramidal cells. However, only a subpopulation of pyramidal cells are labeled. This selective labeling is reflected in substantial regional variations in the number of cells labeled (see Campbell et al, this volume). In every region of monkey cortex that we have studied, the laminar distribution of SMI 32 labeled cells correlates with the known laminar origin of the long corticocortical projections emanating from that area, as well as with tangle distribution in the same area of AD brains. In addition, in monkey and human hippocampus, precisely that region of subiculum and CA1 which is devastated in AD (Hyman et al, Science, 1984) has a high density of SMI 32-labeled pyramidal cells, whereas the neighboring regions which are spared in AD, have a very low density of labeled cells. In AD specimens, as compared to normal human cortices, the density of N-PNFP immuoreactive neurons is greatly decreased in the subiculum, as well as in the superior frontal cortex. Transport studies are in progress to determine the degree to which corticocortical projections originate from N-PNFP-positive cells. However, the present observations suggest the following: 1) the distribution of N-PNFP-positive cells in monkey appears to correlate with important long association projections that may be compromised in AD, 2) the careful analysis of such distribution patterns in combination with connectivity studies in monkey neocortex and hippocampus is very likely to aid in the accurate interpretation of pathological findings in human, and 3) SMI 32 may be a molecular marker for the cells which are particularly vulnerable in SDAT. Supported by AG05131, MH00519, AA07456, AA06420, and the MacArthur Foundation.

259.3 TAU HISTOCHEMISTRY REVEALS WIDESPREAD CYTOSKELETAL DISORGANIZATION IN ALZHEIMER'S DISEASE. Neil W. Kowall and Kenneth S. Kosik. Dept. of Neurology, Mass. General Hosp. & Harvard Medical School, Boston MA 02114.

The microtubule-associated protein (MAP), tau, is the major antigenic component of purified Alzheimer neurofibrillary tangles (NFT) (Kosik et al., PNAS, in press). Histochemical studies of rat brain showed that tau is normally located only in axons (Binder et al., J. Cell Biol.,101:1371). We studied the distribution of tau in normal and Alzheimer's diseased human cerebrum and cerebellum with a monoclonal antibody (5E2) specific for tau on Western blots.

In normal human cerebral cortex, tau positive fibers emerged from densely immunoreactive white matter to form vertical bundles which closely resembled those seen with traditional myelin stains. These 15-30 micron thick bundles penetrated the cortex at regular intervals of 30-50 microns. Individual bundles were composed of both thick (4-6 microns) and thin (0.5-2.0 microns) fibers. Structurally similar horizontal bundles were distributed in a laminar manner which also corresponded to classical myelin staining. The resulting complex fiber patterns varied widely throughout the cortex. In cerebellum, fibers emerged from white matter, coursed through the granule cell layer, and terminated in the Purkinje cell layer. No cell somal or dendritic staining was seen in either cerebral cortex or cerebellum.

The distribution of tau immunoreactivity was strikingly altered in Alzheimer's disease. Very dense staining was seen in both NFT and the neuritic portion of senile plaques. Senile plaques were often embedded in vertically oriented tau immunoreactive fiber bundles. A diffuse pattern of fine curly fibers was seen throughout cortex even in areas with no senile plaques or NFT. Curly fibers were contiguous with NFT at times hence their density was increased in regions with large numbers of NFT. Increased curly fiber density was not seen in the vicinity of senile plaques.

Our findings show that the subcellular compartmentalization of at least one regionally specialized MAP is altered in Alzheimer's disease. Furthermore, the existence of an extensive meshwork of tau immunoreactive curly fibers indicates that the cytoskeletal disorganization seen in Alzheimer's disease is more widespread than apparent from the distribution of NFT and senile plaques alone.

259.4 ALZHEIMER'S-LIKE NEUROPATHOLOGY IN CORTEX AND HIPPOCAMPUS INDUCED BY LONG-TERM NUCLEUS BASALIS MAGNOCELLULARIS (nBM) LESIONING IN THE RAT. V.M. Panzarino*, G.W. Arendash, P.R. Mouton*, and A. Gonzalvo* (SPON: D. Cahill). Division of Physiology and Development, Dept. of Biology, University of South Florida, Tampa, FL 33620 and Pathology Lab., Tampa Gen. Hospital, Tampa, FL 33606.

Alzheimer's Disease is characterized by cholinergic hypofunction and neuritic plaques/neurofibrillary tangles in cortex and hippocampus, as well as by cell losses in the cortex. Considerable evidence indicates that a death or dysfunction of cholinergic neurons, originating in the nucleus basalis of Meynert (NBM) and projecting to the cortex, occurs in Alzheimer's Disease. An involvement of this "NBM to cortex" cholinergic pathway in the pathogenesis of Alzheimer's Disease is suggested from studies showing a positive correlation between cell death within the NBM, the degree of dementia, and the number of cortical neuritic plaques.

The present study was designed to determine if elimination of the analogous "nBM to cortex" cholinergic pathway in rats will induce neuropathological changes similar to those seen in Alzheimer's-diseased brains. Adult male Sprague-Dawley rats were given infusions of ibotenic acid (5 ug/1 ul) into the nBM and sacrificed at 1½-14 months after lesioning. Brain sections were processed for Thionin staining (to determine cell densities), Congo Red staining (for cerebral amyloid), or King silver impregnation (for neural fibers and neuropathological markers).

All forebrain regions outside of the ibotenic acid infusion area appeared normal until 4½-5 months after lesioning. At that time, as well as at 10 and 14 months after nBM lesioning, cell losses ranging from 20-50% were observed in layers II-VI of the frontoparietal (FP) cortex. Atrophic cells were particularly evident at 10 months post-lesioning. Additionally, fiber staining was decreased and often patchy in appearance within the cortex and hippocampus. The most dramatic cortical cell losses were observed at 14 months after lesioning; cell losses were also detected at that time in the hippocampus, where surviving neurons were atrophic and densely packed with dark-staining neurofibrils. Anterograde transneuronal degeneration could explain the above cell losses. Also at 14 months after lesioning, neuritic plaque-like structures were seen primarily in the cholinergic-denervated cortex. Amyloid was not associated with these structures and was not apparent intraparenchymally at any time after nBM lesioning.

These data suggest that a dysfunction of basal forebrain neurons within the NBM is critically important in the etiology and pathogenesis of Alzheimer's Disease. Since destruction/dysfunction of rat nBM neurons induces memory deficits, cortical cholinergic/somatostatinergic dysfunction, and neuropathological changes in cortex/hippocampus, the nBM-lesioned rat may be an appropriate animal model for Alzheimer's Disease.

259.5 DECREASED HIPPOCAMPAL RNA IN ALZHEIMER'S DISEASE BRAIN. B.E. Boyes*, P.L. McGeer and E.G. McGeer (SPON: S.C. Sung). Kinsmen Laboratory of Neurological Research, Dept of Psychiatry, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Postmortem brains of individuals with senile dementia of the Alzheimer's type (SDAT) show accumulation of neurofibrillary tangles and senile plaques, as well as a marked loss of cholinergic forebrain neurons and of cortical choline acetyltransferase (ChAT). It has previously been reported that cortical RNA levels are reduced in SDAT brain (Sajdel-Sulkowska and Marotta, Science, 1984), although this finding has been challenged (Morrison, et al., Neuroscience Abstracts, 1984). Using the procedure of Chirgwin et al. (Biochemistry, 1979), we have isolated total cellular RNA from postmortem human brains by homogenization in guanidinium isothiocyanate, followed by sedimentation through cesium chloride. The pelleted RNA was reprecipitated twice from guanidinium chloride with ethanol. The UV spectra of RNA samples isolated in this manner are consistent with highly purified RNA, with $A_{260/280} > 2.1$ and $A_{260/230} > 2.25$. Analysis of the RNA by electrophoresis through formaldehyde/agarose gels suggests it to be intact, based on the prominent 28S and 18S rRNA bands, and the absence of low molecular weight material. The polyA^+ RNA prepared from these isolates serve as efficient templates for both in vitro translation and reverse transcriptase. We have compared the yields of hippocampal RNA samples from postmortem SDAT and neurologically normal individuals. The clinical diagnosis of SDAT for 5 individuals was confirmed by histological examination. Cortical ChAT was determined in 7 brain regions, then averaged (frontal pole, temporal tip, occipital pole, Broca's area, mid-temporal, pre- and post-central gyri). RNA content was determined assuming A_{260} = 38µg/ml.

	Mean ± S.D.		Two-tailed
	SDAT(n=5)	Controls(n=7)	t-test
Avg. Cortical ChAT (µmol/100mg protein/hr.)	0.29±0.11	0.94±0.49	$p < 0.05$
RNA (µg/g wet weight)	110±29.8	209±73.0	$p < 0.05$
Age (yr)	79.0±12.9	77.8±10.2	n.s.
Postmortem delay (hr.)	2.65±0.68	3.57±0.78	$p < 0.10$

These results support the previous suggestion that RNA metabolism may be altered in the SDAT brain. (Supported by a grant from the A.D.R.D.A.; tissues were provided by the Alzheimer's Disease Program under an MRC Canada grant.)

259.6 NEUROVIROLOGIC AND NEUROIMMUNOLOGIC CONSIDERATIONS IN ALZHEIMER'S DISEASE. Joseph Rogers, Robert H. Singer, Judith Luber-Narod, and Gary J. Bassell*. Departments of Neurology and Anatomy, University of Massachusetts Medical School, Worcester, MA 01605.

We have developed an in situ hybridization approach to detect virus-specific DNA and RNA sequences in human postmortem tissue. By taking advantage of genomic homologies within a given virus family, it is possible to employ a single probe to mark several different members of that family. Similarly, a presently unknown member of the family may also be marked. Our most recent results with this approach indicate significant herpesvirus hybridization in 5 of 5 Alzheimer's patients, and no hybridization in 2 of 2 control patients. Probes for other virus families (e.g., retroviruses) are negative. Further studies of viral distribution in a particularly severe AD brain reveal clear herpes family hybridization in the pathology-rich association cortex, but not in the pathology-free cerebellum. By counterstaining with thioflavin, the hybridization signal appears to be co-localized with AD plaques and tangles, though not exclusively.

We have also sought neuroimmunologic correlates of possible viral involvement in AD. Normally, brain is sequestered from the immune system both by the blood-brain barrier and by a paucity or lack of immunocompetent cells. In particular, HLA-DR antigens, necessary for T cell recognition and antigen presentation, have been observed in human brain only in a few multiple sclerosis patients. We now report that morphologically distinct microglia and astrocytes in AD brain are heavily labelled by an antibody to human HLA-DR. Thus, these cells may be capable of presenting antigen and mediating an unfavorable immune response in AD brain tissue.

Cultured cells of CNS origin are known to express HLA-DR after exposure to interferon. In turn, interferon production is a known concomittant of viral infection, perhaps providing a link between our neuroimmunologic and neurovirologic findings.

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259.7 CHARACTERIZATION OF THE ALZ-50 ANTIGEN. B. L. Wolozin[1] and P. Davies*.[1,2] Departments of Pathology[1] and Neuroscience[2], Albert Einstein College of Medicine, Bronx, New York, 10461.

Alz-50 is an antibody recognizing a 68K protein that is abundantly present in neurons in brains from Alzheimer cases and virtually absent in brains from neurologically normal cases. What little immunoreactivity that is present in normal brains gives a Western blot with a lower molecular weight (Science, 232:648-650, 1986).

The protein recognized by Alz-50 has been purified almost to homogeneity from Alzheimer brain by a several step column chromatography protocol followed by immunoaffinity chromatography. Polyacrylamide gel electrophoresis followed by Western blot analysis confirms earlier results showing that in reduced form the major immunoreactive band has a molecular weight of 68K. In non-reduced form, though, the protein exists as a high molecular weight complex which does not enter the 6% acrylamide stacking gel. Isoelectric focusing of the protein from Alzheimer brain followed by Western blotting reveals a cluster of bands grouped about pH 5.0 ± 0.2. This pI does not change upon reduction of the protein. These results are consistent with the hypothesis that the high molecular weight nonreduced complex is a homogeneous aggregate of the 68K protein. Protein from brains of neurologically normal patients also appears as a faint band in the same pH region.

The function of the antigen is being investigated. The purified antigen preparation has protein kinase activity. In addition, electron microscopy reveals the antigen to be filament associated in the Alzheimer brain.

Immunoreactivity has been detected in Alzheimer CSF using a partial purification followed by polyacrylamide gel electrophoresis and Western blot analysis. The presence or absence of antigen in other neurologic diseases is being studied using biochemical and immunocytochemical methods.

This investigation was supported in part by NIH Training Grant T2 GM-07288 from the National Institute of General Medical Sciences, The McKnight Foundation, The Joyce Mertz-Gilmore Foundation, and The Commonwealth Fund.

259.8 GLUTAMATE DEPLETION OF THE PERFORANT PATHWAY TERMINAL ZONE IN ALZHEIMER DISEASE. B.T. Hyman, G.W. Van Hoesen, and A.R. Damasio. Depts. of Anatomy and Neurology, University of Iowa, College of Medicine, Iowa City, IA 52242.

Hippocampal afferents from widespread association and limbic cortices, as well as some subcortical areas, converge on the cells that form the superficial layers of the entorhinal cortex. These, in turn, give rise to powerful glutamergic excitatory projection (the perforant pathway), that form a final common link for a prominent cortical-hippocampal neural system. Entorhinal lesions lead to depletion of glutamate in the perforant pathway terminal zone in the experimental animal. We have noted that the cells of layer II and the superficial part of layer III of entorhinal cortex are affected selectively by neurofibrillary tangles in Alzheimer's disease (AD), leading to extensive cell loss, and, in many cases, their total disappearance. This cell specific entorhinal pathology causes deafferention of the hippocampal formation. Thus, we predicted that this deafferentation would be associated with loss of glutamate in the terminal zone of the perforant pathway. The terminal zone of the perforant pathway was microdissected from the outer portion of the molecular layer of the dentate gyrus, and assayed for free glutamate in AD and in age-compatible, neurologically normal controls. The glutamate content of controls (n=4) was 3.05 ± 0.60 nM/mg wet weight, whereas the glutamate content from AD cases (n=6) was only 0.51 ± 0.20 nM/mg wet weight. There was no correlation of glutamate content with age or post mortem interval before measurement. This represents an 83% depletion in AD ($p<.005$). The results reveal a major involvement of a glutamergic excitatory pathway in the pathophysiology of AD, and contrasts with previous reports of no change in glutamate content from measurement of cortical glutamate in AD. The difference between our results and others' may be in the anatomic specificity of the sample chosen, in that we have measured glutamate content in a specific terminal zone populated largely by presynaptic endings of known origin and neurochemistry. These results suggest that glutamate is among the neurotransmitter substances altered greatly in AD, and support the hypothesis that neurochemical deficits in AD are correlated closely with the dysfunction or destruction of specific populations of neurons. Therefore, the magnitude of the decrease in glutamate in the perforant pathway termination zone further substantiates the destruction of this crucial cortico-hippocampal system. Insofar as the perforant pathway and hippocampal formation are important for normal learning and memory, this pathology and transmitter alteration contributes to the memory impairment of AD. (Supported by NS 14944 and PO NS 19632, the Mathers Foundation, and a University of Iowa Committee on Aging Grant).

259.9 MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN ALZHEIMER'S DISEASE
S. G. Younkin, T. Golde*, M. Usiak*, and L. H. Younkin, Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106

Acetylcholinesterase (AChE) exists as monomers (G_1), dimers (G_2), and tetramers (G_4) of the basic catalytic subunit and in three asymmetric forms that consist of 1 (A_4), 2 (A_8), or 3 (A_{12}) tetramers attached to a collagen-like tail. In 26 patients with Alzheimer's disease (AD) and 14 age-matched controls, we analyzed AChE in the nucleus basalis of Meynert (nbM) and in Brodmann area 21 cerebral cortex. In control nbM, AChE was 18% soluble G_1 and G_4 forms, 72% membrane-associated G_4 forms, 3% asymmetric forms, and 6% non-extractable enzyme. In AD nbM, AChE was 21% soluble G_1 and G_4 forms ($p<0.05$), 68% membrane-associated G_4 forms ($p<0.01$), 4% asymmetric forms, and 7% non-extractable enzyme ($p<0.05$). In the nbM of the AD cases, there was a significant 61% decrease in the number of cholinergic neurons, an insignificant 19% decrease in AChE activity, and a significant 366% increase in the mean AChE/cholinergic neuron. In Brodmann area 21 cerebral cortex of the AD cases, there was no change in soluble G_1 and G_4 AChE, a significant 40% decrease in membrane-associated G_4 AChE, and significant 345% and 410% increases in A_{12} and A_8 AChE respectively. Thus there are marked alterations in the distribution of the various molecular forms of AChE in AD. Our provisional working hypothesis is that these changes are primarily due to altered axonal transport in diseased cholinergic neurons. Compensatory changes in surviving healthy cholinergic could also account for some of the changes observed. In 5 AD cases and 5 controls, globular and asymmetric forms of AChE were analyzed in parallel in the cortex and subcortical white matter of Brodmann area 21. In the cerebral cortex of these 5 AD cases, globular forms decreased and asymmetric forms increased relative to controls as in the larger series. In the subcortical white matter of these 5 AD cases, both globular and asymmetric forms decreased significantly ($p<0.02$). The fact that asymmetric forms do not increase in subcortical white matter indicates that the increased asymmetric forms in AD are associated with cortex-specific structure(s) - possibilites include senile plaques, degenerating cholinergic nerve terminals, cholinergic axons that are sprouting within cerebral cortex, and cortical neurons undergoing trophic alteration.

259.10 DIMINISHED RATES OF CORTICAL GLUCOSE UTILIZATION IN ALZHEIMER'S DISEASE ARE NOT RELATED TO PLASMA BETA-HYDROXYBUTYRATE CONCENTRATIONS
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The brain is able to derive energy from the oxidation of ketone bodies supplied from the blood and cerebral ketone body utilization rates are directly related to plasma levels. The most important ketone body is B-hydroxybutyrate (BOHB), which has the highest plasma concentration. It has been suggested that changes in the stoichiometry between glucose utilization and oxygen utilization in aging are due to changes in circulating ketone body concentrations (Sokoloff, Res. Publ. Assoc. Nerv. Ment. Dis. 41:237-254, 1966). In order to determine if global or regional changes in the cerebral metabolic rates for glucose (CMRglu) found in Alzheimer's disease (AD) are related to elevated plasma BOHB levels, we collected plasma samples at the time of PET for BOHB assay. We examined 11 subjects with probable AD (mean age 64 years, SD 6.7) and 7 healthy aged controls (mean age 63 years, SD 3.0). PET was performed using the Donner 280-crystal tomograph and 5-10 mCi of (18F)-2-fluoro-2-deoxy-D-glucose (FDG). Plasma BOHB levels were assayed enzymatically. Subjects were fasted for 4-8 hours before FDG injection.

In the AD group CMRglu for entire cortex was 5.38 mg/100g/min (SD 1.12) and plasma BOHB concentration was 219.9 n moles/ml plasma (SD 207.0). In the control subjects CMRglu for entire cortex was 6.31 (SD 0.74) and BOHB concentration was 187.8 n moles/ml plasma (SD 154.4). Bitemporal-parietal hypometabolism was found in the AD group as previously described by our laboratory and by others (Friedland et al, Neurosci. Lett. 53:235-240, 1985). There were no statistically significant relationships between global CMRglu values and plasma BOHB concentrations in either group. We also looked for correlations between BOHB levels and rCMRglu in 45 brain regions. In the control group a negative correlation was found between rCMRglu in left perisylvian cortex at the midventricular level and BOHB concentration ($p = 0.007$, Spearman), which we attribute to chance. There were no other statistically significant correlations between BOHB levels and rCMRglu, or between BOHB levels and age. The lack of a relationship between plasma BOHB concentrations and age in humans has been previously reported (Duara et al, Ann Neurol 16:702-713, 1984). These results suggest that circulating BOHB is not an important determinant of the rate of cortical glucose use in AD.

259.11 ESTROGEN LEVELS IN POSTMENOPAUSAL WOMEN WITH SENILE DEMENTIA-ALZHEIMER'S TYPE (SDAT) ARE SIGNIFICANTLY LOWER THAN MATCHED CONTROLS.
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Estrogen plays a role in normal brain function via trophic and activational effects on estrophilic neurons. We have proposed that neuronal estrogen deficiency is related to the development of SDAT in susceptible postmenopausal women. Furthermore, estrogen replacement therapy appears to have beneficial effects in SDAT in elderly postmenopausal women (Fillit et al, Psychoneuroendocrinol., 1986). We report here serum estrogen levels measured by radioimmunoassay in elderly (>70 years) postmenopausal women with probable SDAT (n=12) and matched controls (n=20). No differences between the groups were found for age (mean age: controls = 76.7 +/- 3.3; SDAT = 78.1 +/- 4.6), body weight, serum total protein, albumin, hemoglobin, calcium, phosphorus, or urea nitrogen. The lower limits of detection of our estradiol assay was 10 pg/ml. 11/12 SDAT patients had undetectable levels. 7/10 controls with undetectable serum estradiol came to clinic because of complaints of memory loss. Comparing all controls with SDAT patients (Mann Whitney Rank Sum test), significant differences were found between the groups for both serum estradiol ($p< 0.05$) and total estrogen levels ($p< 0.05$). Mean estradiol levels (pg/ml +/- S.D.) for controls = 24.8 +/- 20.4; for SDAT= 12.2 +/- 7.0. Mean total estrogen (pg/ml +/- S.D.) for controls=53.8 +/- 26.9; SDAT (n=11)= 33.8 +/- 8.6. This difference was statistically significant (T-test on unpaired data; $p< 0.01$). These results support the hypothesis that estrogen deficiency may play a role in the development of postmenopausal dementia of the Alzheimer's type in susceptible women. Nutritional status as judged by body weight and various relevant serum parameters did not account for the observed differences in estrogen levels. The mechanism(s) responsible for decreased estrogen levels in patients with SDAT require further investigation.

259.12 VARIABILITY OF OLFACTORY DYSFUNCTION IN ALZHEIMER'S DISEASE.
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A range of histological and neurochemical evidence suggests that olfaction may be affected in Alzheimer's disease (AD). Two experiments behaviorally assessed the nature of possible olfactory dysfunction in the disease.

The first experiment evaluated olfactory discrimination in 15 probable AD patients and 6 healthy age-matched controls. Ten pairs of common smells were judged to be the same or different on 30 presentations. A visual discrimination task using the same paradigm served as a control task. Healthy aged subjects performed better than AD patients on all tasks. However, mild AD patients (N=6) did not differ significantly from moderately impaired AD patients (N=9) on olfactory discrimination. Studies with positron emission tomography (PET) using (F-18)-2-fluoro-2-deoxy-D-glucose (FDG) performed on 11 of the AD patients showed that those AD patients with relative left temporo-parietal hypometabolism did not differ from those with relative right temporo-parietal hypometabolism on odor discrimination although the two groups differed significantly on visual discrimination. Thus, although performance on visual discrimination was lateralized, performance on olfactory discrimination was not. Whereas olfactory dysfunction was present in all AD patients, the extent of the impairment was variable and not directly related to the degree of cognitive deficit.

The second experiment, currently underway, explores the time of onset of the olfactory deficits in view of the recent claim that "the olfactory pathway may be the site of initial involvement in the disease" (Pearson et al., 1985, Proc. Natl. Acad. Sci, USA, 82, 4351-4534) and seeks to define these deficits more explicitly. Ten very mild probable and possible AD patients, ten moderately impaired probable AD patients, ten multi-infarct dementia patients and ten healthy controls, all males, are compared on three measures of olfactory function: thresholds and direct scaling of intensity for pyridine, and recognition on a broad spectrum of odors with the University of Pennsylvania Smell Identification Test. For comparison, thresholds and judgments of suprathreshold intensity are obtained for two taste substances (sucrose and citric acid). Preliminary data extend the results of the first experiment and show that there are measurable olfactory deficits in the very early stages of the disease. However the deficits range from total anosmia to near normal performance, suggesting that olfactory impairment in early AD may be highly variable.

260.1 DOPAMINERGIC SYSTEMS IN OCTOPUS RETINA: EVIDENCE FOR INTRINSIC DOPAMINE-CONTAINING CELLS AND DOPAMINE D_1 RECEPTORS. M.H. Makman*, I. Berrios*, S. Pratt*, R.T. Hanlon* and G.B. Stefano. Depts. of Biochemistry and Molecular Pharmacology, Albert Einstein Col. of Med., NY, NY 10461; Marine Biomedical Inst., Univ. of Texas Medical Branch, Galveston, TX 77550; Dept. of Biological Science, State Univ. of New York Col. at Old Westbury, Long Island, NY 11568.

The retinas of several species of Octopus have been shown to contain dopamine (DA) but not other catecholamines or serotonin. We have reported that O. bimaculatus retina contains DA and also DA-stimulated adenylate cyclase. Since DA is present in optic nerve, retinal DA has been proposed to be in terminals of neurons having cell bodies in the optic lobes. We report here the localization of catecholamine in retina and optic lobe by formaldehyde fluorescence histochemistry (Stefano et al, Science 194: 539). Studies were carried out in O. bimaculatus, O. Maya, O. digueti and O. vulgaris. Fluorescence was specific for catecholamine as determined by fading characteristics, requirement for the paraformaldehyde step and quenching by borohydride. Branches of the optic nerve and the inner nerve plexus region of the retinas exhibited green (DA) fluorescence. Interestingly, small regularly spaced green fluorescing cell bodies were noted within the pigment/photoreceptor layer of the retina just at the inner aspect of the basal pigment and basal membrane region. These cells were evident by fluorescence but not by phase contrast alone. They appeared to be multipolar, with branches extending inward toward the plexus region as well as into the pigment/photoreceptor-outer segment region. In order to further assess these retinal DA-containing cells, O. maya, O. digueti and O. vulgaris were examined 1 week following unilateral knife cut lesion of the optic nerve. Following lesion, fluorescence was lost or markedly decreased in the optic nerve branches and plexus region of the retina; also lost were intensely green-fluorescent regularly spaced cell bodies in the outer region of the optic lobe. In contrast, the fluorescence intensity of the intrinsic retinal cells increased, revealing a fine fiber pathway between these cells (running parallel to the retinal surface). Based on these studies Octopus retina contains two DA systems: one arising from the optic lobe and another intrinsic to the retina and more closely associated with photoreceptors. We find that retina also contains binding sites for ^{3}H-SCH23390, a ligand that binds to DA D_1 receptors positively coupled to adenylate cyclase. Following optic nerve lesion, these binding sites decrease by about 50% in retina. In addition, a portion of the ^{3}H-SCH23390 binding sites is located in the outer segment fraction of Octopus retina. Possibly DA released from the intrinsic DA neurons interacts with these receptors, in order to integrate some aspect of inner-outer segment function, such as regeneration of unbleached rhodopsin and/or pigment granule movement. (Supported by USPHS EY 04633, ADAMHA-MARC 17138 and DHHS RR01279)

260.2 MULTIPLE SYNAPTIC ANTAGONISTS FROM THE VENOM OF AN ORB WEAVER SPIDER. M.E. Adams, F.E. Enderlin* and D.A. Schooley*. Dept. of Entomology, University of California, Riverside, CA 92521 and Research Laboratories, Zoecon Corp., 975 California Ave., Palo Alto, CA 94304.

Although it is relatively inactive against vertebrates, Orb Weaver spider venom causes rapid paralysis of prey insects. Reports in the literature suggest that the selectivity of these venoms results from actions at putative amino acid-mediated neuromuscular synapses in invertebrates (Kawai, et al., Brain Res. 247: 169, 1982; Bateman, et al., Brain Res. 339: 237, 1985). We report here on the isolation of a family of paralytic toxins from the Orb Weaver spider, Argiope aurantia which cause rapid but reversible block of skeletal nerve-muscle transmission.

Argiope venom caused paralysis in all insects tested, including grasshoppers, cockroaches, flies, and moths. Active components of the venom fall into two different molecular size ranges based on gel permeation chromatography: low (<1000 daltons) and high (>5000 daltons). The low molecular weight fraction separates into 5 paralytic toxins on reversed-phase liquid chromatography. We refer to these as "Argiotoxins", or AR1, AR2, etc. AR1 is in highest abundance or about 3-5 times that of AR2-5 based on UV absorbance. One or more higher molecular weight toxins eluting much later are referred to as "Big Argiotoxins." Potencies of either crude venom or the Argiotoxins are not appreciably affected by boiling for 10 minutes. Structural studies indicate that the Argiotoxins are novel "pseudopeptides."

The actions of crude venom and purified Argiotoxins were tested on two skeletal nerve-muscle junctions in insects. Contractions and neurally-evoked EPSP's (putative glutamatergic) of larval Drosophila melanogaster and Musca domestica are antagonized by bath application of crude venom or purified AR1 in a dose-dependent manner (threshold: 1:10,000 to 1:40,000 dilution in saline). The time course of antagonism is accelerated and the effects prolonged by nerve stimulation, indicating that toxin binding is enhanced in the presence of released transmitter. Argiotoxins also antagonize neurally-evoked contractions at a cotransmitter nerve-muscle synapse in the coxal depressor muscle of the cockroach. Neurally-evoked phasic (putative glutamatergic) and tonic (putative proctolinergic) contractions are abolished. The Argiotoxins appear to be potent reversible antagonists of amino acid- and peptide-mediated synaptic transmission at insect nerve-muscle junctions.

260.3 VOLTAGE CLAMP ANALYSIS OF RESPONSES TO EGG-LAYING HORMONE AND ALPHA-BAG CELL PEPTIDE SHOWS IDENTITY WITH RESPONSES TO BAG CELL DISCHARGES IN APLYSIA. Rene F. Jansen and Earl Mayeri. Department of Physiology, University of California, San Francisco, CA 94143.

The bag cells of Aplysia californica release several peptides derived from a common precursor protein. Two of the peptides, egg-laying hormone (ELH) and alpha-bag cell peptide (a-BCP) are putative transmitters mediating prolonged excitation of left lower quadrant (LLQ) and inhibition of left upper quadrant (LUQ) neurons, respectively. We report here on the voltage-clamp analysis of ionic mechanisms involved in the responses to focal pressure application of ELH and a-BCP, and the responses evoked by a bag cell burst discharge. Most data were obtained using slow (15 sec) depolarizing ramps between -110 and -10 mV to generate quasi steady-state current-voltage relations.

Application of a-BCP mimics the changes that occur in the LUQ's during the first few minutes of a bag cell discharge. At membrane potentials less negative than -70 mV an outward current develops that results in a decrease in the negative slope region that is characteristic of pacemaker activity. Additionally, a small increase in K^+-conductance (g_{K+}) occurs, as indicated by a reversal potential of approx. -80 mV. Responses to a-BCP application are blocked in the presence of 30 mM cobalt or 2 mM cadmium, indicating that these currents are calcium dependent. ELH has no effect on LUQ's.

In the LLQ's a bag cell discharge evokes two simultaneous long-lasting changes in membrane current. These changes are mimicked by ELH. At membrane potentials less negative than -70 mV a slow inward current occurs that effectively initiates pacemaker activity. In addition, at membrane potentials below -80 mV an increase in g_{K+} can be seen. Changes in the ELH-evoked K^+ current resulting from alterations in the external $[K^+]$ indicate that this current is inwardly rectifying. Although a-BCP is not the principal transmitter for the LLQ's, it produces a slow outward current at membrane potentials less negative than -70 mV, which opposes the effect of ELH in this membrane potential range. All the effects of ELH and a-BCP are blocked by the Ca^{++}-channel blockers nickel and/or cobalt.

The results indicate that the ionic mechanisms of action of ELH and A-BCP are apparently identical to those produced by the bag cell discharge, and that both peptides act in part by modulating Ca^{++}-dependent pacemaker currents. The peptides therefore meet an additional criterion for transmitter identification.

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260.4 EVIDENCE FOR EXCITATORY AUTOTRANSMISSION IN APLYSIA BAG CELL NEURONS MEDIATED BY ALPHA-, BETA-, AND GAMMA- BAG CELL PEPTIDES. Richard O. Brown and Earl Mayeri. Div. of Neuroscience, Dept. of Physiology, Univ. of California, San Francisco, CA 94143.

The bag cells in the abdominal ganglion of Aplysia are neuroendocrine cells which synthesize and release at least six peptides derived from a common precursor protein. Two of these peptides, egg-laying hormone (ELH) and alpha-bag cell peptide (alpha-BCP), are putative neurotransmitters. The four other peptides, including the pentapeptides beta-BCP and gamma-BCP, have no previously described biological activities. The bag cells are usually silent, but episodically fire synchronous prolonged, intense bursts of impulses which release bag cell peptides and initiate egg laying behavior. Previous work (Rothman et al., PNAS 80:5753, 1983) showed that alpha-BCP depolarizes the bag cells, and it was hypothesized that it acts as an autoexcitatory transmitter which provides positive feedback to sustain the regenerative, all-or-none bag cell burst. We report here further evidence which supports and extends this hypothesis.

Intracellular stimulation of an individual bag cell with a train of depolarizing pulses produced slow, depolarizing responses in the stimulated cell as well as in other bag cells, and often led to a prolonged burst discharge. When the perfusion medium (containing protease inhibitors) was collected from one abdominal ganglion during a bag cell discharge, and perfused into a second abdominal ganglion, the bag cells in the second ganglion were depolarized, and sometimes fired burst discharges. This suggests that an autoexcitatory factor is released from the bag cells.

Alpha-BCP, beta-BCP, and gamma-BCP, which have the common internal sequence Arg-Leu-Arg-Phe, all have dose-dependent depolarizing effects on bag cells when perfused into the abdominal ganglion along with protease inhibitors. Alpha-BCP[1-8], alpha-BCP[1-9], and beta-BCP were approximately equally potent, with thresholds in the range of 10^{-8} to 10^{-7} M, while gamma-BCP had weaker effects. The depolarizing response had a time course similar to that of the depolarization produced by intracellular bag cell stimulation, and at higher peptide concentrations it often exceeded the threshold for initiation of a bag cell burst discharge. Depolarization was also seen when peptide was applied by pressure ejection directly onto bag cells in isolated bag cell organs, suggesting that these effects are direct, acting through excitatory autoreceptors.

These results suggest that alpha-BCP, beta-BCP, and gamma-BCP all act as excitatory autotransmitters on the bag cells. The indication of biological roles for beta-BCP and gamma-BCP brings to four the number of peptides from the ELH/BCP precursor which are thought to act as bag cell transmitters.

This work was supported by NIH Grant NS16490.

260.5 FMRF-AMIDE IS PRESENT IN THE ARC MUSCLE OF *APLYSIA* AND DEPRESSES ITS CONTRACTIONS. K.R. Weiss, P.E. Lloyd, E.C. Cropper, M. Frankfurt and I. Kupfermann. Cntr. for Neurobio. & Behav., Columbia Univ. and NYS Psychiat. Inst., New York, NY 10032. Dept. of Pharmacol. & Physiol., Univ. of Chicago, Chicago, Il. 60637.

Feeding behavior of *Aplysia* is controlled by a variety of motivational variables. We have been studying the neural basis of the behavioral expression of one of these variables, namely food induced arousal. In our previous work, using the accessory radula closer (ARC) muscle of *Aplysia* as a model system for studying the peripheral aspects of behavioral arousal, we found that behavioral arousal is expressed, in part, via an enhanced strength of the muscular contraction. We found that this increase of the strength of contractions is mediated by serotonin and SCP which act to increase muscle contractility. We now report that in addition to this up-regulation, muscle contractions may be down-regulated, and that this down-regulation may be mediated by FMRF-amide.

ARC contractions produced by firing cholinergic motoneurons B15 or B16 were depressed reversibly by superfusing the muscle with FMRF-amide. The threshold for this effect was below 0.01 uM of FMRF-amide. The depression of ARC contraction was accompanied by a reduction of the size of EJPs recorded intracellularly. Since the ARC is a non-spiking muscle whose contraction size depends on the magnitude of its synaptic depolarization, the depression of EJP size is likely to account for the depression of the contraction size. FMRF-amide is therefore a candidate inhibitory modulator of the ARC.

The presence and localization of FMRF-amide in buccal muscle was investigated using biochemical and immunocytological techniques. HPLC fractionation of the material extracted from buccal muscles combined with a bioassay was used to detect and measure the levels of FMRF-amide. We found that FMRF-amide was present in the ARC (76 picomole/g wet weight). Light microscopic examination of ARCs stained with an antibody directed against FMRF-amide revealed that immunoreactivity was found in a series of interconnected varicosities, suggesting that it may be localized to varicosities in nerve fibers.

In conclusion, our combined biochemical, immunocytological and physiological studies suggest an inhibitory modulatory role for FMRF-amide in the ARC muscle. It is possible that FMRF-amide may be involved in mediating forms of dearousal that are observed in sensitized or satiated animals.

260.6 LOCALIZATION OF PEPTIDES IN BUCCAL CHOLINERGIC MOTOR NEURONS B15 AND B16: EVIDENCE FOR A MODULATORY SYSTEM INTRINSIC TO THE MOTOR CIRCUIT. E.C. Cropper, P.E. Lloyd, W. Reed*, I. Kupfermann and K.R. Weiss. Cntr. for Neurobiol. & Behav., Columbia Univ. and NYS Psychiatric Inst., New York, NY 10032. Dept. of Pharmacol. & Physiol., Univ. of Chicago, Chicago, Il. 60637.

We have hypothesized that a food-induced arousal state in *Aplysia* is mediated by the interaction of multiple modulatory systems. One such system is extrinsic to the basic sensory-motor circuit that drives the behavior, whereas, another is intrinsic. To investigate this hypothesis, we have studied a model system consisting of two buccal motor neurons, B15 and B16, and a buccal muscle, the accessory radula closer (ARC). Previous studies indicated that the B15-B16-ARC system is modulated by extrinsic input from the serotonergic metacerebral cell. It has also been demonstrated that the molluscan peptides, SCPa and SCPb, are bioactive in this system, i.e., they increase the size of the ARC contractions produced by stimulation of B15 or B16. It was, therefore, hypothesized that SCPa and SCPb may function as intrinsic modulators in the B15-B16-ARC system and may be present in B15 and B16.

In our experiments, biochemistry and immunocytochemistry at the electron microscope level have been used to demonstrate that SCPa and SCPb are present in B15. Specifically, we have found that B15 contains SCP-immunoreactive (SCP-IR) vesicles. Furthermore, B15 incubated with radiolabeled methionine, a SCP-precursor, synthesizes labeled peptides which precisely coelute with synthetic SCPa and SCPb in a reverse phase HPLC system using two different counter-ions. We have also shown that SCPa and SCPb are not present in B16, but have found that B16 contains a different bioactive peptide. B16 has dense core vesicles but they are not SCP-IR. B16 incubated with labeled methionine synthesizes a peptide which does not coelute with synthetic SCPa and SCPb. The bioactivity of this peptide was investigated by sequentially chromatographing material extracted from ARC muscles with radiolabeled peptide HPLC purified from B16, and assaying the effects of the resulting fractions on the contractility of the ARC muscle. Fractions of muscle extract which coeluted with the B16 peptide increased the size of ARC contractions.

In conclusion, we have demonstrated that both of the main cholinergic motor neurons innervating the ARC contain bioactive peptides which may function as intrinsic modulators, and that the peptides are different for the two motor neurons.

260.7 FMRFamide ACTIVATION OF A VOLTAGE DEPENDENT CHLORIDE CURRENT IN APLYSIA NEURON L2. P. Ruben* and S. Thompson (SPON:J.M. Hardman). Hopkins Marine Station, Stanford Univ., Pacific Grove, CA 93950.

The neuroactive peptide FMRFamide is widely distributed in molluscan and other nervous systems. Its prevalence has prompted investigation into its effects on a variety of cell types. Two electrode voltage clamp was used to study the response of the bursting neuron L2 to FMRFamide. The peptide was applied by pressure ejection from a micropipette onto the surgically isolated soma of L2. At a holding voltage of -40 mV the response is a conductance increase to chloride ions. The ionic identity of this current was determined by partial replacement of external NaCl with Na-isethionate and by substitution of KCl electrodes with KAc electrodes. Changes in external potassium concentrations produce no change in the current, indicating that the response in L2 to FMRF-amide is not due to the activation of a potassium inward rectifier current. In addition, the reversal potential for the current is about -40 mV which is more positive than the reversal potential for potassium in this cell. During voltage steps to hyperpolarized potentials, the conductance dramatically increases. The voltage dependent current develops exponentially to a steady state from which there is no inactivation for at least 1 minute. Upon return of voltage to -40 mV, the current deactivates exponentially with kinetics that are very similar to the inactivation kinetics for the fast transient potassium current, IA. The voltage dependence of the FMRF-amide current is also similar to that for IA inactivation. The summation of the FMRF-amide inward current with IA results in a decrease in the amplitude of IA. Therefore, FMRF-amide can modify the excitability of L2 by activating a chloride current that masks the hyperpolarizing effect of IA.

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260.8 VOLTAGE CLAMP ANALYSIS OF ELH, 5-HT, AND SECOND MESSENGER EFFECTS ON THE IDENTIFIED MOTONEURON B16 OF APLYSIA. M.D. KIRK AND R.H. SCHELLER. Dept. of Biol. Sci., Stanford University, Stanford, CA 94305.

The identified buccal ganglion motoneuron, B16, innervates the accessory radula closer muscle in Aplysia californica. The neuropeptide egg-laying hormone (ELH) and the biogenic amine serotonin (5-HT) excite B16 at nanomolar and micromolar concentrations respectively. The prolonged time courses of the responses (minutes) and the absence of desensitization suggest that these effects are mediated by second messengers. Using the voltage clamp technique, we had previously shown that ELH and 5-HT induce a voltage-dependent slow inward current in B16 that is carried by Na^+. We have now confirmed and extended these results by testing for interactions between these transmitters, and by testing the affects of second messengers on voltage-dependent currents in B16.

ELH and 5-HT were bath applied while an axotomized B16 was voltage clamped using two electrodes. High concentrations (500 nM) of ELH fail to occlude the slow inward shift in holding current in response to 5-HT suggesting that the two transmitters probably act through different receptors. However, high concentrations of 5-HT (100 μM) do occlude the response to ELH suggesting that portions of their effects, such as the induction of the Na^+-dependent slow inward current, are in common, and that some portion(s) of the second messenger pathways involved can be saturated. We then bath applied 8-bromo-cyclicAMP (250 μM), forskolin (100 μM), IBMX (1mM), and intracellularly injected cyclic AMP. All of these treatments induced or enhanced the voltage-dependent slow inward current. It was also found that 8-bromo-cyclicGMP had similar effects and this is consistent with the previous results of Connor and Hockberger, 1984. Intracellular or extracellular applications of 8-bromo-5'-AMP or GMP had no effect. Finally, 8-bromo-cyclicGMP occluded the response to 8-bromo-cyclicAMP and the inward current response to 5-HT, however, under these conditions, 5-HT elicited an outward current that was associated with an increase in conductance suggesting that 5-HT may also act through yet another unidentified second messenger system.

260.9 MULTIPLE MECHANISMS OF BURSTING IN A CONDITIONAL BURSTER NEURON. R. M. Harris-Warrick and R.E. Flamm. Sect. of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

The AB neuron is a conditional burster in the stomatogastric ganglion (STG) of the lobster, *Panulirus interruptus*. With intact modulatory inputs from other ganglia, it exhibits large bursting pacemaker potentials (BPPs) underlying burst of action potentials. When modulatory input is eliminated, it falls silent and cannot be induced to burst by current injection. Three endogenous neuromodulators, dopamine (DA), serotonin (5HT) and octopamine (OCT), induce rhythmic bursting in the AB cell. However, the amplitude, shape and burst frequency are very different with each amine: DA induces larger, high frequency BPPs, 5HT is intermediate, and OCT induces small, low frequency BPPs. We have analyzed the ionic mechanisms of burst induction by these amines in AB neurons that are isolated from all synaptic input by a combination of sucrose-TTX block of descending inputs from other ganglia and 5,6-carboxyflourescein photoinactivation of pre-synaptic neurons within the STG. Differences in amine-induced BPPs arise from different contributions of ionic currents during the burst First, when sodium entry is reduced by 10^{-7}M TTX or 50% reduction of extracellular sodium, BPPs induced by 5HT or OCT are rapidly eliminated, while DA-induced BPPs continue with little decrement in the absence of action potentials. This suggests that burst initiation in 5HT or OCT requires sodium entry through TTX-sensitive channels, while DA-induced bursts do not. Second, when extracellular calcium is reduced to 25% or lower, DA-induced BPPs are rapidly eliminated and the membrane potential remains depolarized near the peak of the former BPP. 5HT- and OCT-induced BPPs are only slightly affected by 25% calcium. However, their bursts cease upon complete removal of calcium or addition of cadmium or cobalt, and again the cell remains depolarized near the peak of the BPP. This suggests that the mechanism for terminating the burst is dependent upon calcium entry, and 5HT and OCT differ quantitatively from DA in their lower dependence on calcium entry to terminate a burst. Third, addition of 5mM TEA to reduce potassium current enhances the amplitude of BPPs induced by all three amines, and the OCT-induced BPP is now nearly the same amplitude as the DA- and 5HT-induced BPPs. This suggests that the lower amplitude of OCT-induced BPPs under normal conditions may be in part due to a greater density of potassium current during the bursts than with 5HT or DA. Fourth, the value of the undershoot separating bursts varies with extracellular potassium in a Nernstian fashion for DA and 5HT, but is much less than Nernstian for OCT. This suggests that potassium currents dominate the undershoot for DA and 5HT, but other currents are also active during the undershoot in OCT. These experiments show that a single cell can burst by more than one combination of ionic currents. The AB neuron has an important role in timing and phasing of the pyloric circuit in the STG. Multiple bursting mechanisms in the AB will allow subtle modulation of this neuronal circuit and its related behavior. Supported by NIH #NS17323.

260.10 EVIDENCE FOR STIMULATION OF MUSCLE PHOSPHATIDYLINOSITOL METABOLISM BY AN IDENTIFIED SKELETAL MOTONEURON
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The slow motoneuron to the extensor tibialis of the locust hind-leg (SETi) releases two transmitters, the peptide proctolin (Arg-Tyr-Leu-Pro-Thr) and an amino acid transmitter (probably L-glutamate). Neurally-released proctolin has two types of actions, direct and modulatory. Proctolin acts directly on the muscle to cause a slow and sustained "catch" tension which does not depend on the presence of extracellular calcium. Proctolin also modulates the action of the second transmitter (L-glu). This transmitter acts classically, producing a muscle EJP and transient twitch contraction. Proctolin produces a long term increase in the amplitude, rate of contraction and rate of relaxation of these transient contractions. These modulatory effects can be mimicked by activating the SETi motoneuron in a brief high frequency burst; SETi-evoked twitch contractions subsequent to a SETi burst are stronger, develop more rapidly, and relax more rapidly than twitch contractions preceding the burst. This indicates that proctolin released during high frequency burst activity modulates the synaptic actions of the amino acid transmitter with which it is co-released.

The direct contractile effect of proctolin does not depend on the presence of extracellular calcium, suggesting the involvement of a second messenger. Proctolin does not elevate levels of cyclic nucleotides in the extensor muscle. To investigate the possibility that proctolin contracts muscle via the inositol phosphate second messenger system extensor muscles were pre-incubated with tritiated myo-inositol and exposed to proctolin. Water soluble inositol phosphates were extracted and separated by anion-exchange chromatography. In the presence of 10 mM LiCl, proctolin triggers a significant increase in inositol trisphosphate (IP_3), a soluble second messenger which liberates calcium from internal stores in other systems.

This evidence suggest that SETi initiates muscle contraction by releasing two neurotransmitters which activate separate intracellular signalling systems; the peptide receptor-stimulated metabolism of inositol phosphates which liberate internal Ca^+ and the amino acid receptor-stimulated influx of Ca^+ ions. The modulatory effects of co-released proctolin might result from the interaction of these two pathways at the cellular level.
(Supported by Swiss National Fund No 3.181.0.85)

260.11 STEROID REGULATIONOF THE PHOSPHOPROTEINS DETERMINING PEPTIDE SENSITIVITY IN THE CNS OF MANDUCA SEXTA. D.B. Morton* and J.W. Truman (SPON. J.L. Witten), Department of Zoology, University of Washington, Seattle WA 98195.

The final step of each molt in insects is the shedding of the cuticle of the previous instar. This process (termed ecdysis) is triggered by a peptide, eclosion hormone (EH), acting directly on the CNS. An intriguing aspect of the action of EH is that it will only trigger ecysis during the few hours immediately preceeding the normal time of ecdysis. The appearence of this window of sensitivity depends on the prior exposure of the CNS to the steroid hormone, 20-hydroxyecdysone (20-HE).

EH acts on the CNS via an increase in cGMP, which in turn stimulates the phosphorylation of two 54kD proteins. The availablity of these proteins for phosphorylation determines the onset of behavioral sensitivity to EH (Morton and Truman (1985), Soc. Neurosci. Abstracts 11;324).

The present report details the regulation of the appearence of the 54kD proteins, by 20-HE, at pupal ecdysis in Manduca. By ligating prepupae between the thorax (the source of ecdysteroids) and the abdomen, the abdomenal CNS is deprived of its normal steroid titers. In animals treated in this way the 54kD proteins fail to appear, as judged by their ability to incorporate ^{32}P. Infusion of ligated abdomens with20-HE for 12 hrs, caused the appearence of the 54kD proteins by 60 hrs after the end of infusion. Behavioral sensitivity to EH requires not only the rise in ecdysteroids but also its subsequent fall. The 54kD proteins also have this requirement. If the CNS is removed 24 hrs before ecdysis, when the 54kD proteins are absent and the ecdysteroid levels are falling, and the CNS is kept in culture for 24 hrs, the 54kD proteins become available for phosphorylation. If the fall in ecdysteroids is prevented by culturing in the presence of 20-HE for 24 hrs, the 54kD proteins do not appear. The timing of the action of 20-HE and of the protein synthesis inhibitor cycloheximide, leads us to believe that the steroid priming of the CNS which renders it responsive to EH comes about by induction of the synthesis of the 54kD proteins.

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260.12 NEUROPEPTIDE SYNTHESIS AND ITS REGULATION IN AN INSECT MODEL SYSTEM
S. Hekimi* and M. O'Shea. Lab. of Neurobiology, Univ. of Geneva. CH-1211 Geneva 4, Switzerland

The locust Schistocerca gregaria possesses two peptide hormones with adipokinetic activity AKH I: (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) (Stone et al., Nature 263 (1976) and AKH II (pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH_2) (Siegert et al., Biol. Chem. Hoppe Seyler Vol. 366 (1985). They are found in high amounts (500 pmoles/ 100 pmoles) in the glandular lobes of the corpus cardiacum (C.C.), a major insect neurosecretory structure. We show that the biosynthesis of AKH I and AKH II, functionally and structurally highly similar peptides, is regulated differently during development. The ratio of the amounts of AKH II versus AKH I found in the C.C. drops throughout post-embryonic life, from almost equal amounts in the first larval stage to the adult condition [AKH II/ AKH I = 18%]. Furthermore the rate of synthesis of both peptides is dramatically increased during adult maturation.

We have developped an in vitro organ culture system in which incorporation of ^3H Trp into the proteins and peptides manufactured by the gland can be monitored. Incubations of glandular lobes from animals at different developmental stages showed that the shift in the AKH II/AKH I ratio is the consequence of differential synthesis.

We also used the in vitro system to characterize the biosynthetic pathways leading to AKH I and II. Several lines of evidence, including time-course of label incorporation, pulse-chase, and cycloheximide-translation block experiments, have allowed us to identify, characterize and purify a 12 kd primary protein precursor of AKH I (ProAKH). Currently we are engaged in the purification of sufficient ProAKH for gas-phase microsequencing.

Using synthetic peptide analogs to AKH I and II we have generated rabbit antisera which specifically recognize and discriminate between AKH I and II. These antibodies also bind to the primary AKH precursor and to the smaller peptides we believe to be intermediates leading to the AKHs. Radioimmunoassay studies suggest the presence of multiple copies of AKH I-related peptides in Pro-AKH.

Immunocytochemical studies show that AKH-like peptides are not confined to the glandular lobe of the C.C. There is a small population of AKH-IR neurons in the CNS. These last findings suggest a neurotransmitter role for the AKHs or AKH-related peptides.
(Research supported by SNF No 3.181.0.85)

260.13 IMMUNOCYTOCHEMICAL MAPPING OF NEUROTRANSMITTER CANDIDATES IN THE ANTENNAL LOBES OF *MANDUCA SEXTA*. U. Homberg*, R.A. Montague, S.G. Hoskins, K.S. Kent, and J.G. Hildebrand. Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

The antennal lobes (ALs) of the hawkmoth *Manduca sexta* are the primary brain centers of olfactory integration. In the glomeruli of the AL, axons of the olfactory receptors of the antenna synapse upon CNS neurons. The somata of these neurons are arranged in 3 groups bordering the AL neuropil: one antero-ventral (ca. 13 somata), one medio-dorsal (ca. 180 somata), and one ventro-lateral (ca. 900 somata). Based upon morphology, 2 major types of central neurons can be distinguished in the ALs: *local interneurons* (LNs) with somata in the ventro-lateral group and arborizations in many glomeruli, and *projection neurons*, which can be further classified: (a) ca. 400 neurons with somata in all 3 groups innervate single glomeruli and project in the inner antenno-cerebral tract (ACT) to the mushroom bodies (MBs) and the lateral protocerebrum (LPC) of the brain; (b) ca. 100 neurons with lateral somata project via the middle ACT to the LPC; and (c) ca. 260 neurons with lateral somata project via the outer ACT to the protocerebrum.

As part of our studies of the distribution of putative neurotransmitters in the brain of *Manduca* [Maxwell et al., *Comp Biochem Physiol* 61c:109 (1978); Hoskins & Hildebrand *Soc Neurosci Abstr* 9:216 (1983); Homberg et al. *Soc Neurosci Abstr* 11:942 (1985); Hoskins et al. *Cell Tiss Res* in press (1986)], we have used immunocytochemical techniques to study the distribution of GABA, 5HT, dopamine, ACh, and several neuropeptides in the ALs. While the distribution of putatively dopaminergic and cholinergic neurons is currently under investigation, GABA-like immunoreactivity (GLIR) has been detected in the majority (150-180) of LNs in the AL, ca. 50 neurons in the middle ACT, and a few fibers in the outer ACT. 5HT-like immunoreactivity has been detected in only one, anatomically unique neuron that has arborizations in the contralateral AL and in the LPC. Besides these neurotransmitters, several peptides have been detected immunocytochemically in the lateral cell group: 1 soma immunoreactive to anti-cholecystokinin, 10-15 somata immunoreactive to anti-locust adipokinetic hormone, ca. 20 somata with weak immunoreactivity to anti-substance P and anti-FMRFamide, and ca. 100 somata immunoreactive to anti-corticotrophin releasing factor (CRF). Many of the peptide-immunoreactive neurons are probably LNs. CRF-like immunoreactivity, however, has also been detected in fibers of the outer ACT. Experiments in which adjacent sections were exposed to different antisera revealed that most antibodies tested so far recognize different sets of neurons in the AL. It is possible that peptide(s) and GABA are colocalized in a few AL neurons. The present findings suggest that both GABA and neuropeptides are prominent transmitters in local as well as output neurons of the ALs. [Supported by NIH grant AI-23253 and NSF grant BNS 86-41145.]

260.14 MOLECULAR CLONING OF A TYROSINE HYDROXYLASE GENE IN DROSOPHILA MELANOGASTER. W.S. Neckameyer*, D. Chikaraishi* and W.G. Quinn, Jr.* (SPON: T. Tully) Whitaker College and Department of Biology, M.I.T., Cambridge MA 02139.

A partial cDNA probe containing coding sequences for rat tyrosine hydroxylase (D. Chikaraishi et. al., CSH 48:309-318, 1983) was used to isolate Drosophila cDNA and genomic clones on the basis of hybridization at reduced stringency. Partial sequencing of one Drosophila cDNA clone gives a deduced amino acid sequence which is remarkably well conserved between species: within a region of 140 amino acids in the apparent catalytic region of the protein, 72% of the residues are identical (T.H. Joh et. al., CSH 48:327-336, 1983; B. Grima et. al., PNAS 82:617-621, 1985). Northern blot analysis of this Drosophila partial cDNA clone to total RNA from adult wild-type flies suggests that the major transcript is 3.2 Kb, and that it is at least 20-fold more abundant in head tissue than in bodies. The Drosophila cDNA clone hybridizes to polytene chromosomes at region 65B on the third chromosome. This indicates that the Drosophila tyrosine hydroxylase gene maps quite far from the previously investigated diphenol oxidase gene cluster on the second chromosome (E.S. Pentz et. al., Genetics 112:823-841, 1986).

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ACTION POTENTIALS AND ION CHANNELS VI

261.1 A POST-FERTILIZATION DECREASE OF AN INWARD CURRENT IN THE EGG OF THE MOLLUSC MEGATHURA CRENULATA (GIANT KEYHOLE LIMPET). K.L. McKeown, S.J. Krasne* and J.H. Ye*. Department of Physiology, UCLA School of Medicine, Los Angeles, CA 90024.

Unfertilized eggs (100 μ diameter) obtained from Megathura were prepared for recordings by removing the vitelline layer with brief exposure to a lytic agent from sperm of Megathura followed by exposure of eggs to acidified natural sea water (NSW) to remove egg jelly. In vitro fertilization was performed with a dilute sperm suspension in NSW. The two microelectrode (2M K Acetate filled, 20-25 MΩ resistance) voltage-clamp technique was used to record from unfertlized eggs, fertilized eggs and individual cells of the 2-, 4- and 8-cell embryonic stages at ambient temperature (20-22°C).

The following distinct ion currents were observed in unfertilized eggs: 1) An inward-rectifying K^+ current (anomalous rectifier) elicited by hyperpolarizations was observed in control artificial sea water (ASW: 470 mM NaCl; 10 mM KCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM Tris, pH 7.7) or ASW containing 60 mM Ca^{++} as the only divalent and was blocked by 0.5-1 mM Ba^{++};** 2) An inward component of current elicited by large depolarizations was observed in ASW in which all divalents (60 mM) were replaced by equimolar Ba^{++} (60 Ba-ASW) and was blocked by 100 μM Cd^{++};** 3) An outward-rectifying K^+ current which developed slowly during large depolarizations was observed in ASWs having Ba^{++} at a conc. of $\geq$1 mM (total divalents constant) but was absent in ASWs containing either 50 mM TEA^+ or 100 μM Cd^{++};** 4) A fast, transient outward K^+ current elicited by depolarizations was blocked by 5 mM 4-aminopyridine (4-AP) or 100 μM Cd^{++}; 5) An inward current elicited by hyperpolarizations was observed in ASWs in which all Ca^{++} was replaced by equimolar Ba^{++} or in 60 Ba-ASW but was blocked by 5 mM 4-AP or 100 μM Cd^{++}. However, appearance of the fast, transient outward current (#4) seemed to be correlated with that of the inward current seen in Ba-ASWs (#5) and both appeared to be blocked by the same levels of Cd^{++} and 4-AP. Relative magnitudes of the five currents described above varied from egg to egg; not all currents were present in all eggs.

Our studies suggest that the outward rectifier (#3) persists at least up until the 8-cell stage. The inward-rectifying K^+ current (#1) was identifiable only when the inward current (#5) was absent; we observed the inward-rectifying K^+ current through the 2-cell stage but were unable to examine whether it was present at later stages. After fertilization, there is a significant decrease in the frequency of the occurrence of the inward current elicited by hyperpolarization in Ba-ASW (#5). The decreased frequency of occurrence of this current (#5) also appeared to be correlated with a decreased frequency of occurrence of the fast, transient outward current (#4) after fertilization. Supported by grants from NIH (HL20254) and the Muscular Dystrophy Association.

**Krasne, S.J. et al., 1986, Biophys. J., 49: 577a.

261.2 VOLTAGE CLAMP STUDIES OF BLASTOMERES FROM THE ASCIDIAN BOLTENIA VILLOSA. M.L.Block* and W.J.Moody* (SPON: S.Hagiwara). Department of Zoology, University of Washington, Seattle, WA 98195

The whole-cell version of the patch-clamp technique was used to study oocytes, fertilized eggs and isolated blastomeres to the 8-cell stage from embryos of the ascidian Boltenia villosa. The unfertilized eggs normally contain three major voltage-dependent currents: (1) an inward sodium current which peaks at -20 millivolts (mV), (2) an inward calcium current which peaks at +10 mV and (3) an inward potassium current commonly called the inward rectifier.

Measurements of total membrane capacitance of the embryo (i.e. membrane surface area) show an increase with each division, as expected when the total cell volume remains constant. If this new membrane is added with no addition of channel proteins, then current densities would decrease at each cell division by a factor of roughly 0.8. In fact, none of the three currents behaves in this way during early embryogenesis.

The voltage-dependent sodium current is prominent in the unfertilized egg, with current density averaging 3000pA/nF (measured from a holding potential sufficiently negative to saturate h_∞). By the two cell stage the sodium current is essentially gone, with a mean current density of 100pA/nF and by the 4-cell stage only one cell out of 15 had any measurable sodium current at all, giving a mean current density of 5pA/nF. This enormous decrease in current density can not be accounted for by dilution due to membrane addition and so must be a result of the masking of channel proteins or loss of these proteins from the membrane after fertilization.

The voltage-dependent calcium and potassium currents have also been followed through the 8-cell stage of embryonic development. Calcium current density measured in 50 mM strontium artificial sea water and the slope-conductance density of the potassium inward rectifier show no significant change between the unfertilized egg and the 8-cell stage embryo. This implies that unlike the sodium channel, these two channels appear to be added along with new membrane in such a way that current density remains nearly constant. Preliminary studies also seem to indicate that there are no differences in current densities for these two channels among blastomeres of different developmental fates in the 8-cell stage of the embryo.

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261.3 TEMPORAL AND SPATIAL CHANGES IN SODIUM CURRENT DENSITY AFTER FERTILIZATION IN THE EGGS OF THE ASCIDIAN, Boltenia villosa. R.E. Hice and W.J. Moody*. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

Spatial distributions of voltage-dependent ionic currents were characterized before and after fertilization using two-microelectrode voltage clamp of paired animal-vegetal halves of eggs (merogones) made surgically. Paired half-cells were used because; (1) current densities vary widely between eggs, requiring that spatial distributions of currents be examined in individual eggs, and (2) it is difficult to directly monitor fertilization-induced changes in Boltenia eggs since the chorion is virtually impenetrable by microelectrodes, but the chorion/follicle cell layer is required for fertilization to occur.

Major voltage-dependent conductances (Na, Ca, and K) were randomly distributed along the animal-vegetal axis of unfertilized eggs. Ca and K currents remained randomly distributed along the animal-vegetal axis after fertilization. In contrast, Na current density was found to be 2-fold lower in the vegetal versus the animal hemisphere when merogones were made 15 to 30 minutes after fertilization. The rapid change in Na current distribution was due to a selective loss of Na current in the vegetal hemisphere after fertilization. This rapid Na current loss is intrinsic to the vegetal hemisphere of the Boltenia egg, and is not spatially-determined by sperm entry which normally occurs near the vegetal pole: a rapid, 2-fold loss of Na current was observed in vegetal merogones fertilized after isolation, while no rapid loss of Na current was observed in animal merogones fertilized after isolation.

A second, larger reduction in Na current was observed in whole, fertilized Boltenia eggs near the time of first cleavage (2 hours postfertilization at 12°C). This late loss of Na current was also observed prior to first cleavage in animal halves fertilized after isolation (which do not show the rapid Na current loss). However, the late loss of Na current was never observed in vegetal halves fertilized after isolation even when electrode impalements were delayed until after first cleavage was initiated.

Our results show that 2 major reductions in the voltage-dependent Na current occur in the time between fertilization and first cleavage in the Boltenia egg, and that these 2 events are temporally and spatially separable. We are now examining the mechanism(s) underlying these phenomena.

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261.4 NGF AND Na CHANNEL DEVELOPMENT IN PC12 CELLS. B. Rudy, B. Kirschenbaum* and L.A. Greene. NYU Medical School, New York, NY 10016.

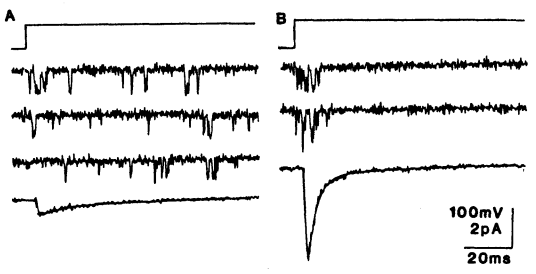
The PC12 clone is a line of rat pheochromocytoma cells that undergoes neuronal differentiation in the presence of nerve growth factor (NGF). In the absence of NGF PC12 cells are electrically inexcitable, when grown in NGF they do not divide, extend long neurite-like processes and become excitable. We have used saxitoxin binding, drug activated Na fluxes through Na channels, and electrophysiological methods to investigate the development of Na channels in PC12 cells. The data indicate that long term NGF treatment brings about a large increase in Na channel density which is sufficient in magnitude to explain the induction of excitability. This increase does not seem to require neurite outgrowth since it takes place in cells grown in suspension and then measured a few hours after plating into polyornithine coated dishes. In this substrate process extension is retarded. The ion flux measurements indicate that these channels are similar before and after NGF, affinities for drugs that act at three different sites on the Na channel are similar. Neurite extension brings about further changes. Cells grown in suspension in the presence of NGF for 2-3 weeks, are plated into polyornithine coated dishes and measured from a few hours to a few days after plating. Similar measurements have been carried out on cells grown in collagen coated dishes in the presence of NGF for 2-3 weeks, then removed from the dish to break off the neurites and then replated into polyornithine coated dishes. Na channel numbers are about 10 times higher per cell than cells grown in the absence of NGF at 3 to 5 hr after plating. Within 24 hr after plating the Na channel number doubles. This increase in Na channel number precedes actual process outgrowth but may be related to cell reorganization. During this time a second type of Na channel appears with a lower sensitivity to the channel blocker tetrodotoxin (TTX). The proportion of TTX-resistant Na channels increases after plating in polyornithine from 0 at 3-5 hr to 5-20% at 48 hr after plating. A similar proportion is found in cultures grown for 2-3 weeks in NGF in collagen coated dishes which thus contain cells with fully developed long neurites.

261.5 SINGLE CHANNEL CURRENTS UNDERLYING SLOW THRESOLD Na^+ CONDUCTANCES IN RAT NEOCORTICAL NEURONS. O.P. Hamill*, J.R. Huguenard, E.F. Enayati* and D.A. Prince (SPON: S. Shotwell). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Within the cerebral cortex, different classes of neurons can be recognized by their morphology, histochemistry, and firing properties. However, there is no information on differences in membrane channel properties that may exist between neuronal classes. To investigate this aspect we have undertaken a patch clamp study of neurons acutely isolated from the cerebral cortex. Here we describe the properties of individual Na^+-channel currents, with attention focused on elementary events underlying threshold, slow, Na^+ conductances.

Isolated neocortical neurons (obtained by a dissociation procedure modified from Numann and Wong, 1984) were visually identified as pyramidal or non-pyramidal. Morphological criteria for discrimination were confirmed by post-staining with anti-GAD. The cell attached patch recording configuration was used and Ca^{2+} and K^+ channels were blocked with pharmacological agents.

Depolarization of membrane patches to threshold potentials, -60 to -50 mV, activated brief events (< 5 ms) of uniform amplitude (1.2-1.6 pA) which occurred throughout the duration of a 100 ms step (figure A). Occasionally, bursts of brief events extending for the duration of the step were observed (not shown). Ensemble averages (e.g., A, bottom trace) displayed double exponential decay with time constants of 3-5 ms and 50-100 ms. With slightly stronger depolarizations, similar brief events occurred usually within the first 20 ms of the pulse (figure B). In the ensemble average, both decay processes were faster and the slow decay component was significantly reduced in amplitude compared to the fast. Similar threshold behavior of Na^+ channels was observed in pyramidal and non-pyramidal cells. Supported by NIH grants NS 06477 and NS 12151.

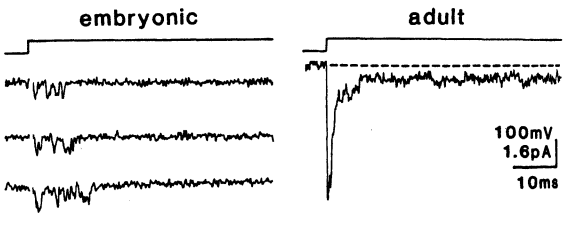


261.6 DEVELOPMENT OF Na^+ CONDUCTANCE IN NEOCORTICAL NEURONS OF THE RAT. J.R. Huguenard, O.P. Hamill*, E.F. Enayati*, and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

During the development of the cerebral cortex, individual neurons undergo dramatic increases in excitability which are evidenced by changes in the action potentials. Included in these changes are an increased rate of rise, decreased width, and increased repetitive firing. We have undertaken a voltage clamp study of individual cortical cells at different stages of development to characterize the conductances which underlie these changes.

Neocortical neurons were obtained from rats (age E18 to P60) by an acute dissociation procedure (see Hamill, et al., this meeting). Neurons (visually identified as pyramidal or non-pyramidal) were studied with the patch clamp method using either the whole cell mode or the cell attached mode. Non-Na^+ conductances were inhibited with either nonconducting ions or pharmacological blockers. Whole cell Na^+ conductance, normalized to membrane area, increased by more than twelve-fold during this time period. No developmental or cell-type specific changes were seen in single channel conductance, activation kinetics, recovery from inactivation or steady state inactivation. However, within the threshold activation range (-60 to -40 mV) there was a developmental increase in the magnitude of a slowly inactivating (up to 100 msec time constant) threshold current, and it was increasingly difficult to fit the inactivation of the Na^+ current to a single decay exponential. These whole cell observations have been confirmed in cell attached patches with single channel records (see figure), as well as ensemble averages. The slow inactivation of the threshold Na^+ current has potential functional significance in promoting repetitive firing in adult neurons. Supported by the NIH grants NS 06477 and NS 12151.

Figure: single channel currents obtained with 50 mV depolarizations in cell attached patches in embryonic and adult cortical neurons (using matched patch pipettes); the adult patch exhibits higher channel density and multiple late openings.



261.7 THE EFFECTS OF AXONAL PRESENCE ON SCHWANN CELL SODIUM CHANNELS. S.Y. Chiu* (SPON: B. Benjamin). Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Previous works (Chiu et al., Nature, 1984) have shown that neuronal-like sodium channels were expressed in newborn rabbit Schwann cells cultured for 2-10 days in which axons have presumably degenerated. In this study, the effects of the presence of intact axons on these Schwann channels were examined. Patch-clamp experiments were performed on collagenase-treated sciatic nerves 2-6 hours after isolation from rabbits of age ranging 5-20 weeks. Schwann cells associated with both unmyelinated and myelinated axons could be seen. Electronmicroscopic studies showed intact axons (less than 1 μm in diameter) were either still enveloped in Schwann cells or peeled off from them. When a patch-pipette was pressed at the body region of a Schwann cell associated with thin unmyelinated axons, a fast inward current was detected under whole-cell recording configuration. This current was identified as a neuronal-like sodium current: it was blocked by TTX, exhibited a sodium sensitive reversal potential, and has neuronal-like sodium activation and inactivation kinetics. Three independent arguments suggest that most of the recordings were from Schwann cells rather than from thin (1 μm) axons. Firstly, no action potentials were observed. Indeed, the peak sodium current density of 22.6 ± 2pA/pF (n=38) is similar to that of 30 pA/pF for cultured Schwann cells. Furthermore, in one rare experiment when recording was made directly on a 1 μm axon seen separated from a Schwann cell, a much larger peak sodium current density of 300 pA/pF was observed. Secondly, the dominance of surface membrane area of a Schwann cell over that of axons in the body region favors giga-seal formation on Schwann membranes. Statistically, 60 out of 61 different recordings yielded a sodium current. Thirdly, if one assumes that the specific membrane capacitance of an axon is 1 $\mu F/cm^2$, then the observed whole-cell capacity is about 4-6 times too big for a 1 μm axon, but is consistent rather with a larger Schwann body with numerous tongues for engulfing axons.

More intriguingly, the expression of this Schwann sodium current appeared to depend on the type of associated axons; sodium currents were detected only on the bodies of Schwann cells associated with small unmyelinated axons, but not from those associated with large myelinated axons. In conclusion, the results suggest that axo-glia interactions lead to an interesting plasticity of ionic channels on both interacting membranes. Not only do Schwann cells affect ionic channels distribution on axons, but conversely, the axonal type also seems to affect expression of ionic channels (for e.g. Na channels) on Schwann cells.

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261.8 **DO GLIAL CELLS ACTUALLY EXPRESS ION CHANNELS *IN VIVO*?** B.A.Barres, L.L.Y.Chun*, and D.P.Corey. Program in Neuroscience, Harvard Medical School, Neuroscience Group, Howard Hughes Medical Institute and Department of Neurology, Massachusetts General Hospital, Boston, Ma. 02114

Glial cells in culture express a surprising diversity of voltage-sensitive ion channels (*e.g.* Kettenman *et al.*, Pflugers Archiv 1984; Shrager *et al.*, PNAS 1985; Bevan *et al.*, Proc. R. Soc. Lond. B 1985; Barres *et al.*, Soc. Neurosci. Abst. 1985). However, it remains possible that channel expression *in vivo* differs from that observed *in vitro* (*e.g.* Ypey and Clapham, PNAS 1984).

We have used the rat optic nerve preparation developed by Raff and coworkers to compare channel expression of glial cells in culture and in suspensions of freshly dissociated cells. Each subclass of glial cells--oligodendrocytes, type 1 astrocytes, and type 2 astrocytes--can be positively identified by a unique surface antigenic phenotype which correlates with a specific morphology in culture. Cells in suspension were prelabelled with antibodies and observed with a fluorescence microscope during electrophysiologic study. Oligodendrocytes were identified by staining for galactocerebroside. Type 1 astrocytes were identified both by staining for the RAN-2 marker, which in optic nerve (but not cortex) persists after papain but not trypsin dissociation, and by their morphology in suspension which is distinct from meningeal cells. This phenotype was confirmed by poststaining for GFAP. Ionic currents were recorded using the whole-cell patch clamp technique.

In all glial cell types we have recorded from in suspension, voltage-sensitive currents have been observed. Oligodendrocytes in suspension express conductances identical to those of their cultured counterparts: an inwardly-rectifying potassium conductance, but not sodium or delayed outward potassium conductances. Type 1 astrocytes in suspension express a delayed potassium conductance and in some cases a sodium conductance. There are in fact some differences between type 1 astrocytes in culture and in suspension: in suspension there is no evidence of a chloride conductance or an inactivating outward potassium A conductance that have been observed in some cultured type 1 astrocytes. Preliminary evidence suggests that type 1 astrocytes in optic nerve suspension may fall into two classes, each expressing different morphologies and densities of sodium conductance. Studies of type 2 astrocytes in suspension are in progress. Optic nerve cell suspensions contain in addition small numbers of beating ciliated ependymal cells. These cells also express both sodium and potassium conductances.

Together, these studies of oligodendrocytes, type 1 astrocytes, and ependymal cells in suspension indicate that glial cells actually express voltage-sensitive ion channels *in vivo*.

261.9 **CHANGES IN K^+-CHANNEL EXPRESSION DURING THE LIFE CYCLE OF MURINE T LYMPHOCYTES.** R.S. Lewis, K.G. Chandy*, S. Gupta* and M.D. Cahalan. Depts. of Physiology & Biophysics and *Medicine, California College of Medicine, Univ. Cal. Irvine, Irvine, CA 92717.

During their lifetime, T lymphocytes progress through a series of proliferative and quiescent states. In mature T cells from mice, quiescence is associated with the expression of small numbers of voltage-dependent K^+ channels similar to the delayed rectifier of nerve and muscle, but treatment with mitogens induces a 10-fold increase in the K^+-channel density prior to the onset of cell division. Blocking these channels inhibits mitogenic responses, suggesting that functional K^+ channels are required for the ability to divide. To test this hypothesis further, we have studied K^+-channel expression in rapidly proliferating, immature T cells in the thymus (thymocytes).

Thymocytes were isolated from the thymus of 4- to 8-wk-old *Balb/C* mice using standard methods. Whole-cell patch-clamp recording was used to characterize ion channels present in thymocyte subsets. Two subsets can be identified on the basis of their affinity for peanut agglutinin (PNA): PNA-binding cells, destined to proliferate and eventually die within the thymus, and PNA-nonbinding cells, destined to proliferate, mature, and be exported to peripheral lymphoid organs such as the spleen. PNA-nonbinding cells were individually selected for recording from populations of thymocytes treated with fluorescein-labelled PNA. Because fluorescent illumination decreased the number of functional K^+ channels in cells that bound PNA, those cells were identified by staining with PNA after recording.

Overall, murine thymocytes possess the same type of K^+ channel that is found in mature splenic T cells, but the level of expression is 10- to 100-fold higher. Using an average single-K^+-channel conductance of 12 pS, whole-cell K^+ conductance (g_K) in 76 thymocytes corresponded to 342 ± 33 channels per cell (mean ± s.e.m.), whereas splenic T cells express only 10 to 15 channels per cell. A comparable difference exists between channel density in the two cell types. Numbers of K^+ channels per cell did not differ significantly between PNA-positive (417 ± 67, n=21) and PNA-negative (425 ± 158, n=8) subsets. Thus, during their lifetime, T cells experience up- and down-regulation of K^+ channels that correlates with their growth state. T cells begin life as rapidly dividing thymocytes with large g_K, enter a mature quiescent state with low g_K, and later become activated by contact with antigen to express high g_K and proliferate. We are extending these studies to mature T cells that are ready for export from the thymus to determine the developmental stage at which down-regulation of K^+ channels occurs. (Supported by an RCDA (MDC) and USPHS grants NS-14609 and AI-21808).

261.10 MATURATION OF OUTWARD CURRENTS IN AMPHIBIAN SPINAL NEURONS DEVELOPING IN CULTURE. A.B. Ribera*, D.K. O'Dowd and N.C. Spitzer. Dept. of Biology, Univ. of Calif., San Diego, La Jolla, CA 92093.

The ionic dependence and duration of the action potential (AP) of Xenopus spinal neurons changes during the first day in culture (Spitzer and Lamborghini, 1976). At early times, the AP is primarily Ca^{++}-dependent and hundreds of msecs in duration; in mature cells, the AP is primarily Na^+-dependent and lasts only a few msecs. The whole-cell voltage clamp technique (Hamill et al., 1981) has been used to study the development of Ca^{++} and Na^+ currents in these cells (O'Dowd, 1983). Here we report the developmental changes in outward currents that occur during the period of maturation of the AP.

Na^+ currents were blocked by TTX; Ca^{++} currents were either (a) blocked by Co^{++} or Cd^{++}, and omission of external Ca^{++}, or (b) reduced by addition of low concentrations of Ca^{++} to the bathing saline. Patch pipets contained a HEPES buffered KCl solution. Neurons were examined between 6 and 29 hours in culture. Two distinct classes of outward current were present: 1) voltage-dependent, calcium-independent (Ikv), and 2) calcium-sensitive, voltage dependent (Ikc). The dependence of both currents on potassium ions was defined by examination of the reversal potential of tail currents. The developmental characteristics of Ikv and Ikc were studied separately.

Ikv was recorded from all cells, even at the earliest times. The mean current density of Ikv increases 3.5 fold during the first day in culture. In addition, the activation kinetics of this current become more rapid during development. Ikv does not inactivate following prepulses to potentials as depolarized as +20 mV for as long as 250 msec.

Ikc was isolated by digital subtraction of Ikv recorded in 500 μM Cd^{++} from the total current recorded without Cd^{++} but in the presence of 100 μM Ca^{++}. This low concentration of external Ca^{++} avoided contamination of the outward current records by appreciable inward Ca^{++} currents. Both an inactivating and a sustained component of Ikc were present in all mature cells examined. However at early stages, the inactivating component of Ikc was frequently absent (5/18 neurons); in contrast, the sustained component of Ikc was observed for all young cells examined. The mean current density of the transient component increased about two fold, while the sustained component acquired an apparent rectification that may have prevented detection of an increase in its amplitude at the most depolarized potentials.

The changes in Ikv and Ikc are likely to contribute to the shortened duration and altered ionic dependence of the AP during development.

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261.11 DEVELOPMENTAL CHANGES IN POTASSIUM CHANNEL PROPERTIES IN CULTURED AMPHIBIAN SPINAL NEURONS. G.L. Harris, L.P. Henderson and N.C. Spitzer. Department of Biology, University of California, San Diego, La Jolla, CA 92093.

The action potential (AP) of embryonic Xenopus spinal neurons undergoes a developmental shift during the first day in culture, from a relatively long duration, primarily Ca^{++}-dependent event to a brief duration, primarily Na^{+}-dependent event (Spitzer and Lamborghini, 1976). In the preceding abstract, Ribera, O'Dowd and Spitzer reported maturational changes in the outward K^{+} currents that contribute to the shift in the ionic dependence and duration of the AP. In the present study we have used excised, inside-out as well as cell-attached patches (Hamill et al., 1981) to examine the voltage-dependent, Ca^{++}-independent K^{+} channels which underlie the purely voltage-dependent whole-cell currents at times before and after the developmental change in the AP.

Neurons were examined either between 6-9 hrs in culture (young cells) or between 15-29 hrs in culture (mature cells). Patch pipets contained (mM): KCl,20; NaCl,60; HEPES,5; $MgCl_2$,5; NaOH,~25. Bath solution for excised patches contained (mM): KCl,100; NaCl,3; HEPES,10; EGTA,10; KOH,~18 (no added Ca^{++}). Data were sampled at 0.1 msec intervals and filtered at 2.5 KHz.

Three classes of Ca^{++}-independent K^{+} channels were found in excised patches from mature cells. Their mean values of slope conductance obtained at membrane potentials between -20 and +40 mV were 14, 28 and 88 pS. The two smaller conductance classes had mean channel open times which ranged from 4-10 msec. In both cases τ was voltage-dependent, being longer at more depolarized potentials. The probability of these channels being open declined with time following depolarizing voltage steps. The large conductance class exhibited briefer channel openings (τ 1-4 msec). The mean channel open time of the large class was independent of voltage, and there was no decline in the open probability following depolarizing voltage steps. Recordings of channel activity from cell-attached patches also revealed three classes of K^{+} channels. In excised patches from young cells there was a lower frequency of channel openings than in patches from mature cells. The two smaller conductance classes of channels had open duration and open probability characteristics which were similar to their counterparts in mature cells. However the large conductance channels were absent from the youngest cells, appearing only after ~9 hrs in culture. Experiments in progress will assess the activation kinetics of these channels in both young and mature cells.

It appears that developmental maturation of the cellular voltage-dependent K^{+} currents is due to changes in the number and populations of K^{+} channels present in the neuronal membrane.

Supported by USPHS postdoctoral fellowship to GLH, MDA postdoctoral fellowship to LPH and NS15918 to NCS.

261.12 **DEVELOPMENT AND MODULATION OF BURSTING IN THE IDENTIFIED NEURON R15 OF JUVENILE *APLYSIA*** E.A. Marcus and T.J. Carew Departments of Biology and Psychology, Yale University, New Haven, CT, 06520.

Intrinsically bursting neurons possess a unique set of ionic conductances that generate rhythmic oscillations in membrane potential. One approach to characterizing these conductances is to examine their expression and assembly during development. The identified burster R15 in *Aplysia* is well suited for such an analysis since it exhibits a gradual developmental progression from non-burster to burster (Ohmori, 1981). In the adult, bursting in R15 is intensified by bag cell neuropeptides (Mayeri et al, 1979). As a first step in analyzing the development of bursting, we asked whether bag cell extract (BCE) might also have modulatory effects on immature, non-bursting R15 neurons.

We first characterized the firing pattern of R15 in Stage 12 juvenile *Aplysia* (0.5-4g, n=15). Confirming Ohmori (1981) we found a developmental progression from non-bursting in younger juveniles to immature bursting in older juveniles. We next examined the effect of BCE on the activity pattern of immature R15's (n=8). In preparations where R15 showed no bursting (n=5), BCE induced bursting; in preparations where R15 showed immature bursting (n=3), BCE intensified these bursts. These effects were specific to BCE: controls (cerebral ganglion extract, n=5) produced no induction or intensification of bursting.

To quantify the effects of BCE we analyzed the distribution of interspike intervals in a 3 min record prior to BCE and compared it to a 3 min record at the peak of BCE effect. We devised an algorithm for calculating a difference score (a "burst induction index") which reflected the effect of BCE on the distribution of the interspike intervals. Induction or intensification of bursting was described by the sum of 3 parameters: 1) an increase in short intervals (a measure of within-burst spike rate); 2) an increase in long intervals (a measure of the number of interburst hyperpolarizations; and 3) a decrease in intermediate intervals (a measure of a decrease in spikes unrelated to bursting). BCE produced a significant burst induction index ($\bar{x}$=52.4, $p<.005$), whereas control extract did not ($\bar{x}$=-2.8, ns). Moreover, there was a significant difference in this index between BCE and controls ($p<.01$). These data show that BCE significantly shifts the distribution of interspike intervals towards a burst pattern.

The developmental progression from non-burster to burster in R15 might suggest that the ionic conductances critical for bursting are absent in early stages. However, our results show that BCE can induce bursting in immature, non-bursting neurons suggesting that these conductances are present and functional before the normal developmental expression of bursting. The next step will be to identify the conductances present in immature R15 neurons and examine how these conductances are expressed and assembled in the development of burst generation.

SENSORY SYSTEMS II

262.1 DEVELOPING VALLATE TASTE BUDS ARE ADDED IN GROWTH ZONES AND MATURE IN TEN DAYS. B. Oakley, M. A. Hosley*, L-H. Wu*, and K. Wilson*. Dept. of Biology, Neuroscience Lab. Bldg., Univ. of Michigan, Ann Arbor, MI 48109.

The rat has a single vallate papilla consisting of a central island nearly surrounded by an elliptical trench. About 600 taste buds develop and mature in the trench walls during the first three months after birth. The objectives of this investigation were: i) to seek growth zones in the vallate papilla where new taste buds might form, ii) to determine taste bud development time from counts of immature and mature taste buds, and iii) to examine properties of immature taste buds that might reveal their mode of development. Ten micrometer, transverse, hematoxylin-stained, serial sections were made of formalin-fixed, vallate papilla in Sprague-Dawley rats at 15, 21, 33, 45 and 90 days. Camera lucida drawings were made of more than 15,000 profiles of taste buds to determine the location and size of taste buds as well as the presence or absence of a taste pore. At 15, 21, 33 and 45 days most immature taste buds (those lacking taste pores) were found at the anterior and posterior extremes of the vallate papilla. Since the trench lengthens for more than two months, it appears that new taste buds form preferentially in the growth zones of the elongating trench wall. The maturation of immature buds led to a geometric increase with age in the number of mature taste buds. Consequently, the number of immature taste buds as a percentage of the mature taste buds declined exponentially with age from 19% at 15 days to 1.8% at 90 days ($r = .94$, $p < .02$). The time required for cohorts of immature taste buds to mature at 15, 21, 33 and 45 days was constant (10 ± 0.9 days, Mean ± S.D.).

It has been suggested that taste buds initially form de novo, but later form by division of mature taste buds. Our observations suggest that the initial mode of taste bud morphogenesis continues throughout development:

1) At later ages, in particular, immature taste buds are clustered together in the anterior and posterior extremes of the lengthening trench wall where there are fewer mature taste buds.

2) At all ages some immature taste buds were found that had no near neighbors from which they might have arisen by division.

3) Some immature taste buds in older animals had spatially disorganized cells unlike the vertically aligned cells expected from division of a mature taste bud.

4) A fixed 10 day maturation time for immature taste buds suggests a single program for development.

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262.2 MONOCLONAL ANTIBODY RAT 203 RECOGNIZES THE PRIMARY OLFACTORY PATHWAY IN PERINATAL AND MATURE RATS. B. Friedman, P. E. Pedersen, G. M. Shepherd, and S. Hockfield. Sec. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The peripheral olfactory system provides a good model to study mechanisms of axon guidance in mammals since olfactory receptor neurons are continually generated throughout the life of the organism. Hybridoma techniques have already provided reagents that distinguish among subsets of mature olfactory primary afferent fibers. The present study aimed to isolate monoclonal antibodies that tag primary afferents at different stages of maturation, with special focus on early axon outgrowth.

Hybridomas were generated by fusion of myeloma cells with nodal lymphocytes from two adult mice. The mice were immunized with homogenized olfactory bulbs from 12-day-old rats that had been perfused with 4% paraformaldehyde. The supernatants secreted by hybridoma colonies were screened on paraformaldehyde fixed tissue sections from P12 rat olfactory bulb. Bound antibody was demonstrated by serial incubation of the tissue with HRP conjugated secondary antibody and DAB. Hybridomas of interest were cloned three times by limiting dilution.

Monoclonal antibody RAT 203 generated from this fusion recognizes olfactory receptor neurons, olfactory nerve, and olfactory glomeruli in P12 rats. RAT 203 also recognizes vomeronasal nerves and accessory olfactory bulb glomeruli. However, this antibody fails to stain axon systems within the olfactory bulb that are recognized by anti-neurofilament antibodies. RAT 203 positive glomeruli are distributed in each quadrant of the olfactory bulb although the intensity of staining among individual glomeruli varies. The pattern of staining within a glomerulus is patchy, resembling that of olfactory axons stained by vimentin (Schwob, Farber, & Gottlieb, 1986) or by silver degeneration methods (Land, Eager, & Shepherd, 1970).

In our analysis of embryonic development, olfactory nerve fascicles are RAT 203 positive at E15. At this time, there is little distinct staining of the olfactory bulb. By E20 the glomerular layer of the olfactory bulb is immunoreactive with especially prominent staining of the modified glomerular complex. Distinct immunoreactive glomeruli are found thoughout the olfactory bulb of P2 rats. Immunoreactivity of olfactory nerve fascicles and glomeruli persists into adulthood.

These findings suggest that RAT 203 is a marker of olfactory nerve at the time of early axon growth as well as at maturity. Studies are in progress to establish the cellular localization of RAT 203 immunoreactivity.

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262.3 INCREASE IN TOTAL NUMBER OF OLFACTORY NEURONS MAY UNDERLIE THE POSTNATAL IMPROVEMENT IN OLFACTORY SENSITIVITY IN RATS.
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In the rat olfactory sensitivity to such odors as amyl acetate is low at birth increasing during the postnatal period by 2 to 10 folds depending on stimulus strength (Alberts & May, 1980). The neural basis of this phenomenon is not clear. It is also well known that the very high convergence ratio of olfactory receptor neurons to mitral cells of the olfactory bulb is one of the bases of high olfactory sensitivity in mammals. To determine whether the postnatal improvement in olfactory sensitivity may be associated with an increase in the convergence ratio, the total number of olfactory receptor neurons and mitral cells of the newborn and weanling rats were estimated, using complete serial sections and morphometric and cell counting methods.

In the newborn, total surface area of olfactory mucosa in each side is about 10 mm2, mean thickness of neuronal layer about 50 um, density of olfactory neurons about $1.4 \times 10^6/mm3$, giving a total number of 800,000 receptor neurons in each side. By day 25, total surface area of mucosa increases by about 5x, mean thickness of receptor cell sublayer increases by about 60%, the packing density remains nearly constant while the number of receptors per unit surface area shows a 70% increase.

These changes result in an average increase of 8 fold in the total number of olfactory receptor neurons during the the suckling period. Since the number of mitral cells in the bulb remains constant during this period (about 60,000) , it follows that convergence ratio of receptor cells to mitral cells increases by about 8 fold in the same age period.

The fact that a proportion of the receptor cells in the newborn and weanling may not be synaptically connected to the mitral cells due to immaturity or turn-over state obviously affects the absolute convergence ratios. However, it is likely that these factors will not markedly alter the relative increase in the ratio from birth to weaning.

The postnatal increase in the convergence ratio, by causing increased spatial summation, would decrease the threshold for mitral cell activation under similar stimulus strength, providing a neurophysiological mechanism for the reported increase in olfactory sensitivity. Our studies indicate that the developmental increase in convergence in the primary olfactory pathway is not limited to the rat, occurring in other altricial mammals such as rabbit and hamster (Meisami, Louie, Hudson, Distel, in preparation; Meisami & Najafi, in prep.).

262.4 PRE- AND POSTNATAL DEVELOPMENT OF THE MEDIAL LEMNISCUS (ML), THE BRACHIUM CONJUCTIVUM (BC), AND THE BRACHIUM OF THE INFERIOR COLLICULUS (BIC) IN RATS.
C. Asanuma, R. Ohkawa, B.B. Stanfield and W.M. Cowan. The Salk *Institute, La Jolla, CA 92037.*

We have examined the development of three of the major ascending pathways to the thalamus in rats using TMB histochemistry after WGA-HRP injections into the dorsal column nuclei, the deep cerebellar nuclei, or the inferior colliculus. By E17, some ML axons have already reached the diencephalon and extend dorsally as a distinct bundle to enter the ventroposterior n.(VP) without, however, ramifying to any significant degree in the nucleus. Other ML axons remain ventral to the thalamus and proceed toward, but not beyond, the rostral end of the thalamus. Terminal ML arborizations are readily detected by E19 and are concentrated within VP, although some extend into adjoining nuclei. At this stage the aberrant ML axons ventral to the thalamus extend into the internal capsule (IC) towards the striatum. During the subsequent week, the arborizations within VP become more elaborate while the density of aberrant axons decreases. By P5, there is a substantial reduction in the number of ML axons rostral to the thalamus, but the terminal arborizations within the thalamus are not yet confined to VP.

Inferior colliculus injections reveal that the initial innervation of the medial geniculate n. (MG) also takes place quite early. Densely labeled terminal arborizations are present within the MG by E19, but there is also a substantial component of BIC axons which proceeds through the ventral nuclei toward the rostral end of the thalamus.

No labeled BC axons can be detected beyond the midbrain at E19, but by P0, terminal ramifications of BC axons have clearly begun to form in the ventral lateral n. (VL). Although most BC terminal arbors are concentrated at this time within the VL, some clearly lie outside the limits of the VL. In addition, like the developing ML, many BC axons remain ventral to the thalamus and proceed rostrally to enter the IC. These aberrant BC axons are less evident after P0 and eventually disappear.

These results indicate that the principal ascending sensory afferents reach the primary sensory relay nuclei of the thalamus quite early in development (E17-19), and suggest that they may precede the innervation of other thalamic relay nuclei. Each major pathway initially projects not only into its appropriate target, but also rostrally beyond the thalamus. Furthermore, although each system arborizes extensively within its appropriate thalamic nucleus shortly after reaching the thalamus, it also contributes fibers to the adjoining thalamic nuclei. Indeed the ascending afferents do not attain their clearly focused and restricted thalamic distributions until some time after the first postnatal week.

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262.5 DEVELOPMENT OF ORDER IN THE WHISKER-TO-BARREL PATHWAY OF THE MOUSE: NEURONAL METABOLIC RESPONSES TO WHISKER STIMULATION
P. Melzer*, E. Welker*, J. Dörfl*, and H. Van der Loos*
(SPON: European Neuroscience Foundation). Inst. of Anatomy, Univ. of Lausanne, 1005 Lausanne, Switzerland.

Female albino mice (ICR-stock) in late pregnancy were checked every 24 hours. The day a litter was detected was defined as postnatal day (P) 0. Infants from P2 to P18 (at least 3 mice per age) and 2 adults (over P 60) were restrained and given [C-14]2-deoxy-D-glucose (DG) i.p. Earlier, small metal rods had been glued to left whiskers C1, C2, C3 and E1; all other whiskers were clipped. The whiskers with rods were moved by magnetic field bursts for 45 minutes (Melzer, P., et al., Brain Res., 348, 1985); the mice were then anesthetized and perfused. Brains were processed for X-ray film autoradiography. Alternate series of sections, stained for cytochrome oxidase or Nissl, were used to correlate areas of stimulus-evoked DG uptake and the representation of the whiskers in the somatosensory relays and in the barrelfield (BF).

At P2, the first signs of stimulus-dependent DG uptake of the three C-whiskers were in the ipsilat. trigem. subnuclei caud. and interpol. At P4, stimulus-evoked DG uptake was seen in the ipsilat. subnucleus oral., nucleus sens. princ. n. trigem. and in the ventrobasal complex of the contralat. thalamus. Only at P7, metabolic activation was found in the BF of the contralat. cortex. In coronal sections, it spread over several barrels in layer IV and extended into layer III; in tangential sections, it covered barrels C1-3 and their neighbors. At P10, in coronal sections high tracer uptake stretched from layers II to VI with a maximum in IV; in tangential sections, it was restricted to barrels C1-3, surrounded by a band of depressed DG-uptake, and E1. The onset of DG uptake in response to stimulation of the single E-whisker followed the same sequence, i.e. from lower to higher stations, as with stimulation of the three C-whiskers, but with a delay of 2-3 days. Up to P10, the areas of stimulus-evoked DG uptake in subnuclei caud. and interpol. were larger than the representations of the whiskers in question. Later, there was a better correspondence between metabolic activation and representation. After P11, DG uptake approached the level of adjacent areas in the subcortical stations but increased in the BF. Are the early DG uptake (that disappears) and the initial mismatch of activated areas and whisker representation in subcortical stations based on transitory connections and/or on subsequent development of inhibitory systems?

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262.6 THE MORPHOLOGY OF INDIVIDUAL THALAMIC AXONS IN SOMATOSENSORY CORTEX OF THE NEONATAL MOUSE.
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Multicellular cytoarchitectonic units (barrels) in layer 4 of the primary somatosensory cortex of adult mice consist of densely cellular walls and densely axonal hollows. Many of these axons have been demonstrated to arise from the ventrobasal thalamic nucleus. From late in gestation until barrel formation in the middle of the first postnatal week, this population of specific thalamic axons are located beneath the cortical plate among the infragranular neurons as demonstrated by anterograde and retrograde transport methods. The morphology and laminar distribution of individual thalamic axons during this perinatal period prior to differentiation of the barrels was investigated by bulk filling with HRP fibers present in the internal capsule and ventrobasal thalamic nucleus in a modified tissue slice preparation. HRP was recrystallized into pellets on micropipette tips and inserted into freshly dissected slices of cerebra removed from newborn to three day old mice. After incubation times from 1-4 hrs. the tissue was immersion fixed and processed for HRP histochemistry with DAB as chromogen and enhancement with nickel-cobalt treatments. Axons were reconstructed from serial 80 um frozen or vibratome sections with a camera lucida drawing attachment.

The majority of HRP-labeled axons course parallel to the pial surface in the white matter and depths of layer 6. At any point along this path axons within these fascicles emit one or more short collaterals. In addition, some axons send one or more longer branches oriented either radially or obliquely toward the cortical surface. These branches travel through layers 5 and 6 but do not penetrate the cortical plate during this developmental period. Branch points as well as growth cone-like structures on the tips of axons in all layers appear to be enlarged in size relative to the size of the main axonal trunk. In both layers 5 and 6 axons decrease in diameter and appear progressively more varicose. These swellings may represent synaptic contacts of growing thalamic axons with infragranular neurons. The relatively simple morphology exhibited by these thalamic axons in the infragranular layers may represent the first stage of axonal differentiation that precedes the explosion of axonal arborization that occurs during barrel formation.
Supported by NIH grant NS20213.

262.7 CHANGES IN TOPOGRAPHY OF SOMATOSENSORY CORTEX AFTER NERVE LOSS IN ADULT AND NEONATAL MARMOSET MONKEYS. M. F. Huerta, J. T. Wall, and J. H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

There is considerable evidence that altering sensory inputs can have a greater or different effect on the developing central nervous system than on the adult system. In the present study somatosensory inputs were altered in young and adult primates and the effects on the topographical organization of somatosensory cortex were examined after chronic survival periods following the alteration. Marmosets were used because they reproduce and mature rapidly.

In marmosets, the median, ulnar, and radial nerves carry somatosensory information from the hand to central pathways which project topographically to cortical area 3b. The topographic organization of the hand representation in area 3b and adjacent cortex was assessed with multiunit mapping methods in ketamine-anesthetized marmosets that had been: (1) reared normally, with all three nerves intact, (2) reared normally, but which had the median nerve cut and ligated as an adult, or (3) reared after having had the median nerve cut and ligated during postnatal week 1-2. Two questions were addressed: (1) How does the topography in the hand representation change when the hand is deprived of median nerve inputs? (2) Are the topographical changes induced by nerve loss in neonates and adults similar or different?

In normal animals, neurons at all or most recording sites were responsive to tactile stimulation of delimited skin areas on the hand, with virtually all surfaces of the hand represented. In contrast, the hand representations of both adult and neonatal denervates were abnormal in that representations of glabrous digit, and palm surfaces on the radial side of the hand were missing. Even though the hand surface was incompletely represented in denervates, cells at all or most recording sites in the hand representations of these animals were responsive to tactile stimuli. While topographic details varied across individuals, the major consistent finding in neonatal and in adult denervates is that deprived cortical zones become responsive to hand inputs from the remaining ulnar and radial nerves.

Thus, cutaneous inputs from surviving nerves substitute for lost inputs in both adults and neonates. This suggests that the central circuits which underlie the observed changes in topographical organization are developed to some degree by postnatal week 1-2 in this primate.

Supported by NIH grants NS21105 (JTW) and NS16446 (JHK).

262.8 DEVELOPMENT OF AUDITORY SELECTIVITY IN A SONG-SYSTEM NUCLEUS DURING SONG LEARNING. S. F. Volman and M. Konishi, Division of Biology 216-76, Calif. Inst. of Technology, Pasadena, CA 91125.

Songbirds, if they are to produce normal song, must be able to hear their own vocalizations during song development. For many species, auditory feedback is used to match a bird's own song to a song model ("tutor song") heard many months previously. In the brains of songbirds, the "song system" is composed of a set of discrete, specialized nuclei. One of these, nucleus HVc in the forebrain, is a site of convergence between auditory and pre-motor activity. Neurons in HVc of white-crowned sparrows (Zonotrichia leucophrys) were recently shown to respond selectively to a bird's own song in preference to other songs, including the tutor song (Margoliash, J. Neurosci. 3: 1039, 1983; Margoliash and Konishi, PNAS 82: 5997, 1985). This selectivity might either reflect the storage of an imperfect copy of the song model or be the consequence of song matching.

In order to distinguish between these two possibilities, we recorded from multiunit clusters in HVc's of juvenile, pre-singing white crowns that had been tutored in the laboratory with a single song model. Auditory responses to this song were compared with similar songs from the same geographic region (same "dialect"), songs of different dialects, reversed song, and songs altered by a computer generated song analysis/resynthesis system. There was no evidence of a consistent preference for familiar song patterns in any of these birds. Single neurons did, however, often respond preferentially to sounds with frequency and amplitude modulations of the sort present in white-crown song. Thus, as in adults, some HVc neurons respond better to song than to tone or noise stimuli. One bird that had begun to vocalize was also tested. The song-like sounds this bird produced were shifted upwards in frequency due to the presence of a glass bead implanted within its syrinx. In this bird, there appeared to be a preference for the distorted sounds.

These results support the hypothesis that, in HVc, selectivity for a bird's own song arises during the process of song matching and not during memorization of the tutor song.

Supported by NRSA Fellowship #NS07714 and NIH grant #MH40455.

SUBCORTICAL SOMATOSENSORY PATHWAYS II

263.1 MYSTACIAL VIBRISSAE AND COMMON FUR BRAINSTEM SENSORY PATHWAYS IN THE RAT. Charles Morgan and Frank R. Sharp, Dept. Neurology V127, UCSF and VA Med. Ctr., San Francisco, CA 94121.

Stimulation of whisker motor cortex activated spinal trigeminal nuclei pars oralis (SpVo) and interpolaris (SpVi) when whiskers were not allowed to touch an external object (Sharp, F.R., and Evans, K., J. Comp. Neurol., 208:1982, 255-287). Tactile sensory stimulation of all of the mystacial vibrissae, however, activated all parts of the spinal trigeminal nucleus including pars caudalis (SpVc), SpVi, and SpVo, as well as the principal trigeminal sensory nucleus (PrV) (Gonzalez, M.F., and Sharp, F.R., J. Comp. Neurol., 231:1985, 457-472). Based upon this data it was hypothesized that different trigeminal sensory receptors might relay to different levels and possibly to different parts of SpV and PrV.

To test this possibility (14 C) 2-deoxyglucose (2DG) was injected into adult Sprague Dawley rats. In one group the mystacial vibrissae in rows A and B were stimulated at 2 to 4 times per second in an anteroposterior direction after prior removal of all other whiskers. In the other group the common fur between rows A and B was stimulated at 2 times per second in an anteroposterior direction after prior removal of all mystacial vibrissae on both sides of the face. Tactile stimulation of the mystacial vibrissae in rows A and B increased 2DG uptake in ventrolateral laminae III and IV of SpVc, and in punctate ventral portions of ipsilateral SpVi, SpVo, and PrV. Ventrobasal n. thalamus and SI and SII of cortex were also activated during mystacial vibrissae sensory stimulation. Tactile stimulation of the common fur between rows A and B increased 2DG uptake primarily in ventrolateral parts of substantia gelatinosa (lamina II) of SpVc, with some spread into adjacent laminae I and III. SpVi, SpVo and Pr5 were not activated by common fur stimulation.

To further delineate maxillary sensory inputs, WGA-HRP was injected into the skin (containing the common fur) or muscle surrounding mystacial vibrissae in rows A and B. After two day survivals, perfusion, and TMB processing, the data demonstrated dense labeling of ventrolateral lamina II with lighter labeling of the magnocellular layers III and IV restricted to SpVc.

The data suggest that the common fur sensory receptors between rows A and B project primarily to lamina II of SpVc with some input to deeper laminae III and IV (seen with WGA-HRP but not 2DG), and that inputs to other trigeminal nuclei must be sparse or not present. The mystacial vibrissae of rows A and B, however, project to all trigeminal subnuclei SpVc, SpVi, SpVo, and PrV.

263.2 A COMPARATIVE STUDY OF THE PRINCIPAL TRIGEMINAL NUCLEUS AND THE DORSAL COLUMN NUCLEI IN THE RAT AND MUSKRAT: PROJECTIONS TO THE VENTROBASAL THALAMUS AND CEREBELLUM. J.P. Romeo and W.M. Panneton (SPON: P.A. Young). Dept. of Anatomy, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Adult Sprague-Dawley rats and muskrats were anesthetized by intraperitoneal injections of 16% chloral hydrate and placed in a stereotaxic apparatus. Either the ventrobasal thalamus (VB) or cerebellum were injected by pressure with a 1µl Hamilton syringe with a micropipette attached. The injection bolus consisted of a 50% solution of horseradish peroxidase (HRP, Sigma type VI) in 1% saponin. Following a survival period of 48-72 hours, the animals were anesthetized by sodium pentobarbital and perfused intracardially with .9% saline followed by a double aldehyde fixative in phosphate buffer. Their brains were removed and stored at 4°C in a 15% sucrose-phosphate buffer solution until the following day when 60µm transverse serial sections were cut with a freezing microtome and divided into two ordered series. One series was treated with diaminobenzidine and the other was treated with tetramethylbenzidine.

The distribution and the morphology of the dorsal column nuclei (DCN) of the common laboratory rat were compared to that of the muskrat. Injections into the thalamus were centered within the ventrobasal complex of both species, but some spread occurred into surrounding areas. In the rat, numerous small and some medium sized neurons were retrogradely labeled in the ventral two-thirds of the principal nucleus after thalamic injection. Numerous neurons also were found in both the nucleus gracilis and nucleus cuneatus throughout their rostrocaudal extent. In the muskrat, similar results were found. Injections into the cerebellum were large and included most of its parts on one side. In both species, numerous neurons were retrogradely labeled in the principal nucleus after cerebellar injections; most were multipolar and of medium size and were distributed throughout the nucleus but most often in aggregations in its middle and ventral parts. Multipolar neurons were also labeled in the dorsal column nuclei, and these were found predominantly in the ventrolateral part of the cuneate nucleus near levels of the obex in both species.

The results of this study show that the projections from the principal trigeminal nucleus and the dorsal column nuclei to thalamus and cerebellum in the wild muskrat are organized similar to those of the common white laboratory rat.

263.3 SOMATOSENSORY PROJECTIONS TO THE MIDBRAIN IN THE MONKEY. M.Wiberg*, J.Westman* and A.Blomqvist (SPON: P.Cancalon) Dept of Anatomy, University of Uppsala, Box 571, S-751 23 Uppsala, Sweden.

The terminal areas and cells of origin of the somatosensory projections to the midbrain in the monkey (Macaca fascicularis) were investigated with anterograde and retrograde tracing with HRP and WGA-HRP. The results show that several regions in the dorsal mesencephalon, such as the periaqueductal gray matter, the intercollicular nucleus, the deep and intermediate gray layers of the superior colliculus, the posterior pretectal nucleus, and the nucleus of Darkschewitsch receive a prominent spinal input, partly somatotopically organized, from lamina I and V of the dorsal horn. Several of these structures also receive an input from the lateral cervical nucleus, the dorsal column nuclei and the spinal trigeminal nucleus, which, with the exception of that from the lateral cervical nucleus, originates from neurons separate from those projecting to the ventrobasal thalamus. The results demonstrate that the organization of the ascending somatosensory projection to the mesencephalon is similar to that previously described in the cat, suggesting that several regions in the primate midbrain have an important somesthetic function including that of pain.

263.4 EFFECTS OF GABA RECEPTOR BLOCKADE IN THE CUNEATE NUCLEUS OF THE RAT. R.J.Weinberg, F.Conti, and A.Rustioni. Depts. of Anatomy and Physiology, University of North Carolina, Chapel Hill, NC 27514.

Most neurons in the dorsal column nuclei (DCN) have small, modality-specific excitatory receptive fields (ERF's). However, classical Golgi studies and recent intra-axonal HRP studies show that primary afferent fibers arborize richly within the DCN; individual fibers may have synaptic boutons distributed over a substantial fraction of the DCN. Recent studies of DCN physiology and plasticity also suggest that under some conditions, DCN cells may respond to stimuli outside their normal ERF borders.

Anatomical evidence demonstrates the presence of many GABAergic cells and synaptic terminals within the rat DCN. Pharmacological study shows that pre- and postsynaptic inhibition within the DCN is blocked by bicuculline, implying that $GABA_B$ receptors are functionally important in this system. To test the hypothesis that this GABAergic innervation enables an anatomically diffuse projection within the DCN to produce small ERF's by means of lateral inhibition, we have studied the responses of single neurons in the rat cuneate nucleus to natural and electrical stimuli during iontophoretic application of bicuculline.

Standard extracellular techniques were used to record from single units in chloral hydrate-anesthetized rats before and after iontophoretic application of glutamate, GABA, and bicuculline methobromide (BMB). To date, useful physiological and pharmacological data have been gathered on 20 neurons. Nearly all cells studied were excited by glutamate, a putative neurotransmitter in this system. GABA inhibited most neurons at currents of less than 50 nA, but inhibited spontaneous activity more effectively than evoked activity. BMB usually blocked the effects of GABA at currents in the range of 10-75 nA.

Contrary to our original hypothesis, BMB in most cases failed to increase the ERF significantly. However, even when no change could be detected in the ERF, post-stimulus inhibition produced either from the ERF center or surround was greatly reduced. Moreover, BMB generally increased the duration of spontaneous or stimulus-produced spike bursts. Our present working hypothesis is that the GABAergic innervation in this system has little influence on static receptive field properties, but plays an important role in dynamic properties, such as adaptation.

This work was supported by HHS grants NS-07132 and NS-12440.

263.5 DO NON-PRIMARY AFFERENTS TO THE DORSAL COLUMN NUCLEI USE SUBSTANCE P AS THEIR NEUROTRANSMITTER ? A.Rustioni, F.Conti. Departments of Anatomy and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514.

In recent studies it was shown that the undecapeptide substance P (SP) is present in the dorsal column nuclei (DCN) of rats, cats and monkeys (Westman et al.,1984; Conti and Rustioni,1986). In a search for the origin of this innervation, we have a) reconstructed the precise distribution of SP fibers and terminals in cats and rats, and b) investigated the presence of SP-positive neurons in the spinal cord and spinal ganglia.

Animals were anaesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2-7.4). The animals in which spinal cord and spinal ganglia were studied received an injection of colchicine in the spinal gray matter. Vibratome cut, 25 µm thick sections were reacted with a commercially available antiserum (Immunonuclear, Inc) using the PAP method.

The main results can be summarized as follows: 1) positive fibers were observed in the fasciculi gracile and cuneate from where they enter the nuclei; 2) the distribution of SP fibers and terminals in the cat is not diffuse. At caudal medullary levels, immunoreactive material was present only in the ventral regions of both gracile and cuneate nuclei and in the lateral regions of the cuneate; at the level of the obex, an additional dorsal shell of SP-immunoreactivity was present; at rostral levels (i.e, above the obex) the reaction product showed a more diffuse distribution; 3) initial observations suggest that in the dorsal horn of the spinal cord of colchicine-treated animals, SP-positive neurons were present in laminae III and IV in addition to laminae I and II; 4) substance P positive neurons in the dorsal root ganglia are of small size and these values do not overlap those of neurons at the origin of primary afferents to the DCN.

These data and the negative findings for double-labeled neurons in the dorsal root ganglia observed in some preliminary double-labeling experiments, suggest that SP may be the neurotransmitter or neuromodulator in the non-primary afferents fibers terminating in the DCN.

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263.6 DISTRIBUTION OF SEROTONIN AND SUBSTANCE P IMMUNOREACTIVE FIBERS IN THE RAT CAUDAL TRIGEMINAL NUCLEUS. J.C. Pearson and L. Jennes. Dept. of Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435.

The processing of nociceptive sensation in the caudal spinal trigeminal nucleus (NTST) is incompletely understood, however it is known that the primary afferent fibers involved are substance P (SP)-immunoreactive, and that inhibition of this pain transmission involves serotonin (5HT) immunoreactive fibers originating from various brainstem nuclei. Possible mechanisms of interaction between SP and 5HT fibers in the NTST are complicated in that some, but not all, 5HT-immunoreactive somata in brainstem nuclei known to project to the NTST also show SP-like immunoreactivity. This implies that some of the 5HT projections involved in pain suppression may contain co-localized SP. However, direct evidence for 5HT and SP in the same terminals in the NTST has not been reported, nor has a projection of co-localized 5HT/SP brainstem somata to the NTST been established.

The present study examines the distribution of SP- and 5HT-immunoreactive fibers in caudal NTST using indirect immunofluorescence histochemistry and an elution-restaining procedure (Tramu et al., J. Histochem. Cytochem., 26:322-324, 1978), as well as an immunofluorescence procedure that allows simultaneous labeling of tissue for two different antigens. Staining procedures were done on adult rats pretreated with colchicine, L-tryptophan, and pargyline. Animals were perfused with 4% paraformaldehyde and brainstems were vibratome sectioned at 40um. SP-immunoreactive fibers consist of varicose strands located in the spinal trigeminal tract and predominantly in the marginal layer (ML) and substantia gelatinosa (SG) of the caudal NTST. 5HT-immunoreactive fibers contain varicosities structurally similar to those of SP fibers. 5HT fibers are prominent in the ML and SG, and are also found in the deeper laminae of the STN. In the ML and SG, fibers immunoreactive for only SP or only 5HT are numerous and are seemingly intermixed within these laminae. In addition, ML and SG contain varicose fibers in which both SP- and 5HT-immunoreactivity are identified. The present findings indicate that a population of neurons with co-localized 5HT and SP projects to the NTST, as well as neurons which contain only SP or 5HT.

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263.7 THE SOURCE AND DISTRIBUTION OF SEROTONERGIC INPUTS TO THE RAT DORSAL COLUMN NUCLEI. J.H. Haring. Dept. of Anatomy, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Studies of the role of serotonin (5HT) in the transmission of somatosensory information have largely focused upon nociception and hence the spinothalamic projection. However, the demonstration of a 5HT fibers plexus within the dorsal column nuclei (DCN) suggests that 5HT also functions in the processing of non-noxious sensory information within the dorsal column-medial lemniscal system. The present study employed 5HT immunocytochemistry both alone and in combination with the retrograde transport of WGA-HRP or diamidino yellow (DY) to identify the origin of the 5HT projection to the DCN, as well as to define the relationship of 5HT axons to lemniscal neurons of the DCN.

Injections of 1% WGA-HRP were made in physiologically identified forepaw and hindpaw regions of the cuneate and gracile nuclei, respectively. These injections resulted in the labeling of a few neurons of the nucleus raphe magnus; however, the majority of labeled cells was located between the pyramidal and the motor nucleus of cranial nerve VII ipsilateral to the injection site. This region has been identified as nucleus paragigantocellularis lateralis (PGL) in the rat and is also part of the B3 group of 5HT-containing neurons. The distribution of labeled cells seen in the PGL overlapped significantly with a cluster of 5HT-immunopositive neurons, suggesting these cells as the source of the 5HT projection to the DCN. This assumption was confirmed by combining the retrograde transport of DY with 5HT immunocytochemistry. The 5HT plexus within the DCN is composed of a sparse feltwork of fine axons which exhibit a moderate number of varicosities. The distribution of 5HT fibers appears to be uniform throughout the rostrocaudal extent of the DCN. The relationship of 5HT fibers to cuneo- and gracilothalamic projection neurons was studied by combining the retrograde transport of WGA-HRP from the thalamic ventrobasal complex (VB) with 5HT immunocytochemistry. A consistent relationship between thalamic projection cells of the DCN and 5HT fibers is not obvious from these studies; however, it is clear that the perikaryon is not the primary target of the 5HT axons. Most frequently, 5HT axons appeared to be unrelated to thalamic projection neurons in a direct manner. Rather, 5HT fibers were seen throughout the neuropil interspersed between neurons labeled from VB. When 5HT-positive fibers were in contact with thalamic projection neurons, they were invariably related to the dendrites of those cells. This observation, together with the apparent random distribution of 5HT axons within the neuropil, suggests that if 5HT fibers are targeting thalamic projection neurons, the distal dendrites (which usually are not filled by WGA-HRP) could be the primary site of synaptic interaction.

263.8 SEROTONERGIC INPUT TO THE RAT DORSAL COLUMN NUCLEI FROM NUCLEI RAPHE OBSCURUS AND MAGNUS. H.H. Willcockson*, S.M. Carlton and W.D. Willis. (SPON: B. Haber). Mar. Biomed. Inst. and Dept. of Anat. & Neurosci., Univ. TX Med. Br., Galveston, TX 77550

The dorsal column nuclei (DCN) are implicated in the central processing of somatosensory information. Anatomical studies demonstrate the DCN receives primary afferent input as well as input from the postsynaptic dorsal column pathway. This information is relayed onto the ventrobasal thalamus. Electrophysiological studies in the cat and monkey have shown that DCN neurons can be inhibited by stimulation in the raphe nuclei. We reported immunohistochemical evidence of serotonin (5HT) containing terminals and varicosities in close proximity to identified DCN thalamic projection neurons in the rat. This suggests a 5HT modulation of somatosensation at the level of the DCN (Soc. Neuro. Abstr., 1984). Subsequently, our retrograde labeling studies (Am. and Can. Assoc. of Anat. Abstr., 1985), have shown that efferent input to the DCN arises from areas known to contain 5HT neurons, such as n. raphe magnus (NRM), n. raphe obscurus (NRO) and n. paragigantocellularis lateralis (PGL). In the present study, a double labeling technique was used to identify those 5HT neurons which project to the DCN in the rat and thus are likely to have an important modulating role on somatosensory processing. HRP gel (30%) or HRP crystals were implanted bilaterally in the DCN of 4 rats. After 24-48 hrs., the rats were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde. The tissue was sectioned on a vibratome (25 µm) and reacted for HRP with tetramethylbenzidine. This was followed by stabilization with cobalt chloride diaminobenzidine to produce a black punctate reaction product. Free floating sections were then immunohistochemically stained for 5HT using the PAP technique. Double labeled cells contained black HRP reaction product and amber colored somata and dendrites. All data were analyzed using 40 and 100X oil immersion objectives. Preliminary analysis of sections verified the presence of double labeled cells throughout NRO with a few cells in NRM. In one animal, within a 200 µm extent of NRO, approximately 4% of the total number of 5HT cells (10 of 237 cells) were double labeled with HRP. In 225 µm of NRM, only 1% of the total number were double labeled (3 of 223 cells). None of the HRP labeled cells in PGL were observed to contain 5HT; however, 4 double labeled cells were observed in the nucleus of the solitary tract. We conclude that somatosensory processing by the neurons of the dorsal column nuclei can be modulated by serotonergic input from the nucleus raphe obscurus and to a lesser extent from the nucleus raphe magnus. Presumably this modulatory system operates in parallel to that concerned in nociception. (Supported by grants NS 09473 and NS 11255 from the NIH).

263.9 REDUCED RESPONSIVENESS OF LOCUS COERULEUS NEURONS TO CUTANEOUS THERMAL STIMULI IN CAPSAICIN TREATED RATS.

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Capsaicin treatment in rats leads to a selective impairment of thermoregulation, involving a loss of various behavioural and autonomic heat defence mechanisms. This thermoregulatory dysfunction in warm ambient temperature has mainly been attributed to an impaired central (hypothalamic) thermosensitivity, allthough a reduced peripheral warm sensitivity has also been suggested. Interestingly, rats treated with capsaicin as neonates, in contrast to rats treated as adults, seem to have an intact hypothalamic thermoregulatory function but have a similarly impaired thermoregulation. This indicates the involvement of extrahypothalamic structures in the reduced heat tolerance after capsaicin treatment.

Recent experiments from our laboratory have demonstrated that brain norepinephrine neurons in the locus coeruleus (LC), a nucleus thought to be involved in behavioural and autonomic functions as well as in sensory processing, are activated by cutaneous thermal stimuli of both non-noxious and noxious character. In the present study, utilizing single unit recording techniques, the LC neuronal responses to cutaneous thermal stimuli were used to evaluate peripheral thermal sensitivity in capsaicin treated rats, as compared to controls. After neonatal as well as adult treatment with capsaicin, the LC activation during thermal stimulation was abolished in the non-noxious temperature range and greatly reduced in the noxious range.

The results suggest that a reduction in peripheral thermal afferent transmission may play a major role in the impairment of thermoregulation following capsaicin treatment. Furthermore, the demonstrated reduction in LC responsiveness to cutaneous thermal stimuli may constitute one underlying mechanism for the previously described loss of normal escaping behaviour in capsain treated animals subjected to warm ambient temperature.

This study was supported by the Swedish Medical Research Counsil (grant 7484), the Medical Faculty, University of Göteborg, and "Knut och Alice Wallenbergs Stiftelse".

263.10 IS SUBSTANCE P THE MARKER FOR A POPULATION OF SPINOTHALAMIC NEURONS? R. Spreafico*, G. Battaglia*, and A.Rustioni (SPON: A.R. Light). Dept. of Anatomy and Physiology, U. of North Carolina, Chapel Hill, NC 27514.

Little is known about the neurotransmitters or neuromodulators involved in the afferent and efferent connections of the VB complex. Substance P (SP) is one of the possible synaptic mediator for ascending connections to VB. Some small primary sensory neurons are, in fact, immunoreactive for SP, and there is some physiological evidence that SP is involved in the transmission of noxious stimuli. The presence of SP+ fibers and nerve terminals in the cat thalamus was investigated in this study. The presence of SP+ neurons in the spinal cord was also investigated under experimental conditions known to maximize the perikaryal content of this peptide. Young adult animals were perfused under deep anaesthesia with 4% paraformaldehyde or with 4% carbodiimide followed by 4% paraformaldehyde. Twenty-four to forty-eight hours prior to the perfusion some animals received colchicine injections in the cervical spinal cord, in some cases accompanied by unilateral anterolateral chordotomy at the same cervical level. Vibratome sections of the thalamus and the cervical spinal cord were reacted with a commercially available antibody against SP using the avidin-biotin (ABC) or the peroxidase-anti-peroxidase (PAP) method.

SP immunoreactive terminals in the thalamus overlap parts of the territory of terminations of the spinothalamic tract, i.e. in VB, posterior complex, n. centralis lateralis, n. submedius, and other nuclei. In the VB complex the SP immunoreactive terminals are confined in regions in which physiological studies have shown the presence of neurons selectively responsive to noxious stimulation. In the cervical spinal segment immediately caudal to the colchicine injection or the anterolateral cordotomy there are SP+ cells in lamina I, lamina V, lamina VIII, i.e. all laminae known to contain cells of origin of spinothalamic fibers. Definite proof that spinothalamic cells contain SP requires double-labeling experiments now in progress in our lab.

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263.11 TERMINATION OF THE POST-SYNAPTIC DORSAL COLUMN (PSDC) PROJECTION IN THE RAT REVEALED USING *PHASEOLUS VULGARIS* LEUCOAGGLUTININ (PHA-L), WITH EVIDENCE THAT FIBERS OF PASSAGE ARE LABELED FOR SHORT DISTANCES. K.D. Cliffer and G.J. Giesler, Jr. Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

As part of a series of studies of the PSDC pathway in the rat, PHA-L was iontophoretically injected (5 uA pulses, 40 uA-min) into the dorsal horn at lumbar and cervical levels. After survival periods of up to a month and subsequent indirect immunofluorescent staining, many axons were labeled in the dorsal columns in segments rostral to the injection, with no clear differential distribution according to depth. At all levels of the gracile (Gn) and cuneate (Cn) nuclei, stained axons were observed with widely distributed branching fibers and terminal-like varicosities after lumbar or cervical injections, respectively. In animals receiving multiple injections in a single transverse plane, the labeled fibers and terminals nearly filled the corresponding nucleus in all dimensions. No labeled fibers were found in the Cn after lumbar injections, and very few were seen in the Gn after cervical injections.

To determine whether PHA-L is transported by axons passing through an injection site, we injected the dorsal or lateral funiculi. Although all such control injections were confined to the white matter, some labeled axons could be followed for approximately 20 mm, with branching and varicosities sometimes present. Following injection into the gracile fasciculus at C6, a few labeled terminals could be seen in the gracile nucleus. Thus, it is unlikely that the observed terminal labeling in control injections was due solely to uptake of PHA-L by nearby cells. In some control injections, labeling of fibers and terminals closer to the injection site was substantial. We conclude that PHA-L does label fibers of passage, but that the problem is minimal at long distances from the injection site, comparable to the distances of the dorsal column nuclei from our spinal cord injections. Relatively close to the injection site (less than 20 mm), interpretation of results from labeling with PHA-L should take into account the likelihood that at least a small percentage of fibers of passage are labeled.

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263.12 CAPSAICIN EFFECT ON REGIONAL GLUCOSE UTILIZATION IN THE RAT CENTRAL NERVOUS SYSTEM. M. Szikszay* and E. D. London (SPON: D. R. Jasinski), Neuropharmacology Laboratory, Addiction Research Center, NIDA, Baltimore, MD 21224.

Capsaicin has widespread pharmacological effects on parts of the peripheral and central nervous systems (CNS), particularly those which are involved in sensation (Fitzgerald, M., Pain 15:109, 1983). The present study was performed to map the in vivo distribution of capsaicin's action in the brain and spinal cord of the adult male Fischer-344 rat. Local rates of glucose utilization (GU), which reflect functional activity in the CNS, were measured using the autoradiographic 2-deoxy-D-[1-^{14}C]-glucose ([^{14}C]DG) method (Sokoloff, L. et al., J. Neurochem. 28:897, 1977).

Rats were injected once daily with capsaicin (10, 20 or 50 mg/kg, s.c.) or the solvent (10% ethanol, 5% Tween-80) for 3 days. The [^{14}C]DG procedure was performed on the fourth day, when rats were prepared with femoral venous and arterial catheters for the i.v. injection of [^{14}C]DG and collection of timed arterial blood samples, respectively. Capsaicin (20 mg/kg s.c.) was administered 10 min before the radiotracer.

The results demonstrated restricted and specific effects of the subacute capsaicin treatment on GU. As expected from the distribution of primary sensory afferent projections to the spinal cord, capsaicin increased rates of GU in the substantia gelatinosa. However, enhanced GU also was observed in the ventromedial aspect of the dorsal horn, extending along the medial border of the dorsal horn. Moreover, enhanced GU was detected in the sensory relay nuclei (n.) in the brainstem, which are related to the ascending dorsal column somatosensory pathway (i.e., gracile n., spinal sensory trigeminal complex, external cuneate n.). No obvious stimulation was seen in the ventrobasal thalamic complex. Interestingly, the spinothalamic pathway showed an increased GU reponse at its termination in the n. submedius. The enhanced GU might be related to the hypermetabolism in the ventromedial aspect of the dorsal horn. Other pain-related structures showed elevated GU as well (red nucleus, including the magnocellular part; paragigantocellular reticular n.). In addition, GU was altered in several other brain areas (e.g. lateral reticular n., medial part of nucleus of solitary tract, lateral preoptic area, septal nuclei and the medial and lateral parts of the bed nucleus of stria terminalis). Most of the latter structures contain detectable levels of markers for substance P neurons; and each of them is involved in either modulating autonomic functions directly or integrating somatosensory and/or affective function with automomic mechanisms.

M. Szikszay is a visiting scientist from the University Medical School, Szeged, Hungary.

EXCITATORY AMINO ACID RECEPTORS: PHARMACOLOGY

264.1 RECIPROCAL RELATIONSHIP BETWEEN THE EXPRESSION OF QUISQUALATE- AND NMDA-RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS DURING *DEVELOPMENT*. H.KASAI* (SPON:M.ITO). Department of Physiology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan.

Development of quisqualate-, NMDA-, and kainate-receptors was studied using the whole-cell voltage-clamp method. Hippocampi from 16-18-day fetal mice were dissected and dissociated into microexplants sieving through 200 μm nylon-mesh, and cultured on plastic dishes. The voltage-clamp experiments were made on 550 neurons cultured for 4 to 28 days. The bath was continuously perfused (140 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgSO_4$, 5 Na-Hepes, 10 Glucose, and 0.2 μM TTX, pH 7.4, 36 °C). Patch-pipettes were filled with a CsCl solution (152 CsCl, 1 $MgSO_4$, 5 Na-Hepes, and 10μM K_2-EGTA, pH 7.4). Quisqualate (0.5 μM), NMDA (250 μM), and kainate (10 μM) were pressure applied (0.5 atm) to somas with triple barrelled pipettes (the inner diameter was 3 μm). Membrane potential was held at -60 mV, and amplitudes of the inward currents elicited by drag applications (I_q, I_n, and I_k for quisqualate, NMDA, and kainate, respectively) were measured when they attained steady-state level. Mean amplitudes of the current were calculated from 10-30 cells in each experiment.

Sensitivities to the three types of glutamate analogs were differently regulated during development. Stage I (day 4-9): glutamate induced currents were very small on day 4 (I_q=15 pA, I_n=30 pA, and I_k=27 pA, mean). They were gradually increased until day 9 (I_q=420 pA, I_n=260 pA, and I_k=270 pA). EPSPs were first detected on day 8. Stage II (day 10-15): I_n and I_k were continued to be increased, while I_q was reduced (I_q=235 pA, I_n=850 pA, and I_k=740 pA, on day 15). EPSPs were most abundant in this stage, and they evoked spikes to the post-synaptic neurons. Stage III (day 16-28): I_n was reduced, while I_q was increased (I_q=650 pA, I_n=330 pA, and I_k=830 pA on day 25). Freqeuncy of EPSPs was reduced in this stage, which might be attributed to decrease in the cell number. Thus, I_k was increased monotonously through out the development, while I_n changed in parallel with the EPSPs and spike activities in stage II and III, and I_q inversely correlated with them. In 5 experiments, spike activities were abolished by adding TTX (3 μM) to the culture medium for two days. I_n was reduced to its half-control level, while I_q and I_k were less affected.

These data indicate that 1) there are at least three types of glutamate receptors in cultured hippocampal neurons, 2) their expressions are differently regulated, and 3) synaptic and spike activities up-regulate the NMDA-receptor. Detailed mechanisms of the up-regulation, including the role of Ca^{2+}, are under investigation, which may correlate the change in the synaptic efficacy in hippocampal neurons in vivo: the long term potentiation.

264.2 PHARMACOLOGY OF EXCITATORY SYNAPSES FORMED BY IDENTIFIED NEURONS IN PRIMARY CULTURES OF RAT VISUAL CORTEX. J. E. Huettner and R. W. Baughman. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Excitatory neurons in the cerebral cortex are thought to use glutamate (Glu) or aspartate (Asp) as their neurotransmitter. When cortical neurons are grown in primary dissociated cultures they establish synaptic contacts with nearby cells and most neurons receive both excitatory and inhibitory synaptic inputs. We have previously shown that cortical neurons in culture possess both NMDA-type and non-NMDA receptors for excitatory amino acids (Huettner and Baughman, J. Neurosci., In press). In the present study we examined the pharmacology of synapses formed by an identified population of cortical cells.

Neurons were dissociated from the visual cortex of 7-10 day old Long Evans rats and plated onto small "islands" of cortical astrocytes. Each island had an area of 1 mm^2 and contained approximately 70-100 neurons. Prior to dissociation, cells in layer five of the visual cortex that project to the superior colliculus were labeled in vivo by retrograde transport of fluorescent latex microspheres (Katz et al., Nature 310: 498 (1984)). After 2-6 weeks in culture, labeled neurons were identified by epifluorescent illumination, and electrophysiological recordings were obtained either with conventional microelectrodes or with gigaseal whole-cell pipettes. For most experiments we recorded simultaneously from a labeled cortico-collicular neuron and a nearby unlabeled cell. Stimulation of labeled neurons with a depolarizing current pulse elicited an action potential in the labeled cells followed by a short latency (1-2 msec) epsp in the unlabeled neurons. Usually both cells showed longer latency synaptic potentials due to polysynaptic circuits. In many cases, stimulation of the unlabeled neurons produced short latency synaptic potentials, either epsps or ipsps, in the labeled cells.

Antagonists of amino acid receptors were added to the perfusion medium and their ability to block evoked synaptic potentials was evaluated. Kynurenate, piperidine dicarboxylate and Y-D-glutamylglycine completely blocked epsps at concentrations of 1-2 mM. These three drugs are antagonists of both NMDA and non-NMDA receptors. The specific NMDA receptor antagonist 2-Amino-5-phosphono valerate, at 20-50 uM, did not block short latency epsps but did reduce the magnitude of long latency, polysynaptic components. Ipsps were completely blocked by 10-25 uM bicuculline methiodide.

In summary, identified cortico-collicular neurons grown in cell culture produce excitatory synaptic potentials in nearby cells. Our pharmacological data suggests that these epsps are mediated by excitatory amino acid receptors. (NIH EY03502, The Milton Fund, Harvard University Society of Fellows)

264.3 KYNURENIC ACID ANTAGONIZES FAST COMPONENTS OF THE RESPONSE TO ACTIVATION OF A SYNAPTIC PATHWAY IN THE RAT SUPRAOPTIC NUCLEUS. V. K. Gribkoff and F. E. Dudek. Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

The excitatory amino acids glutamate and aspartate have been proposed as transmitters at several chemical synapses in the central nervous system. The recent development of effective antagonists of excitatory amino acid receptors, including the tryptophan metabolite kynurenic acid, have permitted direct blockade of postsynaptic potentials in spinal cord (Elmsie and Yoshikami, Br. Res., 330:265-272, 1985) and hippocampus (Ganong, Lanthorn, and Cotman, Br. Res., 273:170-174, 1983). While acetylcholine and norepinephrine have been intensely studied as possible excitatory transmitters in the rat hypothalamic supraoptic nucleus, the involvement of excitatory amino acids in synaptic transmission has been largely unexplored. In the present experiments on rat hypothalamic slices, we have examined the effects of kynurenic acid on responses of intracellularly-recorded supraoptic neurons to stimulation dorsolateral to the nucleus.

Dorsolateral stimulation produces a complex sequence of events in supraoptic neurons (Gribkoff and Dudek, Soc. Neurosci. Abstr., 11:1236, 1985). Single volleys evoke a short latency EPSP, which at higher intensities has a component that can last up to 1 sec. During this prolonged EPSP are several brief depolarizing events, which appear similar to spontaneously occurring EPSP's. Repetitive stimulation of this area results in depolarization during and after the period of stimulation; this is associated with a high-frequency burst of spontaneous PSP's. Bath-applied kynurenic acid (0.5-2.0 mM) significantly and reversibly reduced the short-latency component of single volleys, the spontaneous EPSP's before and after stimulation, and depolarization during stimulus trains. Slow components of the response to single and repetitive stimulation persisted during perfusion with kynurenic acid. These data provide direct pharmacological evidence that excitatory amino acids mediate synaptic transmission in the supraoptic nucleus.

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264.4 3-(2-CARBOXYPIPERAZIN-4-YL)-PROPANE-1-PHOSPHONIC ACID, (CPP) A POTENT ANTAGONIST OF THE NMDA-TYPE RECEPTOR IN VITRO AND IN VIVO. Stephen E. McPherson, J. Lehmann, D.J. Steel J. Schneider, and P. L. Wood, Research Department, Pharmaceuticals Division, CIBA-Geigy Corporation, Summit, N.J., 07901 USA

GABA receptor agonists and NMDA-type receptor antagonists have anticonvulsant activity while GABA antagonists and NMDA agonists are convulsants, suggesting a reciprocal interaction between these two amino acid systems. Moreover, the NMDA-type receptor antagonist, 2-amino-7-phosphonoheptanoic acid (AP7), produces an anticonflict effect in rats (Bennett et al., Abs. Soc. Neur. 15:32.14, 1985), suggestive of anxiolytic potential such as found in the indirect GABA receptor agonist, diazepam. However, AP7 is not potent in vivo, and therefore has limited potential has a therapeutic agent.

We describe here a potent NMDA-type receptor antagonist, CPP. CPP inhibited NMDA-evoked [3H]ACh release from striatal slices with an IC50 of 14 uM, without affecting KCl-evoked [3H]ACh release when tested at 100 uM. This effect was competitive, producing a parallel shift to the right in the concentration-response to NMDA, without affecting the maximal response. The pA2 value for CPP was 5.66, whereas that for AP7 was 5.22.

CPP inhibited also the harmaline-induced increase in cerebellar cyclic guanosine monophosphate (cGMP), an effect previously shown to be mediated by NMDA-type receptors (Wood et al., Neuropharm. 21:1235, 1982). Reduction of cyclic GMP was as follows: D-AP5 at 32mg/kg,i.v. was 60%; L-AP5 at the same concentration was 35%; DL-AP7 at 150mg/kg,i.v. was 80%. CPP, when administered subcutaneously to mice at 2mg/kg reduced cGMP by 50%. This evidence, together with the demonstration of labeling by [3H]CPP of a binding site with a pharmacological profile similar to that of the NMDA-type receptor (Murphy et al., this meeting), support the view that the compound interacts selectively and competitively with NMDA-type receptors. Compounds such as CPP might portend a major new type of therapeutic agent.

264.5 GAMMA-GLUTAMYLAMINOSULPHONATE (GAMS) as a Specific Kainate Antagonist in the Mudpuppy Retina. Paul A. Coleman and Robert F. Miller Washington University School of Medicine, Dept. of Ophthalmology, St. Louis MO, 63110

We applied whole-cell recording techniques to the intact, perfused mudpuppy retina to study the action of GAMS for its effect on synaptic transmission and its ability to antagonize exogenously applied excitatory amino acid (EAA) analogs. At concentrations of 5 to 10 mM, GAMS blocks the light responses of horizontal cells but has no effect on the APB receptors of ON-bipolars. This network-selective effect of GAMS is similar to that of cis 2,3 piperidine dicarboxylic acid and kynurenic acid.

The most significant action of GAMS is evident in the inner retina, among third-order neurons, which have different receptors for kainic acid (KA), quisqualate (QQ), and N-methyl-D-aspartate (NMDA). In these neurons (amacrine and ganglion cells), GAMS selectively antagonized KA, but had little effect on exogenously applied NMDA or QQ. At 5 to 10 mM, GAMS antagonizes 30 uM KA, while leaving the effects of NMDA and QQ relatively intact. The action of GAMS appears to be competetive, because 100 uM KA produces a significant depolarization in the presence of 5 mM GAMS. At the highest concentration tested, GAMS markedly suppressed ON and OFF light evoked activity of all amacrine and ganglion cells tested.

Kynurinic acid (Kyn) is a more potent KA antagonist than GAMS. Kyn (5 mM) can completely block the effects of 100 uM KA, whereas the effects of 50 uM KA cannot be completely supressed by a similar concentration of GAMS. However, what is completely new about GAMS in our hands, is that while it antagonizes KA, it does not effect the action of NMDA or QQ. In this sense the action of GAMS is more discriminating than either Kyn or PDA. GAMS is a specific KA antagonist for retinal neurons, as it is for spinal cord neurons (Davies, et al., 1985). Since GAMS blocks the light evoked responses of third order retinal neurons, at concentrations where it supresses only KA, it seems likely that KA receptors play a pivotal role in synaptic transmission.

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264.6 ACTIVATION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS UNMASKS ^{3}H-TCP BINDING SITES IN BRAIN MEMBRANES: FURTHER EVIDENCE THAT PHENCYCLIDINE BLOCKS THE OPEN NMDA RECEPTOR CHANNEL. G.E. Fagg & J. Baud. Friedrich Miescher Institute, 4002 Basel, Switzerland.

Phencyclidine (PCP) and related substances elicit profound behavioral and psychotomimetic disturbances in man. Recent studies indicate that these drugs potently antagonize neuronal excitation evoked by NMDA (a selective excitatory amino acid agonist) but not that by quisqualate and kainate (Anis et al., Br. J. Pharmac. 79, 565, 1983). Here we report that L-glutamate (Glu) potently stimulates the binding of ^{3}H-TCP (a PCP analogue) to brain postsynaptic densities (PSDs), and that this effect is mediated by NMDA receptors. Our data are consistent with electrophysiological results which indicate that PCP and related drugs act by blocking the open state of the NMDA-activated ion channel (Honey et al., Neurosci. Lett. 61, 135, 1985).

The binding of ^{3}H-TCP to crude PSDs isolated from rat brains was determined as described by Vignon et al. (Brain Res. 280, 194,1983). In the absence of exogenous Glu, specifically-bound TCP was 48% of total binding (5 nM ^{3}H-TCP; non-specific measured using 0.1 mM PCP or dexoxadrol). In the presence of L-Glu, specific binding of TCP was markedly increased, and this binding displayed pharmacological properties typical of "PCP binding sites" (Ki's dexoxadrol 10 nM < PCP < SKF10047 < ketamine < levoxadrol 4 µM). Stimulation by L-Glu was concentration-dependent and saturable, with a Kd for L-Glu of 160 nM and a maximum stimulation of 3.5 times basal TCP binding. Similar effects were observed with NMDA (Kd 2.9 µM) and ibotenate (Kd 3.0 µM), whereas kainate was ineffective and quisqualate was of low potency. The stimulation of TCP binding by 1 µM L-Glu was antagonized by the NMDA receptor antagonists, 2-amino-5-phosphonopentanoate (AP5) and 2-amino-7-phosphonoheptanoate, but not by their 4-, 6- and 8-carbon homologues, nor by the quisqualate/kainate antagonist, γ-D-glutamylaminomethylsulphonate. Dixon analysis of the antagonism by D-AP5 yielded a Ki for this substance of 3.0 µM. Mg^{2+} exerted a biphasic effect on ^{3}H-TCP binding, stimulating at low µM concentrations and inhibiting at mM levels; in the presence of 1 µM L-Glu, lower concentrations of Mg^{2+} were required to stimulate TCP binding.

The pharmacological profile of the stimulation of ^{3}H-TCP binding by excitatory amino acids, and the absolute Kd and Ki values for L-Glu, NMDA, ibotenate and D-AP5, strongly suggest that this response is mediated via activation of the NMDA receptor sub-type. Methodologically, ^{3}H-TCP binding thus provides a convenient means of assessing NMDA receptor function, and of distinguishing between agonists and antagonists. More importantly, our findings suggest that TCP binding sites form a part of the NMDA receptor complex, and are consistent with open channel blockade as a mechanism of action of PCP and related substances.

264.7 MOLECULAR RECEPTORS FOR EXCITATORY AMINO ACIDS ARE DIFFERENTLY INFLUENCED BY AMINO-PHOSPHONOBUTYRIC ACID (APB) CIS–2,3–PIPERIDINE DICARBOXYLIC ACID (PDA) AND AMINO-PHOSPHONOVALERIC ACID (APV). EXPERIMENTS IN THE CAT RETINA. A. Przybyszewski*, N. Sucher*, O.-J. Grüsser. Dept. of Physiology, Freie Universität, Berlin, Germany-West.

Dissolved L-APB, D-APB, PDA or APV (0.5–3mg per eye) was injected into the vitreous body of the cat eye. The electroretinogram (ERG) and single ganglion cell activity were recorded.

The L-APB led at a dose above 0.5mg per eye to a decrease in on-center neuron spontaneous activity and flash-evoked responses, while in off-center neurons the flash-induced inhibitory period was shortened, the postinhibitory and the spontaneous activity slightly increased. These effects began after about 20 minutes and were at maximum 60–90 minutes after injection. D-APB acted similarly but at least 3mg per eye were required to evoke any effect. The racemate DL-APB effect indicated that the D-and L-enantiomeres induced synergistic effects. ERG flash-evoked responses demonstrated a decrease in b-wave amplitude which was approximately parallel to the decrease in on-center neuron activation.

PDA in a dose of 3mg per eye abolished all off-center ganglion cell spontaneous dark activity and reduced the flash-evoked responses of both off-center and on-center ganglion cells. ERG b-wave amplitude was only slightly affected.

APV, in contrast, reduced flash-evoked inhibition in off-center retinal ganglion cell activity at a dose of 2mg per eye. Flash-ERG was reduced by APV-injections into the vitreous body. This observation corroborates the findings obtained in single neurons. The time courses of the various effects are different in both onset and duration but are peculiar to the amino acids injected.

Comparing the effects observed with the substances investigated, we conclude that more than one type of molecular receptors for excitatory amino acids play a role in the signal transmission from photoreceptors to ganglion cells in the cat retina and that these receptors controlling the state of excitation of the retinal neuron membranes are highly selective, requiring specific structures of modified acidic amino acids for interaction.

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264.8 THE VOLTAGE- AND AGONIST-DEPENDENT BLOCKADE OF EXCITATORY AMINO ACID CURRENTS BY THE DISSOCIATIVE ANAESTHETIC KETAMINE. J.F. MacDonald and Z. Miljkovic*. Playfair Unit, Toronto Western Hospital and University of Toronto, Toronto, Ont. M5T 2S8.

In cultured neurones currents activated by N-methyl-D-aspartic acid (NMDA), as well as by L-aspartic acid (L-ASP) and L-glutamic acid (L-GLU) are subject to a voltage-dependent blockade by Mg^{2+} ions whereas currents evoked by kainic acid (KAI) are not. A block with similar selectivity has been reported in the presence of ketamine or phencyclidine (both *in vivo* and using the tissue slice preparation).

Excitatory amino acids (NMDA, L-ASP, L-GLU and KAI) were applied to cultured hippocampal neurones using pressure or iontophoresis. Ketamine (1 to 50 uM) or control recording solution was superfused through the entire bath (single electrode discontinuous switching whole cell voltage-clamp; 20 to 25 kHz). Single applications of excitatory amino acids were made through a range of holding potentials (-100 to +100 mV) and current responses used to construct current-voltage curves. Ketamine selectively depressed currents evoked by NMDA, L-ASP and L-GLU but spared those to KAI. In addition, the block developed only at holding potentials more hyperpolarized than 0 mV. Furthermore, repetitive applications of agonist were required to attain steady-state block. At hyperpolarized holding potentials no recovery from this block could be observed at intervals as long as 5 minutes (also the block was not dependent upon the frequency of agonist application). However, an incremental recovery was observed during repetitive applications of agonist at depolarized potentials. Prolonged washing of the cultures with control solution did not result in recovery from steady-state block until the agonist was repeatedly presented.

This evidence suggests that the voltage-dependent block by ketamine results from a shift of the NMDA channels to a low conductance state. A block of open channels followed by "trapping" of ketamine within the closed channels appears to be a likely mechanism.

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264.9 KYNURENIC ACID, CIS-2,3-PIPERIDINE DICARBOXYLIC ACID, AND GAMMA-D-GLUTAMYLGLYCINE BLOCK GOLDFISH RETINOTECTAL TRANSMISSION IN VITRO. R. B. Langdon and J. A. Freeman, Depts. of Cell Biology and Ophthalmology, Vanderbilt University School of Medicine, Nashville, TN 37232.

In order to assess the efficacy of excitatory retinotectal transmission during various pharmacological treatments, extracellular field potentials were elicited in isolated sections of goldfish tectum by stimulation of the optic tract. Profiles of these were recorded by sampling with glass micropipette electrodes at 50 μm intervals, and profiles of radial current sink-source densities were calculated. We have previously shown that such responses are not sensitive to the presence of nicotinic or other cholinergic antagonists in the bath (Langdon et al., 1985, Inv. Ophth. Vis. Res., suppl. 26: 106, and submitted). We here report the effects of four substances reported to antagonize neurotransmission mediated by excitatory amino acid (EAA) neurotransmitters (such as glutamate or aspartate). Of these, kynurenic acid, cis-2,3-piperidine dicarboxylic acid (PDA), and gamma-D-glutamylglycine (gamma-DGG) reduced response amplitudes by greater than 85% at concentrations of 1 to 2 mM. Subsequent studies were performed in low $[Ca^{++}]$/high $[Mg^{++}]$ media to allow observation of concentration-effect relationships for responses generated by isolated first-order postsynaptic currents. The resulting log concentration-effect curves for all three antagonists were parallel, symmetrically sigmoidal, and somewhat steeper than non-cooperative single site binding isotherms. Partial levels of block progressed no further after 10 to 15 min. All actions were completely reversible. Half-maximal block was observed at 0.15 mM with kynurenic acid, 0.33 mM with gamma-D-GG, and 0.47 mM with PDA. In contrast, 0.25 mM 2-amino-5-phosphonovalerate (APV) had little effect in either normal, low $[Ca^{++}]$/high $[Mg^{++}]$, or Mg^{++}-free media. APV is a relatively potent antagonist specific for EAA receptors of the N-methyl-D-aspartate (NMDA) sub-type.

These data indicate that an excitatory amino acid receptor not of the NMDA sub-type plays an essential role in fast excitatory retinotectal transmission, and would be most consistent with the mediation of most or all excitatory retinotectal transmission by a single class and sub-type of such receptors. (Supported by grants EY07007 and EY01117 (to LAF) from the National Eye Institute.)

264.10 INTERACTIONS OF PCP RECEPTOR AGONISTS WITH EXCITATORY AMINO ACID AGONISTS: ANTAGONISM OF NMDA AGONISTS IN THE MOUSE CEREBELLUM. P.L. Wood, J. Lehmann and D. Steel. Neuroscience Research, Pharmaceuticals Division, Ciba Geigy, Summit, N.J. 07901.

A number of PCP receptor agonists (PCP, ketamine, dexoxadrol and tiletamine) were found to decrease steady state levels of cerebellar cGMP in mice. These actions were stereospecific as evidenced by a comparison of levoxadrol and dexoxadrol. In addition, these agents blocked the increase in cerebellar cGMP elicited by harmaline (50 mg/kg, sc), which specifically activates the climbing fiber system to the cerebellum. This antagonism was complete, even at doses of PCP receptor agonists which did not alter basal cGMP levels.

To examine the observed actions further, we utilized direct intracerebellar injections of the water soluble PCP receptor agonist, tiletamine, along with either NMDA or quisqualic acid. In this paradigm, 0.5 ug of NMDA and 5 ug of quisqualic acid elicited 12- and 10-fold elevations in cerebellar cGMP, respectively. The increases in cGMP after NMDA were antagonized by the coadministration of 1 ug of tiletamine, while the actions of quisqualic acid were unaltered. Tiletamine (1 ug) alone did not alter cGMP levels.

These data indicate that PCP receptor agonists interact with responses mediated by NMDA- but not quisqualate-type excitatory amino acid receptors in the cerebellum. Whether this interaction is: a) directly at the NMDA receptor, b) allosteric via a PCP receptor or c) transsynaptic, remains to be determined.

264.11 REGULATION OF THE N-METHYL-D-ASPARTATE RECEPTOR IN CULTURED CEREBELLAR GRANULE CELLS. J.T. Wroblewski,* F. Nicoletti,* E. Fadda* and E. Costa (SPON: H. Alho) Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington D.C. 20007.

Cerebellar granule cells in primary culture (8-10 days old) express excitatory amino acid receptors which are selective for N-methyl-D-aspartate (NMDA), kainate and quisqualate. We have previously reported that signal transduction at these receptors involves various intracellular messenger systems, as seen by enhanced hydrolysis of membrane phosphoinositides, increased uptake of extracellular calcium ions and elevated levels of cGMP. The activation of the NMDA recognition site stimulates all of the above systems in a manner which is inhibited by physiological concentrations of Mg^{2+} ions. In contrast, the signal transduction at kainic and quisqualic recognition sites is Mg^{2+}-independent. NMDA signal transduction could still occur in the presence of 1 mM Mg^{2+} and a small depolarization induced by 20 mM K^+. The interactions of Mg^{2+} ions with the NMDA receptor do not occur at the level of the recognition site since Mg^{2+} presence did not inhibit the specific binding of ^{3}H-glutamate to membranes isolated from granule cells, but rather at the level of the coupling and/or transducer systems. The signal transduction at the NMDA receptor is strongly and selectively inhibited by phencyclidine (PCP) with IC_{50} values in the range of 30-90 nM. PCP does not affect the binding of ^{3}H-glutamate but shows specific binding to a separate site. Moreover, PCP binding is strongly inhibited by 1 mM Mg^{2+} ions. Our results will be discussed in relation to the hypothesis that the functional expression of the NMDA receptor depends on interactions of the ionic environment, local changes in membrane potential and direct receptor-receptor interactions modulated by putative endogenous ligands for NMDA and PCP recognition sites.

264.12 REGULATION OF PHORBOL ESTER BINDING BY PUTATIVE EXCITATORY AND INHIBITORY AMINO ACID TRANSMITTERS IN CEREBELLAR GRANULE CELLS IN CULTURE. S. Liljequist, F.M. Vaccarino and A. Guidotti. Fidia-Georgetown Institute for the Neurosciences, Georgetown University, Washington, D.C. 20007 and NIAAA, DICBR, Bethesda, Maryland 20892.

Tumor-promoting phorbol esters bind with high affinity to the protein kinase C (PKC) activating this enzyme, hence the binding of radiolabelled phorbol esters may be used to monitor PKC activation and translocation. The effect of cell depolarization, excitatory dicarboxylic amino acids addition and GABA were investigated on ^{3}H-4-beta-phorbol-12,13-dibutyrate (^{3}H-DBu) binding to intact neuronal cerebellar granule cells in primary cultures. The specific ^{3}H-DBu binding (75% of total binding) was determined in the presence of 10^{-5} M 12-0-tetradecanoylphorbol-13-acetate (TPA). This binding was saturable and of high affinity (K_d in nanomolar range). The inactive stereoisomer 4-alfa-phorbol-12,13 didecanoate failed to inhibit ^{3}H-DBu binding. At 25°C the binding rapidly increased with time of incubation and levelled off after 10 min. Cell depolarization produced by 50 mM potassium chloride and 50 uM veratridine enhanced ^{3}H-DBu binding by 400% and 225%, respectively, as compared to controls. Kinetic analysis revealed that the increased binding elicited by depolarization was due to an increase in the number of binding sites (B_{max}) rather than to a change in the affinity (K_D). An enhancement of ^{3}H-DBu binding was observed also following administration of glutamic acid (10^{-4} M; 225% of controls), kainic acid (10^{-4}M; 300% of controls), quisqualic acid (10^{-4}; 150% of controls), and the calcium ionophore A23187 (10^{-6} M; 150% of controls). Mg^{2+}-free assay media resulted in a significant increase in the basal ^{3}H-DBu binding (250% of controls) as compared to Mg^{2+}-containing assay media. NMDA (10^{-4}) and the specific $GABA_A$ receptor antagonist bicuculline (10^{-3} M) which did not alter ^{3}H-DBu binding in the presence of Mg^{2+}, increased ^{3}H-DBu binding by 100% in the absence of Mg^{2+}. This increase was blocked by the specific NMDA receptor antagonist APV. Moreover, the combined treatment of NMDA plus bicuculline caused an additional (about 100%) synergistic enhancement of ^{3}H-DBu binding. Our results indicate that cell depolarization and the administration of excitatory amino acids result in an increased number of recognition sites for ^{3}H-DBu. This increase is modulated by the concentrations of Mg^{2+} and GABA in the medium. Phorbol ester binding may be useful to monitor the functional significance of PKC activation and translocation in intact neuronal cell preparations.

CHARACTERIZATION OF CHOLINERGIC RECEPTORS II

265.1 ACETYLCHOLINE RECEPTOR SUBUNIT ASSEMBLY IN CULTURED CHICK MUSCLE CELLS. A. Ross*, J. Schmidt*, and J. M. Prives*. (Spon. P. Brink). Departments of Anatomical Sciences and Biochemistry, State University of New York, Stony Brook, NY 11794.

The four subunits of the nicotinic acetylcholine receptor assemble posttranslationally in the stochiometry alpha-2, beta-1, gamma-1, delta-1 to form a functional, ligand gated ion channel. Despite the advanced current knowledge of the structural, electrophysiological and pharmacological properties of the AChR, a major unresolved question is how subunit assembly is accomplished. We have studied AChR subunit assembly in primary cultures of chick embryonic muscle cells using two independent assays. The fact that monomeric alpha subunit sediments at 5s in a sucrose gradient, whereas alpha subunit in the assembled oligomer sediments at 9s enables us to measure the rate of assembly by monitoring the sedimentation coefficient of alpha subunit in cultures pulse labeled with 35S-methionine and chased for various time intervals. The sedimentation profile of the alpha subunit is determined by fractionation of cell homogenates from metabolically labeled cultures on a 5-20% linear sucrose gradient, immunoprecipitation of alpha subunit from the fractions by antisera raised against the alpha subunit of adult chicken AChR, separation of the precipitated material by SDS-polyacrylamide gel electrophoresis and quantification of the alpha subunit band by densitometry of the fluorograph. In the second assay we utilize antisera raised against the delta subunit of adult chicken AChR. This antisera, in addition to precipitating the delta subunit; will precipitate that population of alpha subunit that is in physical association with the delta subunit (i.e. partially or fully assembled AChR).

To measure the onset and rate of AChR assembly cultures were pulse labeled for 15 minutes with 35S-methionine and both assays were carried out after increasing chase intervals. The time required for 50% of the alpha subunit labeled during the pulse to shift into the 9s peak or to be precipitated by anti delta antisera was between 40 and 60 minutes of chase. As determined by both assays, all the labeled alpha subunit was in the assembled form within 3-4 hours after translation, indicating that the assembly process is highly efficient in these cells. Moreover, no significant degradation of alpha subunit, either assembled or unassembled, was detected during this interval. AChR assembly was strongly inhibited by treatment of muscle cells with the metabolic inhibitors potassium cyanide and 2,4-dinitrophenol, and moderately impaired by the ionophore monensin, an agent which disrupts the golgi apparatus. In contrast, cyclohexamide did not affect the rate of assembly, indicating that assembly is not dependent on ongoing protein synthesis. Our results are consistent with a model whereby AChR subunit assembly requires continued ATP synthesis and occurs in the golgi apparatus.

265.2 ASSOCIATION OF THE POSTSYNAPTIC 43K PROTEIN WITH ANTIBODY-INDUCED CLUSTERS OF ACETYLCHOLINE RECEPTORS ON CULTURED MUSCLE CELLS. Stanley C. Froehner and Robert Sealock* Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03756 and *Department of Physiology, University of North Carolina, Chapel Hill, N.C. 27514.

The 43K protein, a major component of ACh receptor-enriched membranes from Torpedo, is located on the cytoplasmic side of the electrocyte postsynaptic membrane in a distribution identical to that of the receptor. The precise function of this protein is unknown but it has been implicated in the mechanisms that anchor ACh receptors at synaptic sites. A related protein is found at rat muscle endplates and at receptor clusters that form spontaneously on cultured muscle cells. The association of 43K protein with receptor clusters on Xenopus muscle cells occurs soon after induction of the clusters by polycationic beads (Peng & Froehner, J. Cell Biol. 100: 1698, 1985) or by neurites (Burden, PNAS 82: 8270, 1985). To explore the possibility that the 43K protein is also associated with diffusely-distributed receptors, we have examined the effect of antibody-mediated, rapid redistribution of this population of receptors on 43K protein localization.

Receptors on BC$_3$H-1 muscle cells, which have large amounts of diffusely-distributed receptors but lack spontaneously-formed clusters, were labeled with rhodamine-alpha-bungarotoxin. To induce receptor rearrangement into small aggregates (microclusters) visible under fluorescence microscopy, the cells were incubated with rabbit anti-ACh receptor antibodies and sheep anti-rabbit IgG. The cultures were then fixed, permeabilized with Triton, and incubated with anti-43K protein monoclonal antibodies and fluorescein-labeled second antibodies. The distribution of anti-43K labeling coincided with the receptor microclusters, even those formed after only 30 minutes of antibody incubation. When control antibodies were substituted for anti-receptor antibodies, microclusters did not form. Likewise, there were no detectable microclusters of 43K protein. The 58K protein, located on the cytoplasmic side of the muscle membrane in a diffuse distribution (unpublished results), did not associate with receptor microclusters under identical conditions. Similar results were obtained with primary cultures of rat muscle.

These results show that the 43K-like protein is present in muscle cells that do not spontaneously assemble receptor clusters. Furthermore, it is found in association with clusters of ACh receptors that are formed by rapid, lateral redistribution within the plane of the membrane. While it may be that the 43K proteins assemble with receptor microclusters after their formation, the results are clearly compatible with the possibility that they are associated even with diffusely distributed receptors.

265.3 **RESIDUES AT THE ACETYLCHOLINE BINDING SITE AND AT A NONCOMPETITIVE INHIBITOR BINDING SITE MAY BE CONTAINED IN A SINGLE FUNCTIONAL DOMAIN OF THE RECEPTOR α SUBUNIT**

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We have photolabeled noncompetitive inhibitor (NCI) sites on the nicotinic acetylcholine receptor with [^{3}H]quinacrine azide (QA) [Cox et al. (1985) J. Biol. Chem. 260:7186-7193]. Receptor-rich membrane fragments from Torpedo californica electric tissue were rapidly and continuously mixed with QA and with acetylcholine or hexamethonium. After a delay of 20 ms, the mixture was irradiated for 2 ms with intense light above 290 nm. The receptor subunits were separated by SDS-PAGE and recovered by electroelution. The α subunit was cleaved with CNBr, and the fragments were separated by reverse-phase HPLC and identified by amino acid analysis and sequencing. Specific QA labeling was defined as that both (1) obtained in the presence of acetylcholine but not of hexamethonium and (2) blocked in the presence of other NCIs such as histrionicotoxin, proadifen, chlorpromazine, quinacrine, and bupivacaine. The α subunit contained the predominant site of specific labeling. The dependence of this specific labeling of α on acetylcholine concentration appeared cooperative with a Hill coefficient greater than 1.5. The site of specific labeling was further localized to a 36-residue hydrophobic CNBr fragment, α-208 to α-243, containing the first putative membrane-spanning helix. The region of the NCI binding site is relatively close to Cys 192 and Cys 193 at the acetylcholine binding site [Kao et al. (1984) J. Biol. Chem. 259:11662-11665], and both sites are within a sequence (α-161 to α-239) coded for by a single exon [Noda et al. (1983) Nature 305:818-823]. We suggest that these two sites are contained within a single functional unit of α and are tightly coupled. We suggest further that the NCI site is closely associated with the gate of the receptor channel and is revealed following activation of the receptor by acetylcholine. (Supported by NIH NS07065, MDA, and NY Heart Assoc.)

265.4 RELATIONSHIPS BETWEEN HIGH-AFFINITY BINDING SITES FOR NICOTINIC ACETYLCHOLINE RECEPTOR AGONISTS AND ALPHA-BUNGAROTOXIN ON MEMBRANES FROM RAT BRAIN, TORPEDO ELECTROPLAX, AND THE CLONAL CELL LINES, PC12 AND TE671.

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Controversy exists concerning the relationships between curaremimetic neurotoxin binding sites, high-affinity binding sites for nicotinic acetylcholine receptor agonists, and physiologically functional, neuronal nicotinic acetylcholine receptors (nAcChoR). Curaremimetic neurotoxins, such as alpha-bungarotoxin (Bgt), block the functional responses of some, but not all, neuronal nAcChoR. Binding sites for Bgt do not have the same regional distribution in the mammalian brain as do high-affinity nicotinic binding sites for acetylcholine (ACh). In order to clarify the relationships between these entities, high-affinity binding sites for ^{3}H-labeled ACh (^{3}H-ACh)and ^{125}I-labeled Bgt (I-Bgt) were compared on membrane fractions prepared from rat brain, Torpedo electroplax, or the rat pheochromocytoma or human medulloblastoma clonal cell lines, PC12 or TE671.

d-Tubocurarine-sensitive I-Bgt binding (K_D = 1 nM) to each membrane preparation is fully sensitive to inhibition by ACh with apparent K_I values of 0.1-2 uM. High-affinity ^{3}H-ACh binding (K_D = 10 nM) to Torpedo and TE671 preparations is fully sensitive to blockade (under the appropriate conditions of assay temperature) by Bgt with apparent K_I values on the order of 1 nM. However, only 35% of ^{3}H-ACh binding to rat brain membranes and less than 5% of binding to PC12 preparations is sensitive to blockade by Bgt; half-maximal blockade by Bgt only then occurs at toxin concentrations on the order of 0.1-1 nM. Nevertheless, over 50% inhibition of ^{3}H-ACh binding to rat brain or PC12 cell preparations is effected in the presence of other purified fractions from the venom of Bungarus multicinctus with values of apparent K_I less than 1 uM.

The stoichiometric ratio of I-Bgt binding sites to ^{3}H-ACh binding sites (for concentrations of both radioligands at 10 nM) is near 1 (one) for Torpedo and rat brain preparations, but is about 5 for TE671 cell membranes and 30 for PC12 cell membranes.

These results suggest that high-affinity nicotinic agonist and Bgt binding sites from different tissues are expressed in different proportions and/or have different binding affinities for heterologous ligands. The implications of these results on views of nAcChoR ligand binding site topologies, on postulates of the existence of nAcChoR heterogeneity, and on functional properties of nAcChoR are discussed.

265.5 IDENTIFICATION OF A PUTATIVE ACETYLCHOLINE RECEPTOR ON BOVINE CHROMAFFIN CELLS BY MONOCLONAL ANTIBODIES TO RECEPTOR FROM MUSCLE AND ELECTRIC ORGAN.

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Primary synaptic transmission to bovine adrenal chromaffin cells is mediated by nicotinic acetylcholine receptors (AChRs). Monoclonal antibodies (mAbs) to the "main immunogenic region" (MIR) of AChR alpha subunit from muscle and electric organ identify an antigen on the surface of chromaffin cells in culture that has the properties expected for the neuronal AChR.

Bovine adrenal medulla tissue was enzymatically dissociated into single cells and grown in culture for 1-10 days. The cultures were probed with (^{125}I)mAb 35, an anti-MIR mAb that has been shown to recognize the AChR on chick ciliary ganglion neurons. The amount of binding in chromaffin cultures was proportional to cell number over the range tested (0.25-1.0 x 10^6 cells/16 mm well) and reached saturation within 40 min at 37°. The binding was specific in that it was blocked by other anti-MIR mAbs but not by nonimmune serum or by anti-muscle or electric organ AChR mAbs of the same IgG subclass but different specificity. Scatchard analysis indicated a K_d of about 2 nM for antibody binding. The amount of binding increased with culture age until day 3, reaching a value of about 35 fmol/mg protein which was maintained through at least day 9.

Bgt 3.1, an alpha-neurotoxin that reversibly blocks AChR function on chick ciliary and sympathetic ganglion neurons, has recently been used to confirm the identity of the mAb 35 binding site on the neurons as being the AChR. Preliminary results indicate that bovine chromaffin cells in culture also have a specific binding site for (^{125}I)Bgt 3.1 as do chick ciliary ganglion neurons. Moreover, exposing the chromaffin cultures to 10^{-8} M Bgt 3.1 for 2 hrs at 37° reduces the number of (^{125}I)mAb 35 binding sites by about 25%. The Bgt 3.1 effect can be blocked by co-incubation of the cells with nicotinic cholinergic ligands, as is true for ciliary ganglion neurons. This demonstrates linkage between the mAb 35 sites and Bgt 3.1 sites on the cells as previously shown for AChRs on ciliary ganglion neurons.

These findings suggest that anti-MIR mAbs and Bgt 3.1 will be useful probes for the chromaffin AChR. The adrenal medulla presents opportunities for structural and regulatory studies of the neuronal AChR not possible with the small amounts of receptor present in autonomic ganglia. (Supported by NIH grant NS 12601.)

265.6 **MULTIPLE MUSCARINIC RESPONSES ON THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE RAT**

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When muscarinic agonists are applied to the rat superior cervical ganglion they evoke pharmacologically distinct hyperpolarising and depolarising responses (Newberry et al, (1985) Eur J Pharm 116,191). Their action on the peak amplitudes of the synaptically evoked compound action potential (CAP) and the subsequent hyperpolarisation (AHP, peaking 200-300 ms later) has now been studied. The ganglionic potential was recorded, using a 'greased-gap', from the internal carotid nerve and single stimuli were applied, at 20-30s intervals, to the cervical sympathetic trunk. At submaximal stimulus strengths, carbachol (1μM), muscarine (1μM) and methyl furmethide (0.1-0.3μM) increased the amplitude of the CAP while depolarising the ganglion. A preceding hyperpolarisation and/or an initial reduction in the CAP were sometimes evident. Carbachol and muscarine initially depressed and subsequently enhanced the amplitude of the AHP during the depolarisation. Methyl furmethide, which is selective in that it does not overtly hyperpolarise the ganglion (cf. carbachol and muscarine), simply enhanced the AHP. In the presence of gallamine (10μM), which reduced the AHP itself, the carbachol- and muscarine-induced depolarisations were larger but their depression of the AHP was reduced. Pirenzepine (0.3μM) reduced both the agonist-induced depolarisation and the enhancement of the AHP. The inhibitory action of these compounds on the AHP was hard to demonstrate in pirenzepine since it reduced the AHP more than gallamine.

Muscarinic agonists, therefore, have complex actions on this ganglion and some may be mediated by different receptor subtypes. The actions on the AHP are interesting because it has a similar time course to the calcium-dependent, potassium-mediated hyperpolarisations recorded intracellularly from these cells (McAfee & Yarowsky (1979) J. Physiol., 290 507).

265.7 CHARACTERIZING MUSCARINIC RECEPTOR POPULATIONS IN RAT TRACHEA AND LUNG. H.S. Blaxall*, L.N. Killian*, and R.W. Gardier*, (SPON: J.B. Lucot), Dept. of Pharmacology and Toxicology, School of Medicine, Wright State Univ., Dayton, Ohio 45435.

Parasympathetic innervation of the airway terminates at muscarinic neuroeffector sites on secretory and smooth muscle cells. It would be of particular merit to learn whether there exists a heterogeneity among these populations of muscarinic receptors. Pharmacological differences between these sites will create the potential for new therapeutic approaches to several pulmonary disease states. The non-classical muscarinic antagonist, pirenzepine, divides muscarinic receptors into at least two subtypes, M_1- the high affinity pirenzepine binding site, and M_2- the low affinity pirenzepine binding site (Hammer,R. et al., Nature (London) 283:90, 1980). Previous studies have indicated that gallamine could functionally discern sub-populations of muscarinic receptors in the superior cervical ganglion of the cat (Gardier, R.W., et al., J. Pharmacol. Exp. Ther. 204: 46, 1978). Subsequently, gallamine binding curves in rat striatum determined by displacement of L[^{3}H]- quinuclidinyl benzilate ([^{3}H]-QNB) and obtained by non-linear regression procedure, using SAS Release 79.6, gave a best fit 2-site model, in the presence of agonist (Blaxall, H.S. and Gardier, R.W., Neurosci. Abstracts 8:339, 1982). These multiple binding sites for gallamine were confirmed by a Hill coefficient <1.0. Recent evidence (Cortes, R. and Palacios, J.M., Brain Research 362: 227, 1986) indicates that the gallamine high affinity binding site corresponds to the pirenzepine M_2 receptor site, and that the low affinity gallamine site corresponds to the pirenzepine M_1 receptor site. Thus, the use of these two antagonists can enable a more definitive assessment of the diversity of muscarinic receptors present in extra- and intrapulmonary airways.

Trachea and lungs were removed, following thoracotomy, from anesthetized 500g. male retired breeder Sprague-Dawley rats, after the upper airway was flushed free of blood with cold isotonic sucrose injected via the left heart and drained from the right heart. All tissue was used within 30 days of storage at -80°C. The tissue was divided into three components, trachea, bronchi and bronchioles, and parenchyma. The parenchyma was obtained by placing the lungs on a cold glass plate and by blunt dissection, paring or stripping the soft tissue from the intrapulmonary airway tree. The experimental procedure used for ligand binding was essentially that of Yamamura, S.I. and Snyder, S.H. (Proc. Natl. Acad. Sci. USA 71:1725, 1974). A K_D circa 0.020 nM was determined for all tissue components with the classical antagonist [^{3}H]-QNB, whereas early findings suggest binding of pirenzepine and gallamine to these receptors display non-classical characteristics.

265.8 CHARACTERIZATION OF A CARDIOSELECTIVE MUSCARINIC ANTAGONIST [^{3}H]AF-DX 116 BINDING TO MUSCARINIC RECEPTORS. J-X. Wang,* K. Gulya,* M. Serra,* M. Watson, W.R. Roeske and H.I. Yamamura. Departments of Pharmacology and Internal Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

A novel cardioselective cholinergic antagonist AF-DX 116 (11[2- diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4 benzodiazepine-6-one) was recently synthesized (Chemistry Department at Thomae, W. Germany). We examined its pharmacological and binding profile to muscarinic cholinergic receptors of rat cerebral cortical, cerebellar and heart membranes as well as human neuroblastoma cell (SH-SY5Y) membranes. AF-DX 116 inhibited [^{3}H](-)QNB binding in rat cerebral cortical and heart membranes and SH-SY5Y cell membranes with K_i values of 176 nM, 39 nM and 58 nM, respectively, while it inhibited [^{3}H]PZ binding to putative M_1 sites in rat cerebral cortical membranes and in SH-SY5Y membranes as well with K_i values of 532 nM and 447 nM, respectively. The apparent K_i ratio of [^{3}H](-)QNB(heart)/[^{3}H]PZ (cortex) of 0.073 indicates that AF-DX 116 is cardioselective and a relatively potent inhibitor of putative M_2 class of muscarinic receptors.

[^{3}H]AF-DX 116 was recently custom synthesized (54 Ci/mmole, New England Nuclear). We further evaluated its selectivity towards the cardiac (M_2) subclass of muscarinic receptors by comparing rat cerebral cortical and heart membrane preparations. [^{3}H]AF-DX 116 binding was assayed routinely by a rapid filtration method in different buffered media in the absence (total binding) or presence (nonspecific binding) of 1 µM atropine sulfate at different temperatures. [^{3}H]AF-DX 116 binding (5 nM) to rat cerebral cortical and heart membranes (0.5 % final conc. in both cases) reached steady state within 3 h at 0-4 °C and remained stable up to 24 h at this temperature in both tissues. For inhibition studies 5 nM [^{3}H]AF-DX 116 was routinely used in both cortical and heart membranes. A number of muscarinic agonists (acetylcholine, carbachol, oxotremorine) and antagonists (atropine, pirenzepine, AF-DX 116) inhibited [^{3}H]AF-DX 116 binding in both cerebral cortical and heart membranes consistent with their pharmacologic values. The order of their pharmacological potencies in inhibiting [^{3}H]AF-DX 116 binding in both tissues was atropine sulfate, AF-DX 116, oxotremorine, pirenzepine and carbachol. These studies show that [^{3}H]AF-DX 116 identifies a high affinity muscarinic receptor binding site in the cerebral cortex and heart membranes and labels a subset of [^{3}H](-)QNB labeled sites. Supported by USPHS grants.

265.9 DIFFERENCES IN THE THERMODYNAMIC PROPERTIES OF PIRENZEPINE BINDING TO MUSCARINIC RECEPTORS IN MEMBRANES AND DIGITONIN-SOLUBILIZED PREPARATIONS FROM RAT BRAIN AND HEART.

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The thermodynamic properties of pirenzepine (PZ) binding to muscarinic cholinergic receptors (MAChR) in membranes and digitonin-solubilized preparations from rat brain and heart were studied.

Pirenzepine bound with high and low affinities to MAChR in rat brain membranes. The dissociation constants, measured from PZ/[^{3}H](-)QNB competition experiments, were 5.3 nM, 13 nM and 24 nM for the high affinity sites (M_1), and 120 nM , 200 nM and 220 nM for the low affinity sites (M_2) at 4°C, 25°C and 37°C, respectively. The ratio of the high and the low affinity sites (70% vs 30%) was not altered by changing incubation temperatures. The K_D values for heart membrane MAChR were 280 nM, 400 nM and 430 nM at 4°C, 25°C and 37°C, respectively.

Van't Hoff analysis of the data indicated that the driving force for PZ binding to M_1 receptors in the brain was mainly from an exothermic change of enthalpy ($\Delta H = -8.0$ Kcal·mol^{-1} and $T\Delta S = 2.8$ Kcal·mol^{-1}, at 4°C). In contrast, PZ's binding to the brain M_2 receptors was found under the predominant control of entropy ($\Delta H = -3.0$ Kcal·mol^{-1} and $T\Delta S = 5.51$ Kcal·mol^{-1}, at 4°C). Similar results were obtained for the binding of PZ to heart M_2 receptors ($\Delta H = -2.5$ Kcal·mol^{-1} and $T\Delta S = 6.0$ Kcal·mol^{-1}, at 4°C).

After being isolated from the membranes by solubilization with 0.5% digitonin, K_D values were 8.5 nM, 30 nM and 75 nM in the brain and 10 nM, 50 nM and 70 nM in the heart at 4°C, 25°C and 37°C, respectively. The negative Gibbs free energy for the binding of PZ in both tissues resulted from the change in enthalpy and the change in entropy became unfavorable ($\Delta H = -11$ Kcal·mol^{-1} in both tissues ; $T\Delta S = -9.0$ and -1.0 Kcal·mol^{-1} in the brain and heart, respectively, at 4°C).

The present studies suggest that different molecular interactions are involved in the binding of PZ to putative M_1 and M_2 receptors in rat brain and heart membranes, which may implicate a topographical difference in the ligand binding sites or in the vicinity of the receptors in these two tissues. Solubilization altered the change in entropy and considerably augmented the negative change in enthalpy for PZ binding in both tissues, indicating an important contribution of receptor-associated membrane components to the heterogeneous drug-receptor interactions in these two tissues. Supported by USPHS grants.

265.10 CHARACTERIZATION OF THE MUSCARINIC BINDING SITE HETEROGENEITY REVEALED BY PIRENZEPINE AND N-METHYLSCOPOLAMINE IN RAT BRAIN. Andrew B. Norman*, James H. Eubanks*, Scott N. Blaker* and Ian Creese.
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The novel muscarinic receptor antagonist pirenzepine discriminates multiple muscarinic receptor binding sites homogeneously labeled by [3H](-)QNB in brain. These binding sites have been proposed to represent distinct muscarinic receptor subtypes termed M1 and M2 with respective high and low affinity for pirenzepine. The quaternary muscarinic antagonist (-)N-methylscopolamine ((-)NMS) has also been reported to discriminate multiple [3H](-)QNB binding sites in rat brain. We investigated whether (-)NMS and pirenzepine discriminate the same putative muscarinic receptor subtypes.

The protein modifying reagent N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) acts as an irreversible antagonist of [3H]-(-)QNB binding in rat brain. Following administration of EEDQ (20 mg/kg i.p.) which reduced the Bmax of [3H](-)QNB by 56% in rat cerebral cortex, the competition of pirenzepine for the remaining [3H](-)QNB binding sites revealed an increase in the proportion of lower affinity pirenzepine binding sites. Similarly, (-)NMS discriminates two [3H](-)QNB binding sites in homogenates of cortex and there was an increase in the proportion of [3H](-)QNB binding sites having lower affinity for (-)NMS in tissue from EEDQ-treated rats. Thus, EEDQ acts as a selective antagonist of [3H](-)QNB binding sites having high affinity for both pirenzepine and (-)NMS. Competition of pirenzepine for [3H](-)NMS binding fits to a model assuming two binding sites which have similar affinities for pirenzepine as do those sites labeled by [3H](-)QNB. Thus, (-)NMS does not discriminate the putative M1 and M2 muscarinic receptor subtypes discriminated by pirenzepine. Propylbenzilylcholine mustard (PBCM) also acts as an irreversible antagonist at muscarinic receptors. Treatment of homogenates of rat cerebral cortex with 5 nM PBCM produced an irreversible 60% decrease in the Bmax of [3H](-)QNB binding. The competition of pirenzepine for the remaining [3H](-)QNB binding sites demonstrated that the proportions of the high and low affinity pirenzepine binding sites were unaltered. In contrast, there was a greater proportion of [3H](-)QNB binding sites having low affinity for (-)NMS in the PBCM-treated cortex. Thus, PBCM selectively alkylates [3H](-)QNB binding sites having high affinity for (-)NMS but does not discriminate multiple [3H](-)QNB binding sites having differential affinity for pirenzepine. Interestingly, both (-)NMS and the active form of PBCM possess a positively charged nitrogen moiety and the physiochemical properties may determine the apparent subtype selectivity of these two compounds. Therefore, in homogenates of rat brain there appear to be [3H](-)QNB binding sites having three distinct properties.

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265.11 PROPERTIES OF MUSCARINIC ACETYLCHOLINE RECEPTORS ON INTACT CEREBRAL NEURONS FROM PRIMARY CULTURES OF CHICK EMBRYO BRAIN. M.H. Jalilian Tehrani*, C.L. Thompson*, and E.M. Barnes, Jr. Dept. of Biochemistry, Baylor Coll. of Med., Houston, TX 77030.

The biochemical and morphological characteristics of primary cultures of neurons from the chick embryo cerebrum are similar to those in vivo [Thampy et al. (1983), Dev. Brain Res. 8, 101-110]. Since these preparations contain few non-neuronal cells, monolayer cultures represent an open, experimentally accessible system for investigation of the biochemical, biophysical, and pharmacological properties of cerebral neurons. The expression of muscarinic acetylcholine receptors on intact living neurons has been studied by the binding of [^{3}H]-N-methylscopolamine ([^{3}H]NMS), a membrane-impermeant muscarinic antagonist, to cell monolayers after 8 days in culture. Scatchard analysis of [^{3}H]NMS binding to intact neurons revealed a single binding site with a dissociation constant (K_d) value of 0.11 nM. This value was quite similar to those obtained from crude membrane preparations of cultured neurons (0.25 nM), embryonic brain (16 days in ovo, 0.11 nM), or adult brain (0.17 nM). However, the densities of [^{3}H]NMS binding sites (B_{max} values) on intact neurons (0.14 pmol/mg cellular protein) or crude membranes derived therefrom (0.21 pmol/mg) were lower than those estimated for embryonic (0.48 pmol/mg) or adult (0.57 pmol/mg) brain membranes. From the in situ value, an estimate of 22,000 surface binding sites for [^{3}H]NMS was derived for an average cultured neuron. If crude membranes were used for similar estimates, the number of binding sites per cell was 20,000. The density of [^{3}H]NMS binding sites on cerebral neurons was more than 4 times that reported for N1E-115 murine neuroblastoma cells [Liles and Nathanson (1986) J. Neurochem. 46, 89-95].

The muscarinic antagonists, atropine and pirenzepine, displaced [^{3}H]NMS binding from intact neurons in an apparently monophasic manner with IC_{50} values of 1.5 nM and 280 nM, respectively, while the IC_{50} for gallamine was greater than 10 µM. The cholinergic agonists, carbachol, pilocarpine, and oxotremorine, also displaced [^{3}H]NMS binding with IC_{50} values of 14 µM, 13 µM, and 1.2 µM, respectively. The Hill coefficients for all three agonists were below unity. These results indicate that significant numbers of pharmacologically relevant muscarinic receptors are expressed during development of cerebral neurons in vitro.

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CARDIOVASCULAR REGULATION: AGONISTS, RECEPTORS AND POST-GANGLIONIC

266.1 SIMILARITY BETWEEN CLONIDINE AND METHIONINE ENKEPHALIN ON THE INHIBITION OF RABBIT RENAL NERVE ACTIVITY. H.M. Rhee. Department of Pharmacology, Oral Roberts University School of Medicine, Tulsa, OK 74137

Hypotensive effect of methionine enkephalin (Met-enkephalin) is well established in many anesthetized laboratory animals after an intravenous injection of the peptide. Little is known as to its hypotensive mechanisms and site(s) of action of the peptide. In an effort to understand the hypotensive action of the peptide, effects of met-enkephalin and clonidine were compared on the rate of sympathetic nerve discharge. White New Zealand rabbits (1.5 - 2.0 kg) were anesthetized (pentobarbital 30 mg/kg) and instrumented for the measurement of blood pressure and heart rate. Since the dominant nerve activity is sympathetic in renal nerve of this species as reported previously (Rhee et al., J. Pharmacol. Exp. Ther. 234:534-537, 1985), multiunit renal nerve activity (RNA) was determined with the treatment of either met-enkephalin or clonidine. Blood pressure was processed to obtain systolic, mean and diastolic pressures and RNA was integrated to quantitate numerically.

Met-enkephalin suppressed RNA in peptide dose dependently (3 to 30 µg/kg, i.v.) in parallel with the reduction of blood pressure and heart rate. The peptide not only reduced RNA (the number of integration per sec), but also increased the duration of RNA suppression. Clonidine (2 to 20 µg/kg, i.v.) increased blood pressure initially for 3 to 5 seconds, which was followed by a profound, but gradual reduction of blood pressure and heart rate. At a same time clonidine reduced significantly the rate of RNA discharge (number of integrated RNA per sec) as the case of met-enkephalin. At the high dose, clonidine increased the duration of RNA suppression as the manner that met-enkephalin did. There was no apparent synergistic or antagonistic intereaction between the two drugs in this species. The similiarity between clonidine and met-enkephalin in the suppression of RNA in parallel with the hypotensive and bradycardiac action of the drugs suggests that the mechanism of met-enkephalin action is central activation of α_2 adrenergic receptors.

266.2 **POLYCLONAL ANTISERUM TO A PARA-AMINOCLONIDINE CONJUGATE RECOGNIZES AN ENDOGENOUS CLONIDINE-LIKE SUBSTANCE(S) IN BRAIN.** M.P. Meeley, A.C. Towle*, L.K. Char*, P. Ernsberger and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine, a synthetic imidazole, acts to lower arterial pressure (AP) within the rostral ventrolateral medulla (VLM) (Granata et al., Brain Res., 1986 in press). An endogenous clonidine-displacing substance (CDS) has been isolated from bovine brain (Atlas and Burstein, Eur. J. Biochem., 144 287, 1984). Recently, we have shown that partially-purified CDS 1) potently displaces the clonidine analog ^{3}H-para-aminoclonidine (^{3}H-PAC) from bovine VLM membranes, 2) competes with ^{3}H-PAC for binding at imidazole sites as well as alpha$_2$-adrenergic receptors in VLM, and 3) lowers AP when microinjected into the rostral VLM of the rat (Meeley et al., Life Sci. 38 1119, 1986). We sought to produce antibodies to a PAC-conjugate and to determine whether these antibodies recognize endogenous CDS(s) in brain.

Polyclonal antibodies were raised in adult male Sprague-Dawley rats immunized with PAC coupled to limulus hemocyanin (coupling efficiency, 40%). Rats were injected weekly with PAC-conjugate (35 mg/kg, i.p. and s.c., as 1:1 emulsion with complete Freund's adjuvant) over 4 weeks. Immunoglobulins were precipitated from serum using 50% ammonium sulfate. The binding properties of the PAC antiserum obtained (a-PAC) were examined by incubating in 50 mM Tris-HCl (pH 7.4) with ^{3}H-PAC at 25°C for 1 hr, then 2 hr at 4°C. The reaction was terminated by adding carrier bovine gamma globulin (0.125%) and 7.5% polyethylene glycol to precipitate, followed by rapid filtration. Nonspecific binding was defined using 20 uM unlabeled PAC. Specific binding of ^{3}H-PAC to a-PAC (1:500 dilution) comprised 71 ± 6% of the total binding (at 0.6 nM ^{3}H-PAC; n=3), was saturable and of high affinity. a-PAC exhibited two binding sites with different affinities for ^{3}H-PAC which presumably correspond to at least two antibodies in the serum (K_{DH}=0.8±.3 nM, K_{DL}=348±1000 nM, n=3, LIGAND program). Competition against 1nM ^{3}H-PAC showed that both PAC and clonidine had similar affinities for a-PAC (IC_{50}=3.9±.4 and 4.2±2.7 nM, respectively). Binding of a-PAC was specific: cross-reactivity was not observed (IC_{50}>1 mM) with the transmitters norepinephrine, epinephrine, histamine, GABA, TRH, nor with several imidazole compounds. Naphazoline, a naphthylenyl imidazole, inhibited ^{3}H-PAC binding, but at much higher concentrations (IC_{50}=19±2 µM). CDS, partially-purified from bovine brain (Meeley et al., op. cit.), also inhibited specific ^{3}H-PAC binding to a-PAC (IC_{50} approx. 3.7 Units), but did not affect nonspecific binding.

We conclude that 1) antibodies can be produced in rats against a PAC-conjugate which are of high affinity and specific for PAC and for clonidine itself; 2) a-PAC binds strongly to component(s) in brain extracts containing CDS; and 3) a-PAC may recognize endogenous clonidine like substance(s) in brain thought to participate in brainstem cardiovascular control.

266.3 RAPID DESENSITIZATION OF THE CENTRAL PRESSOR ACTION OF LY171555, A SPECIFIC DOPAMINE D2 RECEPTOR AGONIST, IN THE CONSCIOUS RAT. Y.Igarashi*, Y.F.Chen, J.M.Wyss and S.Oparil, Hypertension Research Program, University of Alabama at Birmingham, AL 35294

Previous studies in our laboratory have demonstrated that intravenous (iv) bolus injection of the specific dopamine (DA) D2 receptor agonist, LY171555 (LY), increases mean arterial pressure (MAP) in conscious Sprague-Dawley (SD) rats through a central DA mechanism and that the central pressor effect of LY can mask the peripheral depressor actions of the drug. To further investigate the contributions of central and peripheral D2 receptors to the regulation of cardiovascular function, cardiovascular responses to sustained infusion of LY and to sustained infusion of LY followed by bolus injections of LY were examined in conscious, unrestrained SD rats. Continuous iv infusion of LY (50μg/50μl/kg/min) for 120 min induced a transient (<60min) pressor response followed by normalization of MAP. A higher dose (100μg/50μl/kg/min) of LY caused a more rapid and abbreviated pressor response, followed by a depressor response and a compensatory increase in heart rate. An iv bolus injection of LY (1-4 mg/kg) 30 min after the end of the infusion of LY at either concentration, induced dose-dependent depressor responses which were blocked by pretreatment with domperidone, a peripheral D2 antagonist. Immediately following 120 min of the low concentration (50μg/kg/min) LY infusion, plasma norepinephrine and epinephrine were significantly elevated above the levels present in rats which were infused with saline.

Table I

	ΔMAP (mmHg), infusion time			
	15 min	30 min	60 min	120 min
Saline (n=6)	0.5±0.7	0.5±1.5	1.3±3.8	-2.1±1.5
LY(50μg/kg/min) (n=10)	13.3±4.0**	19.2±4.0**	4.1±3.7	0.3±3.0
LY(100μg/kg/min)(n=10)	16.9±2.1**	4.7±1.6*	-2.8±2.2	-10.3±1.7**

*p<0.05, **p<0.01, compared to basal value. ++p<0.01, compared to saline control group.

Table II

	ΔMAP (mmHg), dose of LY injection		
	1mg/kg	2mg/kg	4mg/kg
with domperidone pretreatment(n=6)	3.2±0.3**	4.0±0.4**	7.4±0.6**
without domperidone pretreatment(n=10)	11.9±0.8	18.0±1.1	23.3±2.1

**p<0.01, compared to without domperidone pretreatment group.

We conclude that infusion of LY produces only a transient centrally mediated pressor effect and that the peripheral depressor action of LY on D2 receptor is expressed following desensitization of central D2 receptors by continous infusion. Thus central D2 receptors appear to be pressorergic, whereas peripheral D2 receptors are associated with depressor responses.

266.4 EFFECTS OF SINOAORTIC DENERVATION OR NODOSECTOMY ON ANGIOTENSIN II RECEPTOR EXPRESSION IN THE SOLITARY-VAGAL AREA OF THE RAT. D.P. Healy, R. Rettig, T. Nguyen* and M.P. Printz. Department of Medicine, M-013H, University of California-San Diego, La Jolla, CA, 92093.

The solitary-vagal area (SVA) receives primary baroreceptor afferents from the carotid sinus and aortic arch and gives rise to the efferent outflow of the baroreceptor reflex. Injections of angiotensin II (ANG II) directly into the solitary-vagal area of the rat elicit moderate dose-dependent changes in arterial pressure and heart rate (Rettig et al. Brain Res. 364:233, 1986) and inhibit the baroreceptor reflex (Casto and Phillips, Am. J. Physiol. 250:R193, 1986). In addition, the SVA contains both ANG II-like immunoreactive neurons and ANG II receptors (Healy et al., Clin. Exp. Hyperten. A6:1743, 1984). These lines of evidence suggest that an endogenous ANG II neuropeptide system within the SVA may modulate baroreceptor activity. To gain further insight into the relationship between baroreceptor afferents and the endogenous ANG II systems within the SVA, we determined whether ANG II receptors within the SVA were altered following either selective disruption of the baroreceptor afferent input by sinoaortic denervation or complete disruption the vagal afferent and efferent fibers following nodose ganglionectomy (anterograde afferent and retrograde efferent degeneration). Changes in ANG II receptor distribution, density or affinity were analyzed by quantitative in vitro autoradiography. Adult male Sprague-Dawley rats were bilaterally sinoaortic denervated (SAD) in a two-step operation under pentobarbital anesthesia; unilateral denervation with a 7 day survival period followed by contralateral denervation. Additional sets of rats were either unilaterally nodosectomized (NDX) or sham operated. One week following surgery the animals were processed for quantitative in vitro autoradiography using [125I]-sar1,ile8-ANG II and iodinated standards. SAD rats exhibitted a small but significant decrease (15%) in ANG II receptor density within the medial nuclues tractus solitarius (NTS) and dorsal motor vagal nucleus (DMV). In contrast, NDX rats exhibitted a marked decrease in ANG II receptor density ipsilaterally at all levels of the NTS (40%) and the DMV (60%). ANG II receptors within the area postrema were unchanged. These results indicate that ANG II receptors may be localized on some portion of both baroreceptor and general visceral vagal afferent fibers to the NTS and on vagal efferent neurons of the DMV. Moreover, these results suggest that ANG II systems within the SVA are not associated exclusively with baroreceptor afferents, but rather may play a more generalized role in modulating vagal afferent input. (Supported by HL-25457.)

266.5 IONIC CURRENTS OF ISOLATED NEURONS OF SOLITARY TRACT NUCLEUS. D.L. Kunze. Dept. Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

Cells were isolated from the medial and dorsal nuclei of the solitary tract of the medulla of adult guinea pigs in order to examine the electrical properties of neurons in an area which is involved in the control of arterial pressure and heart rate. Predominantly bipolar cells of approximately 10 μ diameter were obtained upon enzymatic dispersion using papain or trypsin. The ionic currents of the cells were studied using the patch technique for whole cell recording. Approximately one third of the cells exhibited spontaneous activity in normal tyrodes solution in voltage recordings. A TTX sensitive Na current, inwardly rectifying, delayed rectifying and calcium-activated potassium currents were identified. Of particular interest were the calcium currents. Action potentials elicited by depolarizing voltage pulses in the presence of 10^{-5} TTX were eliminated by the addition of 1 mM Cd to the bath and thus appeared to be calcium mediated. In all cells studied, two voltage dependent components to the calcium current were identified. In 10 mM Ca a high threshold component was activated at approximately -20 to -10 mV from holding potentials of -30 mV. A second lower threshold component was activated at about -40 mV from more negative holding potentials of -80 mV. The low threshold component was rapidly inactivating while the high threshold current was slowly or non inactivating. The peak amplitudes of the two components were similar and occurred at -10 mV for the low threshold channel and +10 mV for the high threshold channel in 10 mM Ca. Both components were blocked by 1 mM Cd. Both components were present in the soma. A role for the low threshold current in generating repetitive activity in NTS neurons is postulated. Supported by AHA - Texas Affiliate.

266.6 CHOLINERGIC MECHANISMS IN ROSTRAL VENTROLATERAL MEDULLA (RVL) PARTICIPATE IN THE TONIC CONTROL OF ARTERIAL PRESSURE (AP) AND MEDIATE VASOPRESSOR ACTION OF SYSTEMICALLY ADMINISTERED PHYSOSTIGMINE (PS). R. Giuliano*, P. Ernsberger, V. Arango, D. Ruggiero and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

Both functional (JPET 231: 457, 1984; SNA 11: 491, 1985) and neurochemical (Fed. Proc. 45: 3422, 1986) evidence support cholinergic neurotransmission in an area of the RVL containing epinephrine neurons (C1 area). We sought to determine whether cholinergic mechanisms in the RVL (1) involve local neurons and muscarinic receptors, (2) participate in the tonic regulation of AP and (3) mediate the pressor effect of systemic PS. Choline acetyltransferase (ChAT) was immunocytochemically localized to perikarya and varicose processes in this region, with perikarya predominantly localized to the retrofacial-ambiguus complex and medial aspect of the RVL adjacent to the ventral surface. Muscarinic binding sites in RVL labeled with ^{3}H-quinuclidinyl benzylate (^{3}H-QNB) were identified autoradiographically and quantified by image analysis. ^{3}H-QNB binding was corrected for autoradiographic efficiency, determined by a novel method, and expressed as % of grey matter value. Corrected ^{3}H-QNB binding values indicate that the RVL contains a substantial concentration of muscarinic receptors (145±7 fmol/mg). In comparison the n. hypoglossi was highest (234±11), the nucleus tractus solitarii intermediate (159±7) and the pyramidal tract lowest (23±2). In anesthetized (urethane), paralyzed (tubocurarine) and ventilated rats (n=10) PS (100μg/kg, i.v.) evoked an increase in AP (46±4 mm Hg) which was reversed by bilateral electrolytic lesions of the C1 area or local microinjection of GABA (10 nmol/100 nl). Bilateral electrolytic lesions or administration of kainic acid into the C1 area (1 nmol/100 nl) prevented the pressor response to PS (n=12). Bilateral injection of scopolamine (3 nmol/100 nl), but not hexamethonium (3 nmol/100 nl), into the same area, though not into the raphe region, aborted (mmHg: from 130±8 to 102±12, $p < 0.05$) and blocked (19% of control, $p < 0.05$) the pressor effect of PS (n=12). Local pretreatment by microinjection of hemicholinium-3 (HC-3) (3 nmol/100 nl) to deplete C1 area of acetylcholine (n=6) substantially attenuated the hypertension elicited by PS at 100μg/kg (mmHg: 15±4 vs. 57±9, $p < 0.01$) or 1000 μg/kg (mmHg: 47±9 vs. 79±4 mmHg, $p < 0.05$). Resting mean AP fell significantly after bilateral microinjection of scopolamine (24±2 mmHg) or HC-3 (22±5 mmHg), but not after local administration of vehicle (0.9% NaCl, pH 7.4). Pressor responsiveness to L-glutamate microinjection was preserved.

We conclude that 1) intrinsic cholinergic neurons are present in the RVL; 2) the C1 area of the RVL contains a high density of muscarinic receptors; 3) release of acetylcholine within the C1 area contributes to maintenance of resting AP, and 4) the C1 area is a site of action of the hypertension elicited by systemically administered PS.

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266.7 CENTRAL INFUSION OF ALDOSTERONE (ALDO) SELECTIVELY REDUCES THE PRESSOR RESPONSE PRODUCED BY INTRAVENTRICULAR ARGININE-VASOPRESSIN (AVP). P. Janiak, R.L. Dundore and M.J. Brody. Dept. of Pharmacology and CV Center, Univ of Iowa, Iowa City, IA 52242.

Although mineralocorticoid binding sites have been demonstrated in brain, little is known about their physiological role. The purpose of this study was to evaluate the interaction between ALDO and centrally-acting pressor agents. Two cannulae were implanted in the lateral ventricles of male Sprague-Dawley rats (300-400g). The femoral artery and vein were catheterized for pressure measurement and drug administration. On day one (D1), mean arterial pressure (MAP) and heart rate (HR) were recorded in conscious rats during IVT injection of AVP (400ng), angiotensin II (ANG II 150ng) and carbachol (CAR 150ng). An osmotic minipump was then implanted for IVT infusion of ALDO (5ng/µl/hr:a dose that approximates 5% of daily adrenal secretion) or vehicle. Two days later (D3), the agents were repeated and the minipump was removed. On day 5 (D5), the responses were studied again. Central infusion of ALDO did not alter resting MAP or HR. Results are summarized in the following table:

		Increase in MAP (mm Hg)		
		Day 1	Day 3	Day 5
ALDO (n=7)	AVP	45 ± 11	9 ± 3 *	38 ± 9
	ANG II	22 ± 4	20 ± 4	14 ± 2
	CAR	16 ± 5	17 ± 6	15 ± 3
VEHICLE (n=6)	AVP	38 ± 7	79 ± 15	89 ± 19
	ANG II	20 ± 6	22 ± 5	24 ± 6
	CAR	16 ± 5	17 ± 6	15 ± 3

* -Significant difference from both day 1 in ALDO group and day 3 in vehicle group.

ALDO significantly decreased the pressor response to AVP without altering the responses to ANG II and CAR. After removal of ALDO, the pressor response to AVP returned to the pretreatment level. In contrast, the response to AVP was enhanced on D3 and D5 in the vehicle treated group. In a separate experiment, the effect of ALDO on graded doses of centrally administred AVP and ANG II was evaluated along with the responses to intravenously (IV) injected AVP. The selective depression of the pressor effect of AVP was significant at all doses (10,33,100 ng). The action could not be attributed to depressed cardiovascular reactivity since the pressor action of IV AVP was unchanged.

These data indicate that continuous central infusion of ALDO can selectively attenuate the centrally mediated pressor effect of a wide range of doses of AVP. Interactions between ALDO and AVP may be involved in central regulation of cardiovascular function.
(Supported in part by HLB-14388 and Roussel-UCLAF).

266.8 SYSTEMIC AND REGIONAL HEMODYNAMIC EFFECTS OF THYROTROPIN RELEASING HORMONE IN CONSCIOUS RATS. A.-L. Siren*, #C.R. Lake and G. Feuerstein. Neurobiol. Res. Div., Dept. Neurol. and #Dept. Psychiat., USUHS, Bethesda, MD 20814.

The hemodynamic and neural mechanisms underlying the pressor and tachycardic responses to intracerebroventricularly (icv) administered thyrotropin releasing hormone (TRH) were studied in conscious rats (300-340g) chronically instrumented for cardiac index (CI, thermodilution) or blood flow (BF, directional, pulsed Doppler method) measurements. TRH (0.008-8 nmol/kg icv) induced rises of mean arterial pressure (MAP), heart rate (HR) and CI but had no effect on total peripheral resistance index. The BF in hindquarters (HQ) increased while in mesenteric (M) and renal (R) arteries TRH icv produced vasoconstriction. All the effects of TRH were dose-related. More than 1000-fold of the icv dose was needed to produce comparable hemodynamic responses by TRH iv.In sinoaortic denervated (SAD) rats which displayed labile high blood pressure and complete lack of reflex bradycardia to phenylephrine, all the reported effects of TRH icv were markedly potentiated. Elevated circulating levels of catecholamines were found during the pressor/tachycardic response to TRH, whereas plasma renin activity or vasopressin was not effected by TRH. Further evidence for a role of sympathoadrenomedullary system in TRH effects were provided by the findings that phentolamine ($\alpha_1+\alpha_2$ adrenoceptor blocker) blocked the pressor and M vasoconstrictor effects of TRH icv and unmasked a R vasodilatation. The β-blocker propranolol partly blocked the cardiotonic and HQ vasodilator actions of TRH icv. In bilaterally adrenal demedullated rats (ADM-x) the rises of MAP, HR and CI produced by TRH icv were attenuated and the R vasoconstriction abolished while further treatment of the ADM-x with the sympathetic blocker bretylium produced a more complete blockade of all TRH actions. However, a residual TRH response was allways present in ADM-x though TRH had no effect on plasma catecholamines in these rats. Unilateral renal denervation had no effect on the R vasoconstriction by TRH. The present findings provide evidence which together with the neuroanatomical and biochemical data(Sharif, TIPS 6:119-121,1985)support a potential role for TRH in the central cardiovascular control. Our data also suggest a differential involvement of adrenal medulla and sympathetic nerves in TRH actions and call for a role of adrenal medulla in the regulation of RBF.

266.9 EFFECT OF AFFERENT RENAL NERVE STIMULATION ON PLASMA VASOPRESSIN LEVELS. M.M. Caverson and J. Ciriello. Dept. of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Recent electrophysiological studies from our laboratory have shown that single units in the paraventricular nucleus of the cat antidromically activated by stimulation of the neurohypophysis (NH) are excited orthodromically by stimulation of afferent renal nerves (ARN; Can. Physiol., 16:159, 1985), and that putative vasopressinergic and not oxytocinergic magnocellular neurosecretory neurons in the rat supraoptic nucleus are excited by ARN stimulation (Am. J. Physiol., 249:R368, 1985). These data suggest that afferent information originating from renal receptors contributes to neural mechanisms controlling the release of vasopressin (AVP) from the NH. The present study was done to determine whether stimulation of ARN alters circulating plasma levels of AVP. Experiments were done in sinoaortic denervated, vagotomized, chloralose anesthetized, paralyzed and artificially ventilated cats. In the first series of experiments, stimulation of ARN with 10 s trains of pulses (2 ms pulse duration, 500 µA current intensity and frequencies ranging from 10 to 100 Hz) elicited increases in mean arterial pressure (MAP). The MAP response had 2 components; a primary (1°) component which was locked in time to the stimulation period (ΔMAP, +48.7 ± 11.2 mmHg) and a secondary (2°) component which reached its peak after cessation of stimulation (ΔMAP, +45.2 ± 13.4 mmHg). The optimal frequency to elicit the 2° component of the pressor response was found to be 40 Hz. In the second series of experiments, some of the mechanisms involved in the pressor response were investigated. Administration of hexamethonium (30 mg/kg, i.v.) completely abolished the 1° component but did not significantly alter the 2° component of the pressor response. On the other hand, administration of the AVP vasopressor antagonist (AVP_a) d(CH_2)5VAVP (500 ng/kg, i.v.) significantly attenuated the 2° component of the pressor response by 49-72%. In the final series of experiments, the effect of ARN stimulation on plasma levels of AVP was determined. Blood samples were taken 30 min before stimulation and 2 min during stimulation of ARN. All blood samples removed were replaced with an equal volume of 0.9% saline. Blood samples were placed in tubes containing EDTA, centrifuged for 5 min at 3000 g and the plasma was stored at -70°C until assayed for AVP by radioimmunoassay. Plasma levels of AVP were significantly increased from control values of 4.9 ± 2.0 pg/ml to 42.2 ± 17.5 pg/ml during stimulation of ARN. These data demonstrate that afferent information from renal receptors contributes to a reflex pathway by which the kidney may alter the release of AVP from the NH to influence circulatory and body fluid homeostasis.
(Supported by the Heart & Stroke Foundation of Ontario).

266.10 EFFECT OF SYMPATHETIC PREGANGLIONIC STIMULATION ON THE PATTERN OF NEUROPEPTIDE IMMUNOREACTIVITY IN THE STELLATE GANGLION. M. Bachoo*, J. Ciriello, and C. Polosa*. Dept. of Physiology, Univ. of Western Ontario, London, Canada and Dept. of Physiology, McGill University, Montreal, Canada.

We have recently demonstrated that in the presence of the cholinergic antagonist hexamethonium and methylatropine stimulation of the axons of sympathetic preganglionic neurons (SPN) elicited a slow onset cardioacceleration which reached a peak value of 60 to 90% of the peak response obtained in the absence of the cholinergic antagonists. In addition, with continuous stimulation it was observed that the magnitude of the cardioacceleration decreased by 90% in approximately 1 hr. To determine which neuroactive substances contained in SPN may be involved in mediating these effects, experiments were done in pentobarbital anesthetized cats. After the i.v. administration of hexamethonium (30 mg/kg followed by supplements) and methylatropine (1 mg/kg), the pattern of neurotensin-(NT), leucine-enkephalin-(Leu-ENK), substance-P-(SP), somatostatin-(SS) and corticotropin releasing factor-(CRF) like immunoreactivity in the decentralized right stellate ganglion (SG) was analyzed in control conditions (no stimulation) and after 2 hr of 40 Hz supramaximal stimulation of either the preganglionic input to the SG (T_1 and T_2 white rami and sympathetic trunk below T_2) or the postganglionic output (ventral ansa subclavia). Animals were perfused with Zamboni's fixative and 20 µm frozen transverse sections of the SG were placed into antisera for each peptide and processed according to the biotin-avidin immunohistochemical procedure. In the unstimulated SG a dense collection of fibers and presumptive terminals immunoreactive to NT and Leu-ENK were observed. In addition, sparse fibers and presumptive terminals immunoreactive to SP, SS and CRF were found. After preganglionic stimulation, the immunoreactivity to NT and Leu-ENK disappeared in the SG, while that to SP, SS and CRF was unchanged. In contrast, the pattern of neuropeptide immunoreactivity in the SG did not change after postganglionic stimulation compared to control conditions. The loss of NT and Leu-ENK in the SG suggests that these two peptides are released from SPN terminals and their releasable pools are depleted by prolonged stimulation. The association of loss of immunoreactivity to NT and Leu-ENK with the loss of non-cholinergic cardioacceleration suggests that these peptides are involved in the non-cholinergic cardioacceleratory response.
(Supported by Heart and Stroke Fdn. of Ontario, Quebec Heart Fdn. and MRC of Canada).

266.11 **CHARACTERISTICS OF ONGOING AND REFLEX DISCHARGE OF SINGLE RENAL AND SPLENIC SYMPATHETIC POSTGANGLIONIC FIBERS.** R. Meckler and L. Weaver. Dept. of Physiol., Mich. St. Univ., E. Lansing, MI 48824.

Stimulation of visceral afferent nerves can produce unequal responses in multifiber recordings of splenic and renal nerve activity. Splenic nerve excitation exceeds that of renal nerves in response to stimulation of splenic receptors. Conversely, activation of arterial and cardiopulmonary pressor receptors often results in more inhibition of renal than splenic nerve discharge. However, intestinal receptor stimulation causes similar magnitudes of excitation in activity of both nerves. Since multifiber recordings obscure the responses of individual neurons, there may be heterogeneity of responses within any given population of neurons. This study was done to assess the following characteristics of activity of single renal and splenic neurons: 1) correlation of ongoing discharge with phrenic nerve activity (index of central respiratory drive), 2) correlation of ongoing activity with peak systolic arterial pressure (index of relationship to baroreceptor activity), 3) reflex changes in activity elicited by chemical stimulation of intestinal receptors or splenic receptors, and 4) reflex changes in activity caused by stimulation or "unloading" of baroreceptors. In chloralose-anesthetized, artificially respired cats, small fibers were teased from the central cut ends of renal and splenic nerves and were placed on bipolar platinum electrodes for recording electrical activity. Serosal receptors of the small intestine were stimulated by application of 10 μg bradykinin. Splenic receptors were stimulated by splenic arterial injection of 10 μg bradykinin. Baroreceptors were unloaded by i.v. nitroprusside and were stimulated by i.v. phenylephrine. Ongoing discharge rates of renal and splenic axons were similar (range = 1 to 7 spikes/s, mean = 3.4 ± 0.3 spikes/s). Ongoing activity of renal fibers was more strongly correlated with pulse pressure than was that of splenic neurons. Subpopulations of fibers within splenic and renal nerves have activity correlated with peak integrated phrenic nerve discharge. Baroreceptor stimulation or unloading evoked greater changes in activity of renal neurons than that of splenic neurons. The average excitatory responses of these two populations of neurons to reflexes of intestinal origin were similar (increases of 3 ± 0.4 spikes/s) despite pressor responses (increases of 24 ± 3 mmHg). Preliminary results suggest that excitation of splenic neurons by splenic receptor stimulation exceeds that caused by intestinal receptor stimulation. In contrast, renal neurons respond similarly to either visceral stimulus. Whereas excitation of splenic neurons is greater than that of renal neurons in response to splenic receptor stimulation, intestinal receptor stimulation causes similar magnitudes of excitation in renal and splenic neurons. In conclusion, responses observed previously in multifiber recordings of renal and splenic nerves tend to reflect those of single neurons. (HL21436)

266.12 UNEQUAL RESPONSES OF SINGLE MESENTERIC AND RENAL SYMPATHETIC NEURONS CAUSED BY STIMULATION OF INTESTINAL RECEPTORS AND BARORECEPTORS. R.D. Stein* and L.C. Weaver. Dept. of Physiol., Mich. St. Univ., E. Lansing, MI 48824-1101.

Previous studies have shown that stimulation of intestinal afferent nerves with bradykinin produces significant excitation of efferent sympathetic nerve activity, peripheral vasoconstriction, and increases in arterial blood pressure. The excitation of multifiber mesenteric nerve activity is significantly greater than that of multifiber renal nerve activity. These results lead to the following hypotheses regarding the bases for this unequal pattern of neural responses: 1) More mesenteric than renal neurons participate in this reflex, 2) the excitation of single mesenteric neurons is greater than the excitation of single renal neurons, and 3) baroreceptors preferentially suppress the renal nerve responses. These questions were investigated in chloralose-anesthetized cats that were artificially ventilated. The small intestine was externalized via a midline laparotomy and placed in a saline-filled dish. Activity of 14 mesenteric neurons and 6 renal neurons was recorded using standard teased-fiber electrophysiological techniques. Intestinal receptors were stimulated by the application of 10 μg (in 25 ml saline) of bradykinin to the serosal surface of the intestine. Baroreceptors were stimulated by intravenous infusions of 3 doses of phenylephrine (1-8 μg/kg) and "unloaded" by intravenous infusions of 3 doses of sodium nitroprusside (2-8 μg/kg). Relationships between neural activity and changes in arterial pressure were determined by regression analyses. Mesenteric and renal neurons had similar resting discharge rates that ranged from 0.2 to 6.5 spikes/sec (mean = 2.3 ± 0.5 spikes/sec). Whereas renal unit activity was usually correlated with the arterial pressure pulse and inhibited during systole, less than 50% of the mesenteric neurons had ongoing activity correlated to blood pressure. Activity of every renal neuron was sensitive to stimulation and unloading of the baroreceptors. In contrast, activity of only 6 of 13 mesenteric fibers was affected by baroreceptors. Stimulation of intestinal receptors caused significant excitation of 12 of 14 mesenteric neurons (from 2.6 ± .7 to 10.4 ± 2.2 spikes/sec), 4 of 6 renal neurons (from 2.8 ± 1.3 to 4.9 ± 1.7 spikes/sec), and increases in mean arterial pressure of 28 ± 5 mmHg. These results indicate that more mesenteric than renal neurons participate in the reflex response to stimulation of intestinal receptors, and that mesenteric unit excitation is greater than renal unit excitation. In addition, because more renal than mesenteric neurons are sensitive to baroreceptors, it is possible that baroreceptor inhibition of renal neuron activity contributes to the unequal responses of the two nerves. Support: NIH HL21436.

DISEASES OF THE NERVOUS SYSTEM: TRAUMA, TUMOR, BASAL GANGLIA

267.1 EFFECT OF EXPERIMENTAL CONCUSSION ON CHOLINERGIC NEURONS IN SPECIFIC RAT BRAIN REGIONS. S.E. Robinson, A. Saija*, T. Yamamoto*, C.E. Dixon, B.G. Lyeth, M.L. Giebel*, H.H. Stonnington* and R.L. Hayes. Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

Recent data suggest that increased functional activity of cholinergic systems located in the brain stem contributes to components of behavioral suppression following concussion (Hayes et al., Science 233: 301-303, 1984). The present study examines the effect of fluid percussion head injury on the activity of cholinergic neurons, measured by acetylcholine (ACh) turnover, in the dorsal medial pons, as well as in the frontal cortex, hippocampus, thalamus, and amygdala, areas which contain cholinergic cell bodies and/or terminals.

The turnover rate of ACh (TR_{ACh}) was determined by a mass fragmentographic technique that measured the relative rate of incorporation of deuterium label into brain ACh and choline following a constant rate infusion of deuterium-labeled phosphorylcholine through the tail vein. Animals were killed immediately at the end of the infusion by microwave radiation focussed to the skull (8.5KW, 2.0 sec). Male Sprague Dawley rats were surgically prepared 24 hrs prior to injury by implanting a hollow injury screw epidurally over a hole trephined along the sagittal suture, midway between lambda and bregma. Under methoxyflurane anesthesia, the animals were concussed at 1.5 atm using a fluid percussion method modified for rats in our laboratories (Dixon et al., Neurosci. Abst., 1984). TR_{ACh} was measured in injured and sham-injured rats 12 min, 4 hr and 24 hr following injury. ACh content was significantly elevated in the basal forebrain, hippocampus and pons 12 min following injury. At 4 hr following injury, ACh content had decreased so that it no longer significantly differed from control, except in the hippocampus, and by 24 hr ACh content was at control levels in all areas studied. TR_{ACh} was significantly elevated in the basal forebrain and pons 12 min following injury, a time at which the rats were behaviorally comatose, but had returned to control values 4 hrs following injury, at which time the rats were behaviorally responsive but still manifested significant neurological deficits. Interestingly, TR_{ACh} was significantly depressed in the hippocampus 4 and 24 hrs following injury, although it was not affected at the earlier time point. We believe that these findings implicate cholinergic mechanisms in both the early and longer-lasting deficits following concussive head injury.

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267.2 HIGH LEVEL FLUID PERCUSSION IS A MODEL OF BRAIN STEM INJURY. K. Shima*, A. Marmarou*, T. Yamamoto*, A. Unterberg*, B. Andersen*, L. Jenkins*, R. Hayes (SPON: D. Mayer). Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery, Medical College of Virginia, Richmond VA 23298.

Previous studies have shown that the outcome of severe head trauma is heavily dependent upon the degree of brainstem damage. The purpose of this research was to confirm our notion that despite the initiation of a complex series of events associated with impact, high level fluid percussion injury (>3.0atm) produces an irreversible brain stem lesion and may be a primary cause of death in this laboratory model.

Twelve cats were anesthetized with intravenous methohexal and maintained normocapnic using a combination of barbiturate (35mg/kg) and nitrous oxide (70/30). Fluid percussion injury (2.7-3.8atm)was applied to the left temporal region. Short latency brainstem auditory evoked response (BAER) was elicited by binaural click stimulation and was recorded for 8 hours following impact. Cerebral blood flow was also measured using hydrogen clearance technique from bilateral cortex.

Animals were classified into two groups based on the physiologic time course and neuropathologic changes in the brainstem. Group 1 was minimal or slight intraperenchymal and subarchnoid hemorrhage (n=5), and Group 2 had moderate or severe intraparenchymal and subarchnoid hemorrhage (n=7). Immediately after the injury, Group 1 animals demonstrated both transitory and marked suppression in waves 2,3 and 4 of the evoked potential which gradually increased until 60 to 150 minutes post-injury. This was followed by recovery of latency to 95% of control values by 8 hours.

In Group 2, wave 5 was absent immediately after impact. Waves 2,3 and 4 were suppressed in amplitude, prolonged in latency and failed to recover by 8 hours. Cerebral blood flow in both groups followed a similar temporal profile as the evoked potentials. CBF in Group 1 decreased to 40% of pre-injury control and recovered to 89% of control level by 8 hours. CBF in Group 2 animals fell to 39% of pre-injury control and failed to recover. This sustained reduction in CBF was associated with a gradually developing hypotension.

These results support the notion that the fluid percussion model at high levels of impact is primarily a model of brain stem injury.

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267.3 THE EFFECTS OF SCOPOLAMINE PRE- AND POST-TREATMENT ON THE RESPONSES TO CONCUSSIVE BRAIN INJURY IN THE RAT. B.G.-Lyeth, C.E. Dixon M.L. Giebel,* S.E. Robinson, R.J. Hamm, H.H. Stonnington,* H.F. Young* and R.L. Hayes. Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery, and Department of Rehabilitation Medicine, Medical College of Virginia, Richmond, VA 23298-0001.

Data from our laboratory suggest that increased functional activity of central cholinergic systems contributes to components of behavioral suppression following concussion and that pretreatment with scopolamine can attenuate components of the suppression. The present study examined the effects of scopolamine pretreatment on the systemic physiological responses to concussion and the effects of scopolamine posttreatment on acute neurological suppression associated with experimental concussion in the rat.

Rats were surgically prepared under Nembutol 24 hours prior to injury by placing a hollow screw epidurally over a central craniotomy. In experiment one, 12 rats were tracheostomized, ventilated, and femoral artery cannulated under Brevital and nitrous oxide (70% N_2O, 30% O_2) anesthesia. Animals were administered either scopolamine (1.0 mg/kg, i.p.) or saline 15 minutes prior to a 1.5 atm fluid percussion injury. Scopolamine-treated rats exhibited tachycardia within five minutes after drug injection and heart rate remained significantly greater than controls for 60 minutes post-injury. No significant differences were observed between groups in plasma glucose (a measure of the sympathoadrenal stress response), blood gases, pH, or blood pressure responses to trauma. In experiment two, 20 rats were anesthetized with metofane 5 minutes prior to a 1.5 atm, fluid percussion injury. Rats were administered either scopolamine (1.0 mg/kg, i.p.) or saline 30 seconds after trauma. The duration of suppression of several reflexes (pinna, corneal, righting, and flexion) and responses (escape, head support, and spontaneous locomotion) was recorded for up to 60 minutes after trauma. The duration of suppression of the spontaneous locomotion response was significantly shorter for the scopolamine-treated group. All other reflex and response suppression was shorter for scopolamine-treated rats.

These results suggest that the effects of scopolamine pretreatment on attenuating acute behavioral suppression following concussion may not be associated with the acute physiological changes measured here. These results also suggest that posttreatment with anticholinergics may reduce the behavioral suppression associated with concussion. These findings lend further support to the hypothesis that components of behavioral suppression associated with concussion may be mediated by an active cholinergic inhibitory system.

Supported by NIH Grants #NS 21458 and NS 12587 and Richard Roland Reynolds Neurosurgical Research Laboratories.

267.4 EFFECTS OF FLUID PERCUSSION BRAIN INJURY ON MULTIMODAL EVOKED POTENTIALS IN RATS. C.E. Dixon, T. Yamamoto,* B.G. Lyeth, A. Marmarou, M.L. Giebel,* P.G. Newlon,* H.F. Young,* H.H. Stonnington,* and R.L. Hayes. Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery, and Department of Rehabilitation Medicine, Medical College of Virginia, Richmond, VA 23298-0001.

Evoked potentials are useful non-invasive tools for investigating possible changes in neural function associated with various pathological states. Multimodal evoked potentials have been used in the diagnosis of human head injury. However, no animal study has systematically described multimodal evoked potential changes following experimental concussion. The purpose of this study was to characterize the effects of concussive brain injury on multimodal evoked potentials in rats (n=20) injured by fluid percussion techniques which produce brain injury by rapidly injecting saline into the intracranial cavity.

Under ketamine anesthesia, brain stem auditory evoked potentials (BAEP), short and long latency somatosensory evoked potentials (SEP), and corticospinal evoked potentials (CSEP) were recorded prior to and for one hour after a level of injury (1.5 atm) that has been previously shown to produce transient behavioral suppression. BAEPs were recorded at the vertex (active), mastoid (reference), and frontal sinus area (ground). Injury produced immediate latency prolongations and suppression of waves II, III, and IV. Latencies and amplitudes of the altered BAEP components gradually returned to baseline by 30 minutes. Short and long latency SEPs were elicited by electrically stimulating (2-4 mA) the right medial nerve and recorded over the contralateral sensory cortex (active), frontal sinus region (reference) and right forelimb (ground). Injury produced immediate latency prolongation and suppression of waves III, IV, P1, N1, P2, and N2. Gradual recovery to baseline was observed at 30 minutes for waves II and IV and 60 minutes for P1 and N1. However, P2 and N2 latencies were still prolonged at 60 minutes. CSEPs were elicited by electrically stimulating (2-5 mA) the motor cortex with silver ball electrodes and recorded at the dorsal columns (L1, active), adjacent intramuscular region (reference), and frontal sinus region (ground). Injury produced immediate latency prolongations and suppression of all components. Subsequent recovery of latencies and amplitudes was observed 60 minutes post-injury. These data indicate that: 1) recovery is different for different modalities, and 2) a magnitude of injury that produces transient behavioral suppression also produces transient perturbations in multimodal evoked potentials.

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267.5 SODIUM IONIC CHANGES IN INJURED SPINAL CORDS: MECHANISMS OF EDEMA. W. Young and V. DeCrescito. Dept. Neurosurgery., NYU Med. Ctr., NY 10016

In previous studies (Young, W., et al., Brain Research 253:105-114, 115-125, 1982; 365: 42-53, 1986), we have demonstrated large and rapid rises in extracellular K ionic activity ([K]e), falls in extracellular Ca ionic activity ([Ca]e), losses of tissue K ([K]), and gains of tissue Ca ([Ca]) in contused cat spinal cords. We report here that gross derangements of extracellular Na ionic activity [Na]e and tissue Na concentrations ([Na]) accompany the K and Ca changes at the lesion site. Using ion-selective microelectrodes and atomic absorption spectroscopy, we find that [Na]e falls from normal pre-injury levels of 150mM to <50mM while tissue [Na] increases severalfold by 1 and 3 hours after standard weight drop contusions.

These results indicate that extracellular Na ions enter injured cells at the lesion site and that Na ions move into the injured spinal cord from surrounding tissues. Closer analyses of the timing and distribution of the Na shifts, however, reveal two unexpected results. First, unlike [K]e which normalizes by 3 hours after injury, [Na]e behaves more like [Ca]e, remaining severely depressed (<70mM) at the lesion center for 3 hours while it normalizes at the lesion edge by 2 hours after injury. Second, unlike K and Ca which largely redistribute within the spinal cord, [Na] increases both at the lesion site and surrounding cord, indicating a large net entry of Na from blood and CSF into the spinal cord.

Such prolonged depression of [Na]e requires either a profound Na sink or an effective block of Na diffusion to the lesion site. Since tissue [Na] increases at the lesion site during this period, the latter is unlikely. We had earlier proposed that [Ca]e remains depressed at the lesion site because Ca ions entering cells bind to phosphates and other substrates and precipitate. Na ions, however, are much more soluble than Ca and the tissue [Na] does not increase above 150mM. We therefore hypothesize that Na ions enter injured axons at the lesion site and diffuse intra-axonally to surrounding cord. Some of the Na may be transported by axons to extracellular space in surrounding cord and become available for rediffusion back to the lesion site, providing Na ions to exchange with K ions diffusing from the lesion site to surrounding cord and helping to conserve K within the cord.

These findings carry important implications for the mechanisms and treatment of early edema in injured brains and spinal cords. The law of electroneutrality demands that for every cation that diffuses to and from the lesion site, an equivalent charge must be exchanged or an opposite charge must accompany the shift. The extent of ionic exchange will depend on ionic availability at the various sites. Since there is a net K loss and Na gain at the lesion site, differences between the two will determine net anionic shift and consequently water shift, i.e. edema. Our hypothesis highlights several potential sites of drug action to manipulate the ionic movements: (1) membrane stabilization which would reduce ionic flux into and out of injured axons at the lesion site; (2) active transport of Na ions back to extracellular space in surrounding brain or spinal cord; (3) ionic, particularly chloride, permeability or transport between the tissue and the vascular or CSF compartment. The injured spinal cord preparation and the methods that we use to characterize distributions of tissue ions allow us to dissect out these sites of action.

267.6 EXTRACELLULAR POTASSIUM ACTIVITY FOLLOWING CONCUSSION. A MICROELECTRODE STUDY IN THE CAT. A.A.F. DeSalles,* L.W. Jenkins,* R.L. Anderson,* J. Opoku-Edusei,* A. Marmarou,* R.L. Hayes [Spon: R.A. Newton]. Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298-0001.

Events that cause depression of neuronal activity such as hypoxia and ischemia are known to enhance extracellular potassium concentration. Similarly, cerebral concussion is followed by a period of depressed neuronal activity. This suppression may be related to a generalized neuronal depolarization at the time of concussion. We examined this possibility by measuring changes in cerebral extracellular potassium activity after fluid percussion brain injury in the cat. Importantly, this injury model is not associated with hypoxia or ischemia.

Four cats (2.5-3.6 kg) were surgically prepared under a combination of brevital sodium and NO_2 anesthesia. A hollow screw was implanted in the left temporal bone. Potassium-sensitive (Corning 477317) and reference microelectrodes were inserted in the left lateral gyrus cortex through a 0.5 cm opening in the parietal bone and a slit dura mater. The bone opening was filled with cotton and gel foam and repaired with bone cement that also held the microelectrodes in place. Animals were then subjected to a 2.5 to 3.0 atm fluid percussion brain injury. We observed an immediate increase in extracellular potassium activity that peaked within 0.25 sec after percussion. Potassium activity increased from a baseline of 3.7 ± 0.5 mM to 25.7 ± 9.9 mM and returned to 4.5 ± 0.6 mM 15 minutes after injury. At that time animals were sacrificed by KCl i.v. injection. After KCl injection and cessation of cerebral circulation, an increase in cortical extracellular potassium activity from 4.5 ± 0.6 mM to 35.8 ± 14.0 mM was observed (Mean ± SE).

The enhanced cortical extracellular potassium activity observed following fluid percussion brain injury is consistent with a possible generalized brain depolarization following concussion. The level of extracellular potassium activity observed in this study may be associated with the opening of calcium channels. In events such as hypoxia and ischemia, a parallel decrease in extracellular calcium is observed during periods of increased extracellular potassium. Increased intracellular calcium may mediate mechanism of cell damage and release of neurotransmitters. Thus, these initial studies of increased extracellular potassium following concussive brain injury may ultimately provide insight into the molecular basis of cell damage following mechanical brain injury not associated with hypoxia or ischemia.

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267.7 THE ELUSIVE CNS POLAR SPONGIOBLASTOMA: AN ULTRASTRUCTURAL EXAMINATION. L. A. Langford. Neuropathology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Considerable confusion surrounds the recognition and histologic description of the very rare embryonal neuroepithelial neoplasm, the polar spongioblastoma. On purely theoretical grounds this central nervous system neoplasm, if cytologically representative of the migrating polar spongioblasts observed in early central nervous system development, should be composed of bipolar glial cell precursors possessing one large and one long, thin process. Although the polar spongioblastoma's characteristic parallel arrangement and nuclear palisading are readily recognized in the light microscope, the traditional descriptions do not elucidate the nuclear and cytoplasmic characteristics. Ultrastructural descriptions clarifying the precise morphology of these neoplastic cells do not exist. The purpose of this work is to describe the ultrastructure of the cell types comprising the polar spongioblastoma.

This cerebral, intraventricular neoplasm occurred in a 14 month old girl. Tissue was taken for light and electron microscopy at the time of surgery. The tissue for electron microscopy was prepared according to the method of Langford and Coggeshall (Anat. Rec., 197:297-303, 1980). Light microscopic examination shows a cellular, primitive neuroectodermal tumor with the distinctive architecture ascribed to the truly primitive polar spongioblastoma. The cell groups are separated by a delicate and regularly arranged vascular connective tissue. Mitotic figures are frequently encountered. Most of the cells are polar with delicate cytoplasmic processes. The nuclei are oval and often have a nucleolus. Vascular endothelial proliferation is not present.

Preliminary ultrastructural examination reveals the majority of the cells in the palisading areas range from moderately undifferentiated neuroblasts to mature neuronal elements with synapse formation. Many of the more mature cells have a prominent cytoplasmic process; consequently, they appear to be unipolar. Dense core vesicles, often seen in CNS ganglion cell tumors, are present in many processes and at synaptic terminals.

267.8 DEPLETION OF UNILATERAL STRIATAL DOPAMINE IN PRIMATES FOLLOWING THE LOCALIZED PERFUSION OF MPTP: HISTOCHEMICAL AND BEHAVIORAL STUDY. K.Nakai, T.Itakura*, Y.Naka*, K.Nakakita*, I.Kamei*, H.Imai*, and N.Komai*. Department of Neurological Surgery, Wakayama Medical College, Wakayama, Japan 640.

Changes in dopamine (DA) containing neurons in primate following the localized perfusion of 1- methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into unilateral substantia nigra(SN) was morphologically assessed using the glyoxylic acid-induced fluorescence histochemistry.

MPTP (1 or 30mM) was continuously injected through a cannula whose tip was located at the unilateral SN pars compacta of Macaca fuscata for one week. After one day to several months of behavioral observation, monkeys were processed for the fluorescent histochemistry.

At one day after stopping the injection of MPTP, although the monkey behaved almost normally, the intensity of DA fluorescence in the ipsilateral striatum was significantly reduced as compared with that on the opposite side, in contrast to the reported results that in rodent the localized application of MPTP did not cause significant change in striatal DA content (Bradbury et al. Nature 319:56,1986).Numerous swollen fluorescent fibers were found at the dorsomedial portions of SN which correspond to proximal DA axons from SN toward striatum. Somatas of DA neurons in SN appeared to be normal both in fluorescence intensity and dendritic morphology except the area of primary lesion caused by the placement of cannula. In monkeys with longer survival period, striatal DA terminals on the MPTP-injected side became undetectable under the fluorescence histochemistry. Swollen fibers in the early stages were not observed anymore. DA cells in SN showed somewhat less intense fluorescence in the later stage but the number of them had not significantly changed.

These morphological changes in nigrostriatal DA system of primate following the localized perfusion of MPTP, probably due to the interspecies difference between rodent and primate as reflected in the differnt behavioral change to this drug, may suggest a possible contribution of retrograde degeneration from DA terminals to cell bodies in the pathogenesis of MPTP-induced parkinsonism in primate and human.

Although the above treated monkeys showed only minor behavioral changes such as appetite loss and/or impaired motor activity, we observed a purposeless rotational behavior in a monkey treated with repeated injection of MPTP (0.2mg/kg x 5) into the ipsilateral carotid artery in addition to the continuous injection into SN. Histochemical examination of this monkey showed unilateral depletion of DA fluorescence from SN through striatum.

267.9 CEREBRAL GLUCOSE UTILIZATION REDUCTIONS IN CLINICALLY ASYMPTOMATIC SUBJECTS AT RISK FOR HUNTINGTON'S DISEASE. J.C. Mazziotta,* M.E. Phelps, J. Pahl*, S.-C. Huang*, J. Wapenski*, L.R. Baxter*, W. Riege*, D.E. Kuhl*, C. Selin*, R. Sumida*, and C.H. Markham. UCLA School of Medicine, Los Angeles, CA 90024.

Local cerebral glucose metabolism was measured with positron emission tomography (PET) in 44 asymptomatic subjects at-risk (AR) for Huntington's Disease (HD). The age of this group was 37.1 ± 11.6 years. The results were compared with similar measurements in age matched controls (n = 27, age = 44.7 ± 14.4) and drug free, symptomatic (SHD) HD patients (n = 10, age = 56.3 ± 17.4, disease duration = 5 ± 2 years). All control and AR subjects had normal neurological exams and normal gross appearance of the caudates as determined by X-ray CT. Glucose metabolic rate values were determined for the caudate nuclei (Cd) and the cerebral hemispheres (Hem) and expressed as their ratio (Cd/Hem). All SHD patients had marked qualitative and quantitative reductions in LCMRGlc for the caudate nuclei when compared to controls (Cd/Hem:SHD = 0.85 ± 0.13 vs. controls = 1.25 ± 0.04, $p < 10^{-6}$, ANOVA). As a group, the at-risk subjects differed slightly from controls in their Cd/Hem values (AR = 1.19 ± 0.13, $p < 0.05$). However, there was a signficant difference in the variance of the distribution of the values between AR and control subjects ($p < 0.001$, test of inequality of sample variances). Both qualitatively and quantitatively a subgroup of AR subjects (29.6%, 13/44) with reduced metabolic activity for the caudate nuclei could be identified. The reduced values for the Cd/Hem ratio in these 13 AR subjects, whose ages ranged from 16 to 59 years, were outside the 99.5% confidence limits of the control population. Using each at-risk subject's age and the sex of their affected parent, individual risk estimates for developing Huntington's disease were averaged for this subject group and resulted in the prediction that the probability of having the clinically unexpressed Huntington's Disease gene should be 30.0 ± 6.0% for the group. The concordance between the predicted (30.0%) and actual (29.6%) rate of caudate abnormalities indicates that the measurement of glucose utilization may allow the observation of the pathophysiological effects of the Huntington's disease gene during the natural development of the disease. Correlation of these results with genetic linkage marker studies in AR subjects is under way. The results also indicate the potential ability to monitor metabolic responses to experimental therapies during both the clinically asymptomatic and symptomatic phases of the disorder.

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267.10 BRAIN-BEHAVIOR CORRELATIONS IN ASYMPTOMATIC PERSONS AT RISK FOR HUNTINGTON'S DISEASE. W.H. Riege, J.C. Mazziotta, M.E. Phelps, A. Lanto*, J. Pahl*, D.E. Kuhl, and L.R. Baxter*. VAMC Sepulveda and UCLA School of Medicine, Los Angeles, CA 90024.

Persons without motor symptoms but at risk (AR) for Huntington's disease (HD) were evaluated within one day both with neuropsychological and with brain imaging techniques. Neuropsychological tests contained validated measures of learning, memory, problem-solving, and constructional abilities; brain imaging used positron emission tomography with 18F-fluorodeoxyglucose in measures of regional Cerebral Metabolic Rate of glucose use (CMRGlc) which were expressed as ratio of regional to hemisphere CMRGlc to minimize intra-cerebral variabilities. Of 37 AR persons, 25 had complete sets of both measures and had a mean age of 42.7 +/- 14.2 years. They were compared with 21 healthy control persons of similar age range (50.2 +/- 14.9 years). Although both neurological and intellect (Shipley-Hartford) exams of AR persons were normal, their test scores in immediate verbal memory, learning, and delayed recall were lower (approx. $F = 3.98$; $p < 0.003$) than those of controls. The intercorrelations of neuropsychological with adjusted regional CMRGlc measures for AR persons were reliable ($r > 0.55$, $p < 0.01$) between tests requiring organization in verbal recall and problem solving and CMRGlc of insula, head of caudate, putamen, and globus pallidus. In factor analyses, these two sets of 16 measures each were sorted into 5 factors (with eigenvalue > 1.5, accounting for 70% variance). Two factors contributed most to separating the AR persons into 3 clusters with 11 persons in the first two clusters and 3 persons in the third. The clusters differed distinctly on the second ($F = 17.34$, $p < 0.0001$) and third factor ($F = 24.93$, $p < 0.0001$), as verified in step-wise discriminant function by 100% accurate classification. The second factor correlated word fluency with CMRGlc of basal ganglia, while the third factor was characterized by tasks requiring sequential coding and memory and by CMRGlc of temporal and caudate regions. It appears that AR persons of cluster 2 and 3 differed mostly in the third factor from persons in cluster 1. The analyses of results on AR persons reveal a sensitivity of verbal memory and problem-solving tasks to early subcortical dysfunctions before they are symptomatic of HD.

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267.11 CORTICAL NEURONS CONTAINING NADPH-DIÂPHORASE ARE SELECTIVELY RESISTANT TO QUINOLINIC ACID TOXICITY. J. Koh, S. Peters, and D.W. Choi. Dept. of Neurology, Stanford University Sch. of Med., Stanford, CA 94305.

A small subpopulation of mammalian CNS neurons contains high activities of the enzyme NADPH-diaphorase (NADPH-d), the presence of which can be detected histochemically. Such NADPH-d(+) neurons in the cortex and striatum are known to invariably contain immunoreactivity for somatostatin and/or neuropeptide Y, and have been recently reported to be selectively spared in the neostriatum of patients with Huntington's disease (HD). Because of current speculation that the endogenous excitotoxin glutamate (GLU) or quinolinic acid (QUIN) may play a central role in producing the neuronal loss in HD, we decided to directly test the possibility that NADPH-d(+) neurons might be intrinsically resistant to GLU or QUIN neurotoxicity, utilizing the model system of mammalian cortical cell culture.

Exposure of mature (15-23 day old) cultures to 0.5 mM glutamate for 5 minutes, invariably resulted in widespread neuronal disintegration by the following day. Similar brief pulse exposure to 1 mM QUIN had little effect; however, overnight exposure resulted in widespread neuronal loss similar to that seen after glutamate treatment. One day after initiating exposure to QUIN or GLU, cultures were fixed with 4% paraformaldehyde, and subsequently stained for NADPH-d by incubation in a solution containing 1 mM NADPH and 0.2 mM nitro blue tetrazolium at 37°C for 1-2 hours.

In control cultures, NADPH-d(+) cells were sparsely distributed (about 2% of total neurons) but readily identifiable by darkly stained cell bodies and processes. In cultures previously exposed to 1 mM QUIN, NADPH-d(+) cells were largely preserved, in striking contrast to the accompanying reduction in overall neuronal number. However, in cultures exposed to GLU, the number of NADPH-d(+) neurons was reduced in a fashion roughly comparable to the reduction in overall neuronal number. Intracellular recording from NADPH-d(+) cells surviving QUIN exposure confirmed a neuronal identity.

These findings suggest that the biochemically distinct subgroup of cortical neurons which contain NADPH-d may possess an intrinsic, selective resistance to the toxicity of QUIN. In light of evidence that the selective sparing of these neurons is a hallmark of HD, these findings favor the hypothesis that QUIN (or a closely-related compound - perhaps another NMDA agonist?), rather than GLU, may be the actual pathogenic agent in HD.

(Supported by the Wills and Hereditary Disease Foundations, and by NIH grant NS21628. DWC is a Hartford Foundation fellow.)

267.12 THE NATURE COURSE OF PERSISTENT TARDIVE DYSKINESIA. C.C. Huang. Dept. of Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

Twelve moderate to severe persistent tardive dyskinesia (T.D.) patients, 5 female & 7 male, were followed up for 5-8 years. Their medical records were reviewed. The course of their T.D. were studied. It is known that many factors could cause temporary fluctuations of T.D., such as medication change, sleep or wakefulness, daily activity, anxiety, psychotic symptoms and physical health etc. However, despite of these temporary fluctuations of T.D., it appeared that they went through a similar pattern of the course. This includes a period of antipsychotic medication use (incubation), appearance of T.D. (onset), aggravation of T.D. (exacerbation), gradual reduction of T.D. (attenuation) and disappearance &/or diminishing of T.D. (recovery). These patients were mostly schizophrenic and at age 50 or 60's when their T.D. were at the peak. At the time of exacerbation of T.D., they were referred immediately to the author for consultation and their antipsychotic medication was reduced. It usually took 7 to 8 years to reach recovery from the peak of their T.D.

The author postulates that after long term antipsychotic medication use the postsynaptic receptors or neuronal membrane become hypersensitive to dopamine, T.D. symptoms appear, and gradually aggravate; then the neuronal membrane or receptors are able to desensitize and readapt themselve after a long period of time as long as the dosage of antipsychotic medication is maintained constant and not increased.

So the best strategy of managing chronic schizophrenic patient with T.D. is to use lesser antipsychotic medication as possible. This is possible to achieve because after age 60 the schizophrenic patient's psychotic symptoms are attenuated and the dose of antipsychotic medication can be reduced.

CONTROL OF POSTURE AND MOVEMENT II

268.1 Modulation of phasic and tonic reflexes with the skin. M. A. Sabbahi, C. R. Mason*, and E. C. Lou*. Texas Woman's University, School of Physical Therapy, 1130 M. D. Anderson Blvd., Houston, TX 77030.

Previous studies in our laboratory showed that cutanous receptors modulate the excitability of soleus MNP (Sabbahi and Sedgwick, 1976; Sabbahi and DeLuca, 1982). This modulation was dependent on the manipulated skin area. This report discusses the effect of cutaneous receptors afferent discharge on the excitability of tonic and phasic reflexes/motoneurons.

Flexor carpi radialis (FCR) H-reflex and biceps brachii (BB) tonic vibration reflex (TVR) were recorded as an indices for the excitability of phasic and tonic MNP respectively. H-reflex was elicited by 1 msec., 0.2 PPS pulses for the median nerve at the arm and TVR was elicited by applying an air-driven vibrator on the biceps tendon at 80 PPS. FCR and BB EMG and elbow flexion torque were recorded before and after desensitization of the anterior forearm (AF) or posterior forearm (PF) skin areas using topical anesthesia.

Desensitization of AF skin area resulted in significant ($P < 0.05$) facilitation of the FCR H-reflex and significant inhibition of the BB TVR and decreased flexion torque. This effect lasted for 30 minutes post-anesthesia. No measurable changes were recorded in both reflexes or the torque after desensitization of PF skin area or after the placebo.

These results indicate that cutaneous receptors afferent discharge of flexor skin area simultaneously inhibit phasic reflexes/motoneurons, and excite tonic reflexes/motoneurons of flexor MNP. It appears that cutaneous receptor afferents play a major role in increasing muscle stiffness and damping responses to perturbation resulting in a more smooth movement performance.

268.2 SYNCHRONOUS BEHAVIOUR OF MOTOR UNITS. G.A. Scala*, D.W. Stashuk*, C.J. De Luca (SPON:J.F. Jabre). NeuroMuscular Research Center, Boston University, Boston, MA 02215.

Synchronous behaviour of motor units is considered as the tendency for pairs of motor units to contract with similar latencies relative to each other, more often than would be expected if the motor units were functioning independently. A study of the interdependence of concurrently active motor units was performed.

Myoelectric signals were detected using a selective needle electrode from healthy subjects during voluntary isometric contractions. The signals were decomposed, by a proven accurate technique, into their constituent motor unit action potential trains (MUAPTs). Pairs of MUAPTS were then analyzed to determine if any interdependence existed. Histograms of the time intervals between the firing of a triggering or conditioning motor unit to the subsequent firing of the alternate or conditioned motor unit of a pair were created. Assuming the motor unit pairs are operating as independent Gaussian point processes the histograms should be flat with an expected value dependent on the histogram bin width and the mean firing rate of the triggering motor unit. Bins containing 1.96 standard deviations more than the expected number of occurrences indicated at a 95% confidence level statistically significant interdependence or synchronization.

The latency at which synchronization was maximum and the range of bins over which it occured were computed. The amount of synchronization was then calculated as the ratio of the average number of firings in each bin in the above described range, minus the expected value to the expected standard deviation. The frequency of occurrence was also measured.

Synchronization measurements of motor units studied in human first dorsal interosseous (FDI), deltoid and tibialis anterior (TA) muscles during a variety of force protocols indicate that more than 60% of the motor unit pairs examined exhibited synchronization. The latency of the maximii were most often within +/-5ms and they had an average width of about 4ms. The deltoid had lower amounts of synchronization than the FDI and TA muscles which had similar levels. Synchronization was not found to be a function of contraction level in the deltoid or FDI. The analysis of motor units in synergist and antagonist muscle pairs revealed interdependence within but not across the muscles.

The results support the hypothesis that synchronization is indeed a phenomenon of most contracting muscles. Confidence in these results is warranted because of the proven accuracy of the decomposition technique used to obtain the MUAPTS and the statistical nature of the investigation.

This work was supported by Liberty Mutual Insurance Co.

268.3 MOTOR UNIT RECRUITMENT THRESHOLD MODIFICATION DUE TO SKIN DESENSITIZATION. De Luca, C.J. and Matsuzawa, T.* NeuroMuscular Research Center, Boston University, Boston, MA 02215

Prior experiments from our group indicated that when the skin above the soleus-gastrocnemius muscles was desensitized the H-reflex dramatically increased in amplitude. Hence, a study was conducted to investigate the behavior of the recruitment characteristics of motor units in this circumstance. The skin on the hand and the back of the hand, the wrist and the distal 10cm of the forearm was desensitized by liberal application of 10% Xylocaine. EMG signals were detected from the First Dorsol Interosseous muscle while the subject generated a force-range reaching 50% MVC in 5 sec. The contractions were performed under isometric conditions. The paradyne was repeated before and then 15, 30, 45 and 60 minutes following the application of the topical anesthetic. The EMG signal was decomposed into the constituent motor-unit action potentials. The force threshold of progressively recruited motor units was determined and compared among subsequent contractions. Considerable effort was taken to ensure that the same population of motor units was detected in each contraction. The experiments were repeated with a placebo to eliminate any non-physiological susceptibility. Three subjects participated in the study.

The results indicated that the force threshold level of motor units recruited below 10% MVC increased, while that of motor units recruited above 20% MVC decreased in response to the skin desensitization. This effect increased up to 45 min post desensitization. When the placebo was used, no probabilistically significant modification was noted in the recruitment threshold.

We speculate that the motor units recruited below 10% MVC are most likely slow-twitch and aerobic, whereas those recruited above 20% MVC contain some fast-twitch, anaerobic units. Thus, it is suggested that activation of receptors in the skin has an excitation effect on the smaller slow-twitch earlier-recruited motor units and an inhibitory effect on the larger, faster-twitch, later-recruited motor units. Such an interpretation is consistent with our previous observations indicating an increase in the amplitude of the H-reflex in normal subjects and augmented mobility in stroke patients when the skin was desensitized.

(This work was supported by Liberty Mutual Insurance Co.)

268.4 CHARACTERISTICS OF NATURAL PREHENSION MOVEMENTS FOR OBJECTS OF VARYING SIZE. R.G. Marteniuk and S. Athenes*. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Three-dimensional movement analyses yielded information on the displacement, velocity, and acceleration characteristics of the reach and grasp components of natural prehension movements. The data were collected from six subjects as they reached for and grasped disks that were two cm thick and varied in diameter, in one cm increments, from one to ten cm.

Movement time increased linearly with decreases in disk size ($r = -.79$) indicating changes in movement precision requirements due to disk size. These longer movement times were the result of an increase in the deceleration phase (time from peak resultant velocity to time of disk contact) of the reach as the acceleration phase (time from reach initiation to time of peak resultant velocity) remained constant. Thus, movement trajectories were specific to the size of the disk.

In terms of the grasp, the maximum grip aperature correlated $r = .99$ with object size indicating a very precise and stable relationship between the visual-hand system and the size of object to be grasped. For the ten objects the closure phase of the grasp began between 70 to 85% of the total duration of the movement. This onset time was independent of the kinematics of the transport phase.

The data support the conclusion that the control of prehension movements arises from a process that is relatively specific to the size of object to be grasped. In addition, the temporal control of the transport and grasp components appears to arise from independent processes.

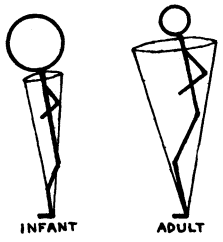
268.5 MATHEMATICAL COMPARISON OF KICKING AND STANDING Gin McCollum, Neurological Sciences Institute, 1120 NW 20th Avenue, Portland, Oregon 97209

During human development, the ability to stand with the feet together changes dramatically in a way that kicking from a supine position does not. Both tasks use the same leg and trunk muscles and, in each case, the mechanical problem involves three joints between four body segments (foot, lower leg, thigh, and trunk) with an end segment as the base of support. The same equations govern the mechanics. Thus, any differences in mechanics result from different parameters. For example, the torso is much longer and heavier than the foot. Therefore, kicking has no stability limits, while postural movements have.

In both movements, angular acceleration caused by gravity increases as the body or leg moves farther from vertical. In the case of kicking, the change in torque as the legs move away from a vertical orientation is not large enough to challenge the stability provided by the torso as base. Kicking movements can freely be made away from the vertical gravitational axis.

For stance with the feet together, gravitation is strong enough relative to the forces acting against it, that humans must remain near erect stance. That is, the axis with no angular acceleration caused by gravity becomes physiologically important as the preferred body position of erect stance.

Freedom of movement about the position of erect stance is limited. Physiological parameters of the body determine values of ankle and hip angle, given a fixed knee angle, beyond which one can not balance without taking a step. These parameters constitute cone-shaped boundaries in the movement space to the efficacy of each type of postural adjustment. As an infant's proportions develop toward adulthood, these cones grow wider, as illustrated in the schematic diagram, thus making it easier to develop strategies of postural stability. The set of automatic postural adjustments is a necessary spatial structure developed by the nervous system to add postural stability to the mechanical metastability of erect stance. Postural adjustments tend to be made with the knees straight, in contrast to kicking, which mixes flexion and extension of the leg with hip bending. Thus the distinguished subspaces of kicking and stance become perpendicular to each other.



268.6 ARM-WRIST COORDINATION IN A CATCHING TASK. F. Lacquaniti and C. Maioli*. Ist. Fisiologia Centri Nervosi-CNR, 20131 Milano, Italy.

Catching a ball involves the coordinated action of several limb muscles within rigid spatio-temporal constraints. A successful performance depends on the ability not only to intercept ball's trajectory in a narrow time interval, but also to adjust limb compliance according to the parameters of ball's impact (e.g. time, force, elasticity) estimated on the basis of sensory information and cognitive inferences. Compliance can be controlled by varying the level of muscle activation preceding impact and by pre-setting the gain of reflex and reaction-time responses to impact.

We studied a simple catching task in which ball's momentum was a manipulated variable. Subjects were asked to catch balls of different mass (200-800 gr) falling freely from different heights (0.2-1.2 m). Upper arm position was fixed, and the hand supinated. A stiff glove was worn to make gripping unnecessary. Elbow and wrist motion was measured goniometrically, and EMG activity of elbow and wrist muscles was recorded.

Muscle activity was modulated over a prolonged time interval preceding and spanning the impact. All subjects estimated accurately both impact time and momentum, as indicated by the co-activation of elbow and wrist flexors and extensors at a constant time interval prior to impact, with a mean amplitude linearly related to momentum. Anticipatory activity peaked before impact and then dropped reaching a relative minimum at impact time, indicating that limb compliance is not simply set at a fixed level, but is continuously modulated through impact.

Reflex responses to impact consisted of multiple peaks at short (20 ms) and medium (50 ms) latencies. In only 50% of the subjects did the mean reflex amplitude increase with momentum; in the other subjects the mean amplitude either was constant or actually decreased with momentum, indicating a pre-setting of reflex gain.

When only an acoustic tone was given to signal the ball's detachment but no visual feedback was allowed, anticipatory activity was absent or very much reduced, while reflex responses were boosted up in amplitude.

268.7 EXECUTION OF SIMPLE MONOARTICULAR MOVEMENTS IN THE PRESENCE OF ANATOMICAL CONSTRAINTS. R. Jaeger[1,3], A. Kralj[1,2,*] and L. Draganich[3,1,*]. [1]Pritzker Inst. Med. Eng., Ill. Inst. Tech., Chicago, IL, 60616; on leave from [2]Edvard Kardelj Univ., Ljubljana, Yugoslavia; [3]Sect. Orthopedics, Univ. Chicago, Chicago, IL.

Among the schemes proposed to categorize movements is the binary classification of ballistic and non-ballistic. In the majority of experimental paradigms, movements well within the anatomic range of motion and the effects of externally applied loads during various phases of the movement have been studied. We have been concerned with the movement of standing up from a sitting posture. There are a variety of endpoints for this particular movement, with all the endpoints normally at or near maximum extension of the hip and knee joints. To better understand the basic mechanisms involved in standing, we are investigating how a movement is braked when the endpoint or target of the movement is at or very near the anatomical limit of the joint. Protective mechanisms to prevent joint damage have been suggested in these situations. One hypothesis for ballistic movements is that the motor control system knows that a constraint is present and takes this into account in the planning and execution of a movement. If this is correct, one might expect a different motor behavior in the case of a ballistic movement approaching a natural joint range of motion constraint as compared to the case where an unexpected artificial constraint is encountered. For slow movements a hypothesis is that sensory receptors exist which signal the arrival of the limb at the constraint, and subsequent motor activity is modified. If this is correct, one might expect a similar type of motor behavior when encountering both natural and artificial constraints. The contributions of the agonist and antagonist during simple monoarticular movement have been controversial for some time. In comparing studies, it appears that speed of movement and instructions to the subject are the most important factors. The motor behavior during a voluntary thumb movement that is unexpectedly halted have shown that the halt did not produce any detectable changes in the first agonist EMG activity (compared to a control movement) but virtually eliminated the antagonist burst (Hallet, J. Physiol. 294:33-50, 1979). In our experiments, subjects produced both ballistic and slow monoarticular movements. We studied both the knee and elbow joints. We found that ballistic movements to the anatomical limit seemed to resemble Hallet's data for unexpected halted movements. On the other hand, in slow movements, antagonistic activity was frequently observed near terminal extension. Our preliminary interpretation of these results is that ballistic movements are planned in the absence of any knowledge regarding anatomical constraints, while slow movements may involve reflex feedback concerning constraints. [Supported in part by NIH (NS20806) and NIHR (G008435046)]

268.8 TIMING FACTORS IN THE COORDINATION OF SPEECH MOVEMENTS. V.L. Gracco. Speech Motor Control Laboratories. Waisman Center, University of Wisconsin, Madison, WI 53705-2280.

A hallmark of many motor behaviors is the coordination of multiple structures. To achieve the necessary coordination, underlying neural signals must be adjusted to reflect peripheral conditions. During speech movement coordination, time-varying articulator positions, interarticulator timing requirements, and biomechanical properties must be considered. To reduce control complexity, the nervous system may establish invariant relations among structures as well as exploit their biomechanical properties. In the present study these issues were addressed using detailed EMG and kinematic analyses of speech movements.

Subjects repeated the word "sapapple" requiring multiple opening and closing movements of the lips and jaw. EMG activity from multiple muscles acting on the lips and jaw were recorded along with upper lip, lower lip, and jaw movements.

Consistent timing relations were observed within the closing and subsequent opening phases. Within the closing phase, movement onsets and times of peak acceleration and peak velocity across structures were consistently ordered and highly related. As movement duration varied, velocity profiles were found to vary proportionally maintaining their invariant timing relations. Similar timing invariances were observed for the subsequent opening movement; however, the ordering of the structures was different than observed during closing. In addition to the within phase timing relations, across phase (closing to opening) timing relationships were also found to be highly related.

EMG analyses, confined to closing muscles of the lips and jaw also reflected invariant relative timing relations. The onset of EMG activity for lip and jaw muscles was found to be highly related maintaining a consistent ordering across trials. Close inspection of the EMG/kinematic relationships revealed that for all closing movements the lower lip and jaw, accelerations were initiated prior to any observed muscle activity. Although subsequent muscle activity closely followed, the initial movement acceleration reversing the downward movement was apparently due to the release of passive forces acting in the opposing direction.

Similar to many multiarticulate motor behaviors such as locomotion, speech production incorporates invariant timing relations in the muscle activity and kinematics. Further, it appears that the passive biomechanical properties assist in generating the kinetic requirements for speech movements. Based on these and previous observations, it appears that speech movement coordination involves sensorimotor interactions, invariant articulator timing, and exploitation of biomechanical properties to facilitate coordination. (Supported by NIH grants NS-20668-03,NS-13274,& HD-03352).

268.9 INTERACTION OF DESCENDING CONTROL AND STRETCH REFLEX IN FAST VOLUNTARY MOVEMENTS IN MAN. L. Stark, B. Hannaford*, and C. Ramos*. Neurology Unit University of California, Berkeley, California 94720

In fast voluntary movements, programmed by descending control pulses, stretch reflex influence must be minimized, especially in the antagonist, to prevent antagonist forces and high resistance force-velocity 'apparent' viscosity from slowing the movement. In control terms, this means that the sampled data control signals produce both a visual feedback and proprioceptive feedback open loop operating conditions during the multipulse controller signal period. Experimentally, EMG shows quite good reciprocal innervation. Reciprocal innervation, by a descending inhibiting control of the motoneuronal pool of the antagonist or reciprocal inhibition, by spinal cord inhibiting mechanisms, or both, may play a role.

When at the end of a movement does the stretch reflex again take control of posture? (See Stark, 1968, Figure 79, page 392) This question may now be put more precisely in terms of the triphasic envelope of EMG. The roles of the three pulses have been assessed and can be designated PA, the action pulse, for first agonist burst, PB, the braking pulse for the antagonist burst, and PC, the clamping pulse for the second agonist burst. (Hannaford and Stark, 1986, in press)

Two approaches have been used by our group: A first approach explores the influence of vibration on each of the three pulses. Continuous vibration produces specific effects---agonist vibration increases PC; antagonist vibration increases PB; PA is unchanged. This suggests that the stretch reflex is open loop for PA and then able to contribute in part to the controller signal during PB and PC. (Hannaford, Cheron and Stark, 1985)

The second approach uses computer simulation of a model of two muscles and load together with spindles and their afferents. Preliminary results suggest that dynamics of experimental movements can be well approximated by stretch reflex supplementation, after PA is completed, with feedback gain for PC relatively elevated (during this late phase) since PB antagonist stretch reflex is enhanced by the actual stretch produced by the movement. (Ramos and Stark, 1986 submitted)

268.10 THE SHAPE OF THE VELOCITY CURVE VARIES WITH MOVEMENT DURATION: EVIDENCE FROM HUMAN ARM MOVEMENT, LOCOMOTION, MASTICATION AND SPEECH. D.J. Ostry*, J.R. Flanagan*, and J.D. Cooke. (SPON: F. Wilkinson). McGill University and University of Western Ontario.

There have been demonstrations that the geometric form of the velocity curve of movements is invariant under manipulations of movement amplitude, path, rate and load. The theoretical importance of these findings has been demonstrated in the case of planar arm movements where invariance greatly simplifies the solution of the inverse dynamics problem (Hollerbach & Flash, 1982). Although the scalar equivalence of velocity curves has been shown in a number of situations, we find it is equally often the case that the form of the velocity curve varies as a function of movement amplitude and duration.

We have examined the form of velocity curves of human jaw movements in both mastication and speech, horizontal movements about the elbow and movements about the knee in locomotion. Although these articulators have dissimilar functions and differ in their biomechanical properties, muscle histochemistry, and reflex and sensory organization, common properties can be identified.

Rate and amplitude were varied for movements about the elbow and for jaw movements in speech. In mastication, the rate of jaw movement and the compliance of the bolus were varied. Movements about the knee were examined at different velocities in both walking and running.

With the exception of movements about the elbow, the symmetry of the velocity curves varied as a function of movement duration. For slow movements of the jaw and slow discrete movements about the elbow, acceleration durations were less than deceleration durations. For slow movements in both walking and running, deceleration durations were less than acceleration durations. In all but one case, as movement speed increased all curves became increasingly symmetrical. The velocity curves in continuous movements about the elbow were symmetrical in shape independent of differences in movement amplitude or duration.

In contrast to previous reports, these findings show that in general the shape of velocity curves is not invariant with changes in movement duration.

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268.11 PREPARATORY SET: INDEPENDENT SPECIFICATION OF DIRECTION AND SIZE IN THE PREPARATION OF RESPONSES TO A RANGE OF TARGETS. M. Favilla*, W.Hening, J.Gordon* & C.Ghez. Ctr. for Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst. New York, NY 10032.

We have previously shown, using a new paradigm, that the amplitude and direction of motor responses to unpredictable targets are specified by independent and parallel processes (Favilla et al., Neurosci. Abstr., 1985). Human subjects were trained to initiate responses in synchrony with a predictable tone and to match one of six visual targets (three amplitudes in flexion and extension) presented at variable intervals before the tone. Specification of response amplitude was gradual, extending from 100 to 300 ms after target presentation, while specification of direction was abrupt and began after 200 ms. The amplitudes of responses initiated at intervals too brief to incorporate target information were clustered in the centers of the ranges of flexion and extension targets. This suggested that specification proceeds from a "default" established through experience. However, we could not determine whether subjects prepared two separate default responses (one for each direction) and made a random choice between them, or whether the amplitude and direction of default responses were prepared independently. The present study was designed to distinguish between these alternatives, and to determine whether the default was simply at the center of the range of targets or weighted by their frequency of occurrence.

Five subjects produced isometric force impulses at the elbow to match four targets, two amplitudes in each direction. Flexion and extension targets were of the same size and were presented in unpredictable sequence. In one direction, the probability of occurrence of each amplitude was biased (1:4 or 4:1), while in the other the two amplitudes were equiprobable. To maximize the effect of the "default" on response parameters, we used a predictable tone to synchronize the initiation of responses at only 120-150 ms after target presentation (one standard deviation below each subject's mean reaction time). We hypothesized that changes in probability of target amplitudes would shift the default responses toward the more probable target. If subjects prepared separate flexion and extension defaults, the amplitudes of the responses would show an influence of target probability only in the direction in which the targets were biased. However, all subjects showed an adaptive shift in mean peak force of both flexion and extension responses, no matter which direction was biased. Responses made to any target, in either direction, were always larger if the large target was more probable and smaller if the small target was more probable. We conclude: (1) that subjects prepare default responses based on the probability of occurrence of the target; and (2) that the amplitude of the default response can be established without regard to its direction.

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268.12 MOTOR CONSEQUENCES OF POSTERIOR PARIETAL-LOBE INJURY. D. Claman and T. Zeffiro. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Electrophysiological and behavioral studies in the monkey have demonstrated the importance of posterior parietal cortex (PPC) for accurate projection of the limb through space. Although misreaching is observed in the human as well as in the monkey following PPC lesions, the human deficit is commonly attributed to a visuospatial impairment; final-position errors are thought to occur when either hand is used to reach into the contralateral spatial hemifield. We hyothesized that in humans, as in monkeys, the misreaching defect observed after PPC lesions involves a motor control defect in addition to the known visuospatial impairment, and predicted that patients would show specific changes in trajectory that could account for their final-position errors.

Ten healthy control subjects, 16 patients with left or right PPC lesions, and 12 patients with unilateral frontal-lobe or cerebellar lesions performed a step-tracking task along the surface of a high-resolution digitizing tablet under two conditions of visual feedback. Hand position was recorded at 100 Hz as subjects reached with a stylus to targets located at various distances and directions from three starting positions. Tangential velocity and acceleration profiles were computed subsequently. ANOVAs were used to evaluate whether feedback condition, the hand used to execute movements, or the spatial hemifield in which movements terminated differentially affected the motor performance of subject groups. Regression techniques were applied to determine whether for patients, as for healthy control subjects, such movement properties as movement duration, the length of the accelerative and decelerative phases, and peak velocity covaried with movement amplitude.

The main results were: 1) PPC lesions affected movements executed with the contralateral hand regardless of the spatial location of the target. 2) Only PPC lesions selectively impaired coordination of the decelerative phase of trajectories. 3) Unlike patients with lesions centered in the superior parietal lobule, patients with visual field defects or damage to the inferior parietal lobule were unable to use effectively the additional information provided by constant illumination of the target position. 4) The motor performance of patients with right PPC lesions was impaired contralaterally, whereas that of patients with left PPC lesions was impaired bilaterally. These results suggest that different mechanisms control different parts of the trajectory and that the PPC may be particularly important for successful execution of the decelerative phase of movement.

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268.13 ANATOMY OF THE TECTOFACIAL PATHWAYS IN THE CAT. P. J. May, P.-P. Vidal and R. Baker. Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave. New York, NY 10016.

In addition to its role in the production of orienting movements of the eyes and head, the superior colliculus also influences movements of the facial musculature that in particular direct the wiskers and pinnae. Using electrophysiological means we previously determined that, in addition to the expected polysynaptic pathways linking the superior colliculus to facial motoneurons, a small, short latency, monosynaptic EPSP is evoked in facial motoneurons following electrical stimulation of either the contra- or ipsilateral superior colliculus. As previous studies had generally not revealed such a projection to the facial nucleus, we consequently investigated these connections anatomically. Utilizing a ventral approach, horseradish peroxidase (HRP) was injected into the facial nucleus of cats and the retrogradely labelled cells were revealed by the tretramethylbenzidene procedure. Labelled neurons were present bilaterally in the intermediate and deep grey layers of the superior colliculus. Contralaterally they were more common in the intermediate than deep layer, while the converse was true ipsilaterally. We have further investigated this question utilizing intra-axonal injection of HRP. Predorsal bundle axons traveling near the midline at the level of the superior olive were penetrated with HRP filled glass micropipettes. They were identified by electrical stimulation of the contralateral superior colliculus and ipsilateral spinal cord with pairs of electrodes, then injected with HRP. Only axons that could be activated at latencies under 0.5 msec, that reliably followed high frequency stimulation and typically displayed pairs of action potentials were injected. Reconstruction of diaminobenzidene labelled, HRP filled predorsal bundle axons revealed the thick parent axons travelling rostrocaudally in the brainstem ventral to the medial longitudinal fasciculus. Numerous thinner collaterals emitted from these parent axons arborized and terminated extensively within the midline reticular formation of the pons and medulla. Ocassionally, long thin axon collaterals reached all the way to the facial nucleus where they produced en passant and terminal boutons within the nucleus and medial to it. Terminals were observed in subdivisions of the nucleus supplying the auriculoposterior and temporozygomatic branches of the facial nerve. The long thin collaterals and the very sparse terminal arbors they produce within the facial nucleus provide an anatomical basis for the physiologic results, which indicate the monsynaptic EPSPs are small (<0.5mV) and inconsistently present. The anatomy of the ipsilateral pathway is presently being investigated in a similar fashion. Supported by NIH Grant # EY05689-03

268.14 ANATOMICAL CORRELATES OF DOPAMINE AGONIST-INDUCED SELF-MUTILATIVE BITING BEHAVIOR IN THE PRIMATE. M. Goldstein, A.Y. Deutch[2], Y. Shimizu, K. Fuxe[3], and R.H. Roth[2]. Dept. of Psychiatry, NYU Medical School, New York, NY 10016; [2]Yale Medical School, New Haven, CT 06510; and [3]Karolinska Institutet, Stockholm, Sweden.

The administration of dopamine (DA) agonists to some monkeys with unilateral lesions of the ventromedial mesencephalic tegmentum (VMT) results in abnormal involuntary movements (AIM), including self-mutilative biting (SMB). The SMB resembles that observed in Lesch-Nyhan disease. The underlying pathology of the AIM may indicate DA receptor supersensitivity in specific telencephalic sites. Certain subsets of monoaminergic neurons are probably involved in SMB, since we have previously shown that 1)DA agonists which interact with D1 receptors induce SMB, and D1 but not D2 antagonists reverse agonist-induced SMB, and 2)Parkinsonian patients do not develop SMB upon administration of DA agonists. We have examined the brains of two African green monkeys which sustained unilateral VMT lesions 10-14 years previously and were subsequently tested for SMB.

In an animal which exhibited agonist-induced SMB the electrolytic lesion occupied the dorsolateral VMT. Tyrosine hydroxylase (TH) immunohistochemistry revealed that DA neurons in the lesion area were lost, as were most DA neurons in the substantia nigra (SN; A9 cell group) and retrorubral field (RRF; A8 cell group), which were damaged by transection of their axons. The DA cell loss was most marked in the caudal SN and RRF. Spared DA neurons were observed in the SN and A8 region; these neurons may project to forebrain sites via a supralemniscal pathway. Surviving DA neurons in the ventral SN were greatly hypertrophied. The loss of TH fibers in the putamen was virtually complete, whereas only a slight decrease in the caudate innervation was observed. The caudate loss was restricted to the diffuse DA innervation, and the dotted innervation was spared. In addition to lesion-induced loss of midbrain DA neurons, there was a partial loss of neurons in the A7 cell group of the pons and in the medullary C1 adrenergic cell group. Finally, the density of substance P (SP) fibers in the SN rostral to the lesion was decreased, indicating a SP innervation of the SN from brainstem sites. The lesion in the second monkey was more lateral than that seen in the first monkey, and involved the medial SN but not the VMT. This monkey did not exhibit agonist-induced SMB. Since in both monkeys the DA neurons in the SN lateral to the lesion were lost, as were most A8 DA cells, it would appear that the critical locus for DA cell loss in cases of agonist-induced SMB may be the VMT. Thus, denervation supersensitivity of mesolimbic and mesocortical sites as well as striatal areas may be central to the development of SMB. These data also suggest that other monoaminergic systems, such as the C1 adrenergic neurons and the A7 noradrenergic neurons may be important in agonist-induced SMB and Lesch-Nyhan disease.

269.1 BLOOD-BRAIN BARRIER (BBB) PERMEABILITY TO MACROMOLECULES IN FETAL NEOCORTICAL TRANSPLANTS. J.M. Rosenstein, Dept. of Anatomy, George Washington University Medical Center, Wash. D.C. 20037.

An intact BBB is essential for maintenance of the brain's homeostatic milieu. By selectively limiting the passage of materials from blood to brain, the BBB largely controls the dynamic equilibrium between the CSF and the interstitial spaces of the parenchyma. The BBB to protein is present relatively early in the fetal vertebrate brain. How might transplantation and ensuing angiogenesis affect barrier function within a fetal graft? Parietal cortex from rat donors between embryonic day 15 to postnatal day 2 was grafted into either the IV ventricle or directly into the cortex of young adult rats. Postoperative times were between one month and one year. A single injection of HRP (mw 40,000) or a coupled human IgG-HRP (mw 180,000) was made in the femoral vein and allowed to circulate for periods up to 50 minutes. Vitratome sections were processed for HRP using highly sensitive TMB. All neocortical transplants were permeable to the systemically administered proteins. Much of the reaction product appeared initially at host-graft interface zones. By the latest time period, HRP had diffused through almost the entire transplant. Owing to its larger size, IgG-HRP did not permeate quite as far. The blood-borne protein traversed the transplant and escaped into the cerebrospinal fluid. Other uninjected hosts were aldehyde perfused and plastic sections of the transplant and surrounding brain stained with anti-serum to the endogenous blood protein rat serum albumin (RSA) (provided by Dr. B. Trapp). Anti-RSA was found within the cortical transplant parenchyma mostly at the interface region. Other areas of host brain were free of RSA. The results indicate that fetal cortical transplants have a permanent BBB dysfunction. Blood-borne compounds, either injected or naturally occurring such as immunoproteins, can have direct access to the interstitial spaces of CNS transplants and could conceivably affect function. It is suggested these permeability changes might be due to angiogenesis and alterations in capillary phenotype. (Supported by NS-17468).

269.2 INNERVATION OF EMBRYONIC NEOCORTICAL CELL SUSPENSIONS BY THALAMOCORTICAL AXONS OF DIFFERENT AGED HOSTS. F.F. Ebner and R. S. Erzurumlu, Brown University, Providence, RI.,02912

Transplanted embryonic neocortex is innervated by only a few thalamic fibers after differentiating in adult host cortex. Many lines of evidence indicate that glial cells in cortex change dramatically at postnatal day 8, a time after the thalamic and commissural fibers arrive in cortex, but before the majority of cortical synapses are formed. The present experiments were carried out to characterize the degree of innervation of postmitotic embryonic day 18 (E18) neuroblasts by thalamic fibers before, during, and after the time thalamic fiber synapses are formed in host cortex. Pieces of E18 (BALB/c) mouse parietal cortex were collected in Eagle's Medium with 0.1% trypsin, washed in Eagle's Medium and suspended by repeated gentle pipetting through a Pasteur pipette. Injections of 0.2-0.3 ul of cell suspensions were made into the barrelfield region of the SI cortex of newborn (P0), P3 and P30 animals through a fine glass pipette attached to a Hamilton syringe. Thirty days later the host animals were anesthetized and the ventrobasal nucleus of the thalamus was entered horizontally with a Hamilton syringe avoiding any damage to neocortex and injected with 0.02ul of HRP-WGA. Labeled thalamocortical fibers that project to the region of the implanted cells were then charted and analyzed to determine the degree of innervation of the transplanted cells by the host.

In all cases the cell suspensions aggregated into cell groups that could be distinguished from the host cortex only by the irregularities in cytoarchitecture of the donor cells and the host cortex. No sign of glial reaction was detectable anywhere around transplants in hosts younger than P8. Transplants in P0 hosts were large and usually occupied the depth of cortex. They contained numerous, very fine caliber HRP labeled axons that were collected into dense bands of labeled terminals often traveling parallel to linear arrays of donor cell bodies, but orthogonal to the layers of host cortex. Many retrogradely labeled neurons were also present deep within the transplants of P0 hosts which without further labeling cannot be identified with certainty as donor cells that project to the host thalamus. P3 host transplants were consistently smaller in size than in P0 hosts and often contained fascicles of host axons growing completely across sections through the transplants. Myriads of fine caliber labeled thalamic fibers were present throughout these transplants. Only a few labeled thalamic axons were observed in the transplants of P30 hosts.

We conclude that a dramatic change takes place in cortex within the first week of life which disallows the thalamic fibers from elongating into and innervating transplanted cells. Our ongoing studies are aimed at precisely defining this time point and at identifying the nature of the change that disallows later innervation of the transplant by thalamic fibers. (Supported by NIH grant #NS-13031 and the Mathers Foundation)

269.3 ANTERIOR HYPOTHALAMIC TRANSPLANTS: IDENTIFICATION OF PARVICELLULAR NEURAL POPULATIONS DERIVED FROM THE ANLAGEN OF THE SUPRACHIASMATIC AND PARAVENTRICULAR NUCLEI. S.J. Wiegand and D.M. Gash. Dept. of Neurobiology and Anatomy, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Fetal anterior hypothalamus survives transplantion to the third ventricle/median eminence of adult rats with congenital or experimentally induced diabetes insipidus, and an amelioration of symptoms observed in some host animals is correlated with the presence within the grafts of large neurons containing the antidiuretic hormone, vasopressin (VP) or its associated neurophysin (Marciano and Gash, Brain Res. 370:338, 1986). However, neurophysin immunoreactive (NPir) neurons identified within the transplants are not a uniformly magnocellular, but express a variety of morphological phenotypes. In the present study NPir neurons contained in anterior hypothalamic grafts were characterized on the bases of their morphology, cytological organization, connectivity and association with other peptidergic cell types. A particular goal of this experiment was to evaluate possible homologies between NPir cell groups identified in the grafts and parvicellular NP-containing cell populations ordinarily present within the adult anterior hypothalamus.

Anterior hypothalami obtained from normal Long Evans rat fetuses at 16 or 17 days post conception were transplanted to the lateral, third or fourth ventricles of adult, VP-deficient Brattleboro rats. Animals were sacrificed 4-6 weeks post transplantation, and brain sections were processed for the immunohistochemical localization of NP. Alternate series of sections were processed for localization of VP, oxytocin (OT), vasoactive intestinal polypeptide (VIP) and/or corticotropin-releasing factor (CRF). Fetal hypothalami survived readily at each transplantation site studied. Numerous parvicellular NPir neurons are present in nearly all grafts, and can be classified as belonging to one of three distinct subtypes.

The most frequently encountered parvicellular NPir subtype (Type I) is comprised exclusively of VPir neurons. Type I cells exhibits many features characteristic of the suprachiasmatic nucleus (SCN) *in situ*. These neurons are organized into well defined clusters and in association with VIPir neurons of a similar small size (9-13μ), and VPir fibers of very fine caliber ramify extensively around the cell bodies of origin and in their immediate vicinity. Type I neurons project, almost exclusively, to areas of the host brain that are normally innervated by the VP neurons of the SCN. A second type of parvicellular NPir neuron often identified within the transplants also expresses VP, but not OT. However, Type II cells are slightly larger (12-18μ) and less densely packed than the SCN-type, and they project to vasculature. Type II parvicellular neurons (or a morphologically similar companion population) also contain CRF, but are not associated with VIPir cells. Similar characteristics are exhibited by neurons in the medial parvicellular division of the paraventricular nucleus (PVN). A third type of parvicellular NPir neuron identified within the grafts was comprised of fusiform and multipolar cells 10-25μ in diameter. In marked contrast to the other parvicellular NPir cells identified within the grafts, Type III parvicellular neurons were predominantly oxytocinergic, as are the NPir neurons of the posterior and dorsal parvicellular subnuclei of the PVN.

Thus, all of the principal parvicellular NPir cell groups present in the normal anterior hypothalamus are represented in anterior hypothalamic transplants. Furthermore, although the fetal donor tissue is incompletely differentiated at the time of transplantation, the various subclasses of NPir cells develop their characteristic associations with other chemically identified cell types contained within the grafts, and maintain their distinct affinities for appropriate target tissues.

Supported by NIH Grant NS 19900

269.4 NEURAL GRAFTING IN THE ENDOCRINE HYPOTHALAMUS, D.E. Scott, Dept. of Anatomy and Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23501.

The present investigation is the continuation of a program initiated in 1980. To date 137 rats have been processed and examined. Under deep anesthesia Brattleboro host rats of the Long-Evans strain with chronic autosomal homozygous diabetes insipidus (DI) were stereotaxically implanted with fragments of anterior hypothalamus derived from normal 17 day post-coitus fetal brains. Host rats were killed by nembutal overdose between days 3 and 120 post-surgery and were prepared for routine scanning-electron microscopy coupled with immunocytochemistry with antisera against arginine vasopressin (AVP), neurophysin (NP), tyrosine hydroxolase (TH), and laminin (LA). Neural grafts in DI host rats were observed to grow vigorously and became well vascularized within 72 hours following their implantation into the third cerebral ventricle of DI hosts. The process of vascularization was multiphasic and appeared to involve the fusion of invading extrinsic mesenchymal elements from the host with intrinsic endothelial primordia of the neural graft. Substantial neural and ependymal ingrowth occurred between the opposing edge of the graft and the ventricular wall of the host. Most invading vessels from the host were of the unfenestrated variety. Fenestrated vessels that arose from the hypophyseal portal system in the underlying median eminence were also seen to penetrate overlying fetal neural grafts. However, having once entered the parenchyma of the graft, portal vessels lost both their perivascular spaces and fenestra and assumed the anatomical appearance of typical cerebral capillaries. Grafts reacted with antisera against AVP demonstrated small numbers of magnocellular perikayria despite substantial numbers of AVP positive varicosities as well as NP positive multipolar nerve cell bodies. Between days 9 and 20 post-surgery, remarkable accumulations of supraependymal neurons (SEN) were observed to emerge upon the floor of the host cerebral ventricle in anatomical juxtaposition to transplants. The cell line was also observed upon the ventricular surface of neurografts as well. Immunocytochemical investigations employing antisera against TH suggests that a substantial proportion of these SEN are catecholaminergic. This phenomenon of SEN emergence is discussed with respect to the influence of fetal neurotropic factors upon neuronal plasticity and reorganization in both neural transplants and DI host rat brains.

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269.5 **Synaptic Innervation of Grafted Hypothalamic Neurons By Host Catecholamine Fibers In Rats.** W.F. Silverman, P.F. Aravich, T.J. Collier, and J.R. Sladek, Jr. Dept.of Neurobiology and Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, New York.

An important issue in transplant neurobiology is the degree to which neural grafts are integrated into the host brain. To explore this question, ultrastructural immunocytochemistry for tyrosine hydroxylase (TH) was applied to fetal rat hypothalamic grafts transplanted adjacent to the medial forebrain bundle (MFB) of adult hosts. The grafted tissue, containing the supraoptic (SON) and paraventricular (PVN) nuclei, as well as other portions of the mediobasal hypothalamus is normally richly innervated by noradrenergic fibers from the (MFB). Hypothalamic tissue from fetal day 17 Long-Evans rats was dissected out and stereo-taxically implanted into the lateral diencephalon of young adult hosts. Following survival periods of 6, 12, and 20 weeks, the host animals were sacrificed by intracardiac perfusion of 5% acrolein and 4% paraformaldehyde. Sample vibratome sections (50 µm) were stained for Nissl substance to locate the grafted tissue and remaining sections were then processed for pre-embedded TH immunocytochemistry and electron microscopy. Some sections were stained for vasopressin-associated neurophysin (NPII) to assess the extent of survival and function of transplanted hypothalamic neurons. At the light level, NPII-positive neurons and fibers in the grafts were observed at each of the survival times. Twelve and 20 week subjects exhibited in addition, a dense plexus of TH-positive fibers resembling that normally found in the SON or PVN. Ultrastructurally, few labelled TH-labelled terminals were observed in the 6 week subjects. In contrast, 12 and 20 week transplants exhibited numerous TH-positive terminals, many of which appear to make synaptic contact with dendrites and neuronal somata in the grafts. Because of the paucity of TH-positive cell bodies in the transplanted tissue, it is likely that these terminals originate in ascending noradrenergic fiber systems which were in close proximity to the transplanted tissue. These findings suggest that ectopically placed neurons may receive a relevant synaptic input from the host brain.

269.6 RAPHE TRANSPLANTS REVERSE SLEEP DEFICITS INDUCED BY NEONATAL ADMINISTRATION OF 5,7-DIHYDROXYTRYTAMINE A. McRae-Degueurce, G. Gandolfo*, L. Glin*, and Cl. Gottesmann*, Laboratoire de Psychophysiologie, Nice, and INSERM U-259, Bordeaux, France

Serotonin (5-HT) has been suggested to play a role in the initiation and maintenance of sleep-waking states in rodents. Here we extend this hypothesis by demonstrating that transplanted 5-HT neurons can reverse sleep deficits in adult animals induced by neonatal administration of 5,7-dihydroxytryptamine (5,7-DHT). Neonatal animals (4 days old) received an intracisternal administration of 5,7-DHT (100 ug). An aged matched sham group received a vehicle injection. Three months later the animals were implanted for sleep-waking studies. Seven behavioral states were dissociated using an automatic sleep-waking scoring system. The electrophysiological recordings established that paradoxical sleep decreased from 10.5% to 6.4% ($P<0.01$) whereas slow wave sleep increased from 21.9% to 28.7% ($P<0.01$) in the 5,7-DHT treated rats compared to the sham group. The other sleep states were not modified. The 5,7-DHT treated rats were then injected a suspension containing dissociated 5-HT neurons (16-day-old embryos) in the cisterna magna. One month later sleep recordings were again performed and demonstrated that both slow wave and paradoxical sleep were restored to normal levels. Immunocytochemical observations revealed numerous 5-HT immunoreactive neurons and processes in the tissue surrounding the IVth ventricle. This transplant paradigm employed in conjunction with this sleep model provides a means to selectively investigate neurobiochemical correlates of sleep-waking states which may be associated with specific neurotransmitter deficits.

269.7 FUNCTIONAL ACTIVITY OF RAPHE NEURONS TRANSPLANTED IN THE HIPPOCAMPUS AND CAUDATE-PUTAMEN. A COMBINED NEUROCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY IN THE ADULT AND AGED RAT. A.Beek*, H.W.M.Steinbusch, A.Frankhuyzen*, [a]J.Tonnaer*, [b]F.Gage and [c]A.Björklund (SPON:G.V.Lees). Dept.Pharmacology, Free University, Amsterdam, [a]Organon Int.,Oss, The Netherlands; [b]UCSD, San Diego,USA and [c]University of Lund,Lund, Sweden.

Fetal rat brain cells, transplanted into the adult and aged rat brain, survive and form functional synaptic connections. Significant changes in behaviour of the host animal have been related to the release of specific neurotransmitters from the transplanted tissue. Adult (3 months) and aged (28 months) rats with unilateral 5.7-dHT lesions in the lateral ventricles received grafts from cell-suspensions taken from the rhombencephalic (RR) or mesencephalic (MR) raphe cell areas selected from embryonic stages E10 till E20. These cell-suspensions were implanted unilaterally into the rostral part of the hippocampus or the caudate-putamen for immunohistochemical and neurochemical studies. Both the MR and the RR cell-suspensions appeared to have the potency to regenerate serotonergic fibers in the previously denervated adult and aged brain areas. However, the RR cell-suspension also resulted in the outgrowth of noradrenaline- and substance-P-immunoreactive fibers. In studying the functional activity of the serotonergic reinnervation, we have combined immunohistochemistry and neurotransmitter release studies on adjacent hippocampal slices of adult rats. Results showed that after a survival time of 10 weeks the serotonergic innervation of the hippocampus was greatly restored and moreover that the depolarization (20 mM K+) induced Ca2+ dependent release of serotonin amounted to about 80 % of normal values. There appeared to be a striking similarity between the immunohistochemical and neurochemical data regarding the increase in the number of newly formed serotonergic fibers, the increase of the release of radiolabeled serotonin and the extent of the outgrowth in the hippocampus. These results give supplementary evidence that newly formed serotonin-containing fibers not only reinnervate the denervated hippocampus but in addition that these fibers can release serotonin upon depolarization.

269.8 EARLY INTERACTION OF HOST AND DONOR IN XENOGENIC SEPTO-HIPPOCAMPAL TRANSPLANTS. J. Wells, B. Vietje*, D. G. Wells*, R. J. McKeon and M. Fiala*. Dept. Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Xenogenic septo-hippocampal transplants have been shown to be effective in the restoration of structure and function following fornix lesions. In previous experiments we have shown that embryonic septal-basal forebrain cells could be labeled with HRP in order to follow the distribution of the injected cells in the host hippocampus. We found in these transplants between species that the surviving labeled cells were located in the host tissue outside of the injection site by 6 days after transplantation. In order to study when the outward migration and differentiation occurred in xenogenic transplants, and preliminary to studies using immunosuppressants, we have looked at the transplants between different species during the early phases of its interaction.

Cell suspensions of embryonic mouse septal-basal forebrain region were labeled by HRP and injected into the hippocampal formation of adult rats. The host received a partial lesion of the fornix at the same operative session. The host brain was analyzed at 1, 2 and 3 days after transplantation. The brains were stained by DAB.

Labeled cells were seen within the injection site at 1, 2 and 3 day survival times, but at 1 day most were obscurred by the peroxidase positive hemorrhage. A variety of labeled cell types were observed within the injection site - pyramidal cells, elongated cells with polar dendrites, and multipolar cells. None of these cell types were present in the injection site at 6 days. Over the first three days the cells became more differentiated with dendrites developing more complex branching patterns. Even at one day, a few dendrites were seen eminating from the injection site. The number of labeled cells that appeared outside of the injection site increased during the first 3 days. When the results were compared to homogenic transplants, they suggested that the early xenogenic interaction selectively effects the survival of certain cell types and the distribution of the surviving cells. Supported by PHS-23266.

269.9 **FACTORS AFFECTING SURVIVAL AND OUTGROWTH FROM TRANSPLANTS OF ENTORHINAL CORTEX** Robert B. Gibbs and Carl W. Cotman. Department of Psychobiology, U. C. Irvine, Irvine, CA. 92717

We previously showed that transplants of entorhinal cortex would innervate appropriate regions of the hippocampal formation when placed into the entorhinal region of adults (Gibbs et al., J. Comp. Neurol., 237:47-64, 1985). In the present study we examined the extent to which transplant survival and outgrowth is dependent upon (1) introducing a delay between the lesion and implant surgeries, and (2) removing native entorhinal projections prior to transplantation.

Transplants consisted of embryonic entorhinal cortex (E17-E18) placed into the left entorhinal region of adult rats (Sprague Dawley). The importance of the delay was examined by comparing grafts transplanted immediately (ECX-no delay) with grafts transplanted 8-10 days (ECX-delay) after severing the angular bundle. The importance of destroying native entorhinal projections was examined by comparing grafts in the ECX-delay group with grafts transplanted (1) in the absence of any prior lesion, and (2) 8-10 days after producing a lesion of occipital cortex, medial septum, or fimbria fornix. Eight to ten weeks post-transplantation, graft-derived innervation of the hippocampal formation was examined by injecting wheat germ agglutin-horseradish peroxidase (WGA-HRP) into the left hippocampal formation and counting the number of retrogradely labeled cells in the transplant. Transplant volume was determined from adjacent sections stained with cresyl violet, or for the detection of acetylcholinesterase (AChE).

Grafts in the ECX-delay group (n=14) were large (average = 12.9 mm^3) and contained many HRP-labeled cells (average = 1943 cells/graft, 144 cells/mm^3). When no delay was used (n=6), grafts were small (average = 1.1 mm^3) and contained no HRP-labeled cells. In many of these cases (4/6), a cavity was observed in the transplant area suggesting that the majority of the transplant had degenerated.

Grafts transplanted without severing the angular bundle were smaller than those in the ECX-delay group (average = 2.8-5.0 mm^3), but integrated well with the host tissues, and received AChE-positive innervation from the host. Graft-derived innervation was observed following transection of the fimbria (average = 365 cells/graft, 85 cells/mm^3) which, in addition to removing non-entorhinal hippocampal afferents, removed some crossed entorhinal projections due to partial destruction of the dorsal hippocampal commissure. Few HRP-labeled cells were observed in grafts transplanted in the absence of a prior lesion (n=14), or following lesions which did not damage entorhinal fibers (average = 12-33 cells/graft, 5.0-17.9 cells/mm^3).

These data suggest that (1) introducing a delay between the lesion and implant surgeries significantly enhances the survival of entorhinal grafts and is necessary for the establishment of transplant-to-host projections, and (2) fibers from entorhinal transplants compete selectively with native entorhinal projections while innervating the host.

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269.10 **RECOVERY OF FUNCTION AFTER A COMPLETE FIMBRIA-FORNIX LESION : INTERACTION OF FETAL SEPTAL TRANSPLANTS AND POST-OPERATIVE ENVIRONMENT.** B.WILL, C.KELCHE AND J.C.DALRYMPLE-ALFORD. Lab. de Neurobiologie des comportements, Université Louis Pasteur, 7 rue de l'université, 67000 Strasbourg, FRANCE.

The aim of this study was to examine whether the behavioral effects of intracerebral grafts in rats with brain lesions can be modulated by post-operative housing conditions. Sixty-six young adult female rats of the Long-Evans strain were used. Twenty-two rats were sham-operated (S) and 44 rats sustained a complete aspirative lesion of the fimbria-fornix and of the supracallosal striae (L). The cavity thus prepared was used in half the rats as the implantation site for a fetal septal graft (LT). The implants were taken from 16 to 17 day-old embryos. Two days after implantation, half the rats of each group (S, L and LT) were housed in an "enriched" environment (SE, LE and LTE); the other half remained in standard conditions (SS, LS and LTS). Two and ten months later, the rats were tested on the 12 standard Hebb-Williams maze learning problems, one problem per day and 6 trials per problem. Initial and repetitive errors were scored.

Two and ten months after surgery, we noted that: 1) S rats made significantly fewer errors (*) than L rats. 2) SE rats committed significantly fewer initial errors than SS rats. 3) In non-grafted rats with lesions, "enriched" housing did not affect recovery of function, confirming our previous results.

Ten months after surgery: 1) The implants promoted recovery of function but **only** in rats housed in an "enriched" environment (LE/LTE:Fl/16=5.55; P<.05) and **not** in rats housed in standard condition (LS/LTS:Fl/19=1.84; P=.29). 2) Performance of LT rats placed in an "enriched" environment was significantly improved when compared with that of LT rats housed for the same period in standard conditions (LTE/LTS:Fl/16=16.2; P<.01).

These data show that both the environment and implant were unable **alone** to promote behavioral recovery but,**applied together**, they reduced the deficits produced by destruction of the intrinsic septo-hippocampal pathways. The interaction of post-operative "enriched" environment and fetal septal implants on the recovery of behavioral function after fimbria-fornix lesions in adult rats will be discussed in the light of anatomical (Nissl staining) and histochemical (acetylcholinesterase) analyses of the grafted brains.

(*) Indicated differences concern total (initial+repetitive) errors, if not alternatively mentioned.

269.11 **SEPTAL GRAFTS RESTORE RHYTHMIC SLOW ACTIVITY (THETA) IN FIMBRIA-FORNIX TRANSECTED HIPPOCAMPUS** G. Buzsaki[1,3], A. Bjorklund[2], and F.H. Gage[3]. Dept. of Physiology, Pecs, Hungary[1], Dept. of Histology[2], Lund, Sweden, Dept. of Neurosciences[3], UCSD, La Jolla, CA.

Solid pieces of the fetal septal region (SG) or hippocampus (HPC) were implanted in a cavity formed by aspiration of the fimbria-fornix (FF) and the overlying cingulate cortex in adult rats. In other lesioned animals cell suspensions obtained from the fetal septal area (SS) or the locus coeruleus region of the brainstem (LC) were injected stereotaxically into the deafferented host hippocampus. Six to nine months after transplantation the animals had chronic recording electrodes implanted into both hippocampi. EEG and unit activity were monitored during running in a wheel, drinking and immobility. Unilateral fimbria-fornix lesions abolished rhythmic slow activity (RSA or theta) in the ipsilateral hippocampus and no recovery was seen up to 9 months later in either the control FF-lesioned animals or in the rats with LC suspension grafts. Recovery of RSA, however, was observed in all animals with solid septal grafts and in some rats with solid HPC grafts. Similar to normal rats, RSA was present only during running and absent during drinking and sitting still. Coherence measurements of RSA between the transplanted and intact hemispheres resulted in high values (0.70-0.95). Concurrent with RSA, interneurons and granule cells in the host hippocampus fired rhythmically at RSA frequently (6-9 Hz). The depth profile and the antero-posterior distribution of the power of RSA correlated with the amount and distribution of the graft-induced acetylcholinesterase-positive reinnervation of the host hippocampus. In contrast to the animals with solid septal grafts, placed within the FF lesion cavity, the rats with intrahippocampal septal suspension grafts displayed only short duration bursts of RSA, and mainly during immobility. Based on these findings it is suggested that at least a proportion of the RSA "pacemaker" cells of the host septum survives the transection of the fimbria-fornix fibers and that a graft of fetal septal or hippocampal tissue implanted into the lesion cavity may be capable of relaying this pacemaker activity to the host hippocampus. This effect may be due to the ability of the *grafted tissue* to promote the regeneration of new, direct or *indirect*, *septo*-hippocampal connections across the lesion *cavity*.

269.12 **HIPPOCAMPAL TRANSPLANTS: A METHOD FOR STUDYING THE NEUROTOXIC MECHANISMS OF TRIMETHYLTIN.** Y. Théorêt*, D.A. Lindemann*, T.W. Bouldin* and M.R. Krigman. Dept. of Pathology, Univ. of North Carolina at Chapel Hill, NC 27514

The selectivity of the neuronal lesion induced by trimethyltin (TMT) in the different subfields of the hippocampal formation (HF) suggests that intrinsic and/or extrinsic neuronal pathways may play a role in the pathogenesis of toxicity. We have used the HF transplant technique as a means to isolate hippocampal neurons from their normal cortical and sub-cortical connections.

Fetal hippocampal tissue (E18-21 days) was transplanted into the cerebellum (adult or weanling Long Evans rats) or near the HF (2-4 day old neonates). TMT was administered 6 to 13 weeks after transplantation. Animals received TMT either as a single oral dose of 8 mg/ml/kg or as 4 consecutive daily doses of 4 mg/ml/kg. Saline (vehicle) controls for each model were included. The animals were perfusion fixed 7 days after the single TMT dose or one day after the fourth dose in the sequential regimen. Nissl or Timm's stained brain sections were examined by light microscopy.

Following the single TMT dose, neuronal necrosis was seen in the CA1 pyramidal layer and in the temporal granule cells of the host HF. The granule cells of HF transplants in cerebellum showed more extensive necrosis than those of the host HF. Granule cells of HF transplants which established apparent contact with the host HF showed a comparable or a lesser degree of necrosis than the host granule cells. In contrast, granule cells of HF transplants which failed to establish apparent contact with the host HF exhibited neuronal necrosis comparable to that observed in HF transplants to the cerebellum. Four daily doses of TMT produced primarily neuronal necrosis of host granule cells (irrespective of the septotemporal location) with little or no effect on the CA1 pyramidal neurons. The susceptibility of transplant HF in the 4 dosage model was similar to the single dose model with respect to granule cells. No obvious difference was observed in the vulnerability of pyramidal neurons to TMT between the transplant and the host in the neonate group. The general laminar organization of CA3 and CA1 pyramidal neurons was virtually absent from the HF transplants within the cerebellum.

The results suggest that the location of the transplanted HF influences its sensitivity to TMT. The lack of neuronal extrinsic connections to the transplants, the absence of potential target cells for neurons within the transplant (e.g. pyramidal and/or GABAergic neurons for granule cells) and/or the development of aberrant fiber connections (associational projections from pyramidal neurons to to the distal dendrites of granule cells) are factors which could affect the neuronal susceptibility to TMT. (Supported by ES01104 and IRSST fellowship to YT)

270.1 VISUAL-FRONTAL DISCONNECTION IN LEARNING BY MONKEYS. D. Gaffan and S. Harrison. Exptl Psychology, Oxford University, OX1 3UD, England.

Visual association cortex in the inferior temporal lobe projects directly to the ipsilateral inferior frontal lobe. To investigate the behavioural function of this projection, we disconnected it by making crossed unilateral ablations of the inferior temporal lobe in one hemisphere and of the inferior frontal lobe in the other hemisphere. The learning ability of animals with this disconnection was compared with that of control animals having the two lesions in the same hemisphere, and also with that of fornix-transected animals.

One task was visuo-motor conditional learning with "hold" and "tap" responses. The animals had to learn which of these two manual reponses to make to each of a set of visual discriminative stimuli. Here visual-frontal disconnection produced a learning impairment but fornix transection did not. The second task was straightforward two-choice simultaneous visual discrimination learning in an automated apparatus. Here also, visual-frontal disconnection produced a learning impairment but fornix transection did not. The third task was visuo-motor conditional learning with "approach" and "withdraw" responses. Here visual-frontal disconnection impaired learning, and so did fornix transection. The fourth was visual discrimination learning in the WGTA. This was not impaired in any group, even in a group in which visual disconnection both from frontal lobe and from amygdala was combined with fornix transection.

Direct cortico-cortical sensory interaction with the frontal lobe, bypassing medial temporal lobe limbic structures, plays a general, important and hitherto under-emphasised role in learning and memory in the monkey.

270.2 THE BED NUCLEUS OF THE STRIA TERMINALIS IN THE RHESUS MONKEY: CONNECTIONS WITH THE AMYGDALA AND THE MEDIAL DORSAL NUCLEUS. R.C. Saunders, D.L. Rosene and M. Mishkin, Laboratory of Neuropsychology, NIMH, Bethesda, MD, 20892, and Dept. Anatomy, Boston University School of Medicine, Boston, MA, 02118.

Connections between the bed nucleus of the stria terminalis (BNST), the amygdala, and the medial dorsal nucleus (MD) were investigated as part of an attempt to understand the organization of amygdaloid efferent connections and their contribution to memory function, and to compare these connections with those of the hippocampal formation, another structure important for memory. Patterns of connections were investigated in the monkey (Macaca mulatta) using both anterograde and retrograde tracers. Tritiated amino acids were injected into the amygdala in eight monkeys and into the BNST in two monkeys. Retrograde tracers, either horseradish peroxidase or fluorescent dyes, were injected into the BNST in two animals, into the amygdala in two animals, and into MD in four animals. Both the ventral amygdalofugal pathway and the stria terminalis were found to carry nonreciprocated projections from the amygdala that terminate in both the BNST and in the magnocelluar part of MD (MDmc). Dense labelling was also found throughout MDmc after amino acid injections into the BNST. The label after the amygdaloid injections appeared to have a topographical organization and a patchy, 'peri-cellular' appearance, whereas the label after the BNST injections was uniform throughout MDmc. On the basis of injections of retrograde tracers amygdaloid projections to MDmc and the BNST were found to arise primarily from the medial basal and the accessory basal nuclei, although retrogradely labelled cells were found in most other parts of the amygdaloid complex as well. Cells with retrograde label were also found in the BNST after MD injections. The amygdala thus has both a direct and an indirect route to the thalamus. The BNST, as a major recipient of amygdaloid efferents and with substantial output to MDmc, makes up an important component of amygdalo-thalamic connections.

These connections of the amygdala with the BNST and MDmc seem to parallel those of the hippocampal formation with the mamillary bodies and the anterior thalamic nuclei. Like the amygdala, the hippocampal formation has both a direct and an indirect route to the thalamus. The subiculum of the hippocampal formation sends fibers through the fornix directly to the anterior thalamic nuclei as well as to the mamillary bodies. The mamillary bodies in turn send an extensive projection to the anterior thalamic nuclei. Thus, the mamillary bodies and the BNST appear to occupy comparable anatomical positions within seemingly parallel hippocampal and amygdaloid efferent systems, respectively. This anatomical correspondence suggests that the BNST and the mamillary bodies may serve analogous functions within the two limbic memory systems. (Supported in part by NIH grant NS 19416)

270.3 OBJECT RECOGNITION MEMORY FOLLOWING DIENCEPHALIC LESIONS IN MONKEYS E.J. Holmes and C.E. Schroeder*. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

For many years, clinical studies have shown that patients with diencephalic damage involving either the mammillary nuclei of the hypothalamus and/or the mediodorsal nuclei of the thalamus demonstrate profound and lasting anterograde amnesias. Using nonhuman primates as models, we have been able to demonstrate that bilateral lesions of either the mammillary or mediodorsal nuclei in monkeys markedly impairs the short-term retention of spatial location over periods of from 10 to 30 secs. (Neurosci. Abstr., 11:460, 1985). The purpose of the present experiment was to expand these studies in order to determine the extent that these lesions would affect the short-term retention of object features.

The subjects were 11 cynomolgus (M. fascicularis) monkeys. Four monkeys received bilateral lesions of the mediodorsal nuclei, 3 monkeys received bilateral lesions of the mammillary nuclei, and 4 monkeys served as sham operated controls. All training and testing was conducted in a Wisconsin General Test Apparatus.

During initial training and testing, each trial consisted of a pre-trial and a test-trial. On the test-trial, the subjects were required to choose a novel object when paired with a familiar object shown immediately before on a pre-trial (two-choice task). Subsequently, the animals were successively presented with 2 objects on two consecutive pre-trials and had to choose a novel, third object when it was presented simultaneously with the two objects shown before (three-choice task). For both paradigms, a different set of objects was used on each trial (from a total pool of approx. 300 objects) and the retention periods were gradually increased from a minimum of 10 secs. to a maximum of 3 mins. During the retention periods, the subject's view of the stimuli was obscured by an opaque screen.

In the two-choice task, none of the animals with diencephalic lesions were noted to differ from the controls in recognition accuracy (approx. 90% correct) for the novel object with retention periods from 10 secs. to 2 mins. and only 3 of the 7 experimental animals showed any decline in performance with retention intervals of 3 mins. In the three-choice task, however, both of the diencephalic lesion groups were noted to show marked declines in performance as the retention interval approached 3 mins. with the controls remaining at approximately 90% correct performance. Moreover, the magnitude of the retention impairment could be increased significantly by increasing the interobject similarity of the object set on each trial.

Thus, lesions of either the mammillary body or mediodorsal nuclei may impair the short-term retention of objects as well as the short-term retention of spatial location in nonhuman primates in a manner analogous to that reported with humans following diencephalic damage.

270.4 THE EFFECTS OF FORNIX TRANSECTION AND OF CINGULATE CORTICAL ABLATION IN MONKEYS ON SPATIAL DELAYED NONMATCHING-TO-SAMPLE IN A T-MAZE. E.A. Murray, M. Davidson*, D. Olton, D. Gaffan, and S. Suomi*, Lab. Neuropsychology, NIMH, Bethesda, MD 20892, Dept. Psychology, Johns Hopkins Univ., Baltimore, MD 21218, Dept. Expt. Psychology, Univ. Oxford, Oxford, England, and Lab. Comparative Ethology, NICHD, Bethesda, MD 20892

Most studies of the role of the hippocampal system in spatial learning and memory in monkeys have been limited to situations in which the monkey makes arm movements to the left or right side of a test tray. The present study was undertaken to examine spatial behaviors in monkeys that were locomoting rather than reaching.

Rhesus monkeys were trained preoperatively on delayed nonmatching-to-sample in a T-maze. In this task, the monkey was first given a sample run through the maze with only one of the arms of the "T" open in order to obtain a grape in the goal box and then, one minute later, was given a test run with both arms of the "T" open. The monkey was required to go into the arm previously not entered in order to obtain five more grapes. Daily testing consisted of four such trials separated by intertrial intervals of about 1 1/2 hours. The monkeys were trained for five or six days per week to a criterion of 36 correct responses out of 40 trials. After learning, they were tested for two days at each of two longer delays, 5 minutes and 15 minutes, that were imposed between the sample and test runs; these two delays were spent in the start box and in the home cage, respectively. Upon completion of testing, monkeys received one of three operations: i) transection of the fornix, the major fiber bundle connecting the hippocampal formation with the thalamus and basal forebrain; ii) ablation of the cingulate cortex, a region that serves not only as a target of hippocampo-thalamo-cortical projections, but also as a source of inputs into the hippocampal formation; and iii) a sham operation. After surgery the monkeys were trained in the same manner as before, except that they were given five trials per day instead of four and were tested for 10 days at each delay instead of two.

Preoperatively, seven monkeys required an average of 73 trials and 32 errors to attain criterion. Postoperatively, there were no group differences in relearning; the two control monkeys, two with fornix transection, and three with cingulate ablation averaged about 88 trials and 33 errors to relearn. By contrast, the experimental groups were severely impaired with the imposition of longer delays. Control animals averaged about 87 and 81% correct responses on the 5 and 15 minute delays, respectively, whereas monkeys with cingulate ablation averaged 69 and 61%, and those with fornix transection scored only 60 and 44% correct responses.

These preliminary results show that the hippocampal system, including its cingulate component, is critical for spatial memory in monkeys in tasks requiring locomotion.

270.5 THE EFFECTS OF SINGLE AND COMBINED LESIONS OF THE AMYGDALA AND HIPPOCAMPUS UPON RECOGNITION MEMORY IN THE RAT. J.P. Aggleton* and J.N.P. Rawlins* (SPON: W.J. Heitler) Dept. of Psychology, Durham Univ., Durham DH1 3LE, U.K. and Dept. of Exp. Psychology, Oxford Univ., Oxford OX1 3UD, U.K.

Research into the effects of limbic lesions upon recognition memory in monkeys has led Mishkin to propose that combined damage to the amygdala and hippocampus is necessary to produce global amnesia (Mishkin, M., Nature, 273: 297, 1978). In order to determine the generality of this finding we have conducted parallel research in rats and have reported that recognition memory can survive extensive hippocampal lesions (Aggleton, J.P., Hunt, P.R., and Rawlins, J.N.P., Behav. Brain Res., 19: 133, 1986). We have now examined the effects of amygdaloid lesions and combined hippocampal and amygdaloid lesions upon the same task.

The amygdaloid lesions (AMX), which were subtotal, were made with ibotenic acid while the hippocampal lesions (HPC) were made by aspiration and involved an additional small region of cortex (CORT). Following recovery from surgery the rats (8 AMX, 5AMX + HPC, 6 AMX + CORT, 7 SHAM) were trained in a Y maze to select between two distinctive goal boxes. One goal box was familiar as it resembled the start box while the other was unfamiliar. Selection of the unfamiliar box was rewarded. This then became the start box for the next trial in which the animal was again rewarded for selecting an unfamiliar goal box in preference to one which resembled the new start box. Nearly all of the rats were able to acquire this nonmatching task to a criterion of 80% correct (over 5 consecutive days, 10 trials a day) and no consistent differences were observed between the four groups. On reaching criterion the animals received 150 trials in which a delay of either 0 (training condition), 20, or 60 sec, was imposed before selection of the unfamiliar from the familiar goal box. Animals with combined AMX + HPC lesions were only impaired under the delay conditions (ave. performance for the 20 s and 60 s delays combined; SHAM 69%, AMX 75%, AMX + CORT 73%, AMX + HPC 60%). The effects of hippocampal lesions combined with more extensive amygdaloid lesions will also be reported and the similarity of the above findings to those obtained with primates will be considered.

270.6 **DIFFERENT EFFERENT PROJECTIONS OF THE GENICULO-AMYGDALA PATHWAY MEDIATE AUTONOMIC AND BEHAVIORAL CONCOMITANTS OF CONDITIONED FEAR: POSSIBLE COMPLETION OF THE CONDITIONED RESPONSE CIRCUITS.** J. Iwata, J.E. LeDoux, and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., N.Y., N.Y.

The increases in arterial pressure and emotional behavior elicited by a tone paired with footshock both critically depend upon projections from medial portions of the medial geniculate body to the dorsal nuclei of the amygdala (LeDoux et al., Neuroscience 17, 615-627). The aim of the present study was to determine whether the autonomic and behavioral responses could be separately disrupted by damage to brain areas efferent to the geniculo-amygdala pathway.

Using ibotenic acid (IBO), intrinsic neurons were destroyed while preserving axons of passage in the amygdala or areas to which the amygdala projects. Rats were anesthetized (halothane) and IBO (0.15-0.3 ul; 15 ug/ul) was microinjected into the dorsal amygdala (n=6), lateral hypothalamic area (LH, n=7), ventromedial nucleus of the hypothalamus (VM, n=5), bed nucleus of the stria terminalis (BN, n=5), or central grey (CG, n=15). Controls were unoperated. After 14d, the animals were instrumented for computer-assisted recording of arterial pressure (AP) and subjected to classical fear conditioning. The next day, CS elicited changes in AP and the duration of activity suppression ("freezing") elicited by the acoustic conditioned stimulus (CS) were measured during extinction trials presented in the animal's home cage. Subsequently, the rats were sacrificed and the brains removed and processed histologically.

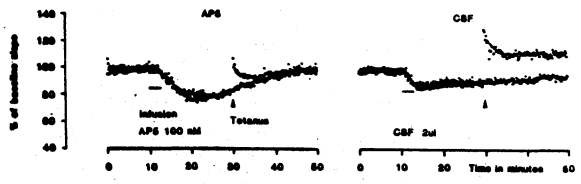
In unoperated controls, the CS elicited increases in AP (18$\pm$ 4mmHg) and induced freezing (118$\pm$1 sec). These responses were both significantly reduced by bilateral injection of IBO into the amygdala (AP: 7$\pm$3, $p \leq .05$; freezing: 16$\pm$5, $p \leq .05$). Injections in LH reduced the AP response (7$\pm$2, $p \leq .05$) but not CS elicited freezing (114$\pm$4, ns). Lesions in the CG significantly reduced freezing (50$\pm$12, $p \leq .05$) but not the AP response (14$\pm$1, ns). In contrast, injections in the VM or BN did not affect either the AP or freezing responses. None of the lesions interfered with the expression of unconditioned AP changes elicited by the footshock.

These data, together with our earlier findings, suggest a tentative completion of the circuits mediating conditioned fear. Transmission of the acoustic CS through the auditory system to the MG and from there to the amygdala is necessary for the expression of both the autonomic and behavioral conditioned emotional response. Projections from the amygdala to the LH, which in turn projects to brainstem and spinal autonomic effector neurons, moderate the autonomic responses, whereas projections from the amygdala to the central grey, which projects to spinal skeletal motor neurons, underlie conditioned emotional behavior. Supported by NIMH, NIH, and the Am. Heart Assoc.

270.7 **ELEMENTS OF A HYPOTHESIS CONCERNING THE PARTICIPATION OF HIPPOCAMPAL N-METHYL-D-ASPARTATE RECEPTORS IN SPATIAL LEARNING.** R.G.M.Morris* (SPON: ENA). MRC Cognitive Neurosci. Group, Univ.St.Andrews, St.Andrews, Fife, KY16 9JU, SCOTLAND.

N-methyl-D-aspartate receptors are known to be abundant in the mammalian hippocampus (HPC) (Olverman et al., Nature, 307:460, 1984). They are not involved in normal fast synaptic transmission, but do participate in the induction of long term potentiation (LTP) (Collingridge,G., J.Physiol., 334:19, 1983). Importantly, their associated ion channels have unusual voltage dependency (Mayer et al, Nature 309:261, 1984). At physiological concentrations of $Mg2+$ occurring in vivo, inward current to transmitter activation at the resting potential would be minimal, but would rise if the cell had previously been partially depolarised. Thus, paired activation of a cell should allow the second input to cause an influx of $Ca2+$ ions through the now activated NMDA receptors, triggering long-term changes in synaptic efficacy (eg. Lynch and Baudry, Science, 224:1057, 1984). Physiological studies are consistent with such a mechanism (Wigstrom et al., Acta Physiol.Scand., 126: 317, 1986), and have shown it to be sensitive to the selective NMDA antagonist +2-amino-phosphonovaleric acid (AP5). Furthermore, Kelso et al's findings (PNAS, in press) that HPC cells can process their input according to a "pseudo-Hebbian" conjunction rule are consistent with the proposed mechanism of association.

How may these ideas be applied to spatial learning? Entorhinal cortex (EC) lesions cause a severe deficit in declarative spatial memory (Schenk,F. and Morris,R., Exp.Brain Res., 58:11, 1985). "Cooperativity" amongst afferents from EC is necessary for the induction of synaptic enhancement (McNaughton et al., Brain Res., 157:277, 1978). Assume the latter reflects (1) the AP5 sensitive mechanism described above, and thus (2) the processing of spatial "associations". It follows that AP5 should, as Lynch, Baudry and I have confirmed, cause a selective impairment of spatial learning without effect upon visual discrimination (Morris et al., Nature, 319:774, 1986). More recently, Halliwell and I (Poster, this meeting) have found that infusion of nanomolar amounts of AP5 into HPC, sufficient to cause a blockade of LTP but only minimal effect upon



baseline field potentials (Fig 1), also causes an anterograde spatial amnesia. These findings extend earlier work (McNaughton et al, J.Neurosci., 1986) implicating hippocampal synaptic plasticity in learning, and are consistent with the hypothesis that NMDA receptors participate in, at least, spatial learning.
Supported by an MRC Fellowship Grant (United Kingdom).

270.8 CENTRAL PATHWAYS IN CONDITIONING. T.J. Voneida, D. Christie and R. Stoll, Department of Neurobiology, Northeastern Ohio Univ. College of Medicine, Rootstown, OH 44272.

Cats were trained to flex their right forelimb to a tone CS, in both instrumental and classical paradigms, using shock as the UCS. Previous studies in this laboratory have shown that sensorimotor cortical lesions contralateral to the trained limb result in a profound (in some cases complete and permanent) loss of the CR, with no loss of the UCR. Pyramidal tract section (uni- and bilateral), immediately caudal to the pons produced no deficit in performance level.

In an effort to determine more precisely which pathways and structures are critically involved in both instrumental and classically conditioned responses, trained animals underwent section of the cerebral peduncle immediately rostral to its entrance into the pons. Preliminary results suggest that this (corticopontocerebellar) system is not involved in the mediation of a conditioned response of the type described. All operated animals responded at the 80 - 100% level on the first postoperative training day, and attained criterion level (3 consecutive days at 80% or above) during their third session.

A number of recent studies from several laboratories (Thompson; Yeo & Glickstein), along with an earlier postulation by Voneida strongly implicate the cerebellum as a crucial part of the associative learning pathway. In addition, the studies of Tsukahara, Smith, Moore and others, point to the red nucleus as a critical part of the conditioning pathway. Finally, Yeo & Glickstein have implicated the dorsal accessory olive as part of the network. In an effort to reconcile some of the above results, we are presently subjecting trained cats to surgical section of the corticorubral and the olivocerebellar systems. We are suggesting the possibility that the red nucleus, sensorimotor cortex and cerebellum represent crucial focal points in associative learning, and that the rubrospinal system may constitute a final pathway for its expression.

270.9 LONG-TERM HABITUATION OF ACOUSTIC STARTLE FOLLOWING LESIONS OF THE CEREBELLAR VERMIS OR CEREBELLAR HEMISPHERES. R. N. Leaton and W. F. Supple, Jr. Department of Psychology, Dartmouth College, Hanover, N.H. 03755

We have shown that the cerebellar vermis is part of an essential circuit for long-term habituation of the acoustic startle response in rats (Leaton & Supple, Science, 1986). Lesions of the vermis abolish long-term habituation but leave short-term habituation unaffected. While the effects are at least partially response specific (Leaton & Supple, 1985; Supple & Leaton, 1986) anatomical specificity has been assumed from historical rather than current comparisons. The present experiment compared the effects of lesions of cerebellar vermis with lesions of cerebellar hemisphere on habituation of the acoustic startle response. The cerebellar hemispheres were considered important as comparison lesion sites as a control for general cerebellar damage and because of their importance in the classically conditioned eye-lid response to an auditory CS in rabbits (Thompson and associates).

Startle response was measured to 114-dB, 100-msec, white-noise bursts on a 60-sec interstimulus interval (ISI) in 10 daily 6-trial sessions. Following these tests responsivnesss to a systematic variation of ISI was tested over intervals of 5, 10, 30, 60, and 120 sec. Three groups of 12 rats were tested; one following aspirations of the cerebellar vermis, one following bilateral aspirations of the cerebellar hemispheres, and one following sham operations.

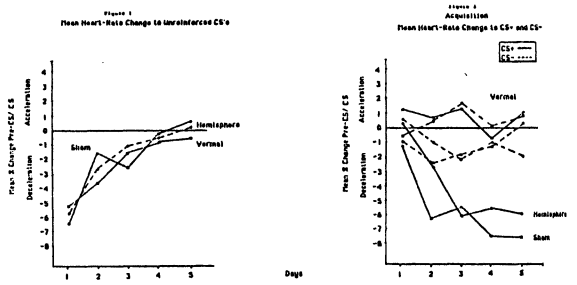
The outcome of the daily testing replicated previous results showing that lesions of the cerebellar vermis blocked long-term habituation. The hemisphere group, while showing significant long-term response decrements in contrast with the vermal group, was intermediate between the vermal and sham groups and was not significantly different from either in the daily tests. However, the differences among these groups were brought out clearly during the systematic ISI manipulations. The vermal group showed significant within-session habituation at all ISI values from 120 to 5 sec. The sham group did not show significant decrements at either the 120 or 60 sec intervals, reaching significant decrements at the 30 sec and shorter intervals. The hemisphere group did not show significant decrements until the ISI was shortened to 10 sec. The data reflect the absence of long-term, between sessions habituation in the vermal lesioned rats and suggest a heightened sensitization in the hemisphere lesioned rats.

270.10 LESIONS OF THE CEREBELLAR VERMIS BLOCK CLASSICALLY CONDITIONED BRADYCARDIA IN RATS. William F. Supple, Jr. and Robert N. Leaton. Department of Psychology, Dartmouth College, Hanover, N.H. 03755.

Preliminary results indicate that the cerebellar vermis is necessary for the development of heart-rate conditioned responses in rats. Vermal lesions severely attenuated simple conditioning without altering unconditioned heart-rate responses (Supple and Leaton, 1986).

In the present experiments rats were tested in a differential conditioning procedure following either cerebellar vermal, bilateral cerebellar hemispheric, or sham lesions. They were tested restrained in a tube and heart-rate was recorded following unreinforced tone CS presentations and tone-tail shock pairings. The groups showed a similar decelerative orienting response which habituated across days (Figure 1). In the differential conditioning procedure one tone (CS+) was always followed by shock and the other (CS-) was never paired with shock. Both the sham- and hemispheric-lesioned groups developed reliable discriminatively conditioned bradycardia. The vermal-lesioned group showed no differential conditioning (Figure 2). These data indicate that cerebellar vermal lesions selectively disrupt associate heart-rate changes without affecting unconditioned heart-rate responses to the stimuli used in training.

In a second experiment, cerebellar vermal lesions disrupted accelerative HR CR's in unrestrained rats without affecting classically conditioned freezing responses.



270.11 **CEREBELLAR LESIONS THAT PREVENT ACQUISITION OF CONDITIONED NMRs DO NOT EFFECT REFLEX FACILITATION OF THE UNCONDITIONED RESPONSE** J.J. LoTurco and D.J. Weisz, Department of Psychology, Yale University, New Haven, CT., 06520

Rabbits with bilateral lesions of the dentate-interpositus nucleus in the cerebellum and operated controls received counterbalanced tone/light discrimination training of the classically conditioned nictitating membrane response (NMR). On CS+ trials tone (4000 Hz, 80 db, 500 msec) or light (28 lx) coterminated with an air puff to the cornea (2.0 psi, 100 msec). The interstimulus interval was 400 msec and the intertrial interval was 50 sec. On CS- trials the tone (T) or light (L) was presented in isolation. Interspersed with discrimination training were three types of test trials-- the CS+ followed by an air puff, the CS- followed by an air puff, and an air puff presented in isolation. There was one CS-/air puff test trial for every nine CS- trials presented during discrimination training. The test trials were used to determine the effectiveness of the CS+ and CS- in modifying the unconditioned NMR. Reflex modification was determined by comparing NMR amplitudes and latencies during CS+ and CS-/air puff test trials with response amplitudes and latencies during air puff-alone test trials.

There were no differences in the acquisition rates in operated control animals receiving T+/L- or L+/T- discrimination training. All controls acquired conditioned NMRs by day 4 and exhibited criterion discrimination performance also by day 4; however, lesioned animals did not acquire conditioned NMRs after 9 days of conditioning. Tones increased the amplitudes of unconditioned NMRs for both the lesion and control animals when the tone was the CS+ but not when it was the CS-. The mean facilitation on Day 1 for the lesion (55%) and the control (61%) groups was not significantly different. Therefore, lesions to the cerebellar deep nuclei that disrupted acquisition of the conditioned NMR did not disrupt reflex facilitation of the unconditioned response. In both the lesion and control groups reflex facilitation of the unconditioned responses by the tone decreased both within days and across days with 82% facilitation on the first block of Day 1 and 28% at the end of Day 1, and 75%, and 40%, at the beginning of Days 2 and 3 respectively. There were no effects of any of the manipulations on the latencies of the unconditioned responses.

The light stimulus used in this experiment did not facilitate the unconditioned NMRs in either the lesion or control group. Therefore, auditory and visual stimuli that conditioned at the same rate had different effects on reflex facilitation. While the tone CS+ did not result in faster CR acquisition, CRs to the tone (7.2 mm) were significantly larger than CRs to the light (4.5 mm).

270.12 CEREBELLAR LESIONS PREVENT OPTIMAL EXECUTION BUT NOT ACQUISITION OF THE CONDITIONED NICTITATING MEMBRANE RESPONSE. J. P. Welsh*, I. Gormezano and J. A. Harvey. Departments of Psychology and Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

It has been reported that lesions of the anterior dentate-interpositus (D-I) region of the cerebellum abolish the retention and acquisition of ipsilateral conditioned nictitating membrane responses (NMRs) in the rabbit (McCormick and Thompson, J. Neurosci. 4, 2811-2822, 1984). The purpose of this study was to determine in greater detail both the critical locus of damage in the D-I region and the associated effects on the response characteristics of the NMR. Naive rabbits (n = 38) first received 16 daily conditioning sessions consisting of 66 pairing of a 250-msec, binaural tone conditioned stimulus (CS) and a 100-msec air-puff unconditioned stimulus (UCS) to the right cornea. The CS-UCS interval was 285 msecs and the mean intertrial interval was 60 sec. A CS-alone test trial occurred after every 10 conditioning trials. All animals had reached postasymptotic levels of CRs by day 16. After day 16, lesions were aimed at the anterior portions of the right D-I region. After 21 days recovery conditioning resumed for 12 days with the UCS directed at the right eye (days 17-28). All animals with lesions encompassing the anterodorsal D-I region (n = 15) showed a significant reduction in CRs on conditioning trials and no evidence of reacquisition. UCRs were not affected. Of these 15 rabbits, 9 demonstrated responding to the tone CS that was at or below baseline levels. The critical region for these lesion effects encompassed a small region of the anterodorsal aspects of the nucleus interpositus and overlying white matter. Subjects that did not possess lesions of this area did not demonstrate severe CR reductions. Analysis of test trials revealed acquisition of CRs whose response characteristics were obscured by the occurrence of unconditioned responses evoked by the UCS on paired trials. The 9 subjects that had failed to demonstrate CRs during the 250 msec of tone CS presentation did demonstrate longer latency responses during test trials. These responses occurred within 250 msec after CS offset and demonstrated a significant acquisition over days 17-28 from 9 to 22.1%. These CRs were also further characterized by their low amplitudes (1.32 mm) and prolonged rise times (406 msec). These data have confirmed the important role of the cerebellum in classical conditioning but suggest that the cerebellum is not as essential for learning as it is for the optimal execution of the learned response. These findings are consistent with the classical interpretation of cerebellar involvement in the regulation and adaptive tuning of learned movements (Holmes, Brain, 40, 461-535; Brooks, Exp. Brain Res. (Suppl.), 9, 170-183, 1984).
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271.1 STRUCTURE OF THE GENE FOR HUMAN GLUTAMIC ACID DECARBOXYLASE (GAD): ATTEMPTS TO FIND MOLECULAR LESIONS ASSOCIATED WITH NEUROGENETIC DISEASES. D.L. Kaufman*[1], M.G. Erlander*[2], E.M. Askanas*[1], A.M. Wong*[1], J.H. Menkes*[3], and A.J. Tobin[1,4,5]. [1]Department of Biology, [2]Neuroscience Program, [3]Departments of Pediatrics and Neurology, [4]Molecular Biology Institute, and [5]Brain Research Institute, University of California, Los Angeles, CA 90024.

Glutamate decarboxylase (GAD) catalyzes the production of gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the central nervous system. Because movement disorders and seizures are often associated with altered GABA levels in brain and CSF, the gene for brain GAD has been suggested as a "candidate gene" for a variety of neurogenetic diseases. In order to identify possible lesions in the GAD gene, we have undertaken to characterize the gene for human GAD and to develop methods for detecting point mutations within its coding sequence.

We have used a cloned cDNA for feline GAD (Kaufman, et al., Science, in press) to isolate human GAD cDNAs and genomic DNAs. The human cDNA clones contain the entire coding sequence of GAD. We have used a subclone of one of these cDNAs to direct the in vitro synthesis of GAD. To accomplish this, we placed the human GAD cDNA into a transcription vector (pIBI76), transcribed it with SP6 RNA polymerase in the presence of GpppG, and translated the resulting capped RNA in a reticulocyte extract. The RNA directed the synthesis of a single polypeptide of 64 kD. This polypeptide was precipitable with antiserum to GAD.

To define the gene structure of GAD we are now mapping overlapping lambda and cosmid genomic clones. The coding region of the human GAD gene spans at least 40 kb.

In order to identify possible changes in the GAD gene associated with hereditary seizure disorders, we have isolated DNA from a number of patients with familial forms of epilepsy. We are scanning these DNAs for insertions, deletions, and polymorphisms in the GAD gene by Southern blotting. We are also applying and extending the method of Myers, et al. (Science 230, 1242, 1985) to detect point mutations in the coding region of the GAD gene.

This work was supported by grants to JHM from the Dystonia Medical Research Foundation and to AJT from the Epilepsy Foundation of America and NIH (#NS 20356 and NS 22256). DLK is supported by a USPHS Training Grant in Cell and Molecular Biology (#GM 07185).

271.2 ISOLATION AND CHARACTERIZATION OF THE GENE ENCODING RAT THYROTROPIN RELEASING HORMONE K. Stewart, S. Lee*, G. Mandel*, and R.H. Goodman* Division of Molecular Medicine, New England Medical Center, Boston, MA 02111

Although thyrotropin releasing hormone (TRH; pyroGlu-His-Pro-NH_2) was the first of the hypophysiotropic hormones to be chemically characterized, relatively little is known about its biosynthesis. Studies of TRH biosynthesis have been problematic due to the small size of the peptide and its low concentration in various brain regions. We have recently isolated a cDNA clone encoding the TRH precursor from a rat hypothalamic lambda gt11 library (*Science* 231: 159,1986). The structure of the cDNA indicates that the precursor is a polyprotein containing five copies of TRH sequence. We have now used the TRH cDNA to examine TRH biosynthesis in rat brain and to isolate the rat TRH gene.

The TRH cDNA was cloned into the expression vector pSP64 in reverse orientation with respect to the SP6 promoter. ^{32}P-labelled antisense RNA generated from the expression vector was used in Northern blot assays of mRNA from various regions of rat brain. TRH mRNA was located in the paraventricular nucleus and lateral portions of the hypothalamus, the olfactory bulb, and the median raphe nucleus. All of these regions are known to contain immunoreactive TRH. TRH mRNA was also identified in the thalamus, a region not previously shown to produce TRH. TRH mRNA from the thalamus was identical in size (1800 nucleotides) to that found in other brain regions.

Southern blotting analysis of DNA from rat liver indicated that there is a single copy of the TRH gene in the rat genome. Hybridization analysis suggested that the TRH gene is highly conserved between rat and human but diverges significantly in amphibia. To isolate the rat TRH gene, we screened a genomic library cloned in Charon 4A bacteriophage with the nick-translated TRH cDNA. DNA from several individual bacteriophage plaques hybridized to the radiolabelled probe. These bacteriophage were isolated, amplified, and the DNA inserts were subcloned into plasmid vectors. The structure of the gene and its promoter region was determined by restriction endonuclease analysis, nucleotide sequencing, and S-1 nuclease mapping.

In summary, we have used the TRH cDNA to characterize the regional expression of the TRH gene within the rat brain and to determine the structure of the gene. TRH mRNA was found in all brain regions known to produce the TRH peptide as well as in the thalamus where TRH production had not been recognized. The structure of the TRH gene is similar to that of other neuropeptide precursors.

271.3 ISOLATION OF ENZYME cDNA CLONES USING A NOVEL TECHNIQUE (EIDA): ISOLATION OF A PEPTIDE ACETYLTRANSFERASE. J.H. Eberwine*, J.D. Barchas, W.A. Hewlett* and C.J. Evans Dept. of Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305.

The biological activity of proteins and peptides can be profoundly affected by enzyme-catalyzed covalent modifications such as acetylation, sulfation, glycosylation or amidation. This article describes the cloning of such an enzyme, a peptidyl-acetyltransferase from rat brain which catalyzes the N-terminal acetylation of endorphins and perhaps other substrates in vivo.The acetyltransferase was cloned using a novel strategy whereby a cDNA expression library was screened using a solid-phase enzyme activity assay; this technique combines the use of substrate coupled to a solid support and subsequent recognition of product using a specific antisera. We have called this method the enzyme immuno-detection assay (EIDA). The EIDA should prove useful in the isolation of other clones for proteins which possess enzymatic activity upon expression in bacterial hosts.

We have looked at brain region distribution of the peptide acetyltransferase mRNA and find it expressed in many regions. The size of the mRNA localized in the brain is approximately 1.9 kilobases. AtT20 tumor cells (mouse pituitary tumor cell line) also express the peptide acetyltransferase. Using the same stringency of hybridization and washing, the size of this mRNA was found to be approximately 2.1 kilobases. Whether this reflects a species difference or a tissue difference is currently being investigated.

Supported by NIDA grant #01207 and NIMH grant #23861.

271.4 MOLECULAR CLONING AND STRUCTURAL ANALYSIS OF THE mRNA AND GENE THAT ENCODE NEUROPEPTIDE Y. J.M. Allen, J.B. Martin and G. Heinrich Depts. of Neurology and Medicine, Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114.

Neuropeptide Y (NPY) is a 36 amino acid member of a larger family of peptides that includes pancreatic polypeptide (PP). Although closely related structurally, these peptides are discretely and uniquely distributed. Thus, the NPY gene is exclusively expressed in neural tissue, whereas PP and PYY are restricted to pancreatic and intestinal endocrine cells respectively. In addition to a high degree of cell specificity, expression of each of these genes may be regulated in a developmentally specific fashion and may yield diverse peptide products derived from proteolytic processing of a larger precursor.

We wish to investigate the molecular mechanisms that regulate expression of this family of genes and, therefore, have begun to analyze the structure of a mRNA and gene that encode rat NPY. To obtain cloned cDNAs that represent NPY, a rat hypothalamic cDNA library constructed in bacteriophage lambda gt11 (R.H. Goodman, Tufts University Medical School) was screened with a synthetic oligonucleotide hybridization probe based on the nucleotide sequence of human NPY mRNA. Restriction enzyme mapping and nucleotide sequence analysis of specifically hybridizing clones revealed that the largest clone contained 60 nucleotides of 5' untranslated region and a typical polyadenylational signal AATAAA followed within 23 nucleotides by a poly-A tail. Thus the clone of approximately 700 nucleotides appears to be a full length representation of rat NPY mRNA. The structure and organization of this mRNA is very similar to that of human NPY mRNA and encodes a similar precursor or pre-pro-NPY.

The same oligonucleotide probe was used to screen a rat genomic library constructed by Land et al in bacteriophage Charon 4A. Four specifically hybridizing clones were obtained. Restriction enzyme and Southern blot hybridization analysis revealed that all the clones contain large extensions of the 5' flanking region of the NPY gene but only two contain the 3' flanking region. Our present structural analysis suggests that the rat NPY gene has at least 2 introns and therefore appears similar to the PP gene in organization.

These molecular clones of rat NPY mRNA and gene will be useful in further structural studies in investigations of the molecular mechanisms that confer developmental and tissue specificity on NPY gene expression and will provide important tools for further studies of NPY biosynthesis, biology and pharmacology.

271.5 CLONING AND SEQUENCE ANALYSIS OF cDNA FOR BOVINE CARBOXYPEPTIDASE E (ENKEPHALIN CONVERTASE). L. D. Fricker and E. Herbert*, Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland OR 97201 USA

Enkephalin convertase (carboxypeptidase E, or CPE) is the carboxypeptidase B-like enzyme involved in the biosynthesis of the opiate peptides, as well as other peptide hormones and neurotransmitters. This enzyme has been purified to homogeneity from bovine pituitary, and a partial amino acid sequence has been determined. Oligonucleotide probes corresponding to the amino acid sequence have been used to isolate a cDNA clone from a bovine pituitary cDNA library. The predicted amino acid sequence of the cDNA clone contains several pairs of basic amino acids, and displays some homology with carboxypeptidases A (CPA) and B (CPB). Although the overall homology is low, all of the amino acids thought to be essential for the catalytic activity of CPA and CPB have been conserved in CPE. Major differences between CPE and the other carboxypeptidases include an additional 120 amino acids at the C-terminal of CPE, and numerous changes in amino acids near the substrate binding sites. These changes may reflect the different specificities of the enzymes: CPE is very specific for C-terminal basic amino acids, whereas CPB and CPA both have fairly broad substrate specificities. The homology between the carboxypeptidases suggests a common evolutionary origin, which supports the theory that the peptide processing enzymes evolved along with the first neuropeptides.

The cDNA encoding CPE hybridizes primarily to mRNA of approximately 3.3 kb in bovine brain, pituitary, and adrenal medulla. In these tissues, the CPE cDNA probes also hybridize to 2.1 and 2.6 kb mRNA, although these species represent less than 5 % of the 3.3 kb mRNA. The shorter mRNA's appear to arise from alternate polyadenylation sites in the 3' untranslated region. Restriction fragment analysis of bovine genomic DNA indicates a single gene encodes CPE. Results of studies on the regulation of this gene will be presented.

271.6 **IDENTIFICATION AND CHARACTERIZATION OF GENOMIC CLONES FOR TYROSINE HYDROXYLASE.** J.M. Carroll, S.E. Hyman, H.M. Goodman*, T.H. Joh. Lab. of Molec. Neurobiology, Cornell University Medical College, New York, N.Y. 10021 and Dept. of Molec. Biology, Massachusetts General Hospital, Boston, Mass 02114.

Tyrosine hydroxylase, the enzyme catalyzing the rate limiting step in catecholamine biosynthesis, is subject to a variety of physiological stimuli. In response to stress, for example, neural activity causes a rise in TH by processes that increase both the number and activity of enzyme molecules. Recently, increased mRNA has been observed in adrenal medulla (Wallet et al., 1983), superior cervical ganglia (Black et al, 1985) and locus coeruleus (Biguet et al., 1986) in response to reserpine, a pharmacological agent thought to simulate transsynaptic induction. In addition, increases in mRNA have been measured in PC12 cells (Baetge et al., 1982; Lewis et al.,1983) and bovine chromaffin cultures (Naranjo et al., 1986) treated with glucocorticoid or cyclic AMP. These reports suggest that transcriptional mechanisms participate in TH regulation.

Analysis of the gene should provide a better understanding of the molecular processes controlling TH expression. Toward this end, we have isolated TH genomic clones from a rat library constructed in the EMBL3 vector (generous gift of G. Scherer). From 9 x 10^9 recombinants, screened with a 600 bp probe containing sequences from the 3' end of the coding region, seven positives were identified. Upon rescreening with a 180 bp PstI fragment derived from the 5' end of the cDNA, two independent isolates, carrying inserts of approximately 20 kb, were selected. We are currently determining whether the complete gene is present in either of the clones.

Restriction analysis of the clones revealed a 1600 bp Bam HI fragment which hybridizes to the 5' 180 bp cDNA probe. This fragment is being sequenced to determine the extent of upstream flanking region present. We plan to construct plasmids containing the 5' flanking region of the TH gene and, by transfection into mammalian cells, examine promoter and enhancer elements responsible for tissue specific and regulated expression of TH.

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271.7 ISOLATION OF A cDNA CLONE ENCODING HUMAN BRAIN GLUTAMATE DEHYDROGENASE. C. Banner[1*], S. Silverman[1*], L. Vitkovic[1*], J. Thomas[1], K. Lampel[1*], and R. J. Wenthold[2] (SPON: M. R. Martin). [1]Lab. Mol. Biol. and [2]Lab. Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892.

Glutamate is thought to be a major excitatory neurotransmitter in the brain. Mammalian glutamate dehydrogenase (GDH, EC 1.4.1.3), which catalyzes the reaction alpha-ketoglutarate + NAD(P)H + $NH_4^+ \rightleftharpoons$ L-glutamate + NAD(P)$^+$ + H_2O, may play an important role in the metabolism of this neurotransmitter. In addition, only glutamine synthetase and GDH are thought to play major roles in detoxifying NH_4^+ in the brain. Recent immunocytochemical evidence suggests that glial cells of the rat cerebellum are enriched in GDH (Wenthold, R. J. et al., submitted). As part of a program to study the regulation of gene expression in neural-glial interactions, we have isolated a cDNA clone for human brain GDH. We screened a human brainstem lambda gtll expression library (kindly provided by R. Lazzarini) with polyvalent and affinity purified antibodies to GDH. One clone was isolated which expressed a GDH-beta-galactosidase fusion protein of 136 kD. Partial protease digestion of the fusion protein and genuine bovine liver GDH followed by immunodetection with the GDH antibody revealed many common peptides. The cDNA insert of approximately 1.8 kb was subcloned for sequencing and cRNA probe synthesis. In rat brain mRNA, the cRNA probe detected a transcript of about 3.6 kb. By nucleotide sequencing we showed that 257 amino acids of the carboxy terminal of human GDH are encoded by the cDNA.

271.8 STRUCTURE OF THE MOUSE MUSCLE ACETYLCHOLINE RECEPTOR α SUBUNIT GENE. K.E. Isenberg, C.M. Crowder* and J.P. Merlie. Dept. of Psychiatry and Pharmacology, Washington University Sch. of Med., St. Louis, MO 63110.

We are interested in transcriptional regulation of mouse muscle acetylcholine receptor (AChR) expression. Expression of the AChR changes during development and following denervation. Changes in receptor level following denervation are accompanied by increases in mRNA levels sufficient to account for the increase in AChR. Although the increase in mRNA may be affected by a number of mechanisms, increased transcription of AChR subunit genes is an attractive and testable hypothesis. As a first step in our study of α subunit transcriptional regulation, we have isolated clones from λ genomic libraries using radiolabelled mouse α subunit cDNA as a probe. We determined the location of coding sequence within the clones with exon specific oligonucleotides. The restriction enzyme maps for the clones overlap each other but no clone contains the complete coding sequence for the mouse AChR α subunit gene. A composite map of the gene constructed from the restriction enzyme maps of the clones shows the coding sequence contained with a 15 kb region. In addition, we have 10 kb of flanking sequence on either side of the coding region. We chose to locate putative regulatory regions by mapping DNase I hypersensitive sites in restricted mouse genomic DNA. DNase I hypersensitive sites are correlated with regulatory regions in other genes. We have mapped DNase I hypersensitive sites to 5' and 3' regions of the α gene chromatin. The genomic clones and derived restriction map along with the location of DNase I hypersensitive sites will permit the direct study of transcriptional regulation of the α subunit gene.

271.9 HUMAN MUSCLE N-CAM: ISOLATION AND CHARACTERISATION OF cDNA CLONES. J.G. Dickson*, R.D. Cox*, C.A. Quinn*, W. Putt* and F.S. Walsh. Institute of Neurology, Queen Square, London WC1N 3BG, England.

The neural cell adhesion molecule (N-CAM) is a cell-surface glycoprotein expressed by neurons and several other cell types such as skeletal muscle, and may play a central role in neural tissue morphogenesis and nerve fibre pattern formation. The availability of cDNA clones for rodent and avian N-CAM sequences has allowed initial studies to be made on the expression of N-CAM mRNAs in brain and skeletal muscle tissues. As one approach to studying the structure and regulation of the human N-CAM gene we have isolated a number of N-CAM-related cDNAs from a human skeletal muscle expression library. The library of 5×10^5 recombinants was prepared in the phage expression vector λgtll, using mRNA from human myotube cultures. Probing the library with a mouse N-CAM cDNA pM1.3 (Goridis et al, EMBO J 4:631, 1985) yielded over 50 positive recombinants, 31 of which were successfully plaque-purified through subsequent screens and inserts ranging from 1.2 to over 6Kb were represented. Confirmation that these clones corresponded to muscle N-CAM transcripts was obtained by immunoscreening: 5 out of the 31 recombinant phage could be induced to express β-galactosidase fusion protein reacting specifically with antiserum to human N-CAM.

One cDNA clone, coded HFM-1 has been analysed in detail following subcloning into PUC and M13 vectors, and shown to contain a 1.3 Kb insert sharing homology with pM1.3 over a 600 base region. In Southern blot analyses of genomic DNAs both these probes identify common bands in mouse and human samples. Using clear distinctions in restriction fragment patterns between mouse and human DNA, a panel of DNA samples from mouse-human somatic cell hybrids was analysed by Southern blotting to identify the chromosomal location of the human gene. With both probes, the presence of human DNA restriction fragments was correlated with the presence of chromosome 11 in the hybrid cells confirming the map location of human N-CAM previously indicated by in situ hybridization studies (Nguyen et al, J Cell Biol 102:711, 1986).

The panel of human muscle N-CAM cDNAs we have produced will be of value in isolating the human N-CAM gene from genomic libraries, in examining the function and regulation of N-CAM expression in skeletal muscle and in defining the relationships between muscle N-CAM transcripts and those expressed in human brain.

271.10 cDNA CORRESPONDING TO A BRAIN CALMODULIN-BINDING PROTEIN: SEQUENCE AND STRUCTURAL FEATURES OF THE BINDING SITE. J. M. Sikela* and W. E. Hahn. Dept. of Cellular and Structural Biology, Univ. of Colorado School of Medicine, Denver, 80262.

Several brain proteins located at synapses and elsewhere in neurons bind calmodulin in the presence of calcium. In order to isolate genes encoding these proteins we have used labeled calmodulin as a probe to screen λgt11 expression libraries representing whole brain (mouse) mRNA. One recombinant we isolated (λICM-1) produced fusion protein that bound calmodulin in a Ca^{2+}-dependent manner (K_d 2-10 nM). The size of the fusion protein indicated the cDNA had an open reading frame (ORF) sufficient to encode ~150 - 200 amino acids. In order to gain more precise information on the ORF and attempt to identify the calmodulin binding site the cDNA insert was sequenced. The 583 bp cDNA insert contained a single long ORF of 155 amino acids. The recloning of a battery of restriction fragments of this cDNA showed that only recombinants containing the 5' end sequences of the cDNA produced fusion protein that bound calmodulin. This molecular genetic mapping narrowed the calmodulin binding site to a 37 amino acid domain. Structural analysis of this domain showed that the likely binding site (as based on features in common with defined peptides that bind calmodulin) is composed of amino acids 3 - 20, a region predicted to be in the form of a basic, amphiphilic alpha helix. Contained in this region is a cluster of basic residues adjacent to a hydrophobic stretch. While the exact amino acid sequence seems unimportant, some or all of the above features are thought to be characteristic of calmodulin binding sites (Cox et al., J. Biol. Chem. 260:2527, 1985; Blumenthal et al., Proc. Natl. Acad. Sci. 82:3187, 1985). The sequence of the predicted binding site is Asn-Ala-Arg-Arg-Lys-Leu-Lys-Ala-Ala-Val-Lys-Ala-Val-Val-Ala-Ser-Ser-Arg. These sequences share some homology to the calmodulin binding sequence in myosin light chain kinase (Lukas et al., Biochem. 25:1458, 1986). For example the sequence Ala-Arg-Arg-Lys is shared.

Northern blot analysis shows λICM-1 is complementary to relatively abundant brain mRNA species of ~2 and 3 kilobases respectively. It does not hybridize to mRNA from liver or kidney. Several lines of circumstantial evidence suggest λICM-1 might correspond to the Ca^{2+}/calmodulin-dependent protein kinase II, the most abundant protein kinase in brain. λICM-1 is complementary to a shorter cDNA clone that was selected on the basis of detection with antibodies to the kinase. While there is some amino acid homology between λICM-1 and myosin light chain kinase the Northern data indicate that the mRNAs corresponding to λICM-1 are not of sufficient size to encode a protein the size of this kinase (M.W. ~130,000). Also the size, abundance, and tissue distribution of the mRNAs that hybridize to λICM-1, as well as the affinity of the fusion protein for calmodulin, are consistent with λICM-1 as corresponding to the Ca^{2+}/calmodulin-dependent protein kinase II. Definitive identification is currently underway.

271.11 IDENTIFICATION OF CDNA CLONES FOR THE HUMAN MICROTUBULE ASSOCIATED PROTEIN TAU AND CHROMOSOMAL LOCALIZATION OF THE GENES FOR TAU AND MICROTUBULE ASSOCIATED PROTEIN 2. R.L. Neve, P.H. Harris*, K.S. Kosik*, D.M. Kurnit* and T.D. Donlon*. Genetics Div., The Childrens Hospital, Boston, MA 02115.

The microtubule associated proteins tau and microtubule associated protein 2 (MAP2) display complementary patterns of distribution in the brain: tau is associated with the axons of neurons, while MAP2 is present in neuronal soma and dendrites. Both molecules share epitopes with the neurofibrillary tangle (NFT), a major structural feature of Alzheimer's disease. Isolation of human cDNA clones for these proteins will enable us to analyze their molecular relationship to one another, and to study their spatial and temporal expression in the normal and diseased states. Such studies may provide insight into the nature of the perturbation of the neuronal cytoskeleton that accompanies formation of the NFT.

We have previously identified a partial human cDNA for MAP2, and we have now isolated human cDNA clones for tau. One million phage from a human 20-22 week fetal brain cDNA library in lambda gt11, consisting of 7.5×10^6 independent phage, were screened with the mouse tau cDNA clone pTA2 (Drubin et al., J. Cell Biol. 98: 1090, 1984). Of 75 positively hybridizing clones on the primary screen, seven were chosen for purification; the two largest of these putative tau cDNAs, p9TAU and p16TAU, with insert lengths of 2.9 kb and 1.7 kb, respectively, were selected for further analysis. To demonstrate the neural specificity of these clones, we hybridized them to RNAs from ten human fetal tissues. Both 9TAU and 16TAU hybridized to a single RNA species of approximately 6 kb present only in brain. Hybridization of the cDNAs to RNAs from rodent brains showed that the RNA species recognized by these cDNAs exhibits a developmental shift in size. Both clones hybridized to a 6 kb RNA species in the fetal (embryonic day 16) rat brain and neonatal (postnatal day 5) mouse brain, and to a 6.5 kb RNA species in the adult brain of rat and mouse.

We also report the human chromosomal localization of the MAP2 and tau cDNAs by both "spot blot" hybridization to individually sorted human chromosomes and in situ hybridization to chromosomes. Because of the observation that individuals with trisomy 21 (Down syndrome) exhibit unusually early development of NFT, which share epitopes with tau and MAP2, it has been suggested that chromosome 21 may carry coding sequences whose dosage may affect formation of the NFT. However, we show that the genes for MAP2 and tau are not among these postulated chromosome 21-specific sequences. The MAP2 gene is localized to chromosome 2q34-35; the tau cDNAs hybridize to chromosome 17q21, and one (p16TAU) also recognizes a region of homology on chromosome 6p21.

271.12 ANTIBODIES TO HUMAN CHOLINESTERASES ELICITED BY BACTERIAL-EXPRESSED PRODUCTS OF HUMAN CHOLINESTERASE cDNA. Hermona Soreq, Rivka Zisling*, Dina Zevin-Sonkin*, Catherine Prody*, and Haim Zakut*[1] Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot, and Dept. of Obstetrics and Gynecology, The Edith Wolfson Hospital and the Sackler Faculty of Medicine, Tel Aviv University, Israel.

Cholinesterases (ChEs) are rare, highly polymorphic and multisubunit serine esterases that rapidly hydrolyze the neurotransmitter acetylcholine in neuromuscular junctions and cholinergic synapses. To study the composition and regulation of various ChEs in humans, we have isolated a cDNA clone coding for a human ChE from fetal brain using oligodeoxynucleotide probes[1]. These were prepared according to a consensus peptide sequence present in the active site of both human serum pseudocholinesterase (acylcholine acyl hydrolase, EC 3.1.1.8, ΨChE) and Torpedo electric organ "true" acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7, AChE). The fetal brain cDNA clone, which codes for a polypeptide containing a putative signal peptide and both the N-terminal and active site peptides of human serum ΨChE, was used to elicit efficient anti-ChE antibodies. When subcloned into a pEX bacterial expression vector in conjunction with β-galactosidase, this human ChE cDNA produced a partially digested fusion polypeptide which reacted in protein blots with antibodies to β-galactosidase, human erythrocyte AChE and Torpedo electric organ AChE. The bacterial-produced protein encoded by this human ChE cDNA was purified by preparative gradient gel electrophoresis, electroelution and lyophilization and was injected into rabbits together with complete Freund's adjuvant. Rabbit serum was prepared following three successive injections of 300 μg of this protein at two weeks intervals. Immunoblot analysis revealed that this serum contains antibodies that interact at 1:10,000 dilution with amounts as low as 20 ng of highly purified 70 Kd AChE from erythrocytes, as well as with proteins of ca. 90 Kd in extracts from fetal brain and liver, both rich in AChE, and of ca. 60 Kd in fetal muscle and liver, both rich in ΨChE. A 200 Kd fetal muscle protein and a 35 Kd fetal brain protein were also detected in this analysis. The nature and interrelationships between these four proteins are currently being investigated.

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[1]Prody, C., Zevin-Sonkin, D., Gnatt, A., Koch, R., Zisling, R., Goldberg, O. and Soreq, H. (1986) Use of synthetic oligodeoxynucleotide probes for the isolation of a human cholinesterase cDNA clone. J. Neurosci. Res., in press.

272.1 IMMUNOCHEMICAL RELATIONSHIP AMONG NEUROFIBRILLARY TANGLES, NEURITIC PLAQUE CORE AMYLOID AND CEREBROVASCULAR AMYLOID IN ALZHEIMER'S DISEASE

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Three major cerebral lesions occur in Alzheimer's disease (AD): neurofibrillary tangles (NFT), neuritic plaques with various degrees of core amyloid [plaque core amyloid (PCA)] and cerebrovascular amyloid (CVA). These lesions give birefringence with congo red staining, leading to the belief that they are composed of proteins arranged in a twisted β-pleated sheet conformation. To study the immunochemical interrelationship of these lesions, we purified CVA from a case of familial amyloid angiopathy (without clinical or pathological features of AD) and from an AD brain. Meninges were stripped and homogenized in 2% SDS buffer, incubated for 2 hr at room temperature, boiled for 5 min and spun at 10,000 x g for 15 min. The pellet was rehomogenized in SDS-buffer, applied to a sucrose gradient of 1.0, 1.2, 1.4 and 2.0 M sucrose layers and spun at 200,000 x g for 90 min. The 1.4/2.0 M interface was highly enriched in amyloid as judged by congo red polarization microscopy.

An antiserum raised to the cerebrovascular amyloid from familial amyloid angiopathy immunolabeled all three lesions: NFT, PCA and CVA. Absorption of this antiserum with its immunogen abolished the staining of all three lesions. However, when the antiserum was absorbed with highly purified plaque core amyloid from AD brain, it failed to immunolabel CVA and PCA but still stained NFT, suggesting the presence of more than one type of antibody in the serum. Absorption with a heat-stable microtubule-associated protein (MAP) prep highly enriched in the MAP tau reduced the NFT staining significantly without altering the staining of CVA or PCA. An antiserum to AD cerebrovascular amyloid immunolabeled CVA and PCA in AD brain sections with no staining of NFT. Absorption of this serum with purified amyloid cores abolished the staining of both CVA and PCA. These findings suggest that 1) an immunochemically shared antigen is present in CVA and PCA (both of which contain extracellular 5-10 nm amyloid fibrils) and that it is not detectable in the intraneuronal NFT, which are principally composed of tau-rich 15-20 nm paired helical filaments; 2) non-AD CVA (familial amyloid angiopathy) shares an epitope or determinant with AD CVA and PCA.

272.2 CHOLINERGIC SEIZURES PRODUCE NEURONAL NECROSIS IN NORMOXEMIC RATS. D.G. Fujikawa, H.H. Itabashi* and C.G. Wasterlain*. Epilepsy Res. Lab., VA Med. Ctr., Sepulveda, CA 91343 and Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024, and Dept. of Pathology, Harbor-UCLA Med. Ctr., Torrance, CA 90509.

Limbic status epilepticus (SE) in the rat can be induced with systemic pilocarpine (PC) and produces neuronal injury in many brain regions, including CA3 of the dorsal hippocampus (Turski, W.A. et al., Behav. Brain Res., 9:315, 1983). We have found that even more widespread damage is seen in dorsal hippocampus if rats recover for 72 rather than 24 hours and that this damage occurs in the absence of hypoxemia, hypotension and hypoglycemia.

Five to 7 days after EEG electrode placement, adult male Wistar rats were given 10 mg/kg methylatropine i.p., followed by 400-500 mg/kg PC i.p. A separate group of rats (n=5) had arterial cannulations and were allowed to recover from anesthesia for 4 hours prior to baseline EEG recording and PC. Prior to and during seizures, EEG, mean arterial blood pressure (MABP), arterial blood gases and plasma glucose were monitored.

Of 23 rats, 14 (61%) had EEG seizure discharges, together with clonic jerks of head and limbs, rearing and falling. Of the 14 with EEG seizures, 7 (50%) had continuous seizures for at least 3 hours, at which time they were given 10 mg/kg diazepam and 60 mg/kg phenytoin i.p. This stopped the behavioral seizures but did not eliminate EEG seizure discharges. Rats which survived 3 days had their brains examined histologically; rats with EEG seizures (n=4) were compared to those given PC without seizures (n=3).

In rats with EEG seizures, edema and irreversibly damaged neurons were present in CA1, CA2 and CA3 of dorsal and ventral hippocampus, dentate gyrus, olfactory cortex, amygdaloid nuclei, entorhinal cortex, multiple thalamic nuclei and substantia nigra. Septal nuclei and cerebral cortex (layer 3) were less severely involved. The animals without EEG seizures had no brain damage. The seizure animals with physiological monitoring maintained MABP (C:122 ± 6, SE:128 ± 4 mm Hg; mean ± S.E.M.), arterial pO2 (C:83.1 ± 3.2, SE:98.3 ± 1.6 mm Hg) and plasma glucose (C:10.8 ± 2.1, SE:14.1 ± 1.3 mM).

Our results suggest that this seizure model produces extensive neuronal damage without adverse systemic changes. Moreover, it is the seizures themselves and not PC which is responsible for the damage. The more extensive damage to dorsal hippocampus seen in our animals 3 days after SE but not in Turski and co-workers' animals 24 hours after SE could have been caused by more severe seizures. However, it could also represent a form of delayed neuronal death analogous to that reported in hypoxia-ischemia (Kirino, T., Brain Res., 239:57, 1982).

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272.3 SENSITIVITY OF CANINE SUBSTANTIA NIGRA TO 1-METHYL-4-PHENYLPYRIDINE (MPP+) IN ORGANOTYPIC CULTURES. B. Christie-Pope*, R.S. Burns*, and W.O. Whetsell, Jr. Departments of Pathology and Psychiatry, Vanderbilt University School of Medicine, Nashville, TN 37232.

In canines, intravenous administration of low doses (2.5 mg/kg) of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces almost complete destruction of nigral dopamine neurons (Burns et al, Soc. Neurosci. Abstr., 1984). In contrast, rats are resistant to toxic effects of systemically administered MPTP. The susceptibility of rat substantia nigra neurons in organotypic culture to MPTP or its pyridinium metabolite MPP+ (Mytilineou et al, Neurosci. Lett., 57:19, 1985) indicates that possible kinetic or metabolic factors are protective in the rat. To study whether differences in sensitivity to these agents may exist in dog and rat at the cellular level in the central nervous system, effects of MPTP and MPP+ in cultured dog nigral neurons were evaluated histologically and ultrastructurally.

Organotypic cultures of substantia nigra from newborn mongrel dogs (Whetsell et al, J. Neural Trans., 52:149, 1981) were maintained for up to 80 days in vitro (DIV). At 30 DIV, substantia nigra cultures were chronically exposed to MPTP (10^{-5}M or 10^{-7}M) or to MPP+ (10^{-5}M, 10^{-7}M, 10^{-9}M, or 10^{-11}M). After exposures to these different levels for various periods, cultures were fixed and examined by light and electron microscopy; untreated sibling cultures of identical ages served as controls. Neurons in experimental cultures were destroyed by exposure to 10^{-5}M MPTP for 7 days and 10^{-7}M MPTP for 21 days. Treatment with 10^{-5}M or 10^{-7}M MPP+ uniformly destroyed the cultures by 3 days; cultures incubated with either 10^{-9}M or 10^{-11}M MPP+ contained some surviving nerve cells for up to 30 days.

The least exposure of dog substantia nigra cultures to MPP+ which produced complete nerve cell destruction was 10^{-7}M MPP+ for 3 days. Results reported in rat substantia nigra cultures (Mytilineou et al, 1985) indicated destruction of dopaminergic neurons after exposure to 10^{-5}M MPP+ for 7 days and destruction of 65% of dopamine fibers with 2.5 X 10^{-6}M MPP+ for 7 days. Comparisons of these in vitro studies suggest that substantia nigra neurons in the dog are more sensitive to cell destruction by MPP+ than substantia nigra neurons in the rat and therefore that species differences in susceptibility to this toxic agent exist at the cellular level.

272.4 **NERVE CELL ATROPHY AND LOSS IN THE DEEP CEREBELLAR NUCLEI OF 'PURKINJE CELL DEGENERATION' (pcd) MUTANT MICE.** J. Norton*, L.C. Trisrhou and B. Ghetti (SPON: A.N. Siskotos). Depts of Pathology and Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

'Purkinje cell degeneration' (pcd) mutant mice lose all cerebellar Purkinje cells between postnatal days 17 and 45. Thus, the pcd mutant represents a model of complete deafferentation of the deep cerebellar nuclei. We examined these structures in normal C57BL/6J and pcd mutant mice to investigate whether the loss of Purkinje cells affects the stability of nerve cell number in the mature cerebellum. Mice were perfused transcardially with 1/4 strength Karnovsky fixative and tissues were processed and embedded in paraffin. Serial parasagittal sections were cut at 10 μm and stained with gallocyanin Nissl. At 23 days of age, the average corrected estimate of neuronal populations in the left hemisphere was 10,167 cells in control mice and 9,436 in mutants. At 300 days, controls had an average population of 10,429 cells, whereas mutants had 7,424 cells (difference 29%, $P<0.01$). The difference between young and old mutants was 21% ($P<0.05$). The mean neuronal diameter at 23 days was 16.4 μm in controls and 15.7 μm in mutants. At 300 days, diameters were 15.6 μm in controls and 13.5 μm in mutants. The difference between old mutants and old controls, and that between old and young mutants were both highly significant ($P<0.001$). These data are pertinent to the understanding of the mechanisms regulating neuronal numbers in degenerative diseases and in aging. (Supported by PHS grant RO1-NS14426 to B.G.).

272.5 NEUROMUSCULAR DEVELOPMENT IN THE CROOKED NECK DWARF MUTANT (CN/CN) CHICK EMBRYO. R. W. Oppenheim and D. Prevette*. Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

The crooked neck dwarf mutant is due to an autosomal recessive gene that is lethal in homozygotes. Owing to severe muscle degeneration and the absence of motility cn/cn embryos don't hatch. Muscles fail to develop beyond the myotube stage, muscle connective tissue is abnormal and following an initial stage of normal segregation of muscle masses into individual muscles, the mutant muscles subsequently fuse into large masses of unpatterned muscle. Owing to the absence of movement the joints of the vertebral and appendicular skeleton of mutant embryos become fused. Although previous studies reported that the cn/cn embryos are indistinguishable from controls up to embryonic days (E)9-11, we have found that the mutant embryos never exhibit either spontaneous or evoked motility; normal embryos begin neuromuscular activity (motility) on E3.5, whereas cn/cn embryos are non-motile from this stage until the end of the incubation period (E20). Chronic treatment of normal chick embryos with paralyzing doses of neuromuscular blocking agents results in neuromuscular and skeletal defects similar to those seen in cn/cn embryos. Such treatment also results in a reduction or prevention of naturally-occurring motoneuron death. We have found abundant peripheral nerves in mutant muscle and injection of HRP into limb muscles retrogradely labels spinal cord motoneurons. Thus, muscle degeneration in mutants does not appear to be due to a failure of innervation. Motoneuron numbers (lumbar) are normal (23,000) in mutant embryos prior to the onset of normal cell death on E5. By E8 when motoneuron numbers in controls are reduced to 15,000, mutant embryos retain approximately 22,000 motoneurons. Between E8 and E15, when muscle mass becomes reduced in mutant embryos by more than 80%, motoneuron numbers gradually decrease to near control values: E15 control = 11,880; E15 cn/cn = 13,200. Although it is surprising that normal motoneuron numbers can be maintained in the face of such a greatly reduced muscle mass, it seems likely that the absence of muscle activity in the mutant may actually be saving some motoneurons from dying but that the drastic reduction in target tissue partly offsets this resulting in little if any net change in motoneuron numbers. Studies are underway to further characterize the progressive changes in neuromuscular development in cn/cn embryos and to identify the major site(s) of gene action. The cn/cn mutant may be a useful model for understanding the role of targets, target activity and neurotrophic interactions in regulating motoneuron survival.

272.6 **NERVE GROWTH FACTOR TREATMENTS SUBSEQUENT TO BILATERAL FORNIX/FIMBRIA LESIONS PREVENT RETROGRADE NEURONAL DEATH IN THE RAT SEPTUM.** Lawrence F. Kromer, Department of Anatomy & Cell Biology, Georgetown University, Schools of Medicine/Dentistry, Washington, DC 20007.

Considerable evidence has now accumulated to support the hypothesis that nerve growth factor (NGF) or an NGF-like molecule, present in the rodent hippocampus, may serve as a neurotrophic substance for cholinergic septal neurons. Moreover, when a series of NGF injections are given both before and after partial lesions of the septo-hippocampal pathway survival of septal cholinergic neurons is enhanced (Hefti, Soc. Neurosci. Abstr. 11: 660).

Thus, the present study was undertaken to determine whether NGF treatment, which was delayed for various periods of time following complete bilateral lesions of the dorsal septo-hippocampal pathways, also would be effective in preventing retrograde neuronal death of cholinergic and non-cholinergic septal neurons. For these experiments all rats received bilateral aspiration lesions of the supracallosal stria, dorsal fornix, and fimbria either 0, 24, or 48 hrs prior to initiating NGF or cytochrome C treatments. Some rats also received bilateral lesions but no treatment and additional unlesioned control animals either were untreated or received NGF treatment. Intraventricular infusions were accomplished by using a cannula (stereotaxically positioned in the right ventricle) that was attached to an Alzet minipump containing either 2.5s NGF or cytochrome C (250ng/ul saline) which were infused at a rate of 0.5ul/hr for 14 days. Prior to perfusing the animals, the residual NGF solution was removed from the cannula-pump assembly and assayed for biological activity. Adjacent cryostat sections (25um) through the septum were processed for acetylcholinesterase (AChE) histochemistry, Nissl staining, and the immunocytochemical identification of choline acetyltransferase (CAT).

Results from this study indicate that: 1) 100% of the original biological activity of the NGF solution is still present at the termination of the treatment, 2) NGF infusions in normal unlesioned control animals do not increase the number of CAT+ or AChE+ septal neurons compared with untreated control animals, 3) treatment with cytochrome C has no effect on the survival of septal neurons following axotomy, 4) NGF treatment begun immediately after axotomy results in a highly significant increase in the survival of septal cholinergic neurons (approx. 300%) with some increased survival of non-cholinergic neurons, and 5) a prolonged delay in initiating NGF treatment results in a reduced survival of cholinergic septal cells. Current evidence suggests that the postlesion time course for the effect of NGF on cholinergic cell survival correlates with the loss of NGF receptors (Koh et al,'86, Anat. Rec. 214: 67)on these septal neurons following axotomy. (Supported by the March of Dimes Birth Defects Foundation)

272.7 NGF PROTECTS ADULT SENSORY NEURONS AFTER SCIATIC NERVE INJURY. K.M. Rich*, J.R. Luszczynski*, P.A. Osborne* and E.M. Johnson,Jr (SPON: V. Yip) Depts.Neurological Surgery and Pharmacology, Washington University Sch. of Med., St. Louis, MO 63110

The reaction of dorsal root ganglia (DRG) neurons to axotomy and its alteration by locally supplied nerve growth factor (NGF) was examined in adult rats. Specially designed silicone chambers were surgically attached to the severed tip of the sciatic nerve and acted as reservoirs capable of providing prolonged access of NGF to the site of injury. The chambers were filled either with NGF/0.9% saline (1mg/ml) or 0.9% saline. The volume of the chambers was approximately 70 microliters. The time course of NGF activity within the chambers was determined by using the standard NGF chick DRG bioassay. The fluid from chambers filled with the NGF/saline solution maintained NGF activity for periods up to six weeks after implantation. By nine weeks, however, the fluid from most chambers failed to show any NGF activity on the bioassay. The fluid from the control chambers (filled only with 0.9% saline) did not show any NGF-like activity on the bioassay at any of the time points.

Experiments were designed to compare the response of the sensory neuron to injury with the neurons either receiving chambers filled with NGF/saline or only with saline. The total neuronal counts in the lumbar fourth and fifth DRG at three weeks and six weeks after sciatic nerve section showed 22% and 16% cell death, respectively, in those injured neurons receiving saline-filled chamber implants. The animals that received chamber implants which contained an NGF/saline solution showed no cell death in the ipsilateral DRG at either three weeks or six weeks after injury. Morphometric analysis of injured DRG neurons showed evidence of atrophy in the injured neurons which did not receive NGF. The degree of atrophy was significantly decreased in those injured neurons receiving NGF among all cell sizes. The mean volume of injured neurons three weeks after section and not treated with NGF was decreased by 28% as compared with only a 13% decrease in neurons treated with NGF. Similar findings were noted in the six week studies. NGF treatment did not alter the incidence of classic changes of chromatolysis (e.g. swelling, nuclear displacement, or dispersal of Nissl substance) in the groups of injured neurons either receiving NGF/saline-filled chambers or only saline-filled chambers.

272.8 INCREASED EXCITABILITY OF THALAMIC RELAY NEURONS IN THE VENTROBASAL NUCLEUS FOLLOWING ABLATION OF THE SI CORTEX IN THE ADULT RAT. D. T. Ross and F.F. Ebner, Center for Neural Sciences, Brown University, Providence, RI 02912

Increased excitability of thalamic relay neurons in the ventrobasal nucleus (VB) has been recorded immediately after ablation of the somatosensory (SI) cortex of adult rats (Angel and Clarke, 1975). The present study was undertaken to characterize the temporal pattern of alterations in excitability that precede retrograde degeneration of specific sensory thalamic relay neurons. Adult Long-Evans rats were anesthetized with 20% urethane, mounted in a stereotaxic frame, and prepared for extracellular unit recording. A low impedence carbon fiber microelectrode was lowered through the right SI cortex at an angle of 45° on a trajectory that advanced into the left thalamic ventrobasal complex. Receptive fields were mapped for all VB units encountered. A second carbon fiber electrode was lowered into the left SI cortex to map the location of cells with receptive fields corresponding to those of the thalamic units. This insured that the cortical ablation included the area which received input from the identified VB units. Spontaneous activity and responses to cutaneous stimulation at 1, 5, and 10 Hz were recorded extracellularly from a single thalamic unit prior to ablation and at 30 minute intervals following ablation. The second electrode was moved to the right SI cortex prior to ablation where it was used to monitor the depth of anesthesia throughout the remainder of the experiment.

VB relay neurons exhibited a high frequency (50 Hz) injury discharge that lasted for 20-30 seconds when their terminal arbors were severed. For the next 45 to 90 minutes the axotomized relay neurons showed lower than normal levels of spontaneous activity, but responded normally to cutaneous stimulation at 1 to 10 Hz. From 90 minutes to 6 hours after ablation the VB units showed very pronounced increases in both spontaneous and evoked activity. The relay neurons which normally show very low levels of spontaneous activity (5 spikes/sec) increased their activity to 20-40 spikes/sec. Spontaneous bursts (5-12 spikes) were common, but bursts with spindle rhythmicity were rarely observed. Cutaneous stimulation at low frequencies often did not drive the injured neurons during this time. When the units did follow at low frequencies, multiple afterdischarges were often seen. The damaged neurons responded to stimulation at 5-10 Hz with high frequency bursts, but after 5 seconds they fired continuously for 1 second then stopped completely. These results support the hypothesis that progressive overexcitation may contribute to the process of thalamic retrograde degeneration following cortical ablation. Experiments are in progress to 1) characterize alterations in excitability that occur between 6 hours and 5 days after cortical ablation, and 2) examine whether intrathalamic administration of excitatory amino acid antagonists decreases the activity of the injured neurons. (Supported by NIH Postdoctoral fellowship NS074919 to DTR and NIH grant NS13031.)

272.9 MOTONEURONAL DEATH IN THE HUMAN FETUS. Nancy G. Forger & S.M. Breedlove, Dept. Psych., Univ. California, Berkeley, CA 94720.

We have recently identified a sex difference in the number of motoneurons in a human spinal nucleus (Soc. Neurosci. Abstr. 11, 531, '85). Sexual dimorphism of motoneuron number in the homologous nucleus of rats involves rescue of motoneurons in males by androgen secreted during the period of normal cell death. Although naturally occurring neuronal death has been demonstrated during early development in a number of species, it is not known whether cell death regulates motoneuron number in the human CNS.

Spinal cords of male and female human fetuses from the Yakovlev collection of the Armed Forces Institute of Pathology were examined. Each cord (or the entire fetus) was embedded in celloidin, cut transversely at 35 µm, and every 10th section was mounted and Nissl-stained. Motoneurons were first distinguishable at about the 10th week of gestation. Bilateral counts of motoneuronal nuclei in 11-26 week old fetuses were made throughout the entire rostro-caudal extent of the spinal cord at a sampling ratio of approximately 1/40. Nuclei of 15-25 motoneurons for each subject were traced with the aid of a computer imaging system; counts were adjusted according to the sampling ratio and corrected for split nuclei by the method of Konigsmark. The relative abundance of degenerating (pyknotic) cells was also noted.

A progressive decline in motoneuron number was observed in 11-26 week old human fetuses ($r = -.823$, $p<0.002$):

Gestational Age (weeks)	N	Motoneuron # (mean + SEM)
11	2	178,572 ± 25,840
14 - 16.5	4	141,169 ± 3,474
25 - 26	5	117,699 ± 2,271

Motoneuron number declined by 21% between 11 and 14-16.5 weeks ($p<0.05$) and 34% by week 26 ($p<0.01$). The decline from 16.5 to 26 weeks was also significant ($p<0.001$). Although small sample sizes preclude definitive conclusions, we did not detect a sex difference in total motoneuron number. Pyknotic cells were evident in 11 and 14-16.5 week old fetuses, while few or none were present by week 26. Preliminary cell counts in older fetuses suggest that motoneuron number does not change significantly after the 26th week.

Testosterone is secreted by the human fetal testis between weeks 10-20 of gestation, with peak concentrations seen at 11 to 16 weeks (Kaplan and Grumbach, 1978). Androgen secretion thus overlaps with the period of motoneuronal death observed here. Androgen may engender sexual dimorphism in developing humans by enhancing survival of selected motoneuronal populations, as it does in rats.

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272.10 MORPHOLOGICAL CHANGES IN AXOTOMIZED OCULOMOTOR MOTONEURONS OF THE MONKEY. John D. Porter. Dept. of Anatomy, Univ. of Mississippi Medical Center, Jackson, MS 39216.

The present study was designed to examine the chronological sequence of motoneuron structural alterations which occur subsequent to oculomotor neurectomy. The oculomotor nucleus was examined at intervals of 3-56 days subsequent to transection of the oculomotor nerve in the vicinity of the cavernous sinus. Light and electron microscopy were used in conjunction with histochemical demonstration of acetylcholinesterase (AChE). Chromatolysis of the extensive aggregations of granular endoplasmic reticulum (GER), which normally characterize oculomotor motoneurons, represented the initial response to neurectomy. In contrast to those responses seen in spinal motoneurons, dispersion of the GER began at the periphery of the neuron and proceeded inward. By 7 days after nerve transection, the GER was heavily condensed around the nucleus. Mechanisms responsible for synthesis of proteins for export were altered as evidenced by a drastic reduction in GER luminal AChE content. Presumably, the neuronal synthetic machinery shifts away from production of substances involved in neural transmission to produce structural elements in an attempt to survive. Additionally, some indications of soma swelling were apparent, often accompanied by migration of nuclei to positions adjacent to the neurolemma. The "rounding" of neuronal somata and peripheral migration of nuclei were widespread by 14 days post-axotomy. At this time GER was largely absent from oculomotor neurons. In the ensuing 42 days, oculomotor motoneuron subgroups largely disappeared from the brainstem. Apparently intact axon collaterals, exhibited by approximately 60% of oculomotor motoneurons in the cat (Evinger et al., '81), are insufficient to provide for neuron survival.

Present findings, which indicate a failure of oculomotor motoneurons to survive axotomy placed proximal to the orbit, are in accordance with data obtained in newborn cats (Baker et al., Soc. Neurosci. Abstr. 11:973, '85). It is interesting that axotomized abducens and trochlear motoneurons of the cat survive to reinnervate the extraocular muscles, while oculomotor motoneurons, in both cat and monkey, fail to survive axotomy. Factors which govern the survival of axotomized brainstem motoneurons and their potential for reinnervation of target structures remain to be determined.

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272.11 TERMINAL DIFFERENTIATION OF THE INTERSEGMENTAL MUSCLES OF THE TOBACCO HAWKMOTH Manduca sexta, L.M. Schwartz, K.S. Bloom* and B.K. Kay*. Biol. Dept., University of North Carolina, Chapel Hill, N.C. 27514

During the course of adult development, the intersegmental muscles (ISM) of Manduca undergo a developmentally programmed cell death. Three days before adult eclosion, the ISM enter the ATROPHY phase which is characterized by a 40% loss of mass without a signifcant change in physiological properties, including, force/cross-sectional area, resting potential, pCa_{50} and Hill coefficient. Then coincident with eclosion, the muscles begin the DEGENERATION program which results in the rapid loss of mass and function during the subsequent 36 hrs. Progression through these developmental stages is controlled by the declining haemolymph ecdysteroid titer. We have sought to define the molecular events associated with these changes in muscle differentiation by looking at changes in protein and RNA. One dimensional electrophoretic analysis of proteins showed no changes in the pattern of resolvable proteins. As well, the content of RNA within the cells remained constant throughout all stages of muscle differentiation examined. Northern analysis of actin mRNA and rRNA, demonstrated that these transcripts were present at all stages studied. Interestingly, in vitro translation of poly A^+ RNA revealed several transcripts which were developmentally regulated, appearing or disappearing at specfic stages of muscle development. We are in the process of making stage-specific cDNA libraries to these molecules so that their regulation and role in cell death can be determined.

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272.12 INITIAL IMPRECISION IN THE TOPOGRAPHY OF THE CHICK EMBRYO'S ISTHMO-OPTIC PROJECTION. S. Catsicas*, S. Thanos* and P.G.H. Clarke* (SPON: E. Rouiller) Anatomy Institute, Lausanne University Medical School, 1005 Lausanne, Switzerland and Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, West Germany.

We have used the intensely fluorescent carbocyanine dye DiI C18[3] (M.G. Honig and R.I. Hume, J. Cell. Biol., 1986) to map the topography of the projection from the isthmo-optic nucleus (ION) to the retina at different ages relative to the period of naturally occurring neuronal death in the ION, i.e. embryonic day 12 (E12) to E17. In each animal, we made a small incision in the sclera and choroid and inserted a fleck of dye so that it went through the underlying retina. Different parts of the retina received dye in different embryos. Some were incubated (and operated) in petri dishes, others in ovo .

Owing presumably to the nonpolar properties of the dye, it hardly diffused at all in the retina, but labeled a narrow band, 0.1-0.5 mm wide, of fibers running to the optic fissure. These included both anterogradely labeled retinofugal fibers and (fewer) retrogradely labeled fibers that could be traced into the isthmo-optic tract and thence to the ION. Retrogradely labeled neuronal perikarya were found within the ION and around its borders - the so called "ectopic" ION neurons. As previously shown by others, the ION was found to project topographically to the central half of the retina. Its medial part projects temporally within this zone and its lateral part nasally. The ectopic cells could be labeled from more peripheral regions than could the neurons within the ION. Given the large number of ectopic cells labeled from any retinal quadrant, they probably have very widespread terminal fields.

In embryos that received dye at E11 (i.e. two days after the arrival of the fibers in the retina) and were fixed at E13 or E14, wherever the dye was placed, the labeled neurons were most densely packed in a region corresponding to the expected topography; but they did also occur, more sparsely, throughout the entire ION. In embryos operated at E17 and fixed six days later, the sparse projection was largely eliminated. From its timing, this refinement could be due to neuronal death in the ION. Moreover, since several tens of inappropriately projecting neurons were initially labeled from a region smaller than one thousandth of the retina, their total number may be comparable to the 10,000 ION neurons that normally die. Changes of axonal arbors may also contribute, but we have preliminary evidence that cell death may be the main regulatory mechanism. Supported by Swiss N.S.F. grants 3.516 and 3.238 and by a twinning grant from E.T.P.

272.13 DEVELOPMENT OF THE GANGLION CELL DENSITY GRADIENT IN THE RABBIT RETINA. S.R.Robinson*, B.Dreher*, G.M.Horsburgh* and M.J.McCall* (SPON:J.Stone).Depts Anat.& Physiol.,Sydney Univ.,Australia,2006.

In most mammals the density gradient of ganglion cells in the adult retina is sculptured during development from a relatively uniform distribution. Two (not necessarily exclusive) mechanisms commonly proposed to account for this change are the "differential death" of ganglion cells (with a greater proportion dying at the retinal periphery) and "differential retinal growth" (with more growth at the periphery). We investigated the contribution of these mechanisms to the emergence of ganglion cell density gradients in the rabbit.

Albino rabbits aged between embryonic day 23 (E23) and adulthood received massive bilateral injections of 50% HRP into the retino-recipient nuclei. After survival of 18-48hr retinae were processed with Hanker-Yates reagent, wholemounted, coverslipped without dehydration (areal shrinkage < 5%). Optic nerves from pre- and postnatal rabbits were processed for electronmicroscopy. The number of axons decreases from about 750,000 at E23, to 690,000 at E25, 590,000 at E28 and 380,000 at E31 (the last day of gestation). The total number of ganglion cells drops from about 500,000 at E24 to 350,000 at postnatal day 1 (P1). Between P1 and adulthood the numbers of both ganglion cells and axons drop slightly to about 325,000. Between E24 and adulthood the distribution of ganglion cells changes substantially. At E24 the peak ganglion cell density is 18,300 cells/mm^2 at the visual streak and 6,100 cells/mm^2 at the superior and inferior edges of the retina (density ratio = 3:1). At P1 the peak density of ganglion cells is 11,100 cells/mm^2 at the visual streak and 1,500 cells/mm^2 at the superior edge (7.5:1). By P12 the peak density of ganglion cells has dropped to 8,900 cells/mm^2 at the streak and 400 cells/mm^2 at the superior edge (22:1). By adulthood the peak density of ganglion cells is only about 4,800 cells/mm^2 at the streak and 95 cells/mm^2 at the superior edge (50:1). From birth onwards, the density of ganglion cells at the inferior edge of the retina was 1.4-2 times higher than that at the superior edge.

The retinal area increases from 45 mm^2 at E24, to 105 mm^2 at P1, 200 mm^2 at P12 and 510 mm^2 by adulthood. At each age we measured the ganglion cell density in comparable sites (i.e. relative to the site of peak of density in the streak) across the retina. These measurements indicate that the visual streak grows mainly along the nasotemporal axis, its growth being greatest towards the retinal edges. The rest of the retina grows differentially along both the nasotemporal and superior-inferior axes. We conclude that differential ganglion cell death (if it occurs) can make only a minor contribution to the increase in the cell density gradient between E25 and E31. The massive postnatal increase in the ganglion cell gradient is entirely due to differential retinal growth.

DOPAMINE RECEPTORS III

273.1 PHARMACOLOGICAL SELECTIVITY OF THE POTENT D-2 DOPAMINE AGONIST N-0437. D.N. Krause, J. van der Weide* T. Woznichak* and A.S. Horn*. Dept. of Pharmacology, Univ. of Calif., Irvine, CA 92717; Nelson Research, Irvine, CA 92715; and Dept. of Pharmacy, Univ. of Groningen, The Netherlands.

N-0437, a noncatecholic aminotetralin, is one of the most potent D-2 dopamine receptor agonists evaluated to date, exhibiting in vitro activity at sub-nanomolar concentrations. This compound also has been shown to be highly selective for the D-2 versus the D-1 subtype of dopamine receptor (Beaulieu, M. et al, Eur. J. Pharmacol. 105:15, 1984). Because of the potential experimental and therapeutic utility of N-0437 for potent, selective D-2 receptor stimulation, it was important to assess the selectivity of N-0437 relative to other, non-dopaminergic, receptor sites.

N-0437 was evaluated using in vitro radioligand binding assays for the following receptors: D-2 dopamine (^{3}H-spiperone), α_2-adrenergic (^{3}H-para-aminoclonidine), serotonin-1 (^{3}H-serotonin), A-1 adenosine (^{3}H-cyclohexyladenosine) and muscarinic cholinergic (^{3}H-quinuclidinyl benzilate). While N-0437 was extremely potent at displacing ^{3}H-spiperone binding (K_D=0.15nM; Horn, A.S. et al, Pharm. Weekbld. Sci. Ed. 7:208, 1985), it was about 1000-fold less potent at α_2-adrenergic sites, over 10,000-fold less potent at serotonin-1 receptors, and virtually inactive at A-1 adenosine and muscarinic receptors. Evaluation of N-0437 in other receptor binding assays is currently in progress.

The specificity of N-0437 on central D-2 dopamine versus α_2-noradrenergic receptors also was assessed in vivo in rats by measuring the drug's effects on dopamine (DA) and noradrenaline (NA) turnover in various brain regions after treatment with α-methyl-p-tyrosine (α-MpT). N-0437 (15μmol/kg,i.p.) significantly antagonized the α-MpT-induced fall in DA levels but had no effect on NA levels.

The specificity of N-0437 for D-2 receptors was further confirmed by pharmacological analysis of the sites to which ^{3}H-N-0437 binds in calf brain homogenates. Only known D-2 or mixed D-2/D-1 dopaminergic agents were potent displacers of ^{3}H-N-0437 binding. Non-dopaminergic agents, including yohimbine, ketanserin, propranolol, prazosin, promethazine, atropine, morphine, diazepam, and GABA, were without exception poor displacers of ^{3}H-N-0437 (IC_{50} values >1μM).

273.2 A DIFFERENCE IN THE SENSITIVITY OF THE DOPAMINE D1 RECEPTORS PRESENT IN RETINA AND IN STRIATUM. J. M. Ackerman and M. E. Gnegy. Neuroscience Program and Dept. Pharmacology, Univ. of Michigan, Ann Arbor, MI 48109.

The retina (RET) contains a large population of D1 dopamine (DA) receptors linked to the stimulation of adenylate cyclase. Equilibrium binding constants to D1 DA receptors using [^{3}H]DA and [^{3}H]SCH 23390 were measured in bovine RET and compared to those obtained in rat striatum (STR). We demonstrated stereospecific binding of high affinity to the DA receptor in a washed membrane (27,000xg) preparation from bovine RET using [^{3}H]DA and [^{3}H]SCH23390, a specific D1 receptor antagonist, using published methods. Saturation analysis of binding gives a K_d of 5.3 nM for [^{3}H]DA and 0.18 nM for [^{3}H]SCH 23390 binding. Both ligands produce comparable B_{max}'s, 267 and 249 fmol/mgP, for [^{3}H]DA and [^{3}H]SCH23390, respectively. Saturation and competition experiments suggest that both ligands are binding to D1 DA receptors, although [^{3}H]DA binding may have a small D2 component.

The K_d values obtained for each ligand in the RET are near those reported for their binding in rat STR, whereas the B_{max} values are higher in STR. On the contrary, the K_i's obtained for DA agonists and antagonists in competition experiments for [^{3}H]DA and [^{3}H]SCH 23390 binding in RET are higher than those reported in rat STR. An exception is the K_i value of 4.3 nM for unlabeled DA in competition with [^{3}H]DA in RET. The difference in K_i values between RET and STR is especially seen for the D1 specific drugs, SCH23390 and SKF38393, a D1 agonist. These drugs competed for both labels in RET with several-fold lower affinity than in STR. The K_i's for competition of [^{3}H]DA binding by SKF38393 and SCH23390 in RET are 55 and 102 nM, respectively. The K_i's for competition of [^{3}H]SCH23390 binding by SKF38393 and DA are 121 and 695 nM, respectively. The lower affinity for SKF38393 is not specific to bovine retina; the IC_{50} for competition of this drug for [^{3}H]SCH23390 binding in goldfishretina is 79 nM. Therefore in competition experiments, there is a difference in sensitivity of the D1 receptor in retina and striatum.

These differences are probably not due to the presence of high levels of endogenous DA and GTP in retina for several reasons. The affinity of SKF38393 was not altered by preincubation of the tissue. The addition of GppNHp shifts the K_i of SKF38393 at [^{3}H]DA binding sites from 55 nM to 2 uM. Finally, preincubation of tissue with GMP to displace GTP from the GTP-binding protein did not increase the sensitivity to SKF38393. The data suggest that: 1) a difference in sensitivity of the D1 DA receptor is present in retina, 2) another factor such as an ion is required for D1 binding in retina or 3) different binding conditions are required to measure D1 binding in retina than in striatum. This study was supported by NIMH 36044-05, the Michigan Memorial Phoenix Society and NRSA 1 F31 MH09291 (JMA).

273.3 INTERACTION OF PERMANENTLY CHARGED AND UNCHARGED DOPAMINE ANALOGS WITH THE D-2 DOPAMINERGIC RECEPTOR. R.A. Wallace*, T. Farooqui*, J. Ares*, Y.A. Chang*, L.J. Wallace*, D.D. Miller* and N.J. Uretsky, Divisions of Pharmacology and Medicinal Chemistry, Ohio State University College of Pharmacy, Columbus, Ohio 43210.

Dopamine (DA) is present in physiological solution in charged and uncharged forms. It is unclear which of these molecular forms interacts with the DA receptor. To answer this question, we have synthesized structural analogs of DA in which the side chain nitrogen is replaced by groups which have a permanent positive charge or are uncharged and have studied their abilities to inhibit ^{3}H-spiroperidol (^{3}H-SP) binding to rat striatal membranes and to inhibit the potassium (K^+)-induced release of ^{3}H-acetylcholine (^{3}H-Ach) from striatal slices.

DA, dimethyl DA (DMDA), and the permanently charged analogs inhibited the K^+-induced release of ^{3}H-Ach, indicating agonist activity (IC_{50} = between 1-20 μM). In contrast, the uncharged analogs were inactive except at extremely high concentrations (1 mM).

Binding of ^{3}H-SP was to a single population of sites with a K_D of 0.075 nM and a B_{max} of 24 pmoles/gm tissue. ^{3}H-SP binding was inhibited by both DMDA and DA. The inhibition curves for both compounds were shallow and were best modelled to two sites (DA, K_H = 9.7 nM representing 60% of total sites and K_L = 354 nM; DMDA, K_H = 24.6 nM representing 50% of total sites and K_L = 720 nM). The permanently charged dimethysulfonium (DMS^+) and dimethylselenonium ($DMSe^+$) analogs of DA inhibited ^{3}H-SP binding; the competition curves were shallow and fit best to a two site model (DMS^+, K_H =1.4 μM representing 47% of sites and K_L = 94 μM; $DMSe^+$, K_H = 0.67 μM representing 70% of sites and K_L = 27.7 μM). The addition of 125 mM NaCl to the incubation buffer decreased the proportion of high affinity sites for the 4 compounds.

The permanently uncharged monomethylsulfide (MMS) and monomethylsulfoxide (MMSO) analogs of DA significantly inhibited ^{3}H-SP binding. The inhibition of ^{3}H-SP binding by the uncharged analogs was complex as they did not produce a maximum inhibition at 1 mM. Consequently, we were unable to reliably estimate their potencies or abilities to bind to two sites. However, 125 mM NaCl decreased the abilities of the uncharged analogs to inhibit ^{3}H-SP binding. This effect of NaCl was qualitatively similar to that seen with the charged analogs. If it is assumed that NaCl decreased the proportion of high affinity sites, then the uncharged analogs can bind to the high affinity site in NaCl-free medium.

These results show that the side chain nitrogen is not essential for binding and activation of the D-2 DA receptor. In addition, positively charged analogs of DA appear to be more effective as DA agonists than permanently uncharged compounds.

273.4 D_2 DOPAMINE RECEPTORS ON ADRENAL CHROMAFFIN CELLS: DETECTION AND CHARACTERIZATION WITH ^{3}H-N-METHYL-SPIPERONE. A.S. Schneider, R.A. Lyon and M. Titeler (SPON: M. Dentinger). Dept. Pharmacol. Toxicol., Albany Medical College, Albany, New York, NY 12208

Although dopamine containing cells are known to be present in sympathetic ganglia, the site of action and role of dopamine in ganglion function remains obscure. We are currently evaluating the interaction of dopamine receptor ligands with particulate membrane fractions from bovine chromaffin cells, a system closely related to postganglionic sympathetic neurons, using the D_2 dopamine receptor radioligand, ^{3}H-N-methylspiperone (^{3}H-NMSP; ref. 1). Scatchard analysis of ^{3}H-NMSP saturation experiments revealed a B_{max} of 24.1 ± 1.6 fmol/mg protein and a K_D of 0.23 ± 0.03 nM in the particulate fraction from adrenal medulla homogenates and a B_{max} of 26.5 ± 2.7 fmol/mg membrane protein and a K_D of 0.25 ± 0.02 nM in the particulate fraction prepared from isolated adrenal chromaffin cells. There were approximately 1000 receptors per cell. There were no detectable levels of specific ^{3}H-NMSP binding in the particulates prepared from adrenal cortical or capsular homogenates. Competition studies with the non-radioactive D_2 receptor antagonists spiperone, chlorpromazine, and (-) sulpiride revealed K_I values of 0.28, 21, and 196 nM respectively. The (+) isomer of butaclamol displayed a 604-fold higher affinity than the (-) isomer. Competition studies with the dopamine receptor agonists dopamine and apomorphine revealed affinities of 3,960 and 417 nM, respectively. A correlation coefficient of 0.96 was obtained in studies comparing the potencies of drugs in inhibiting specific ^{3}H-NMSP binding in bovine adrenal medullary homogenates and in inhibiting specific ^{3}H-NMSP binding to brain D_2 dopamine receptors (1-3). In summary, radiolabelling studies using ^{3}H-NMSP have revealed the presence of D_2 dopamine receptors on bovine adrenal chromaffin cells. Data concerning the regulation of the adrenal chromaffin cell D_2 dopamine receptor by nucleotides and cations, as well as the effects of dopamine agonists and antagonists on chromaffin cell catecholamine secretion will be presented. (Supported by BRSG SO7RR5394-24)

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273.5 INVESTIGATION OF DOPAMINE RECEPTOR AGONIST HIGH AND LOW AFFINITY BINDING SITES IN MtTW15 PITUITARY TUMORS. D. Levesque* and T. Di Paolo (Spon: A. Dupont). School of Pharmacy, Laval University, Québec, Qc, G1K 7P4 and Department of Molecular Endocrinology, Laval University Hospital Center, Ste-Foy, Qc, G1V 4G2, Canada.

It has been shown that the dopamine receptor in a normal anterior pituitary exists under a high affinity state considered as functional which mediates the inhibition of prolactin release and a low affinity state for dopamine and dopaminergic agonists. The pituitary tumor MtTW15 secreting prolactin contains dopamine receptors apparently normal although dopaminergic agonists do not inhibit prolactin secretion. The aim of this study was to investigate the high and low agonist affinity states of the dopamine receptor in MtTW15 tumors. We have confirmed the presence of a high affinity dopaminergic binding site with saturation experiments with the ligand [^{3}H] spiperone. An equilibrium dissociation constant (K_D) for [^{3}H] spiperone binding of 0.11 ± 0.01 nM with a density of binding sites (Bmax) of 40 ± 1 fmol/mg of proteins was obtained. NaCl (120 mM) and Gpp (NH)p (100μM) do not significantly affect [^{3}H] spiperone binding affinity (Kd) and density (Bmax) in MtTW15 tumors. We observe, such as for anterior pituitaries and human prolactinomas, that the interaction of sulpiride is sodium dependent, this benzamide having 8 times greater affinity for the dopamine receptor in MtTW15 tumors in the presence of 120 mM of NaCl. A high (K_D: 15 ± 5 nM, 41%) and low (K_D: 820 ± 190 nM, 59%) affinity sites was observed for apomorphine competition for [^{3}H] spiperone binding in MtTW15 tumor homogenates and is similar to intact tissue. NaCl (120 mM) does not significantly change the affinity of apomorphine (Kd: 14 ± 4 nM, 34% and 665 ± 135 nM, 66%) for [^{3}H] spiperone binding. The guanine nucleotide Gpp (NH)p (100μM) alone or in the presence of NaCl (120 mM) increases, although this is significant only in the presence of sodium, the K_D values of apomorphine while the proportion of the two sites remains unchanged (Gpp (NH)p: K_D = 24 ± 9, 42% and 1690 ± 340 nM, 58%; NaCl + Gpp (NH)p: K_D = 50 ± 15 nM, $p < 0.05$, 53% and 1937 ± 390 nM, $p < 0.05$, 47%). By contrast, in intact pituitary tissue, it was shown (Biochem. Pharmacol. 34, 2459, 1985) that the combination of NaCl + Gpp (NH)p leads to a complete conversion of the high into the low agonist affinity state of the dopamine receptor. Our results thus suggest a defect in the modulation of the high and low dopaminergic agonist affinity states in pituitary tumor MtTW15. Supported by the National Cancer Institute of Canada.

273.6 GUANINE NUCLEOTIDE REGULATION OF DOPAMINE RECEPTORS IN RAT ESTRADIOL-INDUCED PITUITARY TUMORS. P. Falardeau and T. Di Paolo. School of Pharmacy, Laval University, Quebec, Qc, G1K 7P4, and Department of Molecular Endocrinology, CHUL, Quebec, Qc, G1V 4G2, Canada.

Dopamine (DA) receptors in the normal anterior pituitary exists under a high affinity state considered as functional which mediates the inhibition of prolactin release and a low affinity state for DA and DA agonists. Guanine nucleotides have been shown to modulate the conversion of the DA D_2^{high} agonist affinity state to D_2^{low} in striata and normal pituitary (Biochem. Biophys. Res. Com. 1982, 108, 1150; Biochem. Pharmacol. 1985, 34, 2459). We investigated the effect of Gpp(NH)p on the agonist affinity states of pituitary D-2 DA receptors in rat estradiol-induced prolactin (PRL) secreting pituitary tumors. Female rats were injected at 3-week intervals with 2 mg of estradiol valerate (EV) or with vehicle for up to 30 weeks. Rats were sacrificed 1 week after their last injection of EV. EV treatment increased the weight of the pituitary gland 9 fold (control: 18.8 ± 0.6 mg; tumors: 167.1 ± 16.5 mg, $p < 0.001$) and the plasma PRL levels 74 fold (control: 63.3 ± 23.9 ng/ml; tumors: 4631.1 ± 400.0 ng/ml, $p < 0.001$). Plasma estradiol levels were increased from 31.1 ± 1.4 pg/ml in control rats to 1656.1 ± 215.6 pg/ml, $p < 0.001$ after EV treatment. A significant correlation ($R = 0.846$, $n = 34$, $p<0.001$) between pituitary weight and plasma PRL levels was observed whereas no correlation was observed between plasma estradiol and PRL levels or pituitary weight. The effect of Gpp(NH)p on the competition for [^{3}H] spiperone binding by apomorphine (APO), a dopamine agonist, was compared in normal and adenomatous pituitary tissue. The dissociation constant (K_D) of D_2^{high} and D_2^{low} sites as calculated using competition curves of APO for [^{3}H] spiperone binding in the pituitary tumors are unchanged compared to intact pituitary tissue (control: K_D = 5.4 ± 1.7 nM and K_D = 383.0 ± 123.9 nM; Tumors: K_D = 1.5 ± 3.0 nM and K_D = 460.7 ± 85.5 nM). The proportion of D_2^{high} vs D_2^{low} sites is the same for both normal (45% vs 55%) and tumor (42% vs 58%) tissues. Moreover, 100 μM Gpp(NH)p caused complete conversion of high affinity agonist DA D_2 binding sites to the low affinity state in both normal (K_D = 487.0 ± 57.6 nM, 100%) and adenomatous (K_D = 430.1 ± 69.7 nM, 100%) pituitary tissue. These results extend our previous work (Di Paolo and Falardeau, Biochem. Biophys. Res. Com. 1984, 123, 312) showing that rats with primary estrogen-induced PRL secreting tumors have normal pituitary DA receptors. (This work was supported by the National Cancer Institute of Canada).

273.7 DARK ADAPTATION AND CHRONIC BLOCKADE OF D-1 DA RECEPTORS BY SCH 23390 INCREASE ^{3}H-SCH 23390 BINDING SITES IN THE RAT RETINA. M.L. Porceddu*, M.G. Demontis*, E. Ongini, S. Mele* and G. Biggio (SPON: L. Beani). Institutes of Biology and Biochemical Pharmacology, University of Cagliari, Italy.

Chronic administration of SCH 23390 (0.03 mg/kg s.c., three times daily), a selective D-1 dopamine (DA) receptor blocker, increased markedly the ^{3}H-SCH 23390 binding in the rat retina. As revealed by the Scatchard plot analysis of saturation data from retinal homogenates, SCH 23390 increased the total number of binding sites by 55% when compared to tissue from solvent-treated rats but failed to change the apparent affinity of ^{3}H-SCH 23390 for its binding sites. This result is similar to that elicited by the same treatment in the rat striatum and s. nigra (1,2) and indicates that retinal D-1 DA receptors, like striatal and nigral D-1 DA receptors, receive a tonic innervation by DA. This conclusion is further supported by the increase in the sensitivity of retinal DA-sensitive adenylate cyclase elicited by chronic SCH 23390. In fact DA (10^{-7} to 10^{-4}M) produced a higher accumulation of cyclic AMP (from 50 to 100%) in the retina of SCH 23390-treated rats as compared to the accumulation (from 30 to 75%) found in tissue from solvent-treated rats. Several lines of evidence indicate that dark adaptation decreases dopaminergic function in the rat retina. Thus, the influence of environmental lighting on ^{3}H-SCH 23390 binding was studied. After four hours dark adaptation, the density of ^{3}H-SCH 23390 binding sites was higher (35%) than that from light adapted rats. This effect lasted for about 12-16 hours and was reversed by the exposure of rats to light for 30 min. On the other hand, dark adaptation failed to change the apparent affinity of ^{3}H-SCH 23390 for its binding sites. The results indicate that: a) chronic blockade of retinal D-1 DA receptors results in an up-regulation of both ^{3}H-SCH 23390 binding and DA-sensitive adenylate cyclase b) the same phenomenon is induced by a physiological condition such as dark adaptation (4 hrs). The latter finding suggests that, in the rat retina, a short-time decrease in presynaptic dopaminergic function results in parallel rapid up-regulation of the innervated D-1 DA receptors.

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273.8 POSTNATAL DEVELOPMENT OF D-1 DOPAMINE RECEPTORS IN RAT STRIATUM. O. Giorgi[1], M.G. De Montis[2]*, G. Calderini[3], G. Toffano[3] and G. Biggio[1]. Institutes of Biology[1] and Biochemical Pharmacology[2], University of Cagliari, Italy, and FIDIA Res. Lab.[3], Abano Terme, Italy.

Although the postnatal developmental changes in the levels of dopamine (DA) and its metabolites, as well as in the density of D-2 DA receptors and in the activity of D-1 DA receptor-linked adenylate cyclase have been extensively studied, there is no information regarding the ontogeny of D-1 DA receptors assayed with selective ligands for this receptors. Recently, the radiolabelled form of SCH 23390, the most selective D-1 antagonist available to date, has been synthetized and its binding characteristics have been described in rat brain membrane preparations. Therefore, it was considered of interest to study the ontogeny of D-1 DA receptors in the rat striatum using ^{3}H-SCH 23390 as a selective probe. The developmental changes in DA content and DA-stimulated adenylate-cyclase activity were also analyzed. The density of D-1 receptors in newborn rats was between 10-15% of adult levels (3 month old rats, Bmax = 1.2 ± 0.09 pmoles/mg protein; KD= 0.7 ± 0.1 nM). The total number of D-1 sites rapidly increased after birth, reaching 35% and 85% of control values by day 7 and day 14 respectively. The rise in D-1 receptor density continued until day 21, when a plateau was reached that persisted through adulthood. A significant decrease (38%) in the total number of D-1 receptors was found in aged rats (26 month old). Scatchard plots of ^{3}H-SCH 23390 labelled receptors revealed that the affinity remained constant during development and in aged rats. Basal adenylate cyclase activity in striatal homogenates from 7, 15 and 30 day old rats was 18%, 61% and 110% of control (3 month old) values, respectively. Moreover, a sharp increase in the maximum stimulatory effect of DA on adenylate cyclase activity was observed during development. Thus, the increase in cAMP above the basal values induced by 10^{-4}M DA in 7 day old rats was 40% of that obtained in adult animals and the maximal response to DA stimulation reached control levels in 14 day old rats. Finally, striatal DA content in newborn and 30 day old rats was 7% and 62%, respectively, of that found in 3 month old rats (11.62 ± 0.07 µg/g wet weight). These results indicate a delayed development of presynaptic DAergic mechanisms as compared to post synaptic markers of DAergic function in the rat striatum.

273.9 (^{3}H)SPIPERONE BINDING IN THE NEOSTRIATUM AND NUCLEUS ACCUMBENS REMAINS UNCHANGED FOLLOWING ACUTE CHLORAL HYDRATE TREATMENT. H.S. Pan, A. Tien*, J. Blanchard*, and R.Y. Wang. Department of Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

To elucidate effects produced by chronic antipsychotic drug (APD) treatment on dopamine (DA) receptors, it is desirable to use the same APD treated rats for both electrophysiological and neurochemical studies. However, chloral hydrate anesthetic preparation is used for most of the electrophysiological experiments and it is known that anesthetic agents alter DA cell activity. The aim of the present study was to determine whether acute chloral hydrate treatment would affect the DA D_2 binding sites in the neostriatum and the nucleus accumbens (NAc).

Male Sprague-Dawley rats (250-300g) received either chloral hydrate (400 mg/kg, i.p.) or distilled water (1 ml/kg, i.p.) 2 to 3 hours before decapitation. The brains were removed, frozen on powdered dry ice and processed as previously described for receptor autoradiography (Pan et al., J. Neurosci. 3:1189-1198, 1983). Coronal sections (20 µm) were incubated in 0.1 to 2.5 nM (^{3}H) spiperone (89 Ci/mmole, Amersham) for 1 hour at 23°C. Nonspecific binding was defined as binding in the presence of 10 µM 1-sulpiride. The sections were exposed to ^{3}H-Ultrofilm for 2 weeks. Developed autoradiograms were analyzed by computer assisted densitometry.

(^{3}H)Spiperone binding was specific, saturable, and of high affinity; scatchard analyses revealed one binding site. The binding affinities (K_D) and numbers of binding sites (B_{max}) were 0.614 ± 0.069 nM and 160 ± 13 fmol/mg protein for the striatum (n=11), and 0.958 ± 0.216 nM and 120 ± 32 fmol/mg protein for NAc (n = 7) in control rats. The K_D and B_{max} of the two areas in chloral hydrate treated rats (K_D = 0.679 ± 0.105 nM, B_{max} = 171 ± 13 pmol/mg protein, n = 8, for striatum; K_D = 1.143 ± 0.389 nM, B_{max} = 96.1 ± 13.2 pmol/mg protein, n = 7, for NAc) were not statistically different from those in control rats. Both K_D and B_{max} values in the neostriatum were not statistically different from those in the NAc. Nonspecific binding was 0.5-50% of total binding in the striatum and 20-80% in the NAc. The greater amount of non-displaceable binding in NAc may reflect a higher percentage of S_2 binding sites when compared to the neostriatum.

The results of the present study demonstrate that acute chloral hydrate treatment did not affect DA D_2 receptors in either the neostriatum or the NAc. Based on this finding, it is reasonable to use the same preparations for both electrophysiological and radioligand binding studies on the effect of chronic APDs. (Supported by USPHS grants MH-41440 and MH-41696 to R.Y.W.)

273.10 FUNCTIONAL INTERACTIONS OF RAT STRIATAL D1 DOPAMINE RECEPTORS WITH THE EFFECTOR MOIETY ADENYLATE CYCLASE. Ellen J. Hess*, Andrew B. Norman*, George Battaglia and Ian Creese, Dept. of Neurosciences, UCSD School of Medicine, La Jolla, CA 92093. (Spon: S.E. Leff)

We have investigated the functional interactions between rat striatal D1 dopamine receptors and adenylate cyclase (AC). We have previously reported that the irreversible protein-modifying reagent, N - ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) administered in vivo does not result in functional modification of either the guanine nucleotide regulatory protein (Ns) or the catalytic subunit of striatal AC as assessed via guanine nucleotide or forskolin stimulated cAMP production. However, peripherally administered EEDQ markedly reduces [3H]SCH23390 binding to D1 receptors and D1 receptor-stimulated AC in a dose-dependent manner. That these reductions could be prevented in vivo by pretreatment with SCH23390, suggests that EEDQ acts at the D1 receptor recognition site. Interestingly, the loss in D1 receptors does not correlate directly with the observed reductions in dopamine-stimulated AC activity; 40% of D1 receptors may be lost with no significant reduction in the Vmax of dopamine-stimulated AC activity. In contrast, the Vmax of receptor-stimulated AC activity by the partial agonist SKF-38393 is reduced concommitantly with D1 receptor density after in vivo EEDQ treatment. However, we have found that in the presence of 1 uM forskolin, the maximal AC activity stimulated by SKF38393 (10 uM) is identical to that of dopamine (100 uM). Furthermore, in the presence of forskolin, no significant reduction in the Vmax of SKF38393 stimulated enzyme activity was observed after a 28% reduction in D1 receptor density, similar to the full agonist dopamine. These data suggest that forskolin may alter the functional constraints placed on partial agonists.

We have also investigated the time-dependent recovery of D1 receptors and D1 receptor-mediated AC activity after in vivo EEDQ administration. Following the initial EEDQ-induced decreases in receptor density and receptor mediated enzyme activity, recovery of enzyme activity to control levels precedes that of receptors. Furthermore, the receptors remaining appear to be more efficient in their ability to stimulate AC as assessed by calculations of enzyme activity per receptor (i.e. pmol cAMP/min/fmol receptor). Additionally, when the AC and [3H]SCH23390 binding assays are performed under comparable conditions in control tissue, 6 X 10^{-5}M dopamine elicits maximal stimulation of AC activity, while this same concentration of dopamine occupies only 75% of available D1 receptors. Together, these results strongly suggest that the D1 receptor population is not a stoichiometrically limiting factor in agonist-stimulation of AC in tissue from untreated rats or rats recovering from EEDQ treatment.

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273.11 ESTRADIOL MODULATION OF COUPLING OF D_2 DOPAMINE RECEPTORS TO ADENYLATE CYCLASE IN ANTERIOR PITUITARY AND STRIATAL NEURONS IN PRIMARY CULTURE. A. ENJALBERT, P. BERTRAND*, S. DROUVA, C. KORDON, M. MAUS*, H. CHNEIWEISS* and J. PREMONT.
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Peripheral hormones modulate the sensitivity of anterior pituitary cells to various neurohormones. On the other hand, these hormones have also been shown to affect the central nervous system.

In the present work we have studied the effect of estradiol on D_2 dopamine receptors coupled to adenylate cyclase in anterior pituitary cells and in striatal neurons on primary culture. Pretreatment of anterior pituitary cells in presence of 17β estradiol for 5 days did not significantly affect the number or the apparent affinity of D_2 dopaminergic binding sites labelled by ^{3}H spiroperidol. In contrast the dopamine inhibition of adenylate cyclase activity was almost completely suppressed (80 %). This effect of 17β estradiol on dopamine sensitive adenalyte cyclase is dose dependent ($EC50 \simeq 10^{-10}$ M). It is stereospecific since 17α estradiol was ineffective. In addition, other steroids such as progesterone, testosterone or dexamethasone had not effect. This effect seems to involve a genomic mechanism since it was progressively augmented from 15 hours up to 4-5 days. On striatal neurons the same type of effect could be observed on the coupling of D_2 receptors with adenylate cyclase, which can be measured in the presence of a specific D_1 antagonist (Sch 23390) to avoid the stimulation of the enzyme. This effect on neurons presents the same pharmacology than that observed on anterior pituitary cells.

In conclusion these results demonstrate that estradiol uncouples D_2 dopamine receptors with adenylate cyclase both in the anterior pituitary and in the central nervous system.

273.12 ENDURANCE TRAINING EFFECT ON STRIATAL D2 DOPAMINE RECEPTORS AND DOPAMINE METABOLITES IN YOUNG AND OLD FISHER 344 RATS. Richard E. Wilcox (1), Priscilla G. MacRae (2), Waneen W. Spirduso (1 and 3), Greg Cartee (3) and Roger P. Farrar (1 and 3). (1) Neurochemistry Research Program and Dept. of Pharmacology & Toxicology, College of Pharmacy, University of Texas, Austin, Tx 78712 (2) Dept. of Sports Medicine, Pepperdine University, Malibu, CA 90265 (3) Dept. of Health & Physical Education, University of Texas, Austin, TX 78712.

The possible trophic effects of exercise during the lifespan and the role which is played by brain transmitter systems in the adaptive response to exercise are areas which offer exciting promise in the search for an understanding of the mechanisms responsible for the plasticity of the brain. We have previously shown that endurance training increases the binding of 3H-spiperone to striatal dopamine receptors of Sprague-Dawley rats when animals were sacrificed 48 hrs following the last exercise bout. (Gilliam et al., Pharmacol. Biochem. Behav., 20, 863-867, 1984.) In the present study we wished to investigate the effects of an endurance training program (3 mo. duration, at approximately 80% of maximal whole body oxygen consumption, VO2max) in Fisher 344 (F344) rats on the relationship between steady-state levels of DA and its metabolites in striatum in vivo *vs.* the affinity and density of striatal D2 DA receptors in striatum (defined by 2 uM d-butaclamol using a 200-fold excess of ketanserin as a mask for 5HT2 sites) in vitro from the same animals. In order to investigate the interaction of age and exercise in the presenescent older adult, rats were of two ages (3 and 18 mo.) at the start of training. The VO2max of the trained animals was 89.9 ± 2.7 ml/kg x min. *vs.* 75.9 ± 2.4 ml/kg x min. in untrained animals. Rats were sacrificed 7 days following the last bout of exercise. DA metabolite to DA ratios were increased in the old control animals. Endurance training was associated with a slight reduction in DA metabolite levels in the younger rats which reached significance in the 21 mo. old animals. Thus, endurance training may have stabilized DA metabolite levels over a portion of the lifespan.

As expected, the number of D2 DA binding sites was reduced with age (from a Bmax of 429 ± 21 fmoles/mg protein in 6 mo. old rats to 355 ± 20 fmoles/mg protein in 21 mo. old animals) with no change in affinity; significantly decreased binding was observed at each of the four 3H-spiperone concentrations as a function of age. While binding was significantly enhanced at each 3H-spiperone concentration in the young runners, a slight (but nonsignificant) increase in binding affinity rendered the Bmax change in these animals nonsignificant relative to young controls. The Bmax of the old runners was significantly higher (457 ± 38 fmoles/mg protein) than that observed in the old controls; binding of 3H-spiperone was also enhanced in the old runners at each radioligand concentration. Thus, endurance training appears to exert a protective effect on the D2 dopamine receptor during the lifespan. Taken together,the present results suggest that a possible reciprocal relationship exists between changes in DA metabolites and DA binding as a function of age and exercise and that endurance training may ameliorate the effects of age to some extent both on the nigrostriatal dopamine neuron and on striatal D2 dopamine receptors during a portion of the lifespan.

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273.13 DIFFERENCES IN REACTION TIME IN RATS: NEUROCHEMICAL CORRELATES OF THE HIGH- AND LOW-AFFINITY AGONIST STATES OF STRIATAL D2 DOPAMINE RECEPTORS. Elizabeth Mudie* (1), Richard E. Wilcox (1), James A. Severson (2) and Waneen W. Spirduso (1 and 3). (1) Neurochemistry Research Program, Dept. of Pharmacology & Toxicology, College of Pharmacy, University of Texas, Austin, TX 78712. (2) Dept. of Psychiatry, School of Medicine, University of Southern California, Los Angeles, CA 90033. (3) Dept. of Health & Physical Education, University of Texas, Austin, TX 78712.

Over the last several years we have developed and refined an animal model of movement initiation termed reactive capacity (RC) using a task which closely parallels the tasks in man which are so sensitive to the slowed movement initiation apparent in Parkinsonism and normal aging. Within- and between- rat strains, differences in RC are related to the density of D2 DA receptors in striatum such that fast-reacting rats have a higher binding site density than slow-reacting animals. Damage to the nigrostriatal DA system induced by 6-hydroxydopamine impairs RC performance in proportion to changes in striatal DA levels, 3H-DA uptake and 3H-spiperone binding. In addition, DA depletions of as little as 15% are associated with marked decrements in maximal RC performance. Normal aging reduces RC performance proportional to the loss of DA receptors. In addition, an apparent reciprocal relationship between levels of striatal DA metabolites and D2 DA receptor density is related to the RC performance of the animals.

In the present report we wished to evaluate the possible relationship of the high- and low-affinity agonist states of the D2 dopamine receptor in striatum to characteristics of the reaction time in a large population of Sprague-Dawley rats. In experiment #1 we determined the extent to which training on the RC task itself might influence agonist states of the D2 receptor. Groups of 8 rats were trained for 0,1,4, or 7 days on the RC task. Displacement of 3H-spiperone (0.5 nM) to the D2 dopamine receptor (defined using a 2 uM d-butaclamol blank and a 200-fold excess of ketanserin as a mask for 5HT2 sites) by dopamine (20 agonist concentrations from 0.1 nM to 0.1 mM) was conducted under standard conditions. Analysis of agonist binding data by LIGAND indicated that in this population of animals, training of the RC task per se did not alter the affinity of agonist binding to either the high- (Kh= 3.6 nM) or low-affinity (Kl = 2400 nM) agonist state. Similarly, the capacities of the high- (Rh = 128 fmoles/mg protein) and low-affinity (Rl = 98 fmoles/mg protein) agonist state were unaffected by learning of the task. The percentage of receptors in the high-affinity agonist state (%Rh) also remained constant at 55 ± 5%.

In experiment #2 we determined the relationships among RC parameters (percent avoidance, minimal avoidance latencies, total response latencies) to the parameters of the agonist states of the D2 dopamine receptors above and to overall D2 dopamine Kd and Bmax. (1) In a total population of approximately 85 rats for which RC and D2 dopamine binding (nonpooled tissue) values were obtained, there was a more than 5-fold variation in percent avoidance scores for well trained animals. (2) In spite of this variation in behavior all binding data were best described by a 2-site (high- and low-affinity agonist state) model. (3) Preliminary analysis of the binding constants suggests that the extreme animals in the behavioral distribution (corresponding to FR and SR rats) differed both in characteristics of their agonist and antagonist binding to D2 dopamine receptors. (4) Within animal correlations were found for RC components and dopamine binding parameters. These results suggest that an important component of the motor planning within basal ganglia responsible for fast reaction time may be associated with the efficient coupling between striatal DA release and the high-affinity agonist state of the D2 DA receptor. (Supported by NS20827 to REW and WWS and an award from the Texas Advanced Research Technology Program to S.W. Leslie and REW.)

273.14 FUNCTIONAL AND NEUROCHEMICAL STUDIES OF THE THALAMUS: CHARACTERIZATION OF THE D2 DOPAMINE RECEPTOR AND RELATIONSHIP TO LEVELS OF DOPAMINE, DOPAMINE METABOLITES AND THE ACOUSTIC STARTLE RESPONSE IN RATS. Keith A. Young* and Richard E. Wilcox. Neurochemistry Research Program, Dept. of Pharmacology & Toxicology, College of Pharmacy, University of Texas, Austin TX 78712. (Sponsor: Carlton K. Erickson).

The thalamus plays a key role in integrating most sensory information reaching the cerebral cortex. Dopamine (DA) exists independently of norepinephrine (NE) in the thalamus (Oke, A. *et al.*, Brain Res. 269: 180-183, 1983), but to date, DA neurons and their receptors have not been characterized in this brain region. In the present study we first characterized the D2 DA receptor in thalamus (experiment #1). Subsequently, we related D2 DA binding, levels of DA and two of its major metabolites (homovanillic acid, HVA, and dihydroxyphenylacetic acid, DOPAC), and levels of NE within the thalamus to a behavioral response (habituation to acoustic startle stimuli). Habituation to acoustic startle has been correlated to various aspects of DA function (Davis, M. Psychopharm. 86: 31-36, 1985; Swerdlow, N.R. *et al.*, Biol. Psychiatry 21: 23-33, 1986). The integrative role of the thalamus suggests that it may be involved in this dopaminergic modulation of sensorimotor activity.

Experiment #1. 3H-Spiperone concentrations were used to label thalamic binding sites of male, six month old Sprague-Dawley rats. D2 DA receptor binding utilized 2 µM d-butaclamol as a blank for nonspecific binding of the radioligand with a 200-fold excess of ketanserin present in all tubes to occlude binding of the radioligand to $5HT_2$ sites. Binding was linear from 100 to 1000 µg of protein per assay tube. Later experiments utilized 200-400 µg protein per tube. Association of binding reached a plateau by 2 min and was stable for at least 45 min. A pseudo-first order plot of binding over the initial 2 min provided a $k_1 = 2.49 \times 10^7$ M^{-1} sec^{-1}. Dissociation of 3H-spiperone from thalamic D2 DA receptors was assessed over 15 min and a first order plot indicated a $k_{-1} = 2.41 \times 10^{-3}$ sec^{-1}. The Kd calculated from k_{-1}/k_1 was 97 pM. Saturability of 3H-spiperone binding was evaluated using Scatchard analysis and yielded a Kd = 96 ± 24 pM and a Bmax of 6.4 ± 0.7 fmoles/mg protein (compared to Kd = 50-100 pM and Bmax = 400-600 fmoles/mg protein in striatum). Displacement of 3H-spiperone (0.25 nM) from thalamic D2 DA receptors by the following compounds yielded the IC_{50} values calculated by LIGAND: d-butaclamol = 3 nM, l-butaclamol = 10 µM, and spiperone = 0.3 nM. The ratio of IC_{50} values for l-butaclamol/d-butaclamol was 2500.

Experiment #2. Habituation to an acoustic startle response was assessed in 22 Sprague-Dawley rats followed by measurement of D2 DA binding vs. levels of DA, NE, HVA, and DOPAC (using HPLC with electrochemical detection) in the thalamus. For initial analysis of neurochemical data, the 8 slowest adapting (SA) and 14 fastest adapting (FA) rats comprised two groups (SA = 185 ± 31; FA = 39 ± 6 trials to adapt to acoustic startle stimuli, max. = 300). ANOVA indicated no significant differences in D2 DA binding or in levels of DA, NE, HVA, or DOPAC between groups. However, ratios of DOPAC/NE, DOPAC/DA, and (DOPAC + HVA)/DA were all significantly different between FA and SA rats. Within the population of rats measured in the study, significant positive correlations were obtained between DOPAC/DA ratios and startle habituation as well as between (HVA + DOPAC)/DA and startle habituation. These results suggest that dopamine overactivity in this region contributes to the inability of some rodents to habituate to sensory stimuli. Since human schizophrenics display sensory habituation abnormalities that are similar to those observed in rodents (Adler, L.E. *et al.*, Biol. Psy. 17: 639-635, 1982), we speculate that DA hyperactivity in the thalamus may have some relevance in the production of sensorimotor abnormalites in schizophrenic individuals. (Supported in part by NS20827 to REW and W.W.Spirduso and a Texas Technology Grant to S.W. Leslie and REW.)

273.15 PURIFICATION OF BOVINE STRIATAL DOPAMINE D-2 RECEPTOR: REQUIREMENT OF LIPIDS FOR BINDING PROPERTIES. L.K. Srivastava*, S.M.I. Kazmi* and R.K. Mishra. (SPON: Sandra Witelson) Neuropharmacology Laboratory, Departments of Psychiatry & Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Lipids are well-recognized in modulating the functional activity of many hormone and neurotransmitter receptors; however, the question of specific lipid requirements for the maintenance of neurotransmitter receptor functions is still unresolved. Toward this end, we have investigated the role of various phospholipids (PL) in maintaining ligand binding properties of affinity-purified dopamine D-2 receptor from bovine striatum. The D-2 receptor of bovine striatal membranes was solubilized with a combination of sodium cholate-NaCl (0.25%-0.9M) and purified on a haloperidol-linked Sepharose CL6B affinity column as reported earlier (Ramwani & Mishra, JBC, in press). The receptor was eluted from the column either by 1 μM spiroperidol or 0.5M Tris-HCl at pH 8.5. In either case, no appreciable ligand binding properties of the receptor could be detected unless PL (crude soyabean lecithin) was present in the elution medium. In order to understand the role of individual PLs in maintaining the receptor activity, the Tris eluted preparation from the column was reconstituted separately with the purified PLs and assayed for ^{3}H-spiroperidol binding. A sonicated preparation of various PLs was mixed with the eluted receptor at a final concentration of 0.5 mg/ml and after incubation for 30 min at 4°C, the detergent was removed by BioBead SM-2. Except for phosphatidyl choline (PC) which restored about 20% of ^{3}H-spiroperidol binding as compared to that obtained with soyabean lecithin (control), and to some extent phosphatidyl enthanolamine (PE), none of the other PLs had any significant effect. When various combinations of two PLs was used for reconstitution, a PC and phosphatidyl serine (PS) mixture seemed to almost fully restore the binding activity. Furthermore, when PE was also included in the PC,PS mixture, there was about 100% increase in ^{3}H-spiroperidol binding as compared to the control. Substitution of other acidic PLs for PS, either with PC or with a PC,PE mixture, did not result in similar increase in binding, except for a combination of PC,PE and phosphatidyl inositol which was half as effective as PC,PE and PS together. The receptor reconstituted with cholesteryl hemisuccinate and monoolein displayed no spiroperidol binding, indicating that specific PL head groups may be responsible for the expression of ligand binding property of the receptor. The reconstituted receptor exhibited high affinity binding for spiroperidol which was comparable to that obtained with the solubilized receptor. The results, thus suggest a role of specific PLs in preserving the binding properties of purified dopamine D-2 receptor.
(Supported by MRC and OMHF, Canada.)

273.16 ISOLATION OF SITES IN MOUSE STRIATUM IRREVERSIBLY LABELED WITH FLUPHENAZINE-N-MUSTARD (FNM) AND APOMORPHINE. K. Thermos, W. Prozialeck and B. Weiss. Division of Neuropsychopharmacology, Department Pharmacology, Medical College of PA., Eastern PA. Psychiatric Inst., Philadelphia, PA 19129.

FNM irreversibly inhibits the binding of [^{3}H]spiroperidol to mouse striatal homogenates both in vitro and in vivo and irreversibly inhibits the rotational responses to apomorphine in mice with 6-hydroxydopamine induced unilateral lesions of the striatum. FNM was given to mice in a dose (20 umol/kg) that completely blocked the rotational response to apomorphine (1 umol/kg). The rate at which the behavioral responses recovered was then monitored over time. One half maximum recovery of rotational responses occurred four days after the injection of FNM; full recovery took more than one week. By contrast it took just one day for half maximum recovery to occur following the injection of the parent compound fluphenazine, also given in a dose that acutely blocked the effects of apomorphine. To determine whether there is a correlation between the behavioral and biochemical effects of FNM, varying doses of FNM were given to mice, and the inhibition of apomorphine-induced rotational behavior was compared with its inhibition of spiroperidol binding. The ED_{50} for inhibition of rotation was 0.8 umol/kg, whereas the ED_{50} for inhibition of spiroperidol binding was about 100 umol/kg. This suggests the involvement of sites, besides spiroperidol binding sites, in dopaminergic supersensitivity. To identify the sites involved in dopamine mediated behaviors we used [^{3}H]FNM as an irreversible affinity ligand. Striatal homogenates were treated with [^{3}H]FNM (10 uM), and the proteins labeled were separated using SDS polyacrylamide gel electrophoresis. This resulted in the labeling of one major and three minor bands of apparent molecular masses of 14 and 42, 59 and 79 kDa respectively. To correlate more directly the apomorphine-induced rotational behavior with the possible sites responsible for these behaviors, we examined the ability of [^{3}H]apomorphine to become photoactivated and covalently incorporated into striatal homogenates. Incubation of striatal homogenates with [^{3}H]apomorphine (1 uM) and subsequent irradiation at 360 nm resulted in the labeling of protein bands similar to those observed with [^{3}H]FNM (14, 42, 61, 75 kDa). The similarity in the cellular constituents labeled by [^{3}H]FNM with those labeled by the direct acting dopaminergic agonist apomorphine suggests the possible involvement of one of the labeled bands in the mediation of the behavioral responses. Supported by funds from the Dept. of Public Welfare, Commonwealth of PA.

273.17 PHOTOAFFINITY LABELLING OF THE D1 DOPAMINE RECEPTOR COUPLED TO GUANINE NUCLEOTIDE REGULATORY PROTEIN. G.M. Ross, B.E. McCarry* and R.K. Mishra. Depts. of Psychiatry and Neurosciences, and Dept. of Chemistry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Two types of dopamine receptors are currently recognized which may mediate the actions of dopamine in the nervous system. The D1 receptor is positively linked whereas the D2 is negatively linked to adenylate cyclase. Although the D2 receptor has been photoaffinity labelled and partially purified by several investigators, similar work on the D1 receptor has been hampered by the lack of a suitable photoaffinity probe. In order to facilitate the purification of the D1 receptor from bovine caudate nucleus, a photoactive analogue of the dopamine agonist 2-amino-5,6-dihydroxytetrahydronapthalene (ADTN) was synthesized. N-(3-(N'-4-azidobenzamido)-aminopropyl)-ADTN (AzB-AP-ADTN) was prepared by reacting N-(3-aminopropyl-ADTN with the N-hydroxy-succinimidyl ester of p-azidobenzoic acid. The [^{3}H] derivative was prepared from [^{3}H]-succinimidyl 4-azidobenzoate. Both the tritiated and unlabelled products were purified by HPLC. AzB-AP-ADTN demonstrated high affinity for the D1 receptor as measured by inhibition of [^{3}H]-SCH-23390 binding. In competition binding assays, AzB-AP-ADTN competed with SCH-23390 in a biphasic manner, revealing one high affinity (IC_{50} = 27 nM) and one lower affinity (IC_{50} = 0.9 μM) component. Binding of AzB-AP-ADTN to the higher affinity site could be abolished by the addition of GTP or Gpp(NH)p, suggesting the involvement of a guanyl nucleotide binding protein. [^{3}H]-AzB-AP-ADTN was used to label membrane proteins prepared from bovine caudate nucleus. When caudate membranes incubated with [^{3}H]-AzB-AP-ADTN were exposed to UV light, a single band of protein (Mr = 79 kDa) specifically and covalently incorporated the photoaffinity probe. Incorporation of [^{3}H]-AzB-AP-ADTN into the 79 kDa protein could be blocked by specific dopaminergic compounds. SCH-23390 was the most effective in protecting the 79 kDa protein from covalent incorporation, followed by apomorphine, ADTN, SKF-38393, dopamine, spiroperidol and haloperidol, respectively. Serotonin and norepinephrine were ineffective in protecting the 79 kDa protein from labelling. GTP and Gpp(NH)p were also effective in reducing the amount of [^{3}H]-AzB-AP-ADTN incorporated into the 79 kDa band. These data would suggest that [^{3}H]-AzB-AP-ADTN can specifically label the high affinity agonist binding state of the D1 receptor. (Supported by the MRC, Canada.)

273.18 PHOSPHORYLATION OF A 92 KDa STRIATAL GLYCOPROTEIN: RELATION TO DOPAMINE RECEPTOR BINDING SITE SUBUNIT. J.Y.Lew*, K.Y. Lee*, and M. Goldstein (SPON: C.H. Adler). Neurochemistry Research Laboratories, New York University Medical Center, New York, NY 10016.

We have reported that ^{3}H-7-azidofluphenazine covalently labels a striatal 92 KDa peptide which represents the dopamine (DA) receptor binding site subunit (Lew et al., Eur. J. Pharmacol. 113:145, 1985). To determine whether the striatal 92 KDa peptide is phosphorylated, we investigated the incorporation of ^{32}P from labeled ATP into glycoproteins isolated from striatal membranes. The striatal membrane bound proteins were solubilized with CHAPSO, adsorbed on a wheat germ agglutinin agarose column and the glycoproteins were eluted with 0.2M acetylglucosamine (Lew and Goldstein, J. Neurochem. 42: 1298, 1984). The glycoproteins were phosphorylated in a reaction mixture containing the following components: [γ-^{32}P] ATP, 60 mM; protein kinase-catalytic subunit, 1μg; Ca^{++}, 2mM; Mg^{++}, 1mM; K^{+}, 5 mM in a 50 mM Tris buffer, pH 7.7. After 20 min of incubation the reaction was stopped by immersing the tubes in boiling water and tetramethylammonium fluoride was added (final concentration 10 mM). The phosphorylated glycoproteins were separated on a 6-15% polyacrylamide SDS electrophoresis gradient and identified by autoradiography. The SDS gel electrophoresis revealed several ^{32}P-labeled peptides and one with the mobility corresponding to 92 KDa. This peptide was not present in the preparation obtained from rat cerebellum. Our results indicate that the phosphorylated 92 KDa peptide arises from a striatal glycoprotein and its electrophoretic mobility suggests that it may represent the DA receptor binding subunit. It was previously reported that the phosphorylation of a striatal 94 KDa peptide is affected by D_1 DA agonists (Mimo and Hanbauer, Neuropharmacology 23:449, 1984), and it is possible that the 92 KDa and 94 KDa peptides are identical. The 92 KDa peptide probably represents the binding site subunit of the D_1 and D_2 DA receptors, and studies are now in progress to determine whether the phosphorylation of this peptide is linked to both subtypes of the DA receptor. These studies were supported by grants NS 06801 and MH 02717.

273.19 COMPUTER MODELING OF DOPAMINE RECEPTOR TOPOGRAPHY. D.I.Schuster, M.C.Luke*, R.Nodar* and R.B.Murphy. Dept. of Chem., New York University, New York, NY 10003.

In the past, there have been several theoretical models proposed with respect to the topographical nature of the dopamine (DA) receptor. As new dopamine analogues are synthesized and as data on their relative specific binding activities become available, it becomes possible to test the validity of these models and to further refine the receptor topography.

By using computer molecular modeling programs, we have compared the structures of various ligands relative to their specific activity for the DA receptor. From such comparisons, we propose a model of an artificial receptor. This model can then be used to study ligand-receptor interactions, modifying the model to accomodate new data. New structures, not previously synthesized, have been generated for comparison, and their plausible effectiveness in binding to the DA receptor has been examined using ab initio computer modeling and molecular fitting.

Our studies were accomplished by using ChemGraf, via quantitative and visual examinations of computerized molecular models. ChemGraf is a molecular modeling program, especially designed for the purpose of structurally comparing molecules. Quantitative data has been obtained from superimposition of excluded volumes and using logical operations on the surface maps of different structures, from analysis of electrostatic potential maps, and from quantum mechanical minimization using various algorithms. Various aspects of dopamine receptor topography which have been studied systematically in this manner will be presented.

This work is supported in part by a grant from the Sandoz Foundation.

MODULATORS II

274.1 PROGESTINS AFFECT CORTICOSPINAL NEURON RESPONSIVENESS TO ADENOSINE. R.E. Stair*, M. Wolley-Gandara* and J.W. Phillis. Dept. of Physiol., Wayne State U. Sch. of Med., Detroit, MI 48201.

Various findings suggest that steroids can affect nerve cell excitability by actions at the cell surface membrane. The purpose of this study was to test whether progesterone and various progestins could alter neuronal responses to iontophoretically applied adenosine. Corticospinal neurons were selected as a model test system for these studies because their response to adenosine has been well characterized electrophysiologically. Extracellular activity of rat corticospinal neurons was recorded in methoxyflurane: N_2O anesthetized animals using multibarrel glass micropipettes. The responses of spontaneously firing single units to microiontophoretic pulses of adenosine, N-ethylcarboxamidoadenosine (NECA) and GABA were examined before, during and after iontophoretic application of progesterone, pregnenolone sodium sulphate, norethindrone acetate and cyproterone acetate, and after intravenously administered progesterone (200 µg/kg) and cyproterone acetate (50 µg/kg). Whether administered iontophoretically for periods of 1-6 min or intravenously, the four progestational agents potentiated the depressant actions of adenosine on cerebral cortical neuronal firing. Potentiation was apparent within 1-2 min of the onset of application of progestin and was evidenced by an increase in the magnitude, and especially by a prolongation, of the adenosine evoked depressions. The depressant effects of NECA, an uptake resistant analog of adenosine, and of GABA, on cerebral cortical neurons were not enhanced by the progestins. Iontophoretic application of larger amounts of cyproterone acetate resulted in the depression of firing of spontaneously active neurons. These depressions were antagonized by intravenously administered caffeine (20 or 30 mg/kg). Pregnenolone sulphate by itself had a weak excitant effect on cortical neurons, which was not reproduced by application of sulphate ions. Potentiation of adenosine depressions of neuronal firing may be a consequence of the ability of progestational agents to inhibit the uptake of adenosine by rat cortical neurons and glia (Phillis et al., Gen. Pharmacol. 16, 609, 1985) and could account for some of the central actions of these substances. A reduction in purinergic inhibitory modulation of synaptic transmission in the brain, resulting from rapidly declining progesterone levels, could be a contributing factor in premenstrual tension and catamenial epilepsy.
(Supported by NIH RR 08167-08).

274.2 LOCALLY APPLIED SEX STEROIDS ALTER NEURONAL RESPONSIVENESS TO GABA AND GLUTAMATE IN THE CEREBELLUM. Sheryl S. Smith, B.D. Waterhouse and D.J. Woodward, *Cell Biology and Anatomy Dpt., UTHSC, Dallas, TX 75235.*

Ongoing studies in this laboratory have demonstrated that systemically administered sex steroids 17β estradiol (E_2) and progesterone (P) alter cerebellar Purkinje (P) cell responses to amino acid neurotransmitters GABA and glutamate (GLUT) in the urethane-anesthetized, ovariectomized adult rat. In the present study, we have examined the effects of locally pressure ejected E_2 (0.5 µM) and P (.01 mM) on P cell responsiveness to GABA and GLUT. The inactive stereoisomer of E_2, 17α E_2 (0.5 µM), and various metabolites of P (0.01 mM), 5α dihydroprogesterone (5α DHP), 5α-pregnan-3α-ol-20-one (3α OH-DHP) and allopregnanolone, were also tested (Vehicle: 0.01% propylene glycol-saline, pH 7.4). Extracellular activity of single P neurons was recorded using multibarrel glass micropipets. Spontaneous firing rate and neuronal response to microiontophoretic pulses (10s every 40s at 10-50 nA) of GABA and GLUT were examined before, during and after continuous local pressure application of the steroids (1-5 P.S.I., 10-15 min).

GABA responses were markedly enhanced by an average of 113% within 3 min of local P application (6 of 7 cells), unaccompanied by alterations in background discharge. Recovery to control levels of response was observed 6-9 min after termination of pressure application. In addition, locally applied P also resulted in a small decrease in P cell responsiveness to GLUT ($\bar{X}$=40%) by 2 - 4 min post-steroid in 5 of 6 cells tested, with recovery observed 6 - 9 min after termination of steroid application. These results are similar to those seen after systemic injection of P. In contrast, local E_2 administration increased GLUT response by 86% within 2 - 3 min after the onset of steroid application , with no recovery apparent by 30 min after termination of steroid administration (6 of 6 cells). As such, local E_2 application mimicked the effect of systemic injection of this steroid. The inactive estrogen isomer, 17α E_2, failed to significantly enhance GLUT responsiveness, although a slight increase inthe response to this amino acid was observed in 2 out of 6 cells tested. The P metabolites, 5α DHP and 3α OH-DHP, both altered P cell responsiveness to GABA and GLUT in a manner and with a time course similar to P, i.e., to increase GABA and decrease GLUT response. However, allopregnanolone did not produce any alteration in P cell responses to either amino acid.

In summary, this study provides an initial demonstration of local sex steroid actions on neuronal responsiveness in a model extrahypothalamic CNS area. These effects were specific, as 17α E_2 and allopregnanolone did not alter neuronal physiology. However, 2 P metabolites which can be localized in cerebellar tissue, 5 α DHP and 3α OH-DHP, mimicked these local effects of P. The results presented here suggest that the neuronal effects of systemic sex steroids may be mediated by local membrane actions of E_2 and P or their metabolites.
(Supported by MH09010 to SSS; AA3901, DA02338 to DJW and the Biological Humanics Foundation.)

274.3 EFFECTS OF CORTICOSTERONE AND ITS 5α-REDUCED METABOLITE, DIHYDROCORTICOSTERONE, ON LONG TERM POTENTIATION IN THE HIPPOCAMPAL FORM-ATIO. M. Liquornik*, P. Noble* and B. Dubrovsky. (SPON: R. Malmo) Dept. of Psychiat. & Physiol., McGill Univ., Montreal, Que. H3A 1A1

As part of an ongoing clinical experimental study on the effects of steroid hormones on the CNS we examined the effects of corticosterone (B) and its ring A reduced metabolite 5α-dihydrocorticosterone (DHB) on long term potentiation (LTP) of the monosynaptic path from the perforant path (PP) to the granular cells of the dentate gyrus. The reasons for this study were: 1) the fact that the hippocampal formatio is a primary target for corticosterone within the nervous system; 2) the fact that B and DHB have contrasting effects, the former increasing and the latter decreasing neuronal excitability (Dubrovsky et al., J. of Neurosci. Res. 14:117(1985), 3) the fact that memory as well as attention disturbances, among other symptoms, are frequently observed both in Cushing's syndrome patients and in patients with melancholia in whom cortisol levels are also frequently elevated. In both these processes, memory and attention, the hippocampal formatio plays a major role. Methods. Adrenalectomized rats 275 to 350 g were used throughout the study. Anesthesia: Sodium Pentobarbital 65 mg/kg was given I.P. The femoral artery and vein from one leg were cannulated for blood pressure recording and drug infusion respectively. A glass micropipette for recording from the dentate gyrus and a bifocal metal electrode for stimulation of the PP were stereotaxically positioned. Both steroids were dissolved in Nutralipid at a concentration of 1 mg/ml., the only dose used. Once a stable recording (equal latency and amplitude for 10 minutes) response to P.P. stimulation was obtained, the following procedure was followed: 1) either B, DHB or the solvent alone, Nutralipid, were slowly injected (2 minutes) in order to avoid blood pressure changes. 2) 2 series of high frequency trains (10 in each) over a 5.5 min period at 10 and 55 minutes after infusion were given. The train duration was 1 second. Train rate was 0.03 c/sec. Frequency in each train, 400 Hz, pulse duration 100 μ sec each. Current intensity ranged from 200 to 1000 uA. Results: The amplitude of the excitatory postsynaptic potentials (EPSP's) and population spikes (PS's), after a fixed time of stimuli were the parameters evaluated. The data showed a consistent pattern whereby the development of LTP was enhanced by treatment of the animal with B, and impaired by treatment with DHB, both in relation to the values obtained with treatment with Nutralipid, the solvent compound. Further, while B and DHB affected the values of the PS's after the first train of pulses, the changes in the EPSP's values appear more consistently after the second train of stimuli. The results indicate that B and DHB can modulate physiological activity within the hippocampal formatio. Moreover, the different effects of B and DHB on the EPSP's and PS's suggests that hormones may act at more than one site in a neuron.

274.4 EVIDENCE FOR THE PRESENCE IN RAT BRAIN OF AN ENDOGENOUS LIGAND THAT MODULATES DIHYDROPYRIDINE RECEPTOR SITES. E. Sanna, A.G. Wright, Jr. and I. Hanbauer. Hypertension-Endocrine Branch, NHLBI, National Institutes of Health, Bethesda, MD 20892.

Ca^{2+} entry into neurons, cardiac and smooth muscle cells occurs through voltage-sensitive Ca^{2+} channels and can be blocked by dihydropyridines, verapamil and diltiazem. Long-lasting treatment of mice with nifedipine or verapamil causes down-regulation of ^{3}H-nitrendipine binding sites in brain (Panza, G. et al., Neuropharmacology, 24: 1113, 1985) suggesting the possibility of an endogenous ligand (EL) interaction at the Ca^{2+} channel complex. ^{3}H-Nitrendipine is used as a ligand to identify and characterize EL that may be operative in modulating Ca^{2+} channels. Rat brains were extracted in 0.1 M acetic acid at 90°C for 3 min. The defatted extract was passed over Biogel-P-10 equilibrated in 0.1 M acetic acid. ^{3}H-Nitrendipine displacing activity coeluted with a marker of molecular weight 1200. The peak fractions were adsorbed on Alumina, neutral, SEP-PAK cartridge, eluted with 1 M formic acid and further purified on C_{18} reversed-phase HPLC with a linear gradient 0-15% acetonitrile/0.1% TFA in 45 min. ^{3}H-Nitrendipine-displacing activity eluted in one peak with the retention time of 6.20 min. The EL in this fraction failed to decrease the specific binding of ^{3}H-spiroperidol, ^{3}H-imipramine and ^{3}H-muscimol. Pronase treatment of this fraction failed to prevent the decrease of ^{3}H-nitrendipine binding to hippocampal membranes. Scatchard analysis of ^{3}H-nitrendipine binding isotherms in the presence and absence of EL showed a decrease in the number of ^{3}H-nitrendipine binding sites (B_{max} control=250 fmol/mg prot.; B_{max} EL=84 fmol/mg prot.), while the K_D values were similar. The present results show that EL for ^{3}H-nitrendipine binding sites occurs in brain and is a heat-stable, H_2O-soluble, alkaline-instable organic compound. Since it specifically decreases ^{3}H-nitrendipine binding in brain membranes, it is suggested that it may play a role as a modulator of Ca^{2+} channels.

274.5 **MICROIONTOPHORETIC AND BIOCHEMICAL STUDIES OF THE NORADRENERGIC INNERVATION OF RAT VISUAL CORTEX.**

A. Kolta*, L. Diop*, R. Brière* and T.A. Reader (SPON:H.H. Jasper). Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Québec H3C 3J7, Canada.

The rat visual cortex (occipital, areas 17 & 18) receives an important noradrenergic input, as reflected by the levels of noradrenaline (NA), measured by HPLC. This monoamine has been proposed to modulate [1] the excitability of cortical cells, and enhance specific sensory signals. To resolve the modalities and types of responses involved we undertook an electrophysiological characterization of NA receptors, in relation to the in vitro binding isotherms for [^{3}H]prazosin (PRZ; α_1), [^{3}H]idazoxan (IDA;α_2) and [^{3}H]dihydroalprenolol (DHA; β).

Binding of	[^{3}H]PRZ	[^{3}H]IDA	[^{3}J]DHA
B_{max} (fmol/mg P)	166 ± 15	147 ± 16	112 ± 7
K_d (nM)	0.14 ± 0.01	2.09 ± 0.48	1.76 ± 0.25
Hill (n^H)	1.10 ± 0.06	0.99 ± 0.05	0.93 ± 0.04
Contents in	Noradrenaline	Adrenaline	Dopamine
ng/mg P	1.26 ± 0.23	0.03 ± 0.01	0.44 ± 0.03

The firing frequency of spontaneously active (SA) and the post-stimulus histograms (PSH; 1,000 ms duration) of visually-driven (VD) neurons were used to document the effects produced by microiontophoresis (5-25nA; for 10-30 s) of NA, clonidine (CLO), idazoxan (IDA), isoproterenol (ISO) and propranolol (PRO). The main effect of NA was a long-lasting inhibition of the firing of SA (in 28 neurons out of 34) and VD units (47/68), outlasting the ejection [2,3]. For the VD cells, the late components (background firing) of the PSH were inhibited to a greater extent than the earlier (<300 ms) firing. When possible, CLO was tested on the same units and although the neurons were less sensitive the responses were also of inhibitory nature. (SA:15/22; VD:17/38) and long-lasting. The selective α_2 antagonist IDA blocked 50% of the CLO and NA-induced responses, supporting a mediation of effects by adrenoceptors of the α_2-subtype. The responses to ISO and PRO (β drugs) were variable, and slight slow-developing excitations could be documented. These studies favour the hypothesis that NA can increase the signal/noise ratio by reducing background firing and calls upon the participation of α-receptors, probably of the α_2 subtype, in this modulation of excitability.

1] Reader, T.A., Ferron, A., Descarries, L., and Jasper, H.H., Brain Res. 160 (1979) 217-229 2] Reader, T.A., Experientia 34 (1978) 1586-1588. 3) Videen, T.O., Daw, N.W. and Rader, R.K., J. Neurosci. 4 (1984) 1607-1617.

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274.6 CYCLO(LEU-GLY) + HALOPERIDOL: EFFECTS OF DOPAMINE RECEPTORS AND CONDITIONED AVOIDANCE RESPONDING. W.J. Baldy, Jr.*, R.J. Elgin, Jr.*, D.M. Cooper*, A.J. Bean and G.E. Martin. Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477-0776.

Cyclo(Leu-Gly)(cLG) is an enzymatically stable analog of the tripeptide MIF (Pro-Leu-Gly-NH_2). These two compounds have been shown to inhibit the development of tolerance and physical dependence produced by morphine and β-endorphin (Bhargava, JPET, 218(2):404, 1981). Furthermore, cLG can inhibit the development of dopamine receptor supersensitivity induced by either chronic haloperidol treatment, chronic morphine treatment or i.c.v. 6-OHDA lesions in mice (Ritzman and Bhargava, Life Sci., 27:2075, 1980). Since the development of supersensitive dopamine receptors occurs with chronic neuroleptic treatment, and this supersensitivity may contribute to the development of tardive dyskinesias, an agent which prevented the development of supersensitivity without altering the efficacy of a neuroleptic would be of great clinical utility.

In this study we determined whether chronic treatment with cLG, at doses reported to inhibit the development of dopaminergic supersensitivity, has effects on the efficacy and potency of haloperidol (H) in a rat test predictive of antipsychotic activity [discrete trial, one lever, conditioned avoidance response (CAR)].

To confirm the inhibition of H-induced dopaminergic supersensitivity by cLG in vivo, we examined the effect of acute and chronic cLG (acute-2 mg/kg s.c.; chronic-2 mg/kg/day s.c. 14 days via Alza mini pumps) on apomorphine-induced stereotypy in rats which had been given acute or chronic H (acute 0.05-0.4 mg/kg, chronic 1 mg/kg/day s.c. 14 days via Alza mini pumps) or vehicle. Acute treatment with cLG failed to alter apomorphine-induced stereotypy in male rats and did not alter H-induced inhibition of apomorphine-induced stereotypy. However, given chronically concomitant with H, cLG reduced the H-induced supersensitivity to apomorphine as reflected in a rightward shift in the dose response curve for apomorphine. In examining the effects of this dose of cLG (2 mg/kg/day s.c. 14 days) on H efficacy and potency in the CAR assay, no significant change in H potency in blocking rat CAR was observed. One can conclude from these studies that although chronic administration of cLG does inhibit development of dopamine receptor supersensitivity, it does not reduce the efficacy or potency of acutely administered H in the rat CAR assay.

274.7 **ALTERED NEUROCHEMISTRY IN THE SUPERIOR CERVICAL GANGLION OF THE SHR STROKE-PRONE VARIANT.** S.L.Kenny*, J.Kettle*, & M.A.Ariano (SPON: W.G. Bradley). Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

The postganglionic neurons of the superior cervical ganglion (SCG) send axons to the vascular smooth muscle in arteries of the head and neck to control their vasomotor tone. We have examined various neurochemicals within the SCG of the stroke-prone variant of the spontaneously hypertensive rat (SHR), in an attempt to elucidate the possible changes which may underlie this genetic predisposition. Investigations in the SHR and its normotensive genetic control, the Wistar-Kyoto (WKY) rat have proved useful in elucidation of sympathetic responsiveness that occurs during increased systemic blood pressure. Previous studies have demonstrated that there is an increased excitability following intracellular stimulation in the postganglionic neurons of the superior cervical ganglion of the SHR (Soc. Neurosci. 7: 215, 1981). It has also been reported that an elevated level of presynaptic activity can be measured in this sympathetic ganglion (Brain Res. 296: 152, 1984).

We have examined the dopaminergic SIF interneuronal population and the immunofluorescent localization of the second messenger cyclic nucleotides in a series of age-matched pairs of SHR stroke-prone and WKY animals. SCG were rapidly removed from the area of the bifurcation of the carotid arteries and frozen at -25°C for subsequent sectioning at 8 um in a cryostat. Glyoxylic acid was employed for catecholamine condensation and demonstrated a substantial decrement in the frequency of occurrence of SIF clusters in the SCG of the stroke-prone variant of the SHR. Cyclic AMP and cyclic GMP were more readily visualized in the stroke-prone SHR, and were localized within the large-diameter postganglionic neurons. Cyclic AMP immunofluorescence was also found within the small glial-satellite cell population. WKY immunohistochemical staining for the two cyclic nucleotides was apparent, but the large postganglionic neurons (cyclic GMP and cyclic AMP positive) and glial cells (cyclic AMP positive) were not as robustly fluorescent. The pattern of cyclic nucleotide immunofluorescent staining in the WKY was generally equivalent to that seen in the Sprague-Dawley rat, as we have previously reported (Cell. Molec. Neurobiol. 2: 142, 1982).

These investigations suggest that a decrease in the dopaminergic modulation of postganglionic neuronal activity occurs in the stroke-prone SHR animal and that the chronically elevated presynaptic activity in the SCG increased the cyclic AMP and cyclic GMP immunofluorescent staining that can be visualized in these projection somata.

This work was supported by a Vermont Heart Association Grant-in-Aid. MAA is the recipient of RCDA NS 00864.

274.8 MODULATION OF FAST AND SLOW CALCIUM CURRENTS IN FROG SKELETAL MUSCLE FIBERS. J. García*, G. Cota*†and E. Stefani. Dept. of Physiology, Biophysics and Neurosciences, CIEA del IPN, 07000 México, D.F., MEXICO and †Dept. of Physiology, Univ.Pennsylvania, Philadelphia, PA. 19104, U.S.A.

Fast and slow Ca^{++} currents (I_{Ca-f} and I_{Ca-s}) were recorded with intracellular microelectrodes (three-microelectrodes in the fiber end or two microelectrodes voltage-clamp techniques in short dissociated fibers) and with the vaseline-gap technique. Muscles were equilibrated in the following solution (mM): CH_3SO_3-TEA 120, $(CH_3SO_3)_2$-Ca 10, HEPES-TEA 5 and 3,4-diaminopyridine 1 at pH 7.0. In a first series of experiments we found that during repetitive stimulation I_{Ca-f} and I_{Ca-s} increased in size. For example after stimulation to 0 mV from a holding potential of -90 mV at 3.3 Hz with 150 msec pulses, I_{Ca-f} gradually increased from 23 $\mu A/cm^2$ to 40 $\mu A/cm^2$ through the fourth pulse. The time course of activation of I_{Ca-f} did not show major modifications. In two pulse experiments, a 245 msec prepulse to -40 mV potentiated I_{Ca-s} during the following test pulse to 0 mV. The slow I_{Ca} tail increased from 270 $\mu A/cm^2$ to 284 $\mu A/cm^2$. The time course of activation became faster in the potentiated I_{Ca-s}. The half time constant of activation in the same experiment was reduced from 100 msec to 75 msec. In a second series of experiments we found that addition of 10 µM-adrenaline and 2mM-dibutyryl-cAMP increased the size of both I_{Ca}. Figure 1a shows I_{Ca-f} with charge movement and tail current. Figure 1b shows charge movement only, after replacing 10 mM-Ca by 10 mM-Mg, plus 3 mM-Cd added to the recording solution. In Fig. 2, record a is the control and b after the addition of dibutytyl-cAMP. Both I_{Ca} were potentiated by this nucleotide, the effect appeared to be larger for I_{Ca-f}.

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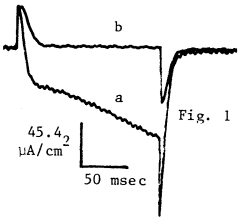

Fig. 1

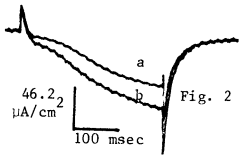

Fig. 2

274.9 BIPHASIC EFFECTS OF ALKYLXANTHINES ON TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. S.R. Barry. Dept. of Phys. Med. and Rehab., University of Michigan, Ann Arbor, MI 48109.

Alkylxanthines affect the activity of excitable cells by at least three different mechanisms. These drugs may release calcium from intracellular stores, may inhibit phosphodiesterases, and may block adenosine receptors located on the extracellular membrane. At the frog neuromuscular junction (NMJ), alkylxanthines were found to have biphasic effects on transmitter release. At millimolar concentrations, alkylxanthines elevated transmitter output while, at micromolar concentrations, these drugs depressed transmitter release. At the lower concentrations, the action of alkylxanthines may be mediated by adenosine receptor blockade.

The frog cutaneous-pectoris nerve muscle preparation was isolated, placed in a recording dish, and continually perfused with frog Ringers. Spontaneous miniature endplate potentials (mepps) were recorded with conventional microelectrodes. A change in mepp frequency, which was not accompanied by a change in mepp size, results from a change in transmitter release. In most experiments, the osmolarity of the Ringers solution was elevated with 30mM sucrose to increase baseline mepp frequency. Drugs were applied by perfusion.

Theophylline, at a concentration of 1mM, enhanced mepp frequency by 79%. In contrast, at a concentration of 10µM, theophylline depressed mepp frequency by 28%. 8-Phenyltheophylline and 8-p-sulfophenyltheophylline, both at concentrations of 1µM, also reduced mepp frequency by 27-39%. Theophylline hyperpolarized the muscle by a few millivolts and caused a small increase in mepp size while the other two drugs had no postsynaptic effects.

At micromolar concentrations, alkylxanthines block brain adenosine receptors but have little effect on phosphodiesterases (Smellie et al. Life Sci, 24:2475, 1979). The effects of these drugs at the frog NMJ may also result from adenosine receptor inhibition. Theophylline and 8-phenyltheophylline blocked the effects of adenosine at the frog NMJ. For example, in one experiment, application of 2.5µM adenosine reduced mepp frequency to 36% of control. In the presence of adenosine and 1µM 8-phenyltheophylline, mepp frequency increased to 53% of control. Furthermore, application of adenosine deaminase at 0.1 and 0.5 units/ml also reduced mepp frequency by 31% and 53% respectively without producing postsynaptic effects. Adenosine deaminase converts adenosine to inosine, an inactive metabolite. Its action at the frog NMJ may result from destruction of endogenous adenosine.

In summary, alkylxanthines at millimolar concentrations may enhance transmitter release from frog motoneurons. At micromolar concentrations, alkylxanthines may reduce transmitter output, perhaps by blockade of adenosine receptors and inhibition of the effects of endogenous adenosine. However, application of adenosine to the frog NMJ also reduced transmitter release. This paradox may be explained if adenosine has more than one action on frog motoneurons. Supported by NSF grant #BNS 8506778.

274.10 IMINO ACID UPTAKE AND RELEASE BY PC-12 CELLS. J.G. Ortiz, R. Velez Soto* and M.L. Alonso*, Dept. of Pharmacology, Univ. of Puerto Rico School of Medicine, San Juan, Puerto Rico 00936.

Imino acids (proline and pipecolic acid) and glycine are normal constituents of the mammalian CNS. The role of glycine as an inhibitory neurotransmitter is well established. On the other hand, recent evidence suggest the possibility that proline (Pro) and pipecolic acid (PA) may act as neurotransmitters/neuromodulators.

The uptake of glycine, proline and pipecolic acid, was examined in PC-12 cells. Confluent cultures were incubated at 37°C with the labelled compound in Earle's balanced salt solution. Cultures incubated at 0-4°C were used as controls in every experiment. At different times thereafter, the incubated medium was aspirated and the cells washed twice with ice-cold phosphate buffered saline. Radioactivity was determined in 0.5 M NaOH cell extracts after neutralization with glacial acetic acid.

The uptake of proline and pipecolic acid in linear for 25 min. These cells are capable of taking up imino acids over a wide range of concentrations (10^{-5} - 10^{-3} M) suggesting the presence of one uptake system that appears to require Na^+ and to be ouabain-sensitive. Kinetic analysis shows that imino acid uptake system is rather specific for proline in this cell line. On the other hand, the uptake of glycine in these cells is linear for approximately 15 min.

Proline can significantly inhibit pipecolic acid uptake and vice-versa. Glycine may also inhibit Pro and PA uptake but a lesser degree (10-20%), suggesting the presence of several uptake mechanisms.

Preliminary release experiments point to the presence of temperature-sensitive proline release mechanism(s), while the release of PA by pheochromocytoma cells appears to be temperature-independent. Further characterization of the release process(es) will be discussed.

PC-12 cells may be useful in claryfing imino acid transport mechanism(s), as well as, in identifying possible sites involved in the etiology of hyperpipecolatemia and/or Zellweger syndrome. (Supported by NIH/MBRS 1S6 RR08224-01).

274.11 EFFECT OF FRONTAL DECORTICATION OF PE N;N METHYLTRANSFERASE (PEMT) ACTIVITY IN RAT STRIATAL SYNAPTOSOMES. M.T. Tacconi*, G. Fisone*, and S. Consolo* (Sponsor G. Calderini). Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy.

Frontal decortication reduces the striatal uptake of glutamate which has been shown to be involved in membrane phosphorylation. Since PEMT activity was suggested to play an important role in the transduction of receptor-mediated signals through the membranes,we studied the effect of decortication on PEMT activity in striatal synaptosomes in basal condition and after pharmacological manipulations. Female CD-COBS rats, b.w. 175 g approx.(Charles River,Italy) were used. Frontal bilateral decortication by undercutting the cortex was performed. Animals were killed 3 and 14 days after lesion and synaptosomes were prepared by gradient centrifugation. PEMT activity was measured by incubating different aliquots of synaptosomes for 30' at 37° in the presence of 1.2 and 10 uM of S-adenosyl (^{3}H)-methyl methionine (SAM), 2.5 uCi/assay. Methylation products (phosphatidylcholine, PC, phosphatidyl N,N dimethylethanolamine, PDME, and phosphatidyl N-methyl ethanolamine, PMME) were extracted, separated by TLC and their radioactivity measured. We found that production of (^{3}H)-PC, (^{3}H)-PDME and (^{3}H)-PMME was increased by 20, 50 and 70% respectively in striatal synaptosomes as early as 3 days after decortication as compared to sham-lesionated rats; 14 days after treatment the increase was still present. Instead no changes on methylation products were found after chemical degeneration with 5,7-DHT of the serotonergic midbrain rafe nuclei. Experiments in which increasing amounts of synaptosomes were used (30 to 140 ug protein) showed a proportional higher increase in (^{3}H)PL production in groups with lower protein content with a shift of the dose/response curve to the left. The addition of dopamine at 10 and 50 uM to synaptosomes stimulated PEMT activity in sham rats but not in decorticated. Haloperidol addition (10 uM) did not affect both groups; at higher doses (100 uM) of the drug both sham and decorticated were equally slightly reduced. In conclusion we have shown that the excitatory cortical striatal pathway plays a role in the regulation of striatal molecular mechanisms.

274.12 GABA-INDUCED RELEASE OF ENDOGENOUS ASCORBATE FROM RAT STRIATAL HOMOGENATES IS UNAFFECTED BY CORTICAL LESIONS. G.V. Rebec, A. Basse-Tomusk, R.M. Wightman and J.C. Bigelow (SPON: G.P. Frommer). Depts. of Psychol. and Chem., Indiana Univ., Bloomington, IN 47405.

GABA has been shown to cause the release of ascorbate (AA) from rat striatal homogenates and this releasing action is mimicked by muscimol and blocked by picrotoxin (Bigelow et al., J. Neurochem., 42:412, 1984). The source of this GABA-induced AA release, however, is unknown. Some evidence suggests that AA may be released from corticostriate terminals. Unilateral cortical lesions, for example, reduce the electrochemical signal for AA in the striatum by 55% (O'Neill et al., Neurosci. Lett. 42:105, 1983).

To investigate the possibility that corticostriate terminals are the source of GABA-induced AA release, we performed bilateral cortical suction lesions extending from 1 mm posterior to bregma to the frontal pole of rats. Four weeks later, AA release was studied with the use of a rapid superfusion system with an on-line amperometric detector. Striatal homogenates from control and lesioned animals were exposed briefly (15 s) to a Ca++ free GABA solution in the presence of a depolarizing agent (40 mM potassium). Under these conditions, an oxidizable substance, which has been shown to be AA, is released (Bigelow et al., J. Neurochem., 42:412, 1984).

Lesions of the cortex failed to alter this release of AA. Thus, it is unlikely that corticostriate terminals are the primary source of the pool from which AA is released by GABA. These and other results (Grunewald and Fillenz, Neurochem. Int., 6:491, 1984) suggest that the striatum contains more than one pool of AA and that at least one of these pools is independent of the corticostriate system.

274.13 GABA INDUCED STIMULATION OF SEROTONIN BINDING TO THE S2 RECEPTOR IN VITRO. S. Kowalik*, R.M. Mangano, G.T.Bautz* and W.D. Horst. Department of Neurobiology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

It is a well established phenomenon that gamma-aminobutyric acid (GABA) can enhance the affinity of the benzodiazepine (BZ) receptor for BZ agonists. Recently we have found that the affinity of the serotonin-2 (S2) receptor for serotonin (5HT) can also be enhanced by GABA. Using a modification of the binding assay of Leysen et al. (Mol.Pharmacol., 21:301- 314, 1982), the affinity of the S2 receptor was determined in rat frontal cortex membrane fragments using ^{3}H-ketanserin. Approximately 200 ug of membrane protein prepared in 50 mM Tris-HCl, pH 7.7, was incubated at 37° C for 15 minutes with 0.7 nM ^{3}H-ketanserin and concentrations of 5HT ranging from 10^{-10} to 10^{-4} M; specific binding was determined by the addition of 1 uM methysergide. The reaction was terminated by vacuum filtration and the filters were washed with 15 ml of the Tris-HCl buffer. Radioactivity was determined and the IC50 values calculated from the data using log-probit analysis.

The results indicate that the addition of GABA to the ^{3}H-ketanserin incubation mixture enhances the affinity of the S2 receptor for 5HT by 544% (IC50 without GABA= 337 +/- 57 nM; IC50 with GABA 62 +/- 15 nM, p <0.01). A similar effect was obtained with the GABA agonist, muscimol; the addition of 10 uM muscimol resulted in a 527% stimulation of 5HT binding to this receptor. When 10 uM bicuculline iodate, a GABA-A inhibitor, was added along with GABA in the incubation mixture, the stimulation by GABA still occured, albeit it was somewhat attenuated (173%). GABA was not able to alter the affinity of the S2 receptor for the S2 antagonist mianserin (IC50 without GABA = 5.5 +/- 1.3 nM; IC50 with GABA=5.1 +/- 1.4 nM) . These findings suggest that GABA may modulate agonist binding to S2 receptors. In addition, further experiments may establish this system to be useful in distinguishing S2 agonists from S2 antagonists.

274.14 MODULATION OF LIGAND BINDING TO MUSCARINIC CHOLINERGIC RECEPTORS. N.M. Spirt* and R.M. Mangano. (SPON: J.H. Hurst) Neurobiology Research, Department of Pharmacology and Chemotherapy, Hoffmann-LaRoche Inc., Nutley, NJ 07110.

Cholinergic neurotransmission plays a central role in cognitive processes of the mammalian brain. Blocking cholinergic receptors with muscarinic antagonists produces cognitive deficits while compounds that facilitate cholinergic activity (agonists, cholinesterase inhibitors) produce improvements in cognitive performance. A cholinergic receptor modulator that would enhance activation of muscarinic receptors by endogenously released acetylcholine might, therefore, be beneficial as a novel approach for treating certain memory disorders. We describe here an in vitro method to determine the ability of compounds to modulate ligand binding to the muscarinic acetylcholine receptor.

The assay utilizes a crude P_2 preparation of synaptosomal membranes (freshly prepared or frozen homogenate) in 50 mM phosphate buffer, pH 7.4. The membranes are preincubated for 60 min. at 37°C under three different conditions:

1. Control - in the presence of vehicle alone;
2. Compound - in the presence of test compound at a concentration of 10 uM;
3. Compound plus Carbachol - in the presence of test compound at 10 uM plus 10 uM carbachol.

The pretreated membranes are isolated by centrifugation and washed three times in ice-cold buffer. The membranes are then used in a receptor binding assay to determine the IC_{50} for carbachol displacement of ^{3}H-QNB from muscarinic receptors.

Preliminary experiments examined the effects of two compounds in this assay: Aniracetam and TRH. Both compounds are active in certain rodent behavioral models of memory but neither binds directly to the muscarinic receptor. TRH, however, is known to facilitate cholinergic neurotransmission. In this assay, membrane preincubation in the presence of 10 uM aniracetam plus 10 uM carbachol results in a 4-fold increase in muscarinic receptor affinity for subsequent agonist binding. Membrane preincubation in the presence of 10 uM aniracetam alone slightly decreases muscarinic receptor affinity. Preincubation in the presence of 10 uM TRH and 10 uM carbachol increases muscarinic receptor affinity 2-fold. These observations suggest that the ability of these compounds to alter muscarinic receptor affinity in vitro may be related to their memory enhancing effects in vivo.

274.15 EFFECTS OF SYSTEMIC ETHANOL ON RESPONSES OF RAT CEREBELLAR PURKINJE NEURONS TO IONTOPHORETICALLY APPLIED NOREPINEPHRINE AND GABA. Z.N. Stowe, R.-S. Lee, S. S. Smith, J.K. Chapin, B.D. Waterhouse, and D.J. Woodward. (SPON: S.M. Sorensen). *Dept. of Cell Biology and Anatomy, The University of Texas Health Science Center at Dallas, TX 75235.*

Acute ethanol intoxication is known to produce substantial impairment of motor function and to result in certain cerebellar signs, such as ataxia. Previous studies from this laboratory have demonstrated that norepinephrine (NE) potentiates GABA-mediated inhibition of cerebellar Purkinje cell (P-cell) discharge. The goal of the present study was designed to test the effect of acute ethanol intoxication on: (1) the response of individual P-cells to GABA and (2) NE-induced increases in GABA-mediated inhibition.

Male Sprague-Dawley rats (230-370 gm) were intubated endotracheally and anesthetized with halothane (2% or 0.75% in oxygen during surgery and electrophysiology, respectively). An intraperitoneal catheter used for administration of ethanol (1.5 to 2.0 gm/kg in saline) was implanted prior the recording session. Extracellular activity of single cerebellar P-cells was recorded using five-barrel glass micropipettes. GABA was administered by microiontophoretic pulses (2-60 nA for 10 sec every 40 sec) before, during and after continuous iontophoretic application of NE (10-45 nA for 4-5 minutes). Spontaneous discharge, GABA responses and NE-GABA interactions in P-cells were monitored before and 1 to 1.5 hours following systemic administration of ethanol. A transient inhibition of spontaneous neuronal activity was observed usually within 4-8 minutes of ethanol injection. This effect lasted 2-4 minutes in 8 out of 15 rats tested. Despite recovery of spontaneous discharge to control levels, GABA responses were slightly increased (8-15%) relative to pre-ethanol values. Between 10 and 60 minutes post-injection, ethanol enhanced direct NE-induced inhibition of spontaneous discharge normally seen at high ejection currents of the drug. In addition, NE augmentation of GABA action, observed at low ejection currents of NE, was further potentiated as a result of ethanol administration. Both the magnitude and the duration of these effects were increased, such that augmentation of GABA inhibition was greater and persisted for 2-13 minutes longer than in the pre-ethanol period. In conclusion, these results indicate that ethanol may enhance inhibitory actions of both GABA and NE on spontaneous discharge. Furthermore, these data suggest that NE-mediated potentiation of GABA inhibition can also be enhanced following ethanol administration. The modulatory actions of ethanol on synaptic physiology described in the present study may provide potential mechanisms for the CNS effects of ethanol intoxication.

Supported by grants AA-3901, DA-02838 to DJW and the Biological Humanics Foundation.

274.16 IMIPRAMINE INHIBITS PKC-INDUCED ELECTROPHYSIOLOGICAL CHANGES IN HERMISSENDA B PHOTORECEPTORS. D.Fisher* & J.Farley (SPON: J.R.Gaddy). Prog. in Neurosci. & Behavior, Princeton Univ., Princeton, NJ 08544.

We examined the ability of imipramine, an inhibitor of the calcium/phospholipid sensitive enzyme PKC (Mori et al., J.Biol.Chem.,1980) in cell-free reaction systems, to antagonize PKC-induced changes in B photoreceptor light response and ionic currents (Farley & Auerbach, Nature, 1986). Peak (0-1 s) and steady-state (25 s) depolarizing light responses of synaptically-isolated B cells were enhanced by a single injection of PKC in 15 of 17 experiments (see Table 1). This enhancement was quite reliable, persistent, and statistically significant [pk and ss response: t(16)'s = 4.43, 3.72; p .05]. In contrast, B cells bathed in 10 M imipramine ASW failed to show increased light responses following PKC injection [t(10)'s = .63, 1.65; ns], with 9/12 cells showing a slight decrease. Comparisons of pk and ss responses prior to PKC injections revealed the response of imipramine-treated cells to be larger, significantly so for the ss response [t(27) = 2.56, p .05]. Voltage-clamp analysis of the early (I_A) and delayed (largely I_{K-Ca}) outward K^+ currents revealed that while PKC injections reduced both currents when cells were bathed in normal ASW, imipramine (10 M) prevented this effect (Table 1). These results suggest that imipramine may be a useful tool to separate PKC-dependent vs. calcium-dependent phosphorylation-mediated changes in B cells during in vitro conditioning studies.

Table 1
PKC effects on B cell in presence (IMIP) or absence (ASW) of imipramine

	Light response (mV) Before pk	Before ss	After pk	After ss		K^+ currents (nA) Before early	Before late	After early	After late
ASW	32.2	22.2	33.8	24.0	(n=4)	12.3	6.3	7.7	1.2
n=17	±1.3	±1.4	±1.2	±1.3		± 1.7	± 1.8	± 1.9	± 0.9
IMIP	34.8	27.6	34.6	26.9	(n=5)	6.7	1.1	9.1	1.7
n=12	± 1.0	± 1.4	± 0.7	± 1.4		± 2.8	± 1.2	± 2.7	±0.8

* p .05, before vs. after
± measured at ~ 10 mV from V_h = -60

Supported by NSF BNS 8316707 for J.F.

274.17 STRIATAL EXTRACELLULAR PHENETHYLAMINE AND p-TYRAMINE MEASURED BY IN VIVO MICRODIALYSIS AND RADIOENZYMATIC ASSAY. L. A. Phebus*, W. L. Russell*, R. R. Bowsher*, D. P. Henry*, and J. A. Clemens, Eli Lilly and Co., Indianapolis, IN 46285.

Using in vivo brain microdialysis coupled with a new sensitive radioenzymatic assay we have been able to measure the levels of the "trace amines" phenethylamine (PEA) and p-tyramine (p-TYM) in the striatal extracellular fluid of anesthetized and awake rats. The levels of these amines were unexpectedly high and, in awake rats, varied greatly from sample to sample.

For anesthetized preparations, male, Sprague Dawley-derived rats were anesthetized with chloral hydrate and placed in a stereotaxic frame. A miniature dialysis probe was lowered into the striatum through a hole drilled in the skull and dialysate samples were collected. For experiments involving awake rats, animals were implanted as above under methoxyflurane anesthesia, the probe was cemented into place and the rats were allowed at least two days to recover from surgery before commencing measurements. On the day of an experiment, rats were connected via polyethylene tubing to a syringe pump and saline was pumped through the dialysis loop at a rate of 1 microliter/minute. Samples were collected at half-hour intervals and frozen until assayed for PEA and p-TYM.

In anesthetized rats, basal PEA levels in the dialysate were 44.3 ± 17.8 picograms per 30 microliter sample. Basal p-TYM levels in these same samples were 3.6 ± 0.34 picograms.

In awake rats, the levels of PEA detected in the dialysate varied markedly from sample to sample. Some samples contained less than 1 picogram of PEA whereas others contained 50 picograms. P-TYM levels varied even more markedly from a low of 1 to a high of 113 picograms/sample. The levels of these two amines varied independently. These levels were often greater than the amounts of dopamine detected in similar samples.

274.18 BINDING CHARACTERISTICS OF 2-[^{125}I]-IODOMELATONIN IN HAMSTER BRAIN MEMBRANES M.J.Duncan* J.S.Takahashi and M.L.Dubocovich (SPON: E.M.Silinsky). Dept. Pharmacology, Northwestern Univ. Med. School, Chicago, IL 60611 and Dept. Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

The pineal hormone, melatonin, mediates photoperiodic regulation of reproduction in mammals. Implantation of melatonin in the suprachiasmatic area of the hypothalamus leads to gonadal regression in photostimulated white-footed mice, suggesting that this region may be the site of action of melatonin (Glass and Lynch, Neuroendocrinol. 35:117, 1982). Melatonin binding sites have been studied in bovine and rat brain using ^{3}H-melatonin (Cardinali et al, Endocrinol. 105:437, 1979; Niles et al, Eur. J. Pharm. 55:219, 1979). Recently, Dubocovich et al (Neurosci. Abstr., 1986) have demonstrated that the new radioligand, 2-[^{125}I]-iodomelatonin, possessing high specific activity, labels melatonin receptors in chicken and rabbit retina. Therefore, we used this radioligand to identify melatonin binding sites in hamster (M. auratus) brain. Binding of 2-[^{125}I]-iodomelatonin (100 pM) was determined in washed membranes prepared from whole brain in 50 mM Tris-HCl buffer (pH 7.7) at 0°C. Specific binding as defined with 10 µM 6-Cl-melatonin was 80% of total binding. Specific 2-[^{125}I]-iodomelatonin binding to brain membranes reached a plateau within 30 minutes and was stable for 150 minutes. 2-[^{125}I]-iodomelatonin binding was completely reversible by addition of 6-Cl-melatonin at 120 min. 2-[^{125}I]-iodomelatonin binding increased linearly with tissue concentration. Saturability of the 2-[^{125}I]-iodomelatonin binding sites was investigated in two ways. Competition experiments with unlabelled 2-iodomelatonin showed that binding was saturable. Scatchard analysis using 2-[^{125}I]-iodomelatonin [0.20-12.00 nM] yielded a Kd=4.3 nM and Bmax=112.8 fmoles/mg protein. The pharmacological profile of the 2-[^{125}I]-iodomelatonin binding site was investigated in competition studies. Monophasic dose-response curves demonstrated that the order of potency (IC_{50}, nM) of several indoleamines is: 6-Cl-melatonin (4) > 2-iodomelatonin (5) > N-acetylserotonin (13), melatonin (20) > 6-hydroxymelatonin (125), 5-methoxytryptophol (225) > 5-methoxytryptamine (1000), N-acetyltryptamine (2225), and serotonin (4000). There was a significant correlation between the potency of 5-methoxyindoles to compete for 2-[^{125}I]-iodomelatonin binding in hamster brain and to inhibit ^{3}H-dopamine release from rabbit retina in vitro (r=0.835; p<0.05), suggesting that 2-[^{125}I]-iodomelatonin may label a melatonin receptor in hamster brain. These studies show that 2-[^{125}I]-iodomelatonin can be used to identify the site of action of melatonin in hamster brain. Supported by grants NS-07909 (MJD); NIMH, NSF and Searle Scholars award (JST) and NSF CCB-8402-007 and NIH RR 05470 (MLD).

274.19 2-[^{125}I]-IODOMELATONIN: A NEW RADIOLIGAND FOR CHARACTERIZATION OF MELATONIN RECEPTORS. M.L. Dubocovich, S.S. Nikaido and J. S. Takahashi. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611, and Dept. Neurobiol. and Physiol., Northwestern Univ. Evanston, IL 60201.

Melatonin inhibits the calcium-dependent release of dopamine from rabbit and chicken retina through activation of a site possessing the pharmacological and functional characteristics of a receptor (Dubocovich, M.L., J. Pharmacol. Exp. Ther. 234: 395, 1985). The aim of this study was to characterize melatonin receptor sites in retina using 2-[^{125}I]-iodomelatonin , which was synthesized as previously described (Vakkuri et al., Anal. Biochem. 142: 284, 1984). The potency of 2-iodomelatonin to inhibit the calcium-dependent release of $_3$H-dopamine from the chicken (IC_{50}: 100 pM) and the rabbit (IC_{50}: 30 pM) retinas was similar to that of melatonin, suggesting that iodination of melatonin did not alter its biological activity.

Binding of 2-[^{125}I]-iodomelatonin (0.025-1.2 nM) was determined in washed membranes prepared from chicken and rabbit retina in 50 mM Tris/HCl buffer (ascorbic acid 0.1%, pH, 7.4) at 0°C. Samples were incubated for 5 h, and the reaction was terminated by rapid filtration. The binding of 2-[^{125}I]-iodomelatonin was time dependent, saturable, of high affinity and increased linearly with tissue concentration. Binding of 2-[^{125}I]-iodomelatonin was inhibited by NaCl (10-200 mM), but was not affected by KCl, $CaCl_2$ or $MgCl_2$. 6-Cl-Melatonin, used to define specific binding, inhibited 90% of total 2-[^{125}I]-iodomelatonin binding. Scatchard analysis of 2-[^{125}I]-iodomelatonin defined with 6-Cl-melatonin (3 uM) resulted in a Kd = 0.43 ± 0.06 nM and a Bmax = 74.0 ± 13.6 fmol/mg protein (n=5) in the chicken retina. Competition experiments showed a monophasic reduction of 2-[^{125}I]-iodomelatonin binding by melatonin analogs with the following pharmacological profile (affinity, nM): 2-iodomelatonin (2.5) > 6-Cl-melatonin (4.0) > melatonin (6.3) > 6-OH-melatonin (74) > 6-OCH_3 melatonin (460) > N-acetyl- tryptamine (1,600) > N-acetyl 5OH-tryptamine (3,000) > 5-OCH_3 tryptamine (4,600). Serotonin, and other indoles lacking the ethyl N-acetyl group were inactive. Drugs acting at *serotonin*, alpha-adrenergic, and dopamine receptors and neuronal uptake inhibitors did not affect 2-[^{125}I]-iodomelatonin binding. The correlations between the potency of melatonin analogs to compete for 2-[^{125}I]-iodomelatonin binding sites and to inhibit the calcium-dependent release of ^{3}H-dopamine both in chicken (r = 0.961, $p < 0.01$, n = 9) and rabbit retina (r = 0.799, $p < 0.01$, n = 8) were significant. We conclude that 2-[^{125}I]-iodomelatonin is a selective and specific ligand for the identification and characterization of melatonin receptors. Supported by grants EY 04877 to MLD, and NIMH, NSF and Searle Scholars to JST.

UPTAKE, STORAGE AND SECRETION II

275.1 EFFECT OF IRREVERSIBLE BETA-ADRENORECEPTOR LIGANDS ON THE NOREPINEPHRINE UPTAKE SYSTEM IN RAT BRAIN SYNAPTOSOMES. K.M. Standifer, C. Sumners, S.P. Baker and J. Pitha*, Depts. of Pharmacology and Physiology, Univ. of Florida, College of Medicine, Gainesville, FL 32610, and GRC-National Institute on Aging, Baltimore, MD, 21224.

Previous studies have shown that bromoacetylalprenololmentane (BAAM) is an irreversible beta-adrenergic antagonist (Baker, S.P. and Pitha, J., J. Pharmacol. and Exper. Ther., 220:247, 1982), whereas bromoacetylmenthylnorepinephrine (BAAN; Baker, S.P. et al., J. Biol. Chem., 260:15820, 1985) and 1-(2-bromoacetamido-isobutyl)-4-[N-(2-(3,4 dihydroxyphenol)-2-hydroxyethyl)-2-amino-isobutyl] piperazine (BHAP) are beta-adrenergic agonists which irreversibly bind to the receptor. These compounds were tested for their ability to block the sodium-dependent uptake of norepinephrine (NE) in rat cortical synaptosomes. BAAM, BAAN, and BHAP were found to inhibit NE uptake (80 nM) in a concentration dependent manner, with IC_{50} values of 0.9, 1.2, and 2.0 µM respectively. In the presence of 5 µM BAAN, there was no change in the V_{max} value for NE uptake, but an increase in the K_M value (control K_M 0.35 µM; K_M in presence of BAAN, 1.3 µM). However, pretreatment of synaptosomes with 5.0 or 50.0 µM BAAN, followed by extensive washing, resulted in a 40 and 77% decrease in the V_{max} for NE up-take without a change in the K_M (0.45 µM). In contrast, pretreatment with either BAAM or BHAP, followed by washing, had no effect on either the K_M or V_{max} value for NE uptake. The ability of BAAN to reduce the V_{max} of NE uptake was time dependent, blocked by sodium thiosulfate (2.0 mM) and dithiothreitol (0.5 mM), and attenuated by concurrent addition of either NE (10.0 µM) or desmethylimipramine (10.0 µM). BAAN seems to be relatively selective for the NE uptake system as incubations with striatal synaptosomes inhibited [^{3}H]dopamine (DA; 70.0 nM) uptake only at high concentrations (IC_{50} value 2.0 x 10^{-4} M). However, preincubations with BAAN followed by washing did not alter the K_M or V_{max} of DA uptake. The data indicate that the alkylating agents BHAP, BAAM, and BAAN are inhibitors of sodium-dependent NE uptake, and, BAAN appears to bind irreversibly to NE uptake sites. Furthermore, BAAN is relatively selective for the NE uptake system. BAAN may be a useful agent for probing the NE transport system.

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275.2 STEREOSELECTIVE UPTAKE OF [^{3}H]ATENOLOL BY ADRENERGIC STORAGE GRANULES ISOLATED FROM PC12 CELLS. E.E. Bagwell*, J.G. Webb*, T. Walle* and T.E. Gaffney* (Spon. S. Katz), Departments of Cell and Molecular Pharmacology and Medicine, Med. Univ. of S.C., Charleston, SC 29425.

Previous studies from these laboratories have shown that the beta receptor blocking drugs propranolol and atenolol are taken up by both peripheral and central adrenergic neurons. In addition, upon appropriate stimulation, the release of these antagonists can be demonstrated both in intact animals and in isolated synaptosomes and ganglion preparations. These observations have led to the development of the hypothesis that the uptake and subsequent release of beta receptor antagonists into the synaptic cleft near effector sites in the central or peripheral nervous system may play a role in the antihypertensive and other therapeutic actions of these drugs. The objective of the present study was to extend these observations to determine whether the uptake of beta receptor antagonists into adrenergic storage granules is mediated by an active, stereoselective transport process similar to that described for catecholamines.

Storage granules were isolated from PC12 cells by the method of Rebois et al. (Biochem, 19: 1240, 1980) and exposed to 0.05 µM (±)[^{3}H]atenolol or (-)[^{3}H]norepinephrine (NE) for 15 min (pH 7.4) at 37°C and 0°C. The uptake of both drugs at 0°C was approximately 20% of that at 37°C and was subtracted in each assay. At 37°C and in the presence of 1 mM ATP uptake of NE was 3.01 ± 0.37 pmol/mg protein and atenolol was 1.1 ± 0.2 pmol/mg protein. In the absence of ATP, NE uptake was reduced by 80 ± 2% and atenolol uptake by 77 ± 3%. Uptake of both molecules was inhibited 90% by either reserpine (1 µM), an inhibitor of the vesicular amine carrier, or by nigericin (2 µg/ml) which dissipates the proton gradient across the granule membrane. In addition, NE uptake was inhibited when incubated with atenolol and, likewise, atenolol uptake was reduced in the presence of NE. Uptake was unaffected by either desipramine (1 µM), an inhibitor of uptake 1, or oligomycin (2 µg/ml), an inhibitor of mitochondrial ATPase.

To examine possible stereoselective uptake of atenolol, aliquots of the granule preparation were exposed to racemic [^{3}H]atenolol at 37°C and 0°C and the (-) and (+) enantiomers subsequently separated by HPLC following chiral derivatization. At 37°C, the uptake of (-) atenolol was 4.3X that of the (+) isomer, while at 0°C the uptake of the enantiomers was not stereoselective.

These results support the hypothesis that beta receptor blocking drugs are stereoselectively transported into adrenergic storage granules by the catecholamine transporter protein where they are stored for stereoselective release during nerve stimulation.

275.3 THE REGULATION OF NOREPINPHRINE UPTAKE BY ECTOKINASE-MEDIATED PROTEIN PHOSPHORYLATION IN PC12 CELLS. J.E. Chaffee, Y.H. Ehrlich, and E.D. Hendley. Depts. of Physiology & Biophysics and Psychiatry, Univ. of Vermont, Burlington, VT 05405.

A cultured, noradrenergic, neuronal cell line (PC12 cells), derived from a rat adrenal medullary pheochromocytoma by Greene and Tischler (PNAS 73:2424, 1976) was used to investigate the potential role of externally located protein kinases (ectokinases; Ehrlich et al., Nature, 320:67, 1986) in the regulation of norepinephrine (NE) uptake$_1$. Hendley, et al (submitted) provided evidence for the potential involvement of a phosphorylation process in the regulation of synaptosomal NE uptake$_1$. These experiments demonstrated that NE uptake was inhibited by the addition of AppNHp, a non-hydrolyzable form of ATP. This AppNHp-sensitive uptake was also shown to be dependent on calcium, but independent of magnesisum. Incubation of the synaptosomes with γ-^{32}P-ATP, followed by SDS-PAGE and autoradiography, showed a correlation between the phosphorylation of a 39Kdalton protein and uptake. Specifically, this protein was phosphorylated in the presence of calcium, not magnesium, and inhibited by AppNHp.

Further investigation of this process required the utilization of an intact cell preparation. PC12 cells were chosen because they appear to possess a high-affinity NE uptake. To ascertain the validity of this model system, it was necessary to demonstrate that NE uptake in PC12 cells was similar to that in synaptosomes and that these cells possess ectokinase activity.

Uptake was determined by measuring the incorporation of ^{3}H-NE into the cells. Preliminary analysis of 5 min incubations in a Kreb's-Ringer solution gave an apparent Km of 0.38 uM. Studies of inhibition of uptake by the specific pharmacological agents nisoxetine and desmethylimipramine gave IC_{50} values consistent with those reported for synaptosomal uptake. Furthermore, incubation of PC12 cells with 10μM extracellular γ-^{32}P-ATP, followed by SDS-PAGE and autoradiography, demonstrated the existence of ectokinase activity. In addition, omission of magnesium or calcium from the incubation medium produced patterns of phosphoprotein labelling that were similar to those seen in synaptosomes, including the presence of a phosphoprotein with an apparent MW of 42-45Kdaltons labelled only in the presence of calcium. (Supported by PHS MH36064 and USAFOSR 84-0331).

275.4 PROTECTION OF THE CATECHOLAMINE TRANSPORTER IN BOVINE CHROMAFFIN GRANULES FROM INHIBITION BY N-ETHYLMALEIMIDE. J.D. Deupree and D.A. Rohn*. Dept. Pharmacology, Univ. Nebraska Medical Center, 42nd and Dewey, Omaha, NE 68105

Catecholamines are transported into chromaffin granules via a carrier-mediated, active transport process. We have previously demonstrated that this transport process can be studied by measuring ^{3}H-norepinephrine transport into and ^{3}H-reserpine binding to chromaffin granule ghosts (J. Biol. Chem. 259: 10907-10912, 1984). Both ^{3}H-norepinephrine transport and ^{3}H-reserpine binding are inhibited by N-ethylmaleimide (NEM) in the 1 μM range. Mg and ATP have been reported by others to protect the transport of norepinephrine into chromaffin granules from the inhibition by NEM. Under our conditions, Mg and ATP (4 mM) shifted the IC50 for NEM inhibition of ^{3}H-norepinephrine transport into and ^{3}H-reserpine binding to ghosts to the right by a magnitude of 400. We postulated that a sulfhydryl group involved with catecholamine transport may be exposed on the outside of the membrane in the absence of a membrane potential. When Mg and ATP are added, the Mg ATPase is activated and a membrane potential develops which produces a conformational change that causes the sulfhydryl group to become buried in the membrane and less accessible to NEM. To test this hypothesis we examined the effects of an uncoupler of oxidative phosphorylation on the NEM inhibition of ^{3}H-norepinephrine transport and ^{3}H-reserpine binding. Our results demonstrated that Mg and ATP still protected the transporter from inhibition by NEM even though the membrane potential was dissipated by the uncoupler. A check of the metal ion and nucleotide requirement revealed that a metal ion was not required for protection of the transporter from NEM inhibition. ATP could be replaced by ADP and GTP but not by AMP, pyrophosphate, inorganic phosphate or cAMP. These results suggest that the ability of ATP to protect the transport of ^{3}H-norepinephrine into and the binding of ^{3}H-reserpine to chromaffin granule ghosts from inhibition by NEM may be independent of its action on the Mg ATPase since neither a metal ion nor a high energy phosphate bond are required. A di- or triphosphate nucleotide appears to be required for the protection of the transporter from NEM inhibition. (Funded by NINCDS # NS15187).

275.5 EXTRACELLULAR MATRIX (ECM) CHANGES PC12 CELL SHAPE AND PROCESSING OF NEWLY SYNTHESIZED DOPAMINE (DA). C.L. Bethea, Oregon Regional Primate Research Center, Beaverton, OR 97006.

PC12 cells cultured on ECM flatten extensively and show an increase in DA release with a decrease in DA content relative to round cells on plastic as measured with a COMT radioenzymatic assay (Mol. Cell Endo. 37:319). To determine if this difference was a result of a change in DA synthesis or a change in post-synthetic processing, cells were incubated with 3H-tyrosine (TY). Newly synthesized DA and NE were determined in medium and cells using Dowex cation exchange chromatography for separation of 3H-TY, 3H-DA and 3H-NE. Cell and medium concentrations of 3H-TY were also determined. The effects of (a) cell shape induced by ECM or plastic substratum, (b) presence or absence of serum and (c) 3H-TY specific activity were assessed from 1-48 hrs. ANOVA and t-test were used for analysis. In the presence of serum, higher levels of 3H-DA were detectable sooner when 3H-TY was added to tyrosine-free medium instead of tyrosine-containing medium (high vs. low spec. act.). 3H-DA release was higher in ECM cultures whereas 3H-DA content was higher in plastic cultures, but total 3H-DA (medium + cells) was higher in plastic cultures. DA synthesis was then examined with high specific activity 3H-TY in the presence and absence of serum. Column validation showed 3H-DA standard retention by Dowex resin was decreased with serum-containing medium. The differences between ECM and plastic cultures were more pronounced with serum-free medium. Again, 3H-DA release was higher in ECM cultures whereas 3H-DA content was higher in plastic cultures, but total 3H-DA was equal in ECM and plastic serum-free cultures which differed from matching cultures containing serum. To verify these results and to control for the tyrosine limitation incurred with tyrosine-free medium, catecholamine synthesis was examined in serum-free medium with high and low 3H-TY specific activity. 3H-DA declined at 48h in ECM and plastic cultures when 3H-TY was added to tyrosine-free medium but not when tyrosine-containing medium was used. However, 3H-DA release was higher in ECM cultures and 3H-DA content was higher in plastic cultures, but total 3H-DA was equal in ECM and plastic cultures regardless of the 3H-TY specific activity. In all of these experiments, 3H-NE was marginally detectable and loosely reflected the pattern of 3H-DA. It was concluded that with serum-free medium (1) determination of catecholamine synthesis with cation exchange chromatography may be more accurate and (2) that PC12 cells of different shapes on ECM and plastic synthesize similar levels of DA but then process it differently in terms of relative secretion and storage. Supported by AM35044 and RR00163.

275.6 ON-LINE DETECTION OF SECRETION FROM MONOLAYER CULTURES OF BOVINE ADRENAL CHROMAFFIN CELLS. T.D. White, J.M. Bourke* and B.G. Livett. School of Biochemistry, U. of Melbourne, Parkville, Victoria, Australia, 3052.

We have developed a method for directly monitoring ATP release, as an indicator of secretion, from 72 hr primary monolayer cultures of bovine adrenal chromaffin cells plated on multiwell, collagen-coated culture trays. Before assay, the culture medium was replaced with 3 changes of 0.5 ml HEPES-Locke's buffer containing 0.25% BSA at 25°C. 20 μl of firefly luciferin-luciferase reagent were added to the test well and the tray covered with reflective aluminum foil. The tray was placed into a light-tight box with the test well positioned directly above an RCA 931B photomultiplier tube. Drugs were added to the well by penetrating the foil with a Hamilton syringe through an injection port in the lid and the box shaken horizontally to provide mixing. The light produced from the ATP-luciferin-luciferase reaction was detected by the photomultiplier tube connected to an Aminco microphotometer and recorded for up to 20 min. This system easily detected 10^{-10} M ATP in 0.5 ml of medium. Nicotine caused a highly reproducible release of ATP from monolayers of chromaffin cells. Surprisingly, nicotine-evoked ATP release had 3 distinct phases: 1) an initial, rapid phase beginning at < 1 sec and plateauing at 1.5-2 min; 2) a second phase beginning at 1.5-2 min and peaking at 3-4 min; 3) a slow, late phase of ATP release which persisted for > 20 min. The dose-response curve for peak ATP release produced by nicotine was bell-shaped with threshold at 10^{-6} M nicotine, maximum at 10^{-5} M nicotine and higher concentrations eliciting reduced responses (e.g. peak release of ATP by 10^{-4} M nicotine was only 72% of release obtained with 10^{-5} M). However, the initial rate of ATP release by 10^{-4} M nicotine was twice that observed for 10^{-5} M nicotine, but by 8-10 sec, ATP release by 10^{-4} M nicotine declined, possibly indicating the onset of receptor desensitization. The triphasic nature of nicotine-evoked ATP release has not been observed with suspensions of chromaffin cells, where release usually terminates by 3-4 min. We believe that plated cultures more closely resemble chromaffin cells in situ. Moreover, most studies of chromaffin cell function, such as amine release, have utilized similar monolayer cultures. This technique should permit temporal comparisons of these various functions to be made. (Supported by the NHMRC of Australia and the MRC of Canada. T.D. White was an MRC Visiting Scientist.)

275.7 ASCORBIC ACID RELEASE FROM PRIMARY CULTURES OF BOVINE ADRENOMEDULLARY CHROMAFFIN CELLS. J. Knoth*, O. H. Viveros and E. J. Diliberto, Jr.* (SPON: F. S. Menniti). Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Ascorbic acid is released by various secretagogues from both intra- and extravesicular compartments in chromaffin cells suggesting a differential release of endogenous catecholamines (CA) and newly-acquired (^{14}C)ascorbate (NA-ASC). Because NA-ASC distributes predominantly into the post-microsomal supernatant fraction, the possibility that ascorbate is labeling a mechanically fragile compartment(s) was investigated by simultaneously monitoring the release of NA-ASC and other subcellular markers such as α-aminoisobutyric acid (AIB) (a non-metabolizable amino acid often used as a cytosolic marker), soluble acetylcholinesterase (AchE), (a marker for the cisternae of endoplasmic reticulum) and CA (a marker for chromaffin vesicles).

Increasing the osmolality of the incubation medium causes a progressive inhibition of secretion for CA, NA-ASC and AIB. Catecholamine secretion is maximal near isoosmotic conditions whereas NA-ASC and AIB secretion progressively increases with increasing hypotonicity. The profile obtained for AIB is nearly identical to that of NA-ASC implying release from the same compartment. We also analyzed release from digitonin-permeabilized cells. Digitonin treatment increases NA-ASC and AIB release in the presence or absence of Ca^{2+} in a concentration and time dependent manner. AchE release was also monitored at various osmolalities and in permeabilized cells. Under these conditions AchE release is very similar to catecholamine but not AIB release implying that NA-ASC and AIB are probably not being released from the endoplasmic reticulum.

We also investigated the effects of various ionic conditions on NA-ASC release. NA-ASC release was measured following complete substitution of external Cl^- with various anions (isethionate, NO_3^-, SO_4^{2-} and glutamate). In all cases NA-ASC release was inhibited at least 40%. Additionally, replacement of external NaCl with either LiCl or sucrose inhibited NA-ASC release approximately 50% and 85%, respectively, suggesting an ionic requirement for NA-ASC release.

To determine if NA-ASC is released from the cell by reversal of its transporter or through an anion channel or another transporter, the effects of ouabain and DIDS were investigated. Ouabain increased the basal release of NA-ASC and potentiated veratridine-induced release. Addition of 100 μM DIDS resulted in approximately 40% inhibition of NA-ASC release. Overall, these results suggest that secretable extravesicular NA-ASC is contained within a presently undefined cytosolic compartment of the cell.

275.8 NEUROPEPTIDE Y RELEASE FROM THE ADRENAL MEDULLA AFTER CHOLINERGIC RECEPTOR STIMULATION T.D. Hexum, E.A. Majane*, L.R. Russett and H.-Y.T. Yang* Dept. of Pharmacol., University of Nebraska Medical Center, Omaha, NE 68105 and *Lab. Preclin. Pharmacol., NIMH, St. Elizabeth's Hospital, Washington, DC 20032

Peripheral nerves as well as chromaffin cells have been shown to contain neuropeptide Y (NPY), a 36 amino acid peptide which exhibits potent stimulatory actions on vascular smooth muscle. Neuropeptide Y-like immunoreactivity (NPY-IR) has been shown to be released into the circulation following sympathetic nerve stimulation. High affinity, saturable binding sites for this peptide have not been demonstrated in the vasculature. Since NPY-IR is stored in chromaffin granules we chose to investigate, using the retrogradely perfused bovine adrenal gland, whether nicotinic cholinergic receptor stimulation results in the secretion of this material. In addition we monitored the release of catecholamines and [Met^5]-enkephalin-like peptides (ME-IR) which have been demonstrated to be co-released from the bovine adrenal medulla. NPY-IR and ME-IR were measured using C-terminal directed antisera prepared from hemocyanin conjugates of the peptides. High performance liquid chromatography with electrochemical detection was used to determine norepinephrine and epinephrine (3,4-dihydroxybenzylamine as the internal standard). Increasing concentrations of acetylcholine (1×10^{-6} M to 5×10^{-5} M), infused in the presence of 1×10^{-5} M physostigmine, produced significant increases in NPY-IR, ME-IR as well as epinephrine and norepinephrine release. Hexamethonium (5×10^{-4} M) antagonized the release of these substances but atropine (5×10^{-7} M) did not. The role of the nicotinic cholinergic receptor in this process was further established by examining the effects of 1,1-dimethyl-4-phenylpiperizinium (DMPP). Increasing concentrations of DMPP (1×10^{-6} M to 5×10^{-5} M) also stimulated the release of NPY-IR, ME-IR and catecholamines. These data demonstrate that NPY-IR can be released through stimulation of the classical receptor mediating exocytosis from the adrenal medulla, i.e., the nicotinic cholinergic receptor. The adrenal medulla appears to be one of the sources for circulating NPY-IR.

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275.9 SODIUM CHANNELS IN BOVINE ADRENAL MEDULLA AND THEIR MEDULATION BY ANTIDEPRESSANT DRUGS. M.Arita*,A.Wada* and F.Izumi(SPON:N.Yanagihara).Dept.of Pharmacol. Univ. of Occupational and Environmental health, Sch.of Med., Kitakyushu 807,Fukuoka,Japan

In adrenal medullary cells, we have reported the presence of three distinct ionic channels; (a) nicitinic receptor-associated Na channels, (b) voltage dependent Na channels which are activated by veratridine, and (c) voltage dependent Ca channels. Carbachol and veratridine cause Na influx and therefore activate voltage dependent Ca channels, whereas high K activates the voltage dependent Ca channels directly. The influx of Ca through the voltage dependent Ca channels is a requisite for the secretion of catecholamines from the adrenal medulla.

In this paper, we examined the effects of tri- and tetra-cyclic antidepressant drugs on the influx of Na-22 and Ca-45, and the secretion of catecholamines in cultured bovine adrenal medullary cells. (1) Influx of Ca-45 and secretion of catecholamines caused by carbachol were inhibited by tricyclic(imipramine, amitriptyline, nortryptiline) and tetracyclic(maprotiline, mianserin) antidepressant drugs. (2) Influx of Ca-45 and secretion of catecholamines caused by veratridine were also inhibited by these drugs. (3) On the contrary, either type of depressant drugs did not inhibit the influx of Ca-45 and secretion of catecholamines induced by high K. (4) Influx of Na-22 caused by carbachol and veratridine was inhibited by these drugs in a similar manner as they inhibited the influx of Ca-45 and the secretion of catecholamines.

These observations suggest that tri- and tetra-cyclic antidepressant drugs inhibit receptor-associated Na channels and voltage dependent Na channels, but not the voltage dependent Ca channels. It seems that antidepressant drugs inhibit the influx of Ca-45 and secretion of catecholamines caused by carbachol and veratridine by the interference of Na influx. The inhibition of Na channels may be comprised in the mechanism by which tri- and tetra-cyclic antidepressant drugs alter central nervous function.

275.10 CALCIUM POOLS IN BOVINE ADRENAL MEDULLARY CELLS: IDENTIFICATION AND PROPERTIES. F.Izumi,M.Okazaki*, Y.Koda*,N.Yanagihara and A.Wada*.Dept.of Pharmacol., University of Occupational and Environmental Health, Sch.of Med.,Kitakyushu 807, Fukuoka, Japan

To locate the Ca-pools in bovine adrenal medullary cells, we investigated (a) distribution of cell Ca, (b) binding of Ca-45 to digitonin treated leaky cells and (c) binding of Ca-45 to the isolated chromaffin granules. The free Ca concentration in the medium was adjusted by mixing calcium chloride and ethylene glycol bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid and expressed as pCa.

(1) The cell Ca was found to be highly condensed in chromaffin granule fraction containing catecholamines. (2) The leaky adrenal medullary cells showed high affinity binding of Ca-45. The half-maximal binding of Ca-45 was observed at pCa 6.75. The binding of Ca-45 saturated above pCa 6.25. (3) The binding of Ca-45 to the leaky cells was dependent on adenosine 5'-triphosphate(ATP) and magnesium. A nonhydrolyzable analogue, AMP-PNP could not substitute for ATP. (4) Binding of Ca-45 was inhibited by carbonyl cyanide p-trifluoromethoxyphenyl hydrazone(FCCP), carbonyl cyanide m-chlorophenyl hydrazone(CCCP) and the sodium azide. (5) Chromaffin granule fraction isolated from adrenal medulla also showed high affinity and saturable binding of Ca-45 with similar concentration-binding curve as in leaky cells. (6) The binding of Ca-45 to the chromaffin granules was also dependent on ATP and magnesium and inhibited by FCCP, CCCP and sodium azide.

Basing on these findings, we suggest that chromaffin granules are the major Ca-pool in adrenal medullary cells in addition to being the site of catecholamine storage. Binding of Ca to the chromaffin granules is an energy dependent process.

275.11 DOWN REGULATION OF PROTEIN KINASE C (PKC) IN NEURONAL CELLS. H. Matthies*, H.C. Palfrey*, L.D. Hirning*, T.M. Perney* and R.J. Miller (SPON: P. Lloyd). Dept. of Pharmacol. & Physiol. Sciences, Univ. of Chicago, Chicago, IL 60637.

Previous results obtained in this and other laboratories suggests a role for PKC in neurotransmitter release and in the regulation of voltage sensitive Ca^{2+} influx. To further define the role of PKC in these processes, we examined the effect of depleting cells of PKC by down regulating the enzyme by long term treatment with phorbol esters.

We observed that in normal and differentiated PC12 cells, the majority of PKC activity is cytosolic and was elevated in differentiated PC12 cells. Treatment of cells for short periods (< 10 min) with phorbol 12 myristate 13-acetate (TPA) or phorbol 12,13 dibutyrate (PBt_2) (10^{-9}-10^{-6}M) caused a dose dependent translocation of the enzyme from the cytoplasm to the particulate fraction. Phorbol esters that do not activate PKC do not produce translocation. If cells were cultured with TPA or PBt_2 (10^{-6}M) for longer periods, there was a time dependent loss of PKC activity so that after 24 hours virtually all Ca^{2+}/phosphatidyl serine stimulated kinase activity in cells extracted with the detergent NP40 was abolished. This was true in both differentiated and undifferentiated PC12 cells. The process was complete by 12 hours of culture. Again phorbol esters that do not activate PKC did not produce down regulation. In contrast to the effects seen with PKC, there was little effect of chronic TPA treatment on cAMP or Ca^{2+} calmodulin stimulated protein kinase activity.

K^+ rich solutions (e.g. 70mM K^+) produce a Ca^{2+} dependent release of ^{3}H-norepinephrine (^{3}H-NE) from PC12 cells. Acute treatment with phorbol esters (10^{-9}-10^{-6}M) synergizes with K^+ to produce an enhancement of 70mM K^+ induced release. Treatment of cells with TPA (10^{-6}M) for one hour eliminated the ability of phorbol esters to synergize with 70mM K^+. Longer treatment (20h) with TPA reduced 70mM K^+ stimulated release to about 50% of control values. Long term treatment with PBt_2 but not with inactive phorbol esters, however, was also effective in reducing 70mM K^+ induced release. Importantly we also observed that the effects of PBt_2 could be reversed on washing the phorbol ester out of the cells with fatty acid free bovine serum albumin.

The effects of phorbol esters on primary cultures of dorsal root ganglion (DRG) and superior cervical ganglion cells (SCG) cultured from 2 day old rat pups were also examined. TPA (10^{-9}-10^{-6}M) enhanced K^+ induced release of ^{3}H-NE from SCG neurones. Treatment of SCG and DRG cells with TPA produced translocation of PKC followed by down regulation of the enzyme. Again there was little effect on cAMP or Ca^{2+}/calmodulin dependent protein kinases.

These results suggest that in PC12 cells and normal neuronal cells, PKC participates in the normal process of exocytosis.

275.12 EVIDENCE THAT PROTEIN KINASE C MODULATES BUT IS NOT NECESSARY FOR Ca^{2+}-DEPENDENT CATECHOLAMINE SECRETION IN DIGITONIN-PERMEABILIZED ADRENAL CHROMAFFIN CELLS. M.A. Bittner* and R.W. Holz. Dept. of Pharmacology, Univ. of Michigan Med. Sch., Ann Arbor, MI 48109

Treatment of cultured bovine chromaffin cells with the phorbol ester phorbol 12-myristate 13-acetate (TPA) for 30 min activated protein kinase C and enhanced the Ca^{2+}-dependent secretion of [^{3}H]norepinephrine in cells permeabilized with digitonin. Continued exposure of cells to TPA for 18-24 hr resulted in a loss of at least 85% of protein kinase C activity in the cells, as measured by the Ca^{2+}- and phospholipid-dependent phosphorylation of histone in vitro. Prolonged incubation with 4-ß-phorbol 12,13-monoacetate, a phorbol ester that does not activate protein kinase C, did not alter the protein kinase C content of cells. After 18 hr preincubation with TPA, the TPA-induced enhancement of secretion was abolished. In these cells secretion stimulated by 0.3-1.0 µM Ca^{2+} was equal to that from cells not incubated with TPA. Secretion stimulated by 10 µM Ca^{2+} was inhibited less than 30% compared to cells not incubated with TPA. TPA pretreatment was accompanied by release of up to 30-40% of [^{3}H]norepinephrine from the cells over 18 hr. This release represents exocytosis, since it was accompanied by the release of soluble dopamine-ß-hydroxylase. Reduction of medium Ca^{2+} concentration from 1.8 mM to less than 0.1 mM caused a 50% inhibition of TPA-induced secretion during a 7 hr incubation, but did not alter the effects of prolonged incubation with TPA on Ca^{2+}-dependent secretion from subsequently digitonin-treated cells. Thus, the loss of secretory granules is unlikely to be responsible for the effects of prolonged incubation with TPA. The data suggest that activation of protein kinase C is not required for Ca^{2+}-dependent secretion in permeabilized chromaffin cells, but that protein kinase C may modulate the secretory response in the presence of phorbol ester or at high (10 µM) Ca^{2+} concentrations.

CYCLIC NUCLEOTIDES

276.1 PHARMACOLOGICAL CHARACTERIZATION OF ADENYLATE CYCLASE-LINKED ADENOSINE RECEPTORS IN PRIMARY CULTURES OF RAT ASTROGLIA AND NEURONS. M.G. Murphy*, C. Moak* and W.F. MacDonald* (SPON: J. Fowler) Dept. of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Distinction between A_1 and A_2 adenosine receptors is currently based on differences in the rank order of potencies of various adenosine analogues in eliciting biochemical and/or physiological responses. To determine whether the A_1/A_2 classification pertains to differential effects of adenosine on adenylate cyclase activity in brain cell cultures, we have examined structure-activity relationships for the stimulation and inhibition of cAMP formation in primary cultures of rat astroglia and neurons. Following 12-21 (astroglia) or 8-12 (neurons) days in culture, cells were incubated in situ for 40 min at 30°C in the presence of Ro 20-1724, and intracellular cAMP was quantitated. Mean basal adenylate cyclase activity in both cell types was 20-40 pmol cAMP formed/mg protein. In glia, stimulation at µM concentrations of agonists was rapid, stereospecific and blocked by theophylline (see Table). Neuron-enriched cultures prepared from embryonic rat (14-16 day's gestation) responded to the agonists with the same order of stimulation and with similar values for Vmax.

	cAMP Production (pmol/mg protein)	
Agonist	Vmax	EC_{50}(µM)
NECA	1000.8	7.3
Ado	1089.4	17.0
(-)PIA	400.3	31.4
CHA	199.3	49.1
(+)PIA	<75	-

Low (nM) concentrations of the adenosine agonists inhibited isoproterenol- and forskolin-stimulated cyclase activities in the glia and neurons, respectively. In the glia, maximum inhibition of stimulated activity (≃8500 pmol/mg protein) was ≃40% with 10^{-6}M Ado, NECA and (+)PIA, and ≃70% with CHA and (-)PIA. Apparent values for IC_{50} for all agonists were between 6-13 nM. By contrast, in the neuronal cultures values for IC_{50} ranged from 1 nM ((-)PIA) to 100 nM ((+)PIA). Maximum inhibition of forskolin-stimulated activity (≃13,000 pmol/mg protein) with 10^{-6}M agonist followed the order CHA > (-)PIA > Ado > (+)PIA = NECA. These studies have demonstrated that the order of potencies for stimulation of adenylate cyclase activity is consistent with that described for the A_2 receptor in both glia and neurons, whereas the pharmacological profile for the inhibitory response is consistent with that described for the A_1 receptor only in the primary neuronal cultures (supported by the Canadian MRC).

276.2 DEVELOPMENTAL CHANGES IN CYCLIC NUCLEOTIDES IN CULTURED RAT NEURONS. A. Novelli and R.C. Henneberry*. Molecular Neurobiology Section, Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

It is known that cyclic GMP accumulates in tissues of the CNS in response to occupancy of specific receptors by excitatory amino acids. We have recently shown that this increase also occurs in rat cerebellar granule cells in primary culture when they are stimulated by excitatory amino acids and depolarizing agents. We show here that this response increases greatly with the age of the cultures. Prior to 3 days in culture, stimulation of cGMP accumulation was not detectable. By day 4, the cells acquired the ability to increase intracellular cGMP levels in response to both excitatory amino acids and depolarizing agents; the magnitude of the response increased with time in culture until a maximum was reached at 8 days. Both basal levels and stimulated levels increased proportionately from day 4 to day 8. These increases did not reflect age-related differences in the dose response to stimulating agents or in the time course of cGMP accumulation after stimulation. At both 4 and 8 days, stimulation by glutamate required Mg^{++} and was blocked by D(-)-2-amino-5-phosphonovaleric acid. Stimulation of cGMP accumulation by both excitatory amino acids and depolarizing agents also dependended on the presence of extracellular Ca^{++}. Moreover, the ionophore A23187, in a Ca^{++}-dependent manner, stimulated the increase in cGMP to the same extent as either glutamate or veratradine at any culture age. Variations in cyclic nucleotide phosphodiesterase activities between 4 and 8 day old cultures do not appear to offer an explanation for these differences; the phosphodiesterase inhibitor isobutylmethylxanthine (0.5 mM) caused proportionate increases in cGMP accumulation in both 4 and 8 day-old cells. The results suggest an increase in the capacity of the granule cells to accumulate cGMP in response to the above stimuli as a function of cell differentiation. The increased capacity appears to involve a component(s) of the guanylate cyclase system different from the neurons' receptors for excitatory amino acids.

276.3 IMIPRAMINE INHIBITION OF ADENYLATE CYCLASE IN CULTURED NEURONS AND GLIOMA CELLS. P.G. Lysko*, A. Novelli, and R.C. Henneberry* (SPON: E.Freese). Molecular Neurobiology Section, Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

Long-term treatment of laboratory animals with tricyclic antidepressants such as imipramine leads to the down-regulation of beta-adrenergic receptors, which suggests an interaction of such drugs with these receptors. We have found that this down-regulation also occurred when rat C6 glioma cells were grown for 24 hours in the presence of imipramine. The number of cell surface receptors decreased without a change in receptor affinity for [^{3}H]CGP-12177. In contrast to these long-term effects, we have also found that imipramine immediately inhibited in a dose-dependent fashion both isoproterenol- and forskolin-stimulated cyclic AMP production in C6 glioma cells and in cultured granule cells from rat cerebellum. Inhibition of forskolin-stimulated adenylate cyclase by imipramine was also observed in C6 cell membrane preparations indicating that the drug acted directly on the regulatory or catalytic subunit of adenylate cyclase, bypassing the receptor. This effect was prevented by addition of the nonhydrolyzable GTP analog 5'-guanylylimidodiphosphate [Gpp(NH)p], which fully activates the guanine nucleotide-binding regulatory protein (N_s). These results suggest that activation of N_s negatively influences the interaction of imipramine at either the regulatory or catalytic subunit of adenylate cyclase due to conformational shifts altering or masking previously exposed imipramine binding sites.

276.4 CHRONIC ESTROGEN TREATMENT IMPAIRS FORSKOLIN STIMULATION AND DOPAMINE INHIBITION OF ANTERIOR PITUITARY ADENYLATE CYCLASE. B. Borgundvaag* and S.R. George, Departments of Medicine and Pharmacology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada.

Prolonged treatment with estrogen is known to result in prolactin cell hyperplasia and tumorigenesis in both male and female rats. This effect in pituitary has been shown to be primarily antidopaminergic in nature, acting directly to block the inhibitory effects of dopamine. The mechanism by which estrogen exerts this effect is not understood. cAMP is an important second messenger in the dopaminergic inhibition of prolactin release from anterior pituitary cells, and agonist interaction with the anterior pituitary D_2 dopamine receptor is known to result in the inhibition of adenylate cyclase activity in this tissue. As chronic estrogen treatment does not have marked effects on the density of anterior pituitary D_2 receptors, we postulate that the effects of estrogen are mediated at post-receptor sites such as the adenylate cyclase system. Female Sprague-Dawley rats (250-300 g) were ovariectomized under halothane anesthesia and implanted s.c. with an empty, sealed Silastic cannula or one containing 2.5 mg beta-estradiol-3-benzoate. Animals were sacrificed by cervical dislocation at 21 days, pituitaries removed, and adenylate cyclase activity determined using the method of Krishna et al. (1968), measuring the conversion of ^{3}H-ATP to ^{3}H-cAMP. Treatment with estrogen resulted in a significant ($p<0.005$) increase in anterior pituitary weight, from 12.72±0.15 mg in controls to 57.52±5.16 mg in the estrogen group. Estrogen produced a 35% decrease in the ability of forskolin (10 uM) to stimulate adenylate cyclase activity, with Vmax values of 60.5 pmols/min vs 39.0 pmols/min for control and estrogen-treated tissues respectively. This decrease in stimulation was accompanied by a 31% reduction in the maximal inhibition of forskolin-stimulated adenylate cyclase activity by the potent dopamine agonist n-propyl-norapomorphine (50 nM). The affinity of the enzyme for ATP was unchanged, with Km values of approximately 0.25 mM. These results suggest that at least in part, the antidopaminergic activity of estrogen in anterior pituitary is a result of interference by this agent of the normal functioning of the adenylate cyclase system in this tissue.

276.5 EFFECTS OF Δ^9-TETRAHYDROCANNABINOL ON ADENYLATE CYCLASE ACTIVITY IN VENTRICULAR MEMBRANES. A.S. Bloom, C.J. Hillard and J.J. Pounds*. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

In previous studies, we demonstrated that the cannabinoids, in particular Δ^9-tetrahydrocannabinol (THC), have effects on adenylate cyclase activities in several tissues. THC and 11-OH-Δ^9-THC, cannabinoids with marihuana-like psychoactive potency, increase glucagon stimulation of adenylate cyclase in liver membranes but have no effect on forskolin, GTP, NaF or Gpp(NH)p stimulated activities (Pharmacologist 27: 165, 1985). In contrast, THC increases GTP-stimulated activity, presumably through prostaglandin release, in cerebral cortical membranes (Eur. J. Pharmacol. 91: 21, 1983). In the present studies, we have investigated the effects of THC on various stimulators of adenylate cyclase in rat ventricular plasma membranes to determine whether the interactions of THC with the enzyme are tissue-specific.

Male Sprague-Dawley rats were sacrificed, hearts were removed, rinsed, quickly frozen in liquid nitrogen and stored at -80°C until use. Membranes were prepared and adenylate cyclase activity was measured at 30°C in an assay buffer containing (mM): HEPES (25), EGTA (1.33), $MgSO_4$ (5), NaCl (150), theophylline (10), 0.75 mg creatinine phosphate, 0.025 mg creatinine phosphokinase, and ATP (1.5). Cyclic AMP was measured using the competitive protein binding assay of Brown (Adv. Cyclic Nuc. Res. (1972) p. 25). THC was solubilized using an Emulphor-ethanol vehicle (J. Pharm. Pharmacol. 25: 345, 1973). The results of these studies are shown below.

Adenylate Cyclase Activity (pmol/min/mg prot)

		THC (μM)			
Stimulator	Vehicle[a]	1	3	10	30
ATP alone	22	29	23	23	23
	(3)[b]	(6)	(4)	(3)	(7)
100 μM GTP	34	32	33	34	35
	(9)	(11)	(9)	(10)	(11)
10^{-6}M ISO[c]	57	85*	66	62	58
	(3)	(2)	(6)	(6)	(8)
10^{-4}M ISO[c]	77	112*	103*	89	72
	(6)	(14)	(7)	(13)	(6)

[a] Emulphor-ethanol vehicle equivalent to 30 μM THC;
[b] S.E.M.; [c] Also contained 100 μM GTP.
* $p < .05$ compared to vehicle using Dunnett's t-test.

These results demonstrate that in vitro THC, at low concentrations, enhances ISO stimulation of adenylate cyclase in heart membranes. The enhancement is probably at the receptor level since basal and GTP-stimulated activities are unaffected. (Supported by Grant DA-03725)

276.6 CHARACTERIZATION OF CHOLERA TOXIN-STIMULATED CYCLIC AMP ACCUMULATION IN RAT BRAIN CEREBRAL CORTICAL SLICES. E.W. Karbon* and S.J. Enna (SPON: B. Ho). Departments of Pharmacology and of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77025.

The effects of cholera toxin on cAMP accumulation in rat brain cortical slices was examined in the presence and absence of various substances known to influence adenylate cyclase activity. The accumulation of cAMP was measured using a prelabeling technique. Cholera toxin alone was found to increase the accumulation of cAMP in a concentration-dependent manner. A delay in the response was noted with this preparation, with maximal accumulation occuring after a 3 hour exposure to a 50 μg/ml concentration of toxin. While neither baclofen, a $GABA_B$ receptor agonist, nor 6-fluoronorepinephrine, an α-adrenergic agonist, had any effect on cAMP accumulation themselves, both augmented the response to cholera toxin. Moreover, phorbol 12-myristate, 13-acetate, a tumor-promoting phorbol ester, enhanced the cAMP response to cholera toxin, with the magnitude of the enhancement being dependent upon the length of time the tissue was exposed to the phorbol ester. A 3 hour preincubation with cholera toxin augmented the cAMP response to forskolin, causing a leftward shift in the concentration-response curve of the diterpene. Finally, cholera toxin enhanced the cAMP response to 2-chloroadenosine, and the adenosine analog eliminated the lag phase normally observed with cholera toxin alone. These results suggest that cholera toxin may be a valuable tool for studying the functional interaction of the components associated with cAMP production in brain slices. In particular, this methodology should be useful for characterizing the involvement of the stimulatory guanine nucleotide binding protein in the action of various drugs, neurotransmitter and modulatory agents. (Supported in part by a grant from the U.S. Air Force).

276.7 PERTUSSIS TOXIN AND 8-BROMO-cAMP REVERSE THE DEPRESSANT BEHAVIORAL EFFECTS OF THE α_2-ADRENERGIC AGONIST ST-91 ON THE ACOUSTIC STARTLE REFLEX. M. Davis, J.H. Kehne, J.V. Cassella, G.K. Aghajanian & J.F. Tallman. Dept. of Psychiat., Yale Univ. Sch. Med., 34 Park St. New Haven, Conn. 06508

It has been proposed that α_2-adrenergic receptors are negatively coupled to adenylate cyclase via an inhibitory guanine nucleotide binding protein (G_i). These actions can be prevented by pretreatment with pertussis toxin which inactivates certain G-proteins including the G_i protein. The present study investigated the possible involvement of cAMP in the depression of a behavioral response produced by stimulation of α_2-adrenergic receptors.

The acoustic startle response in the rat is a simple brainstem and spinal cord reflex that is markedly inhibited by α_2 agonists acting on supraspinal α_2 receptors. Thus, intracerebroventricular (ICV) but not intrathecal administration of the lipophobic α_2-adrenergic agonist ST-91 causes a dose-dependent depression of the acoustic startle reflex which can be reversed by the α_2-adrenergic antagonist idazoxan.

If the depressant effects of ST-91 involve the inhibitory nucleotide binding protein, then pretreatment with pertussis toxin should block this effect. To test this, four groups of 10-12 rats each were placed into special cages equipped to automatically measure the amplitude of the startle reflex elicited by a 105-dB noise burst presented at a 20-sec interstimulus interval. Startle amplitude was measured over a 14-min period prior to and a 30-min period after ICV infusion of either 6.25 μg of ST-91 or artificial CSF in groups pretreated 24 hrs earlier with either ICV pertussis toxin (2 μg) or its vehicle. To evaluate the possible role of cAMP in the depressant actions of ST-91, groups of rats each were infused with either ST-91 or CSF in combination with either 8-bromo-cAMP (50 μg) or its vehicle. To test for specificity, other groups were treated similarly except they were tested with ICV administration of the 5-HT-1B agonist m-chlorophenylpiperazine (MCPP) which is not thought to be negatively coupled to cAMP.

Pertussis toxin significantly increased baseline startle levels and completely blocked the normal depressant effect caused by ST-91 but did not block the depressant effect of MCPP. Administration of 8-bromo-cAMP also elevated startle after ICV infusion and completely blocked the depressant effects of ST-91 but not the depressant effect of MCPP.

These data suggest that the depressant behavioral effects of α_2-adrenergic agonists are mediated by an interaction with a pertussis toxin sensitive G-protein that may be negatively coupled to adenylate cyclase.

276.8 ALTERED GUANINE NUCLEOTIDE REGULATION OF RAT STRIATAL ADENYLATE CYCLASE BY PHORBOL ESTERS. M.C. Olianas and P. Onali. Institute of Pharmacology, University of Cagliari, Italy.

Guanosine triphosphate (GTP) exerts a biphasic effect on striatal adenylate cyclase (a.c.), stimulating at concentrations up to 1μM and inhibiting at higher concentrations. Two different GTP-binding regulatory proteins, termed N_s and N_i, are considered to mediate the GTP stimulation and inhibition of a.c., respectively. We observed that addition of 1μM phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, to striatal membranes markedly enhanced GTP stimulation of a.c. and abolished the enzyme inhibition elicited by micromolar concentrations of the nucleotide. The following evidence support the involvement of protein kinase C activation in PMA effect. Firstly, PMA stimulated a.c. with a potency (EC_{50} 8nM) consistent with an action on C kinase and its effect was mimicked by other activators of C kinase, like phorbol 12,13-dibutyrate and 1-oleoyl-2-acetyl-glycerol, but not by inactive compounds such as 4β-phorbol and phorbol 13-acetate. Secondly, EGTA treatment of striatal membranes reduced whereas addition of 1-α-phosphatidyl-L-serine enhanced PMA effect. Thirdly, PMA did not affect a.c. activity when adenyl-5'-yl-imidodiphosphate, a non phosphorylating analog of ATP, substituted for ATP in the a.c. assay. A series of experiments were performed to ascertain whether N_s and/or N_i function were affected by PMA. Thus, treatment with cholera toxin increased the stimulation of striatal a.c. by low concentrations of GTP whereas the inhibition induced by GTP at micromolar concentrations was unaffected. PMA did not potentiate the stimulatory effect of low concentrations of GTP as expected if the synergistic action of PMA and GTP occurred at the level of N_s, but prevented the enzyme inhibition elicited by high concentrations of GTP. PMA did not affect forskolin (FSK) stimulation of a.c., but reversed the GTP-induced inhibition of FSK-stimulated a.c. In contrast, inhibition of FSK-stimulated a.c. by guanyl-5'-yl-imidodiphosphate was not antagonized by PMA. Under assay conditions similar to those used to detect PMA stimulation of a.c. phorbol esters inhibited a high affinity GTPase activity in striatal membranes with a potency and specificity consistent with their activity on a.c. These results indicate that phorbol esters increase striatal a.c. by counteracting the GTP-induced inhibition of the enzyme. This effect is likely to be mediated by protein kinase C phosphorylation and consequent inactivation of N_i protein.

276.9 **GUANINE NUCLEOTIDE EXCHANGE AMONG GTP-BINDING PROTEINS WHICH REGULATE NEURONAL ADENYLATE CYCLASE.** S. Hatta*, M.M. Marcus*, C.A. Moore*, Y. Hatta*, and M.M. Rasenick. (SPON: C. Melchior) Dept. of Physiology and Biophysics, U. of Illinois Coll. of Medicine, Chicago, IL 60680

Exposure of rat cerebral cortex membranes to hydrolysis-resistant GTP analogs results in inhibition (or stimulation) of adenylate cyclase which persists subsequent to buffer washing. The hydrolysis-resistant GTP photoaffinity probe P^3-(4-azido-anilido)-P^1-5' GTP (AAGTP) can promote a similar persistent inhibition of adenylate cyclase and, after removal of unbound AAGTP and subsequent UV photolysis, AAGTP is covalently linked to the 40 kDa inhibitory GTP binding protein, GNi. Under conditions where the persistent inhibition of adenylate cyclase is overcome by subsequent incubation with guanylyl-5'-imidodiphosphate or NaF, AAGTP bound to the 40 kDa GNi protein is diminished while that bound to the 42 kDa stimulatory GTP-binding protein (GNs) increases. The sum of the AAGTP bound by the GNi and GNs proteins is constant and the transfer of AAGTP to GNs from GNi (before photolysis) is stable to buffer washing. Furthermore, this alteration in the AAGTP labelling pattern corresponds to the shift in adenylate cyclase from inhibition to stimulation. Furthermore, we have polymerized rat brain tubulin, substituting [^{32}P]AAGTP for GTP in the second polymerization cycle. Adding AAGTP-tubulin to synaptic membranes results in the transfer of AAGTP from tubulin to GNi. Subsequent transfer of AAGTP to GNs proceeds as above. If AAGTP binding to tubulin is made covalent prior to addition of AAGTP-tubulin to membranes, no nucleotide transfer occurs. These data raise the possibility that hydrolysis-resistant GTP analogs may be exchanged directly among synaptic membrane GTP binding proteins, and that there exists some interaction among those proteins in the regulation of adenylate cyclase activity.

Additionally, we have identified a 32 kDa protein which binds AAGTP with an affinity similar to that of GNs. This protein does not appear to be a byproduct of proteolysis as demonstrated by S. aureus V-8 protease digestion experiments and it is not a substrate for ADP-ribosylation by bacterial toxins. Furthermore, although this protein appears to be abundant in brain, we have not identified it in non-neural tissue.

276.10 INTERACTIONS BETWEEN INHIBITORY GTP REGULATORY COMPONENTS WITH BOTH ADENOSINE RECEPTORS AND THE ADENYLATE CYCLASE CATALYTIC SUBUNIT IN DETERGENT SOLUTION. D.M.F. Cooper*, S.-M.H. Yeung* and E. Perez-Reyes* (SPON: W.O. Wickelgren). Dept. of Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

The inhibitory GTP regulatory component (Ni), the inhibitory adenosine receptor (Ri), and the adenylate cyclase catalytic subunit (C) were solubilized from rat cerebral cortical membranes with cholate. Interactions between these soluble components was detected directly in detergent solution.

Ni-C interactions were detected by measuring guanine nucleotide mediated inhibition of adenylate cyclase activity. These interactions demonstrated the same functional properties as described in membranes; the specificity for guanine nucleotides was similar: treatments that inactivated Ni function in membranes eliminated Ni activity in the cholate extract; and calmodulin stimulation of basal activity was required in both preparations (Perez-Reyes, E. and Cooper, D.M.F., J. Neurochem., in press). The adenylate cyclase components were fractionated by either sucrose gradient centrifugation or TSK-3000 SW chromatography. Ni activity was detected by assaying the ability of gradient or column fractions to confer guanine nucleotide-mediated inhibition on the resolved C. Labeling of these gradient or column fractions with [32-P]NAD and pertussis toxin revealed that both 39,000- and 41,000-Dalton substrates migrated with the functional Ni. This reconstitution of Ni-C interactions was dependent on both calmodulin stimulation of C and on the concentration of Ni used in the assay. These studies demonstrate that the calmodulin-regulated C from cerebral cortex provides a sensitive assay of Ni activity, which may allow the resolution of which of the pertussis toxin substrates is Ni.

Ni-Ri interactions were detected by measuring guanine nucleotide regulation of [3-H]phenylisopropyl adenosine (PIA) binding in cholate solution. The soluble receptor maintained the functional properties of the Ri-adenosine receptor described in membranes. Since the soluble receptor was stabilized by adenosine agonists, the hydrodynamic properties of the receptor were studied by: prebinding the membranes with [3-H]PIA; solubilization; then either sucrose gradient centrifugation or Sepharose 6B chromatography. Guanine nucleotide regulation of binding was observed in both gradient or column fractions. This receptor displayed a sedimentation coefficient of 7.0 S and a Stokes radius of 7.2 nm. This corresponded to a protein-detergent complex of 254,000-Daltons. We have shown previously by radiation inactivation that the high affinity form of the Ri-adenosine receptor displayed a molecular size of 63,000-Daltons (Frame, L.T. et al. Biochem. J. 235:621, 1986). We suggest that the adenosine receptor in cholate extracts represents an oligomeric species of Ri and Ni.

276.11 DISTINCT MODES OF INHIBITION OF RAT STRIATAL ADENYLATE CYCLASE BY GPP(NH)P AND MORPHINE. M.K. Ahlijanian* and D.M.F. Cooper* (SPON: P. Fuchs). Dept. Pharmacol., University of Colorado Health Sciences Center, Denver, CO 80262.

The inhibition of adenylate cyclase from rat striatal plasma membranes by Gpp(NH)p and morphine was compared to determine whether Gpp(NH)p-mediated inhibition accurately reflected hormone-mediated inhibition in this system. Inhibition of adenylate cyclase activity by Gpp(NH)p and morphine was examined with respect to temperature, divalent cation concentration and the presence of Ca^{2+}/calmodulin. Gpp(NH)p-mediated inhibition was dependent upon the presence of Ca^{2+}/calmodulin at 24°C; the inhibition was independent of Ca^{2+}/calmodulin at 18°C; and inhibition could not be detected in the presence or absence of Ca^{2+}/calmodulin at 30°C. In contrast, naloxone-reversible, morphine-induced inhibition of adenylate cyclase was independent of both temperature and the presence of Ca^{2+}/calmodulin. Ca^{2+} dose-response curves, performed at 24°C, confirmed that Gpp(NH)p-mediated inhibition required the presence of Ca^{2+}/calmodulin, while morphine-induced inhibition did not. Mg^{2+} dose response curves also reinforced the differences in the Ca^{2+}/calmodulin requirement for Gpp(NH)p- and morphine-induced inhibition. Because Gpp(NH)p-mediated inhibition was independent of Ca^{2+}/calmodulin at low basal activities (i.e., 18°C, or below 1 mM Mg^{2+}) and dependent upon the presence of Ca^{2+}/calmodulin at higher basal activities (24°C, or above 1 mM Mg^{2+}), the inhibitory effects of Gpp(NH)p were examined at 1 mM Mg^{2+} in the presence of 100nM forskolin. Under these conditions, both Gpp(NH)p-and morphine-induced inhibition of adenylate cyclase were independent of Ca^{2+}/calmodulin. The results demonstrate that the requirement for Ca^{2+}/calmodulin to observe Gpp(NH)p-mediated inhibition depends upon the basal activity of adenylate cyclase, while hormone-mediated inhibition is Ca^{2+}/calmodulin-independent under all conditions tested. Further, this study shows that Gpp(NH)p-mediated inhibition of adenylate cyclase does not mimic the inhibition of adenylate cyclase produced by a complete Ri-Ni-C interaction in rat striatal plasma membranes.

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276.12 CYCLIC AMP-MEDIATED ENHANCEMENT OF MAUTHNER CELL INHIBITORY RESPONSES. L.R. Wolszon* and D.S. Faber. Dept. Physiology, Div. Neurobiology, SUNY, Buffalo, NY 14214.

Although cyclic nucleotides are known to mediate diverse pre- and postsynaptic effects in the central nervous system, there is little evidence suggesting they can alter the fast synaptic responses mediated by amino acid transmitters. We report that cyclic AMP (cAMP) and one of its analogues unmask and enhance a presumably glycinergic inhibition of the goldfish Mauthner (M-) cell, with evidence suggesting a postsynaptic action.

Intracellular recordings were obtained from the M-cell lateral dendrite, and stimuli were applied to: i) the spinal cord, to antidromically (AD) activate the cell and its powerful recurrent collateral inhibitory network, and ii) a localized region of the posterior branch of the left VIIIth nerve for graded activation of a disynaptic inhibitory pathway. Since the M-cell soma-dendritic membrane does not support spike electrogenesis, inhibition was quantified as the reduction in amplitude of a test AD action potential following AD or VIIIth nerve stimulation. Initially, series of experiments, topical application of 10 to 100 mM dibutyrl-cAMP unmasked a Cl-dependent inhibitory postsynaptic potential (IPSP) evoked by the VIIIth nerve stimulus. To distinguish between a pre- and postsynaptic locus of action, cAMP was iontophoretically injected into the M-cell, using electrodes filled with 200mM cAMP in 20 mM Tris-base and either 4M KAc or 0.5M K_2SO_4 (pH=7.6-7.7). In the absence of any drug (control), the test AD spike height was reduced by approximately 50% following AD stimulation and from 0 to 40% following graded VIIIth nerve stimulation. Within 3 to 15 minutes following cAMP injection, a persistent VIIIth nerve-evoked IPSP was unmasked and/or enhanced by 30 to 75%. In three cases in which weak control stimuli evoked no inhibition, the cAMP induced a 6 to 16% reduction in test spike height. Smaller increases in the AD-evoked inhibition were seen following cAMP injection, but these results are inconclusive, due to the nonlinear relation between spike height reduction and the inhibitory conductance change for large inhibitions. Finally, injection of acetate or sulfate alone did not alter VIIIth nerve-evoked IPSP's in any of the experiments, and the cyclic nucleotide did not appear to alter passive M-cell membrane properties, as indicated by the constancy of resting membrane potential and control spike heights.

We conclude that cAMP injected postsynaptically increases the magnitude of some VIIIth nerve-activated inhibitory inputs and can unmask presumably silent synapses, and suggest it may act as a second messenger in the modification of glycine receptors or receptor-coupled proteins.

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276.13 CALCIUM INFLUX MODULATES A CYCLIC AMP-REGULATED IONIC CONDUCTANCE IN APLYSIA BURSTING PACEMAKER NEURONS. Richard H. Kramer* and Irwin B. Levitan Graduate Dept. of Biochemistry, Brandeis Univ., Waltham, MA 02254

Calcium and cyclic nucleotides function as intracellular messengers in many cells. Ionic conductances regulated by both substances have been extensively studied in Aplysia bursting neurons. Calcium regulates three ionic currents which generate the bursting pacemaker cycle of electrical activity. Cyclic AMP regulates several ionic currents including a K current which activates below the K equilibrium potential ("anomalous rectifier" K current; I_R). We now report that Ca influx both during natural bursting activity, and under voltage-clamp regulates I_R, possibly by affecting cyclic AMP metabolism.

All experiments were done on Aplysia bursting neuron R15. Under voltage-clamp, a train of five 250 msec depolarizing pulses to +20 mV reduced the membrane conductance by 15-25%. The conductance decrease was specific for I_R; it was blocked by addition of 1mM Ba or Cs to the bathing medium, and the conductance decrease shifted to more hyperpolarized potentials in low external K. The reduction in I_R was dependent upon Ca influx during the depolarizing pulses. The decrease in I_R was blocked in Ca-free, Mn-containing bathing medium, and was smaller with depolarizing pulses which approach the Ca equilibrium potential.

I_R was also decreased following spontaneous bursts of action potentials, and this effect was remarkably long-lasting. After a single burst, the conductance decreased by about 20% and recovered over more than 10 min. The slow kinetics suggests that Ca affects I_R via a series of biochemical steps. Experiments with serotonin support the notion that Ca does not directly inactivate I_R channels. Serotonin activates I_R by increasing the intracellular concentration of cyclic AMP. Addition of increasing concentrations of serotonin to the bathing medium increases I_R, however the effect of Ca influx on I_R is supressed above 1 μM serotonin, suggesting that high levels of serotonin-sensitive adenylate cyclase activity interfere with the effect of internal Ca. We are currently investigating the possibility that Ca activates a Ca/calmodulin dependent phosphodiesterase which regulates the internal concentration of cyclic AMP. Supported by NIH grant NS17910.

276.14 FORSKOLIN ACTIVATES LOCUS COERULEUS NEURONS AND GENERATES A HYPERPOLARIZING-ACTIVATED INWARD CURRENT. Y.-Y. Wang* and G.K. Aghajanian, Depts. of Pharmacol. and Psychiat., Yale Univ. Sch. of Med. and the Ribicoff Res. Facilities, New Haven, CT 06508.

Forskolin has been reported to interact directly with the catalytic protein of adenylate cyclase (C) and probably also enhances coupling between C and the stimulatory GTP binding protein (K.B. Seamon, Drug Develop. Res., 6: 181, 1985). By either mechanism, the action of forskolin is to promote the synthesis of adenosine 3',5'-monophosphate (cAMP) and elevate the levels of intracellular cAMP. In this study, we investigated the effect of forskolin on the spontaneous activity of noradrenergic neurons of the locus coeruleus (LC) and the ionic mechanism underlying the observed forskolin-induced activation of LC neurons.

Brain slices containing LCs were obtained from male albino rats and perfused with artificial CSF of the following composition (in mM): NaCl, 126; KCl, 5.0; Na_2HPO_4, 1.25; D-glucose, 10; $NaHCO_3$, 26; $CaCl_2$, 2.0; $MgSO_4$, 2.0. The fluid and chamber were gassed with 95% O_2/5% CO_2. Electrodes filled with 2 M NaCl were used for extracellular recordings. Single-electrode voltage clamping was performed using an Axoclamp-2 and electrodes filled with 2M KCl (30-50 MΩ).

Forskolin (1-10 μM) increased the firing rate of all LC cells tested (n=14, mean increase=179%). The vehicle (up to 0.1% DMSO) had no effect. The activation elicited by forskolin remained in the presence of cobalt (2mM), indicating that the activation was not due to the release of neurotransmitter(s) and/or other endogenous substance(s) nor was it dependent upon calcium influx. The forskolin-induced activation could be suppressed completely by clonidine, an α_2-agonist which is known to block the forskolin activation of adenylate cyclase. Single-electrode voltage clamping at -60/-65 mV and -90/-95 mV revealed that forskolin (10 μM, n=5) induced an inward current. This current was voltage-sensitive with an increase in the hyperpolarizing direction and did not reverse at the potassium equilibrium potential. The inward current generated by forskolin at -90/-95 mV was about twice as large (mean=0.17 nA) as that at -60/65mV (mean=0.07 nA). 8-Br-cAMP (0.5-4 mM), a membrane permeable cAMP analog, and Ro20-1724 (50-100 μM), a preferential inhibitor of cAMP-phosphodiesterase, had similar actions. The spontaneous activity of LC cells was not significantly altered by 8-Br-cGMP (1 mM).

The inward current induced by forskolin and 8-Br-cAMP in LC neurons resembles the cAMP-dependent hyperpolarizing-activated pacemaker current (I_f or I_h) of cardiac Purkinje fibers (DiFrancesco, Prog. Biophys. Molec. Biol. 46: 163-183, 1985). Our results suggest that cAMP may also play an important role in regulating the pacemaker activity of LC cells.

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276.15 CALMODULIN-DEPENDENT PHOSPHODIESTERASE IMMUNOREACTIVITY IN CEREBELLAR PURKINJE CELLS IS ATTENUATED BY 3-ACETYLPYRIDINE-INDUCED DESTRUCTION OF THE INFERIOR OLIVE. C.D. Balaban, M.L. Billingsley, and R.L. Kincaid* Depts. of Anatomy and Pharmacology, Hershey Medical Center, Penn State Univ., Hershey, PA 17033; Lab. of Cellular Metabolism, NHLBI/NIH, Bethesda, MD 20837.

Immunocytochemical studies using an affinity purified polyclonal antibody against calmodulin-dependent phosphodiesterase (PDE) have shown that PDE is localized in the dendrites and somata of specific populations of neurons in the CNS. High levels of PDE are expressed in cerebellar Purkinje cells and in neurons of the deep cerebellar nuclei, but not in stellate, basket, or granule cells. In order to determine whether synaptic input can influence the expression of PDE, the climbing fiber input from the inferior olive was removed using the neurotoxin 3-acetylpyridine (3-AP). Long-Evans and Sprague Dawley rats were injected with 3-AP (75 mg/kg; i.p.) followed 3 hr later by harmaline (15 mg/kg; i.p.) and 15 hr later by niacinamide (300 mg/kg; i.p.); this treatment produced complete destruction of the inferior olive. Animals were sacrificed 1 wk-5 mos post treatment. Several rats were used for immunocytochemical studies, using a biotinylated second antibody and avidin/peroxidase chromogens. The immunocytochemical staining for PDE was virtually absent in cerebellar Purkinje cells at all times following inferior olive destruction by 3-AP; however, the pattern of PDE immunostaining was not altered in specific neocortical cells (pyramidal cells in layers III, V, and VI) nor in other neuronal populations expressing PDE. A specific polyclonal antiserum against calcineurin was also used to stain tissue sections from 3-AP treated rats, and indicated that calcinuerin immunoreactivity in cerebellar and other neurons was not affected by destruction of the inferior olive. Western immunoblot analysis was performed on cerebellar and cortical extracts of control and 3-AP lesioned rats. PDE immunoreactivity was greatly reduced in whole cerebellar extracts from toxin-treated rats suggesting that most cerebellar PDE is contained in Purkinje cells. PDE immunostaining in the cortex of 3-AP treated rats was comparable to the patterns and levels seen in control rats. Analysis of total calmodulin binding proteins in treated and control tissues was performed using a biotinylated calmodulin overlay of Western blots, (Billingsley, M.L. et al. Proc. Natl. Acad. Sci. 82:7585, 1985) and indicated that, with the exception of PDE in the cerebella of treated rats, none of the major calmodulin binding proteins were depleted by 3-AP treatments. These results suggest that the expression of PDE in cerebellar Purkinje cells is under transsynaptic control, and is dependent on the integrity of climbing fiber input. Supported by PHS NS00891 and NS19850 (CDB) and the ILSI Research Foundation (MLB).

276.16 EFFECTS OF DOPAMINE ACTING DRUGS ON THE KINETIC PROPERTIES OF PROTEIN KINASE C IN THE STRIATUM. C. T. Giambalvo. Rhode Island Psychiatric Research and Training Center and Section of Neurobiology, Brown University, Cranston, RI 02920

Protein kinase C (Pk-C) is a calcium, phospholipid dependent enzyme that may play a role in the release of neurotransmitters, particularly of dopamine (DA) in the nigrostriatal pathway (C.T. Giambalvo, Ann. Meeting Neuroscience 11:346, 1985). It was found that injection of drugs which increased DA release in vivo resulted in an increase in particulate Pk-C activity in vitro, while drugs which inhibited DA release led to a decrease in particulate Pk-C activity. Moreover, there was a good correlation between the ability of drugs to affect DA release and their effects on Pk-C activity. In this study, the effect of these drugs on the kinetic properties of Pk-C was examined.

Rats were injected with apomorphine (0.5 mg/kg) or haloperidol (2.5 mg/kg) and sacrificed. The striatum was dissected, homogenized in 20 mM of Tris buffer containing 20 mM Mg Cl_2, pH 7.4) and then analyzed for Pk-C activity. The latter was analyzed by the th iophosphorylation lipids (phosphatidylserine and diolein). The phosphorylated protein was precipitated with TCA, filtered and then counted in the lipid scintillation counter. Activity was expressed as pmoles phosphorylated/min/mg protein minus basal activity (measured in presence of EGTA and absence of calcium and lipids).

Acute injection of apomorphine inhibited Pk-C activity by increasing the Km for lipids, while haloperidol stimulated Pk-C activity by decreasing the Km for lipids. The Vmax was unchanged by either drug. In contrast to this change in the Km, subchronic treatment with haloperidol induced a decrease in the Vmax for lipids without altering the Km.

The ability of these drugs to alter the kinetic parameters of Pk-C for lipids is further evidence that membrane lipids may play a role in the transduction of signals by secondary messengers.

276.17 THE cGMP-PHOSPHODIESTERASE SYSTEM IN THE DEGENERATIVE RETINA OF THE rd MOUSE. D.B. Farber, C. Yamashita* and C. Bowes*. Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024.

A deficiency in cGMP-phosphodiesterase (PDE) activity causes cGMP levels to become elevated in the rd retina 2-3 days before any morphological sign of pathology. This light-sensitive enzyme is present but not activatable in the rd retina. However, it is not known whether its concentration is the same or lower than that in normal retina or if the components of the outer segment necessary for PDE activation are present in rd retina. We have studied the biosynthesis of PDE in normal and rd retinas during development in order to determine the earliest age at which PDE is found in both retinas and whether synthesis is stopped or degradation of PDE increases in rd retina before degeneration of the visual cells. Furthermore, we have hybridized retinal RNA from 11-day-old and adult control and rd retinas with cDNA probes specific for the α, β and γ subunits of G-protein and for rhodopsin to determine whether there are any differences in the sizes of the mRNAs that code for those proteins. Our results indicate that in normal and rd retinas, synthesis of PDE, although minimal, occurs already at 6 postnatal days, it is comparable until day 12 and is decreasing at day 14, possibly as a result of the death of photoreceptors. The adult rd retina does not synthesize any detectable PDE, in agreement with the absence of activity reported previously (J. Cyclic Nucleotide Res. 2:139-148, 1976). In addition, we found no difference between 11-day-old rd and 11-day-old and adult normal retinas in the sizes of the mRNAs for opsin and for the three subunits of G-protein. Our results suggest that the defect in the rd retina does not stem from reduced levels of PDE or from apparent changes in size of mRNAs coding for rhodopsin or G-protein, components required for PDE activation.

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276.18 PURIFICATION OF PCPP-260 (A PURKINJE CELL-SPECIFIC CAMP-REGULATED MEMBRANE PHOSPHOPROTEIN OF M_r 260,000) FROM BOVINE CEREBELLUM G. Weeks*, A.C. Nairn*, S.I. Walaas and P. Greengard. The Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021

PCPP-260 (Purkinje Cell Phosphoprotein of M_r 260,000) is an integral membrane protein which is a specific substrate for the cAMP-dependent protein kinase present in Purkinje cells of the mammalian cerebellum (Walaas et al., J. Neurosci. (1986) In press). PCPP-260 has been purified from a crude particulate fraction of bovine cerebellum using the ionic detergent n-lauryl sarcosine (NLS) as solubilizing agent. Purification was monitored by silver stain and autoradiography of ^{32}P-phosphorylated samples separated by SDS-PAGE. Phosphorylation of the protein by cAMP-dependent protein kinase in the presence of NLS required the addition of Triton X-100 (10-fold excess over NLS). Con A binding to NLS extracts that had been separated by SDS-PAGE and blotted onto nitrocellulose suggested that PCPP-260 was a glycoprotein. PCPP-260 was retained on a column of Con A-agarose and eluted by α-methyl mannoside. Further fractionation on Sephacryl S-400 yielded a preparation that was at least 95% pure and enriched 350-fold relative to the initial NLS extract.

The phosphopeptide maps generated from the purified protein were indistinguishable from those obtained from the membrane-bound protein. Antibodies have been prepared against the purified protein in order to study the detailed cellular and subcellular distribution of PCPP-260 in the cerebellum. In addition, these antibodies will be used to characterize the relationship between PCPP-260 and a closely related 265 kDa membrane phosphoprotein, which is present in low concentrations in other regions of the brain.

276.19 PHOSPHORYLATION OF MICROTUBULE ASSOCIATED PROTEINS BY GUANOSINE 3,5-CYCLIC MONOPHOSPHATE DEPENDENT PROTEIN KINASE. S.N. Ahmad* and H.C. Pant (SPON: I. Tasaki). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism Rockville, MD 20852.

The influence of cyclic nucleotides on the phosphorylation of microtubule associated protein 2 (MAP 2) was investigated. Fractions rich in MAP 2 were prepared from the rat brain homogenate extract by temperature dependent assembly and disassembly of microtubules (Anal Biochem 98:64,1979). Proteins in these preparations were phosphorylated either with γ-^{32}P adenosine 5-triphosphate (γ-^{32}P)ATP or with γ-^{32}P guanosine 5-triphosphate (γ-^{32}P)GTP and separated on 4-11% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). MAP 2 phosphorylation was analysed by autoradiography. Quantitative incorporation of labeled phosphate into individual bands was recorded on an Image Array Processor.

Guanosine 3,5-cyclic monophosphate (cGMP) at concentrations (0-100 uM) increased phosphorylation of MAP 2 in a dose dependent fashion when (γ-^{32}P)ATP was used as phosphoryl donor. No significant increase in the phosphorylation of MAP 2 was noticed with (γ-^{32}P)GTP in the presence of cGMP. Kinetic analysis of the data on the influence of cGMP on MAP 2 phosphorylation, using (γ-^{32}P) ATP as phosphoryl donor, revealed that 10 uM cGMP was required for half maximal phosphorylation of MAP 2. In the presence of higher concentration of cGMP (>50 uM) a decrease in the mobility of MAP 2 band on SDS-PAGE was noticed probably due to the cGMP stimulated phosphorylation of MAP 2. Densitometric analysis of MAP 2 band showed that both MAP 2A and MAP 2B bands were phosphorylated with cGMP dependent protein kinase but the extent of phosphorylation of MAP 2B was higher compared to MAP 2A. This observation suggests that MAP 2 may have an additional role in the regulation of interactions between MAP 2 and other cytoplasmic components. In the presence of cAMP, neither a shift in the MAP 2 band on SDS-PAGE nor preferential phosphorylation of MAP 2B was noticed. These observations suggest that different sites on MAP 2 may be involved in the phosphorylation by cAMP and cGMP dependent protein kinase(s) and different mechanisms may be responsible for the regulation of MAP 2 phosphorylation.

INTERACTIONS BETWEEN NEUROTRANSMITTERS III

277.1 ANATOMIC AND PHYSIOLOGIC EVIDENCE FOR INNERVATION OF NORADRENERGIC LOCUS COERULEUS BY NEURONAL CORTICOTROPIN-RELEASING FACTOR. R. J. VALENTINO, C. I. CHA*, and S. L. FOOTE. Dept. Pharmacology, George Washington Univ., Washington, D. C. 20037 (RJV) and Dept. Psychiatry, Univ. Calif., La Jolla, CA 92093 (CC and SLF).

It has been hypothesized that corticotropin-releasing factor (CRF) acts not only to intiate endocrine aspects of stress responses, but also acts within brain circuits as a neurotransmitter to control certain autonomic and behavioral components of stress. We have previously suggested that the enhanced turnover of brain noradrenaline (NA) observed during stress may be mediated by neurotransmitter CRF released onto NA neurons in locus coeruleus (LC). This suggestion is supported by observations in other laboratories of CRF-immunoreactive axons in rat LC and by our previous finding that rat LC-NA neurons exhibit enhanced basal discharge rates after intracerebroventricular (ICV) or microinjection administration of CRF. We now report that highly arborized CRF-immunoreactive fibers are evident in monkey LC and that CRF alters LC sensory reponses in halothane-anesthetized rats.

The distribution of CRF-immunoreactive axons and cell bodies was studied in the brainstem of two cynomolgus monkeys (Macaca fascicularis). Fine-caliber immunoreactive fibers were evident in many structures. The LC is among the most densely innervated of those areas we have examined. For example, LC is as densely innervated as parabrachial areas. This contrasts with the relative densities in rat, where parabrachial areas are much more densely innervated. In these monkeys, who were not treated with colchicine, immunoreactive LC cell bodies were unusual.

In the electrophysiological studies, ICV administration of CRF (0.3-3.0 ug) resulted in a dose-dependent enhancement of LC spontaneous discharge rates 3 min after injection, with 3.0 ug producing a 47 $\pm$ 12% increase. Ala-14 CRF (3.0 ug), an inactive analog, had no effect. LC cells respond to sciatic nerve stimulation with a brief activation beginning 20-40 msec poststimulus followed by a period of suppressed activity. 1.0 and 3.0 ug CRF attenuated both response components while low-dose CRF (0.3 ug) and Ala-14 CRF (3.0 ug) had no effect. These results confirm and extend, with additional doses and another inactive analog, our previous demonstration of CRF effects on basal discharge rates and indicate that LC sensory responses are also sensitive to CRF effects.

These anatomic and physiolgic observations lend additional credence to the suggestion that CRF, acting as a neurotransmitter, may activate LC neurons in response to stress-inducing stimuli. Supported by PHS Grant MH40008.

277.2 LOCALIZATION OF NEUROTENSIN BINDING SITES TO DOPAMINERGIC AND PUTATIVE CHOLINERGIC NEURONS IN RAT CENTRAL NERVOUS SYSTEM. E. Szigethy*, P. Kitabgi* and A. Beaudet. Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada and Centre de Biochimie du CNRS, Parc Valrose, 06034 Nice, France.

The distribution of ^{125}I-neurotensin (NT) binding sites was studied in relation to dopaminergic neurons in the midbrain tegmentum, and to putative cholinergic neurons in the basal forebrain, using adjacent section radioautography-immunocytochemistry and radioautography-cholinesterase histochemistry, respectively. Serial 5-10 µm-thick frozen rat brain sections taken at the level of the substantia nigra and the basal forebrain were incubated for 1 h at 4°C with 0.1 nM ^{125}I-NT (2000 Ci/mmole) in 50 mM Tris buffer containing 5 mM $MgCl_2$, 0.2% BSA and 20 mM bacitracin in the presence (non-specific binding) or in the absence (total binding) of 0.5 mM non radioactive NT. After several buffer rinses, the sections were fixed with 4% glutaraldehyde and processed for light microscopic radioautography using conventional dipping techniques. Alternate sections from the midbrain were fixed by immersion in Zamboni's fixative and processed for tyrosine hydroxylase (TH) immunocytochemistry according to the PAP method. Alternate sections from the basal forebrain were processed after or without immersion fixation for acetylcholinesterase (Ache) histochemistry, according to the procedure of Genneser-Jensen (1971).

In radioautographs from the midbrain tegmentum, high labeling densities were detected over clustered neurons of the substantia nigra, pars compacta. In some areas, distinctly labeled neuronal perikarya stood out against low to moderate neuropil reactivity. Comparison with adjacent TH-immunostained sections revealed a precise overlap between groups of ^{125}I-NT radioautographically labeled and TH-immunoreactive cells. A number of TH-positive neurons showed unequivocal ^{125}I-NT labeling while others appeared to be devoid of NT binding sites.

In radioautographs from the basal forebrain, selectively labeled perikarya were visible in the ventral pallidum and substantia innominata, as well as along the medial edge of the globus pallidus, bordering the internal capsule (i.e., in an area considered equivalent to nucleus basalis). A large proportion of ^{125}I-NT-labeled cells in this area showed point to point correspondence with neurons positively stained for Ache in adjacent sections. In conclusion, these results demonstrate the presence of specific NT binding sites on a subpopulation of dopaminergic neurons in the substantia nigra, pars compacta. In addition, the present data provides first evidence for co-localization of Ache activity and specific NT binding sites in magnocellular, putative cholinergic neurons of the substantia innominata and medial globus pallidus. It would be of interest to determine if degeneration of cholinergic neurons in this region reported in patients with Alzheimer's disease is accompanied by changes in neurotensin receptor binding. Supported by the MRC.

277.3 DUAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE AND TYROSINE HYDROXYLASE IN RAT NEOSTRIATUM. J. Chan*, M. Carson*, T.H. Joh, and V.M. Pickel. SPON:(B.A. Judd.) Lab. of Neurobiology, Dept. of Neurology, Cornell Univ. Med. Center, N.Y., N.Y. 10021.

Pharmacological studies have recently suggested that the major interactions between dopaminergic afferents and intrinsic cholinergic neurons in the neostriatum may be at the level of axon terminal (Lehman and Langer et al., Neurosci. 10: 1105, 1983). We investigated the anatomical relationship between dopaminergic and cholinergic axon terminals in the rat neostriatum by dual immunocytochemical labeling of the respective synthesizing enzymes, tyrosine hydroxylase (TH) and choline acetyltransferase (CAT). The brains from adult rats were fixed by aortic arch perfusion with 0.1% glutaraldehyde and 4% paraformaldehyde in phosphate buffer. Coronal vibratome sections through the neostriatum were sequentially incubated with rabbit polyclonal antiserum to TH, ^{125}I-donkey antirabbit immunoglobulin (IgG), rat monoclonal antiserum to CAT, rabbit antirat IgG and rat peroxidase-antiperoxidase complex. The labeled sections were processed for light and electron microscopic autoradiography. After an exposure period of 7-14 days, light microscopy revealed a dense accumulation of silver grains throughout the neuropil exclusive of cell soma and bundles of myelinated axons. Peroxidase labeling for CAT was evident in isolated large neuronal perikarya and in varicose processes within the same sections. At exposure periods of 2-4 months, both autoradiographic and peroxidase labels were readily differentiated by electron microscopy. The autoradiographic labeling for TH was exclusively seen in small unmyelinated axons and axon terminals. These terminals formed primarily symmetric synapses with the more distal dendritic processes and the base of dendritic spines which also received asymmetric contacts from unlabeled axon terminals. Within the same section peroxidase labeling for CAT was occasionally detected within large neuronal soma and relatively smooth dendrites. However, the most common localization of CAT was in myelinated and unmyelinated axons and in small axon terminals. Like the TH-labeled terminals, the CAT-labeled terminals usually formed symmetric synapses with distal dendrites and spines. The TH and CAT labeled terminals were frequently found in direct apposition to each other and exhibited synaptic specializations with a common spiny dendrite. These findings support a possible axo-axonic association between dopaminergic and cholinergic terminals and provide morphological evidence for dual regulation of the output from medium spiny neurons in the rat neostriatum. (Supported by grants NSF 8320120 and NIMH 00078.)

277.4 **CATECHOLAMINERGIC NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA RECEIVE DIRECT SYNAPSES FROM TERMINALS WITH SUBSTANCE P AND ENKEPHALIN-LIKE IMMUNOREACTIVITY.** T.A. Milner, V.M. Pickel, T.H. Joh and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. College, New York, NY 10021.

Ultrastructural analysis of catecholaminergic (CA) neurons in the C1 area of the rostral ventrolateral medulla (RVL) has demonstrated that they are postsynaptic to a number of non-CA terminals (Milner et al., Neurosci. Abst. 1985). Light microscopic immunocytochemical detection of substance P (SP) and enkephalin (ENK) in varicose processes in regions containing these CA perikarya suggests that one or both of these neuropeptides are found in the pre-synaptic terminals. Thus, we sought to determine whether SP or leucine (Leu^5)-ENK made synaptic contact with CA neurons in the C1 area of the RVL.

Adult rats were anesthetized and the brains perfused with 3.75% acrolein and 2% paraformaldehyde. Coronal vibratome sections of fixed tissues were incubated in a 1:15,000 dilution of rabbit tyrosine hydroxylase (TH) antiserum followed by ^{125}I-secondary IgG. The sections were then directly processed for light or electron microscopic autoradiography or subsequently incubated with rabbit antiserum to SP (1:1500) or Leu^5-ENK (1:1500 dilution) and processed with peroxidase conjugated IgG followed by autoradiography.

Ultrastructural analysis of the CA neurons in the RVL showed selective accumulation of silver grains for ^{125}I labeling of TH in neuronal perikarya, dendrites, axons and axon terminals. In sections processed for only TH, the TH-containing perikarya and dendrites received symmetric and asymmetric synapses from TH-labeled and unlabeled terminals. Within single thin sections prepared for dual labeling, the peroxidase marker for SP or ENK was differentially localized with respect to autoradiographic labeling for TH. SP and ENK-like immunoreactivity (LI) were localized in axons and axon terminals. Terminals with SPLI were large (0.8-1.4 μm) and contained numerous small clear and 3-10 dense core vesicles. The SP-labeled terminals formed primarily asymmetric synapses with dendrites (0.8-2.0 μm) and their spinous processes containing TH immunoreactivity and with other unlabeled dendrites. Terminals with ENK-LI were medium sized (0.6-1.0 μm), contained numerous small clear and 1-6 dense core vesicles. These labeled terminals formed symmetric synapses with both TH-immunoreactive perikarya and dendrites (1.0-3.0 μm) in addition to unlabeled dendrites. We conclude that CA neurons in the RVL, probably the C1 adrenergic neurons, are directly innervated by both SP and ENK-containing axon terminals which differ in terms of their respective synaptic specializations and distribution on neuronal perikarya reflecting perhaps differences in their functional role.
(Supported by NIH grants HL18974 and MH0078).

277.5 Immunocytochemical Visualization of Dopamine Neurons and Their Target Cells in Reaggregate Cell Culture. L. Won, B.H. Wainer, A. Heller, P.C. Hoffmann J.P. Bolam[a], I. Shalaby[b], H. Hemmings[b], C. Ouimet[b], N. Rosen[b] and P. Greengard[b]. The University of Chicago, [a]Oxford University and [b]The Rockefeller University.

Previous work has shown that dopaminergic (DA) axons form synapses when co-cultured with their target cells in reaggregate cell culture (Won et al., Soc. Neurosci. Abstr. 11:66, 1985). In the present study, immunohistochemical procedures have been used to identify target cells and DA neurons in such reaggregates.

Dissociated fetal murine mesencephalic cells containing DA neurons were coaggregated with dissociated fetal striatal cells in rotatory culture for 20 days. Two neural antigens were localized in the same section using a novel two-color procedure of sequential immunoperoxidase staining (Levey et al., Soc. Neurosci. Abstr. 11:441, 1985). Polyclonal rabbit antisera against tyrosine hydroxylase (TH), and GABA, were used to localize DA neurons and GABA neurons, respectively. A monoclonal antibody was used against the phosphoprotein, DARPP-32, believed to identify dopaminoceptive striatal cells expressing DA_1 receptors, some of which may contain GABA (Ouimet et al., J. Neurosci. 4:111-124, 1984). Reaggregates were stained for visualization of the following antigenic combinations: TH/DARPP-32 and DARPP-32/GABA.

The pattern of DARPP-32 immunoreactivity showed that immunolabeled cells and their processes formed discrete patches within the reaggregate. TH and DARPP-32 immunoreactivities were never found to co-localize within the same neuron, however, small TH-positive varicosities were interspersed within patches of DARPP-32-positive cells and processes. In sections stained for DARPP-32/GABA, some cell bodies contained both DARPP-32 and GABA-immunoreactivity. Cells which were labeled only for DARPP-32 or only for GABA were also observed.

In summary, dopaminergic varicosities associate with dopaminoceptive target cells in reaggregates composed of mesencephalic and striatal cells. A portion of these dopaminoceptive cells also contain GABA and may receive direct DA input. Further examination of neurotransmitter interaction in these reaggregates remains to be studied at the ultrastructural level. (Supported by MH-28942 and L.W. was supported by GM-07151.

277.6 MODIFICATION OF COERULEO-CORTICAL NORADRENERGIC NEUROTRANSMISSION AFTER SEROTONIN DEPLETION: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT. A. Ferron. Centre de recherche en sciences neurologiques (Dép. physiologie), Université de Montréal, Montréal, Canada.

Previous microiontophoretic studies in cerebral cortex have shown a markedly diminished responsiveness to dopamine (DA) and noradrenaline (NA) at relatively short time interval (2-4 days) after serotonin (5-HT) depletion with the synthesis inhibitor parachlorophenylalanine (PCPA) (Ferron et al., Brain Res. 231:93, 1982). Recent biochemical data (Reader et al., Neurochem. Res. in press) suggest that such diminished responsiveness could reflect an adaptive desensitization (down-regulation) of DA and NA receptors secondary to increased endogenous release of catecholamines. The present study was undertaken to determine whether other modifications in the functional properties of coeruleo-cortical neurons also accompanied the 5-HT depletion.

Adult male Sprague-Dawley rats (250-350 g) were used. 5-HT depletion was produced by daily administration of PCPA (500 mg/kg i.p.) for 2 or 3 days. Twenty-four hours after the last PCPA injection and under urethane or chloral hydrate anaesthesia: 1) The spontaneous firing rate of NA neurons was recorded in the locus coeruleus (LC). 2) The sensitivity of NA autoreceptors in LC was measured by determining the effects of single doses of intravenous clonidine (1-6 ug/kg) on spontaneous neuronal activity. 3) The responsiveness of cortical neurons to electrical stimulation of the LC was assessed in sensorimotor cortex by peristimulus time histograms using 0.5 ms pulses at 2 Hz with 400 to 800 uA currents.

By comparison with controls, spontaneous activity of LC neurons was significantly increased in PCPA-treated rats (3.7 ± 0.2 vs 2.6 ± 0.2 Hz; P 0.01). In the same rats, the effect of i.v. clonidine on LC neuron firing was weaker than in the controls (1.33-fold decrease). In cerebral cortex, the number of spontaneously firing units inhibited by electrical stimulation of the LC was also significantly reduced (78% vs 30%).

These findings suggest that, following 5-HT depletion by PCPA, increased firing of NA neurons in LC was associated with decreased sensitivity of NA autoreceptors in this nucleus. Whether this change involved LC nerve cell bodies and dendrites and/or NA and /or other nerve terminals in LC remains to be determined. The fact that the responsiveness to LC stimulation was decreased in cortex suggests that, in the absence of 5-HT, coeruleo-cortical NA neurotransmission was markedly reduced in its efficacy. It remains to be determined whether such a regulation of coeruleo-cortical NA neurons by 5-HT is primarily exerted distally (at the level of NA nerve terminals within the cerebral cortex) or proximally (at the level of cells bodies in LC). (Supported by the Medical Research Council of Canada).

277.7 ROLES OF D_2 AND $5HT_2$ RECEPTORS IN MEDIATING THE EFFECTS OF METHAMPHETAMINE, 3,4-METHYLENEDIOXYMETHAMPHETAMINE AND 3,4-METHYLENEDIOXYAMPHETAMINE ON STRIATO-NIGRAL NEUROTENSIN SYSTEMS. A.A.Letter*, K. Merchant*, J.W. Gibb and G.R. Hanson. (SPON: J.H. Petajan) Department Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112

Numerous studies have demonstrated toxic effects of methamphetamine (METH) and other amphetamine-like drugs on the dopaminergic and serotonergic systems. Recently our laboratory has shown that following treatment with METH, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), there is a significant elevation (200-300% of control) of the concentrations of neurotensin-like immunoreactivity (NTLI) in the striato-nigral regions of the rat brain. The purpose of our present research was to characterize further these changes in neurotensin systems by concurrent administration of a specific D_2 receptor antagonist, sulpiride (80 mg/kg), or the $5HT_2$ receptor antagonist, ritanserine, (1 mg/kg), with each of the amphetamine congeners, METH (15 mg/kg), MDMA (10 mg/kg), or MDA (10 mg/kg). Treatments consisted of 5-doses of the drugs at 6-hr intervals: animals were sacrificed 18-hr after the last dose. Concentrations of NTLI were measured by radioimmunoassay using a highly specific antiserum.

We observed that sulpiride significantly attenuated only the METH-induced increase in nigral NTLI and had no effect on that induced by either MDA or MDMA. Ritanserine, however, produced no NTLI attenuation when given with METH, MDA, or MDMA. In the striatum, sulpiride or ritanserine administered alone, significantly increased NTLI concentrations to 160% and 140% of control respectively, whereas METH, MDMA and MDA increased NTLI to 230%, 217% and 222% of control, respectively. When either METH, MDMA or MDA were given concurrently with sulpiride, synergistic increases in NTLI concentrations to 461%, 481% and 517% of control, respectively, were observed. Unlike sulpiride, ritanserine in combination with the amphetamine-like compounds did not alter the response of striatal neurotensin system to these drugs.

Our studies indicate that unlike MDMA and MDA, the METH-induced increases in NTLI in the substantia nigra are partially mediated through D_2 receptors while $5HT_2$ receptors do not appear to play a significant role. Striatal increases in NTLI induced by these drugs appear to be enhanced by concurrent administration of sulpiride, but not by ritanserine.

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277.8 ANGIOTENSIN II RECEPTORS ARE PRESENT ON CATECHOLAMINERGIC CELLS IN NEURONAL CULTURES. M.K. Raizada*, E.M. Richards* and C. Sumners (SPON: P. Reier). Dept. of Physiology, Univ. of Florida, Gainesville, FL 32610.

In previous studies we have determined that neuronal cultures contain catecholamine (CA) neurons and also specific receptors for angiotensin II (AngII-R). In addition we have shown that AngII can influence CA levels and metabolism in these cultures by acting at its specific receptors, while the AngII-R are themselves regulated by the released CA (Sumners et al., Am. J. Physiol. 250, C236, 1986, and Am. J. Physiol. 246, C502, 1984). The relative anatomical localization of each component is a prerequisite for understanding the discrete neuronal interactions between AngII, AngII-R and CA. In the present study a combined autoradiography and immunocytochemical approach has been utilized to examine the anatomical localization of AngII-R and CA in neuronal cultures. Neuronal cultures were prepared from the hypothalamus and brainstem (co-cultures) of one day old Sprague-Dawley rats as described previously (Sumners et al., Am. J. Physiol. 250, C236, 1986). Ten to fourteen day old cultures which contained 75-80% neuronal cells were used for experiments. Using autoradiographic analysis with [^{125}I]-AngII to visualize AngII-R, we have previously determined that neuronal cells in culture contain specific AngII-R both on the cell bodies and on neurites (Raizada et al., Am. J. Physiol. 247, C354, 1984). Immunocytochemical staining of neuronal cultures for CA cells using an antibody against dopamine β-hydroxylase (anti-DβH, obtained from Dr. R. Rush, Flinders Univ., South Australia) revealed a specific population of neurons which stain positively for DβH. Double labelling experiments for AngII-R and DβH positive cells were performed as follows. AngII binding was done by treating cells with [^{125}I]-AngII (0.5 nM, 60') in PBS (pH 7.2). Following this, the cells were fixed, treated with sodium borohydrate and then DβH immunocytochemistry was performed using the Avidin-Biotin technique. Double-labelled cultures were prepared for autoradiography 2-3 weeks later, and the relative localization of silver grains (AngII-R) and DβH-neurons was observed with phase contrast microscopy. It was observed that AngII-R were present on both DβH- and non-staining neurons. On the DβH cells, AngII-R were present on both the cell bodies and on the neurites. Additionally, some DβH neurons were seen which did not contain AngII-R. These findings show that AngII-R are present on central CA neurons, and further support our observations that the AngII effect on CA levels and metabolism is an intraneuronal event.

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277.9 CHRONIC MORPHINE TREATMENT AND UP-REGULATION OF BENZODIAZEPINE AND DOPAMINE RECEPTORS IN THE MOUSE. Maureen E. Ellis, Robin R. Dean*, and R. Karler*. Dept. of Pharmacology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

Substantial experimental evidence exists for interactions between the endogenous opioid systems and the benzodiazepine-Gaba complex, as well as the dopaminergic system. The purpose of the present study was to determine the influence of chronic morphine treatment on benzodiazepine (BZ) and dopamine (DA) receptors, and on drug responsiveness of these transmitter systems. The experiments were conducted on about 25 gm CF-1 mice that were treated once daily for 20 days with 100 mg/kg of morphine sulfate i.p. Receptors were measured using standard receptor autoradiography techniques with a video camera based densitometry system interfaced with an Apple computer. The ligands employed were flunitrazepam and RO 15-1788 for the benzodiazepine receptors, and SCH 23390 for the dopamine (D-1) receptors. In addition, opiate receptors were determined with dihydromorphine. The receptors were measured in the following areas: for the BZ receptors, the dentate gyrus, CA3, lateral amygdaloid nucleus, paraventricular nucleus of the thalamus and lamina IV of the cortex; for the DA receptors, the caudate putamen and substantia nigra; for the opiate receptors, the anterior hypothalamus, the paraventricular nucleus of the thalamus, and periaqueductal gray. The results indicate that for the brain areas studied there was a 30-50% down-regulation of the dihydromorphine binding, a 1/5-200% up-regulation of the benzodiazepine receptors, and a 200-400% up-regulation of the D-1 receptors. The chronic morphine treatment was also associated with tolerance in the tailflick test, and to the hypothermic effect of morphine; an increase in the anticonvulsant activity of diazepam (0.1 mg/kg) against pentylenetetrazol-induced minimal but not against maximal convulsions; and an increase in the locomotor response to amphetamine (4 mg/kg), cocaine (20 mg/kg), and bromocriptine (5 mg/kg). The data support the following conclusions: 1) chronic morphine treatment can cause down-regulation of opiate receptors; 2) concomitantly both the benzodiazepine and dopamine receptor systems show up-regulation; and, 3) the observed receptor changes are associated with appropriate changes in the pharmacodynamics of drugs ostensibly affecting these receptor systems. (Supported by DA 00346 Research Grant)

277.10 EFFECTS OF SUBSTANCE P ON THE BINDING OF LIGANDS TO NICOTINIC ACETYLCHOLINE RECEPTORS. S.M.Simasko, J.M. Henley, J.A.Durkin* and G.A.Weiland. Dept. of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

It has been shown in a number of systems that substance P (SP) can modulate the effects of compounds which activate the nicotinic acetylcholine receptor (nAChR). To examine if this effect of SP is mediated by an allosteric mechanism on the receptor we have characterized the interactions of SP with the binding of various ligands that have previously been shown to bind to the nAChR. The labeled ligands we have used are [^{3}H]acetylcholine (ACh), [^{3}H](-)nicotine, [^{3}H]phencyclidine (PCP), and [^{125}I]α-bungarotoxin (BGT). We have examined the interaction on *Torpedo* electroplaque membranes, PC12 cells, BC_3H1 cells, and homogenates of brain tissue.

In *Torpedo* membranes SP decreased the initial rate of [^{125}I]BGT binding with an IC_{50} of 10 μM, SP had no effect on the binding of [^{3}H]ACh, and SP appeared to increase the binding of [^{3}H]PCP (EC_{50}≈10 μM). It has previously been shown that carbamylcholine (carbachol) increases the binding of [^{3}H]PCP. Interestingly, SP blocked the stimulatory effect of carbachol on [^{3}H]PCP binding (IC_{50}≈10 μM) but with a low Hill coefficient (n_H<0.5). These results would suggest that SP interacts with a site on the *Torpedo* nAChR that is unique to SP. The mechanism by which SP blocks the carbachol effect on [^{3}H]PCP binding without showing any action at the [^{3}H]ACh site remains to be determined.

In solubilized homogenates of cortex from rat brain, whole chick brain, and whole goldfish brain, SP inhibited the initial rate of [^{125}I]BGT binding up to 50% (EC_{50}≈1 μM). In these same tissue preparations SP had no effect on [^{3}H](-)nicotine binding. On BC_3H1 cells SP did not displace [^{125}I]BGT binding, however, 10 μM SP did displace [^{125}I]BGT binding on PC12 cells to the same degree as 1 mM d-tubocurare or 10 mM carbachol.

The results with [^{3}H]ACh and [^{3}H](-)nicotine indicate that SP does not directly interact with the agonist recognition site on nAChRs from a variety of tissues. However, SP does seem to distinguish nAChRs based on its ability to compete for [^{125}I]BGT binding. BGT does not block nicotinic responses on PC12 cells (thought to be "neuronal-type" nAChRs) but does on BC_3H1 cells (thought to be "muscle-type" nAChRs). SP blocks BGT binding on PC12 cells but not BC_3H1 cells. We have previously shown that SP can inhibit nicotinic responses on both cell lines (IC_{50}s of 1 μM and 10 μM on PC12 cells and BC_3H1 cells, respectively). The observation that SP inhibits only half the [^{125}I]BGT binding to brain tissue would suggest that either nAChRs in brain are heterogeneous or that SP has an allosteric effect on brain [^{125}I]BGT binding. The observation that SP can compete for [^{125}I]BGT sites in *Torpedo* membranes but not BC_3H1 cells would suggest that these receptors are not equivalent. The relationship between the functional aspects of BGT and SP on nicotinic responses and the observations on the effects of SP on [^{125}I]BGT binding on nAChRs from various tissues remains to be determined. This work was supported by NSF grant #BNS-8215572.

277.11 **EFFECTS OF SEROTONIN DEPLETION WITH P-CHLOROPHENYLALANINE ON BETA-ADRENERGIC RECEPTORS IN RAT BRAIN.** K.J. Kellar, J.-M. Mienville*, S.M. McLeskey*, A.M. Martino*, and C.A. Stockmeier. Dept. of Pharmacology, Georgetown Univ. Sch. of Med., Washington, DC 20007.

Selective lesions of serotonin (5-HT) neurons with 5,7-dihydroxytryptamine or p-chloramphetamine (PCA) result in an up-regulation of beta-adrenergic receptors in rat hippocampus and cerebral cortex (Stockmeier et al., Science 230, 1985). The purpose of this study was to determine whether the loss of 5-HT itself is sufficient to increase beta-adrenergic receptor binding or whether additional factors associated with 5-HT axons are involved in the regulation of beta-adrenergic receptors.

Male Sprague-Dawley rats were injected with p-chlorophenylalanine (PCPA) to inhibit 5-HT synthesis. The initial dose was 300 mg/kg on day 1 followed by 50 mg/kg every other day for 33 days. The rats were sacrificed one day after the last injection. The 5-HT and the norepinephrine (NE) content of the hippocampus and cortex were measured by HPLC. Beta-adrenergic receptors were measured with a single concentration of [^{3}H]dihydroalprenolol ([^{3}H]DHA; 6 nM) or of [^{125}I]iodopindolol ([^{125}I]Pin; 150 pM).

Treatment with PCPA reduced 5-HT content in the hippocampus and in the cortex by about 80% compared to saline injected controls. Norepinephrine (NE) content was not affected in either brain area.

In the hippocampus, this treatment with PCPA resulted in a 62% and a 45% increase in the binding of [^{3}H]DHA and [^{125}I]Pin, respectively. In the thalamus, [^{3}H]DHA binding was increased by 38%, while in the frontal cortex and cerebellum binding of [^{3}H]DHA appeared to be unchanged by treatment with PCPA.

In other rats, a lesion of 5-HT axons was produced by injections of PCA (5 mg/kg on days 1 and 3). Beginning on day 5, lesioned rats and nonlesioned controls were injected daily with carbidopa (25 mg/kg) and either 5-hydroxytryptophan (5-HTP; 50 mg/kg, twice daily) or saline for 4 weeks. The rats were sacrificed 14 or 38 hours after the last injection. As expected, the lesion with PCA resulted in a 37% increase in [^{3}H]DHA binding in the hippocampus. The chronic treatment with 5-HTP, designed to increase 5-HT content in brain, did not prevent the PCA lesion-induced increase in [^{3}H]DHA binding in the hippocampus. Nor did chronic treatment with either carbidopa plus 5-HTP or carbidopa alone alter [^{3}H]DHA binding in nonlesioned rats.

Depletion of brain 5-HT with PCPA appears to be sufficient to up-regulate beta-adrenergic receptors in hippocampus. But following 5-HT axon lesions, replacement of 5-HT levels in brain generally by treatment with 5-HTP apparently is not sufficient to prevent the up-regulation of these receptors. It is possible that some other component of 5-HT neurons, or the proximity of intact 5-HT axons to specific target neurons receiving NE innervation is required for complete regulation of beta-adrenergic receptors by 5-HT axons.

277.12 COMPLIMENTARY PATTERNS OF SEROTONERGIC AND NORADRENERGIC FIBERS IN MAIN OLFACTORY BULB OF ADULT RATS. M.T. Shipley, G.S. Aston-Jones, W.T. Nickell, and J.H. McLean, Dept. of Anatomy and Cell Biology, Univ. of Cincinnati College of Medicine, Cincinnati,OH.45267-0521 and Dept. of Biology, New York Univ., New York, NY 10003.

The main olfactory bulb has distinct laminar cytoarchitectural patterns which facilitate studies of afferent connections and development. Previous immunohistochemical and connectional studies have established that in the adult rat, serotonergic input from the dorsal and median raphe terminates predominantly in the glomerular layer of the olfactory bulb. Little is known about the laminar termination pattern of noradrenergic fibers in the bulb. Since recent evidence suggests that serotonergic and noradrenergic fibers form mainly complimentary rather than overlapping patterns of innervation in the neocortex, we compared the terminal distribution of these two monoaminergic systems in the olfactory bulb.

Immunohistochemical studies were performed on adult (>60 days, >300gm.) male rats. Frozen transverse sections from rostral to caudal levels of the main olfactory bulb were floated and incubated in primary antibodies to serotonin or dopa-B-hydroxylase (for noradrenaline) followed by reactions using the ABC and DAB method of antibody visualization. Patterns of antibody distribution were compared to patterns obtained by anterograde tracing studies performed by injecting WGA-HRP into either the raphe nuclei (serotonergic) or locus coeruleus (noradrenergic).

The laminar patterns of fiber distribution of the two monoaminergic systems were remarkably complimentary using both immunohistochemical and anterograde tracing methods. Serotonergic fibers and anterograde tracing from the raphe show heavy labelling in glomeruli of the olfactory bulb with relatively few fibers present in deeper layers of MOB. By contrast, noradrenergic fibers and anterograde tracing from the locus coeruleus are very dense in the infraglomerular layers of MOB, especially the internal plexiform layer. Scant or no label is present in the glomeruli.

These results provide strong support for the notion that these two monoaminergic systems innervate common cortical targets with strikingly different laminar termination patterns. The functional significance of the anatomical differences is unknown but one may speculate that the two systems exert quite different and possibly complimentary actions on bulb circuits.

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277.13 EFFECT OF NEUROPEPTIDE Y ON THE RELEASE OF (^{3}H)-NOREPINEPHRINE FROM THE ANTERIOR HYPOTHALAMUS AND NUCLEUS OF THE SOLITARY TRACT REGION OF RATS. A.E. Ciarleglio*, M.C. Beinfeld* and T.C. Westfall (SPON: L.C. Massopust). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Neuropeptide Y (NPY) is a thirty-six amino acid peptide that has been shown to be colocalized with norepinephrine (NE) in central catecholamine neuronal systems including the A_1, A_2, C_1 and C_2 regions of the medulla oblongata. Many of the catecholamine neurons of the medulla that contain NPY project to nuclei in the hypothalamus. In addition, NPY has been shown to decrease the release of NE from peripheral sympathetic nerves in the perfused rat mesenteric artery (Fed. Proc. 45:398, 1986), suggesting that the peptide participates in the modulation of noradrenergic transmission. The purpose of the present study was to examine the effects of NPY on NE release in vitro in the anterior hypothalamus (AH) and the nucleus of the solitary tract (NTS) region.

Male Sprague-Dawley rats (270-350 g) were decapitated and the brain quickly removed. In a cold room the AH was removed by making an anterior coronal cut through the optic chiasm, a posterior cut through the midsection of the infundibulum, and two diagonal cuts from the mediolateral borders of the pyriform cortex to the anterior commissure. The tissue was sliced at 500 µm thickness, rotated 90° and sliced again. The brainstem was removed, and 1 mm coronal slices were made; slices were placed on a glass slide on a cooled microscope stage and the NTS region was punched bilaterally from three consecutive slices. Tissues were preincubated in Krebs Ringer bicarbonate buffer for 15 min, then incubated in buffer containing (^{3}H)-NE for 30 min. After a 40 min equilibration period, tissues were stimulated twice (S_1 and S_2) using high potassium buffer with a 15 min interval between S_1 and S_2. In a second group of animals, NPY was added after the first stimulation and remained present through the second basal period and stimulation. ^{3}H-NE release was measured using liquid scintillation spectroscopy. To further probe the role of NPY in the modulation of NE release, radioimmunoassay for NPY was used to examine the possibility that NE and NPY are co-released in the AH and NTS region. Methods for this part of the study were as above, except that no exogenous NPY was added. Results were expressed as a ratio of S_2/S_1.

In the NTS region, NPY was shown to decrease the release of ^{3}H-NE significantly: S_2/S_1 control = 0.77 $\pm$ .06, NPY = 0.58 $\pm$ .09, $p < .05$. Similar results were obtained in the AH: S_2/S_1 control = 0.68 $\pm$ .03, NPY = 0.54 $\pm$ .04, $p < .05$. These results suggest that NPY modulates NE release in these brain regions by decreasing the evoked release of NE.

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277.14 **BEHAVIORAL EVIDENCE FOR GABAERGIC SUPERSENSITIVITY IN ADULT RATS LESIONED WITH 6-OHDA AS NEONATES.** G.R. Breese, K.L. Hulebak, T.C. Napier, R.A. Mueller, A.A. Baumeister[1], and G.D. Frye[1]. University of North Carolina School of Medicine, Chapel Hill, NC 27514 and [1]Texas A&M University College of Medicine, College Station, TX 77843.

Certain aspects of the behavioral syndrome produced when striatal dopamine receptors are activated are believed to be mediated by a striatonigral GABA pathway. In the present study, muscimol was administered into the substantia nigra zona reticulata (SNR) of 6-OHDA-lesioned rats to assess if this treatment altered GABA receptor sensitivity. Unilateral microinjection of muscimol (5, 15, or 30 ng) into the SNR caused turning in adult rats lesioned with 6-OHDA as neonates or as adults. The rate of circling was greater in lesioned rats than rates observed following intranigral muscimol in unlesioned animals. These data suggest that SNR GABA receptors in 6-OHDA-lesioned rats are functionally supersensitive. Bilateral administration of 30 ng/per side of muscimol into the SNR induced self-mutilation behavior (SMB) in 2/11 of the unlesioned rats, 0/8 adult-6-OHDA-lesioned rats, and 11/11 neonate-6-OHDA-lesioned rats. The incidence of SMB in the neonatally lesioned rats was dose related. Muscimol induced self-biting was observed in rats lesioned as neonates, but not in those lesioned as adults. Similar results were observed after L-DOPA administration to the 6-OHDA-lesioned rats. These data support the view that lesions of dopaminergic neurons cause a neuronal adaptation of GABAergic output to the SNR as reflected by an increased functional responsiveness of GABA receptors at this brain site. (Supported by HD-03110, HD-18668 and AA-06223).

277.15 EVIDENCE FOR ADENOSINE-DOPAMINE INTERACTIONS IN THE CNS. H. Criswell, G.R. Breese, and R.A.Mueller. Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Patients with Lesch-Nyhan syndrome have disorders of both purine and dopamine metabolism (1). The neonatally 6-Hydroxydopamine (6-OHDA) lesioned rat has been proposed as a model of the dopamine deficiency in Lesch-Nyhan disease (2). In the present study, we examined the relationships between purines and dopamine function. Although the HGPRT deficiency in Lesch-Nyhan disease is associated with a dopamine deficiency, we found that destruction of dopamine-containing neurons did not reduce HGPRT levels. We then tested the effect of adenosine, a purine, on dopamine function. This included the effects of the adenosine antagonist theophylline in neonatal-6-OHDA lesioned rats and the effect of adenosine agonists on behaviors evoked by dopamine agonists in rats made supersensitive to dopamine by neonatal-6-OHDA lesions. Administration of theophylline (5,10 & 20 mg/kg) to rats treated with 6-OHDA as neonates produced a dose-related elevation of locomotor activity which was greater than that observed in unlesioned controls. This increase in locomotor activity could be blocked by alpha-methyl tyrosine (20 mg/kg) and reinstated by l-DOPA (5mg/kg). In normal rats 20 mg/kg of theophylline was required to produce a statistically reliable increase in locomotor activity. When rats lesioned as neonates with 6-OHDA were given l-DOPA as adults, they exhibited a variety of behaviors, including self biting. Microinjection of the adenosine agonist, NECA, into the striatum blocked all of the behaviors produced by l-DOPA in these animals. In contrast, 2-chloro-adenosine, a relatively selective A_1 adenosine agonist, blocked the self biting but did not antagonize other behaviors produced by l-DOPA. NECA produced a similar blockade of behaviors produced by the selective D_1 dopamine agonist, SKF-38393, and the D_2 agonist, LY-171555. These data are consistent with the view that adenosine influences dopamine mechanisms by affecting the release of dopamine and by influencing dopamine receptor mediated events.

1. Lloyd et al. N.Engl. J. Med. 305, (1981) 1106-1111.
2. Breese et al. J. Pharmacol. Exp Ther. 231, (1984) 343-353

277.16 OPIATE MODULATION OF CAPSAICIN INDUCED RELEASE OF SUBSTANCE P FROM CEREBRAL VESSELS. S. Markowitz*, M.A. Moskowitz (SPON:M.J. Brennan) Depts. of Neurosurgery and Neurology, Massachusetts General Hosp., and Harvard Medical School, Boston, MA. 02114.

Cephalic blood vessels play a central role in the pathogenesis of vascular head pain. Indeed, cerebral vessels possess a dense perivascular afferent innervation comprised of axons in which a number of putative neurotransmitters have been identified, including, substance P (SP), cholecystokinin and calcitonin gene related peptide. Recently, we identified dynorphin B (dynorphin 20-32) in perivascular nerve fibers by immunohistochemistry and measured, by radioimmunoassay (RIA), levels of this peptide in this tissue. Since opiates are able to modify release of SP from primary sensory neurons, we considered the possibility that opiates might modulate sensory transmission in perivascular axons as well. Bovine pial blood vessels were obtained immediately after slaughter, placed in an in-vitro superfusion system and perfused with an oxygenated Krebs-bicarbonate buffer. Immunoreactive SP (iSP) was measured by RIA in the eluate using F2 antiserum. Following a period of equilibration, the vessels were exposed to a pulse of capsaicin. Capsaicin (8-methylnonene-(6)-oyl vanillyamide) is the pungent ingredient in hot peppers and is known to release SP from primary sensory neurons. Capsaicin induced a dose-dependent release of iSP from pial blood vessels. Vehicle was without effect. Spontaneous release per minute was 0.06% of total tissue content and averaged 1.6fmols. Release above baseline was measured at doses greater than 10-7M and an increase of 85% above baseline was observed using 10-5M. Capsaicin 10-6M induced an increase of 30% over baseline. Dual pulses in the same experiment released iSP in reproducible amounts, without evidence of tachyphylaxis. The effect of dynorphin B on this release was then investigated. Following a pulse of capsaicin (10-5M for 20 mins.) and an equilibration period (Krebs only), dynorphin alone (10mins.) was superfused followed by a pulse of capsaicin plus dynorphin (20 mins.). Dynorphin inhibited the chemically-induced release of iSP in a concentration dependent manner, but did not effect spontaneous release. At a concentration of 10-6M, but not 10-7M, nor 10-8M, the expected release was reduced by 68%. In a similar set of experiments, lofentanil, 10-5M, also attenuated the capsaicin induced iSP release from perivascular fibers by 70%. The demonstration of a possible functional interaction between an endogenous opiate system and a pathway believed to be important in pain transmission may have important implications for the understanding and rational therapy of vascular head pain.

277.17 COMPARISON OF RESPONSES BY SUBSTANCE P AND SUBSTANCE K SYSTEMS TO METHAMPHETAMINE TREATMENT, G.R. Hanson, A.A. Letter*, K. Merchant*, J.W. Gibb. Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112

We have observed previously that high doses of methamphetamine (METH) significantly elevate the concentrations of substance P-like immunoreactivity in CNS regions associated with the basal ganglia. Recently, another tachykinin, substance K (SK), has been demonstrated to be closely associated with substance P (SP)and synthesized from a common preprotachykinin. While both neuropeptides exert comparable effects when locally injected into different brain regions, there are significant differences in their potencies, apparently due to the relative concentrations of their unique receptors. Due to the controversy which has arisen as to their respective roles within the basal ganglia, we have measured and compared the responses of the striatal and nigral SP and SK systems to METH treatment. Both systems were evaluated by RIA techniques employing highly specific antisera. We observed that multiple high doses of METH (15 mg/kg; q6h/total of 5 doses) increased nigral and striatal concentrations of both neuropeptides in an identical fashion. Our observation that METH treatment did not alter the relative concentrations of SP and SK in either brain tissue suggests that responses of both transmitter systems, associated with the basal ganglia, parallel each other and are sensitive to the same regulatory mechanisms.

This work was supported by U.S. Public Health Service Grants MH 40175 and DA 00869.

278.1 **CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVITY IN EMBRYONIC NEURONS OF THE QUAIL CILIARY GANGLION.**
James Coulombe and Marianne Bronner-Fraser, Developmental Biology Center and Department of Developmental and Cell Biology, University of California, Irvine, Ca. 92717

Our previous studies have shown that some neurons from the quail ciliary ganglion accumulate catecholamines when transplanted into trunk regions of a chicken embryo. Prior to transplantation, neurons were selectively labeled by retrograde transport of fluorescent latex microspheres. These results suggest that cholinergic neurons can express adrenergic traits.

Though ciliary ganglia from 5.5 day old quail embryos are known to have choline acetyltransferase (ChAT) activity, the neurotransmitter phenotype of individual embryonic neurons has not yet been described. In the present study, we have examined the cellular development of the cholinergic phenotype in avian ciliary ganglia. In addition, we have examined whether labeled neurons from 6.5 day old quail ciliary ganglia express a cholinergic phenotype prior to transplantion. The recent availability of antibodies against ChAT has made it possible to examine ChAT immunoreactivity in ciliary ganglia. Whole ganglia were fixed in paraformaldehyde and picric acid, infiltrated with sucrose, and cryostat sectioned. Cell cultures were fixed in paraformaldehyde and picric acid. Either monoclonal or polyclonal antibodies against ChAT (kindly provided by P. Salvaterra and M. Epstein, respectively) were used for immunocytochemistry and visualized with biotin/avidin peroxidase complex (Vectastain) developed in diaminobenzidine and intensified with osmium.

In the intact 6.5 day old quail ciliary ganglion, all cells with large cell bodies which are characteristic of neurons appeared to contain ChAT immunoreactivity. To more accurately distinguish neurons, ciliary ganglia were dissociated and plated onto laminin substrates for 8 hours to allow axonal regeneration. ChAT immunoreactivity was observed in all cells with neuronal morphology (i.e., round cell bodies and processes) in ganglion cultures from 5.5 to 6.0 day embryos. ChAT immunoreactivity persisted at all later stages examined. At these times, no distinctions could be made between ciliary and choroid neurons.

In order to determine if labeled neurons were cholinergic prior to transplantation, ciliary ganglia were retrogradely labeled with fluorescent latex microspheres and processed for ChAT immunocytochemistry. To date, all of the labeled neurons of the ciliary ganglia also possessed ChAT immunoreactivity. These results suggest that the labeled neurons which contained adrenergic neurotransmitters were cholinergic prior to transplantation.

(supported by NSF grant DCB-8502604)

278.2 DISSOCIATED CELL CULTURES OF RAT SUPERIOR CERVICAL GANGLION NEURONS: SEROTONIN SYNTHESIS. D. W. Y. Sah. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Cultures of dissociated sympathetic principal neurons from newborn rat superior cervical ganglia (SCG) contain and release serotonin (5-HT; see Matsumoto et al., Soc. Abs. 11:87, 1985; Sah and Matsumoto, ibid). A major source of 5-HT in the neurons is uptake from the rat serum added to the growth medium, as might be expected from reports on 5-HT uptake in adrenergic neurons in vivo. We were interested in determining if the neurons synthesized 5-HT. Mass cultures of 1000-3000 neurons were used in these biochemical and immunocytochemical experiments. The cultures were grown in 3 types of medium,with or without 5% rat serum:i) L-15 CO_2 medium, ii) depolarizing growth medium ("high K^+") with 20mM KCl, and iii) L-15 CO_2 medium equivalent to 100-150% heart-cell conditioned medium (serum-free CM; Fukada, Nature 287:553, 1980).

To study 5-HT synthesis and accumulation,cultures were incubated with 4μM ^{3}H-tryptophan and pargyline for 17-18 hrs at 37°C, and then extracted for HPLC with fraction collection. Significant amounts of radioactivity co-eluted with 5-HT in extracts of cultures grown in CM (0.18 fmol/neuron),but not of those grown in L-15 CO_2 or high K^+ (<0.008 fmol/neuron). Thus, CM in the growth medium was required for detecting 5-HT synthesis and accumulation.

When neurons were grown in serum-free medium,to eliminate uptake of exogenous 5-HT,only cultures grown with CM contained a substance that co-migrated with authentic 5-HT. A voltammogram of this putative 5-HT peak overlapped that of authentic 5-HT; cultures grown with CM in serum-free medium contained endogenous 5-HT (0.6 fmol/neuron). The distribution of this 5-HT was examined using immunocytochemistry; after growth in CM (but not L-15 CO_2 or high K^+), many cell bodies and processes contained 5-HT-immunoreactivity, presumably as a consequence of 5-HT synthesis and storage.

Happola et al. (J. Auton. Nerv. Sys. 15:21, 1986) recently reported that some principal neurons in the rat SCG transiently contain 5-HT-immunoreactivity in vivo, during the first 5 postnatal weeks. How the situation in culture, where some principal neurons synthesize 5-HT after growth in CM, relates to that in vivo remains to be determined.

CM contains a factor that enhances cholinergic properties while diminishing adrenergic ones (Patterson and Chun,Devel. Biol. 56:263, 1977), so the effect of CM on 5-HT synthesis seemed dissonant with the preliminary electrophysiological result that adrenergic function is required for vesicular storage and release of 5-HT. Two questions are immediately raised:1) is 5-HT synthesis co-expressed with adrenergic function, and 2) is the factor in CM that induces 5-HT synthesis the same as the cholinergic factor, or is an additional factor present? Experiments are underway to address these questions. (Supported by MH 18012, NS 11576, and NS 18316)

278.3 A NEW LOW MOLECULAR WEIGHT ADRENERGIC NEUROTROPHIC FACTOR FOR CULTURED CHICK SUPERIOR CERVICAL GANGLION NEURONS. A.D. Zurn* (SPON: B. Fulpius). Dept. of Biochemistry, University of Geneva, CH-1211 GENEVA 4, Switzerland.

Chick superior cervical ganglion neurons grown for 3-4 weeks in the absence of non-neuronal cells synthesize and accumulate both catecholamine (CA) and acetylcholine (ACh) but the amount of CA produced is several fold higher than the mount of ACh (A.D. Zurn and F. Mudry, Dev.Biol., in press). A new low molecular weight factor has been found that specifically stimulates the growth and development of these neurons. It is present in medium conditioned by chick liver cells in culture (LCM) but not in heart conditioned medium. LCM does not allow neuronal survival in the absence of nerve growth factor nor does it increase survival in its presence. It does, however, increase the neuronal growth and adrenergic differentiation of these cells. CA production is increased several fold whereas ACh production is decreased. Both the growth and differentiation promoting activity are of very low molecular weight (lower than 500 daltons). The effect appears to be specific for sympathetic neurons since LCM does not increase neuronal growth in chick ciliary ganglion, dorsal root ganglion and spinal cord neurons, nor does it induce CA production in these cells.

278.4 DIFFERENTIAL OPIOID EXPRESSION IN CHOLINERGIC CILIARY NEURONS IN VIVO AND IN VITRO. D.B. Gray, M.J. Ford, and G. Pilar. Dept. of Physiology and Neurobiology, The University of Connecticut, Storrs, CT 06268.

The ability of tissue-derived factors to alter or regulate cholinergic and catecholamine transmitter phenotype in culture has been well documented for neurons in the autonomic nervous system. It now appears that such plasticity of expression may extend to endogenous opioid peptides and their receptors in the developing chick ciliary ganglion as well.

Although enkephalin-like immunoreactivity has been shown in the developing avian ciliary ganglion, the antigen has been histochemically localized only in a sub-population of preganglionic synaptic terminals. Ciliary ganglion cell somas and terminal regions in the chick iris do not stain for leu-enkephalin. However, E9 ciliary ganglion neurons cultured for 1 week in DMEM with 10% horse serum and 10% chick embryo extract typically display enkephalin-like immunoreactivity in somas as well as in processes. This result is supported by the observation, at the E.M. level, that ciliary neurons in vitro acquire increased populations of large dense core vesicles relative to neurons in vivo (Crean, G. et al., Neurosci. Abstr. 10: 127.15, 1984).

The same dichotomy may also exist for functional opiate receptors that mediate inhibition of presynaptic release at the neuromuscular junction between the ciliary ganglion and its target musculature. In vivo, chick ciliary neurons make nicotinic synapses with striated muscle of the iris and ciliary body. At hatching, these synapses not only release ACh upon stimulation but are capable of regulating synthesis of transmitter after stimulation-induced depletion. Addition of opiates, such as morphine, or opiate antagonists, such as naloxone, has no effect on these processes. However, ciliary ganglion neurons co-cultured with striated myotubes contain synapses with cholinergic parameters closely resembling those within the in vivo iris and are affected by addition of opiates. Addition of 5 uM morphine during high K+ stimulation significantly decreases neuronal ACh release and completely inhibits release-induced acceleration of ACh synthesis. Co-administration of 5 uM naloxone with the morphine completely prevents these effects.

These results show that the expression of neuronal opioid peptides and functional receptors can be influenced by their developmental environment. Since it is known that exogenous morphine profoundly influences neuronal death in the ciliary ganglion, it is of considerable interest to determine if the in vitro expression represents an amplification of an endogenous opioid system or a novel gene expression for the ciliary ganglion neuron. Supported by NSF grant #BNS 8410581-01, NIH grant #2R01NS10338, and The University of Connecticut Foundation.

278.5 **EFFECTS OF CLASSICAL CONDITIONING PROCEDURES ON DYNORPHIN A(1-8)-LIKE IMMUNOREACTIVITY IN THE HIPPOCAMPAL FORMATION OF RATS.** M. W. Meagher, M.A. Pelleymounter, J.S. Hong and M. Gallagher. Department of Psychology, U. of N. Carolina, Chapel Hill, NC 27514 and Lab. Behav. Neurol. Toxicol, NIEHS/NIH, Research Triangle Park, NC 27834.

Neural activity within the hippocampus is altered by contingent and non-contingent presentation of events during classical conditioning. Paired presentations of conditioned and unconditioned stimuli (CS and US) produce changes in the responses of hippocampal neurons to the CS that are not found when the CS and US are randomly presented. An input to the hippocampus arises from granule cells located in the dentate gyrus. The granule cell/mossy fiber projection to CA3 of hippocampus contains the opioid peptide dynorphin: these neurons appear to be the major source of dynorphin in hippocampus. This research examined the effects of classical conditioning procedures on dynorphin A (1-8)-like immunoreactivity [dyn(1-8)-LilR] in hippocampus. In addition, because the ascending norepinephrine (NE) system would be activated by the training procedures, and the NE system provides a substantial innervation of the dentate region, this research also sought to determine any contribution of NE input to the regulation of dynorphin/granule cell activity. Thus, animals in this experiment had unilateral lesions of the dorsal NE pathway.

Male Sprague-Dawley rats received either paired or unpaired presentations of the CS (20 sec, 85 dB tone) and US (0.5 mA; 1.0 sec footshock) during a training session. Animals used for determination of dyn(1-8)-LilR in hippocampus were sacrificed at either 30 min or 3 hr after training. Additional groups of animals were tested during sessions that followed training to determine that conditioned responses (freezing) to the CS were established. Radioimmunoassays for dyn(1-8)-LilR were conducted as previously described (Hong et. al., In M. Conn (Ed.) Methods in Enzymology, New York:Academic Press, 1984).

The behavioral data affirmed that significant associative learning occurs in the present paradigm. A three-way analysis of variance performed on the measures of dyn(1-8)-LilR in hippocampus yielded a significant effect of training (paired vs. unpaired), a significant effect of time (30 min vs. 3 hr) but no significant effect of brain treatment (NE lesioned vs. unlesioned hemisphere). At 3 hr after training levels of dyn(1-8)-LilR were comparable for paired and unpaired training groups (expressed as Pmoles/g tissue, wet weight, 10.61±.41and 9.64±.61, respectively). At 30 min after training dyn(1-8)-LilR levels were higher in the paired group (14.38±.81) relative to the unpaired group (10.36±1.01).

The results of this experiment indicate that training procedures similar to those that differentially affect the development of conditioned neural activity within hippocampus, also have distinctive effects on the content of dyn(1-8)-LilR, presumably within a major input pathway to hippocampus. These results may be considered in the context of a role for opioid peptides in learning, as well as the differential stress effects of signalled vs. unsignalled shock on opioid peptide function.

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278.6 α_2 RECEPTOR LIGANDS DIFFERENTIALLY REGULATE LOCUS COERULEUS PHENOTYPIC CHARACTERS IN CULTURE. C.F. Dreyfus, M.A. Gillies,* and I.B. Black. Division of Devel. Neurology, Cornell Univ. Med. Coll., N.Y., NY 10021.

To examine regulation of brain neurotransmitter development, we have been studying ontogeny of noradrenergic characters in the mouse locus coeruleus in vivo and in explant culture. Initial studies have indicated that tyrosine hydroxylase (TH) and the specific, high affinity, norepinephrine (NE) uptake system develop in culture, reflecting ontogeny in vivo. In addition, the depolarizing agents, veratridine and elevated K^+, significantly increase TH in locus cultures without affecting NE uptake in the same cells. Consequently, two NE traits, localized to the same neurons, may be differentially regulated by pharmacologic depolarizing agents.

To determine whether locus cells are similarly responsive to specific receptor ligands, we evaluated the effects of piperoxane and clonidine. Both agents are known to interact with α_2 receptors localized to locus neurons. The locus was dissected from mice on embryonic day 14 (E-14) and placed in culture. Exposure to piperoxane (3.7μM) or clonidine (.37μM-3.7 μM) for 7 days resulted in significant increases in TH catalytic activity. To determine whether these agents also influenced NE uptake, cultures were exposed to piperoxane or clonidine, washed exhaustively, and the specific, high affinity uptake process was characterized. Doses of piperoxane or clonidine that increased TH activity failed to affect NE uptake.

Our studies suggest that pharmacologic depolarizing agents or specific α_2 receptor ligands selectively increase TH activity without altering NE uptake. Consequently, presynaptic signals, presumably acting through stimulation of locus α_2 receptors, differentially regulate distinct noradrenergic characters localized to the same brain neurons. We are presently examining the molecular basis of this differential regulation. (Supported by NIH Grant NS 20788 and The American Parkinson's Disease Fdn., CFD is the recipient of a Teacher Scientist Award from the Andrew W. Mellon Fdn.).

278.7 **EXPRESSION OF TYROSINE HYDROXYLASE IN CEREBRAL CORTICAL NEURONS IN CULTURE IS REGULATED BY MUSCLE.** L. Iacovitti, J. Lee, and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We have previously reported that a population of postmitotic neurons from embryonic rat cerebral cortex can express the catecholaminergic (CA) enzyme tyrosine hydroxylase (TH) in vitro but not in vivo (Iacovitti et al., Neurosci. Abst., 1985). Since in the peripheral nervous system, factors derived from muscle can regulate the expression of CA traits in autonomic neurons (Patterson and Chun, Dev. Biol. 56:263, 1977; Lee et al., Neurosci. Abst., 1985), in this study, we sought to determine whether similar factors might also regulate TH expression in central cortical neurons. To test this, cardiac (ventricle), skeletal (gluteus maximus) and smooth (aorta) muscle was removed from rat embryos at day 19-22 of development (E19-E22), dissociated and plated on collagen-coated dishes. Three days later, cerebral cortices from E13-14 rats were dissociated and were grown either alone or in co-culture with muscle. Cultures were fed media containing 10% fetal calf serum. After 2 days in vitro, the cultures were fixed and processed for immunocytochemical staining. Control cultures consisting of only cortical cells contained 1115 ± 201 TH immunoreactive neurons/dish. However, when cortex was co-cultured with skeletal, cardiac or smooth muscle, the number of TH-labeled cortical neurons dramatically increased to 4942 ± 464/dish. This effect of muscle appeared to be specific for the TH-immunoreactive cell population since there was not an apparent 5-fold increase in the total number of surviving cortical cells.

To examine whether the large number of fibroblasts present in these cultures or their fibronectin-rich extracellular matrix contributed to the regulation of TH expression in cortical neurons, cortex (E13-14) was co-cultured with skin-derived fibroblasts (E21-P2) or plated onto dishes coated with 10 ug/ml of the glycoprotein, fibronectin. Under both conditions, the number of TH immunoreactive cortical neurons remained at control levels (1058 ± 140/dish). Finally to test whether the effect of muscle on the expression of TH in cortical neurons, was produced by diffusable or membrane-bound substances, E13-14 cortical neurons were grown in vitro either in the presence of heart conditioned medium or in culture dishes containing a substratum of lysed heart cells. In both cases, control numbers of TH cells (918 ± 196) were observed.

The results of these studies indicate a) that the number of cortical neurons expressing TH increases 5-fold when grown in the presence of cardiac, skeletal or smooth muscle, b) that this effect is specific to muscle since it is not mimicked by fibroblasts or fibronectin, and c) that the factors regulating this effect do not diffuse into the media. These findings suggest that an interaction of cortical neurons with muscle regulates the expression of TH either by increasing the survival of TH-containing cortical neurons or by inducing a greater number of neurons to express TH.

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278.8 MODULATION OF CHOLINERGIC NEURONAL REGENERATION WITH GROWTH FACTORS. N.J. Woolf, M.C. Hernit*, and L.L. Butcher. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, California, 90024.

The effects of intracerebral administration of nerve growth factor (NGF) and somatostatin (somatotropin release inhibiting factor, SRIF) on the regeneration of putative cholinergic fibers in the neocortical mantle were studied. Coronal knife cuts were placed in frontoparietal cortex which resulted in the interruption of acetylcholinesterase (AChE) fibers that course in a rostrocaudal direction as described previously (Wendt and Ayyad, Soc. Neurosci. Abstr., 11, 975, 1985). Stainless steel cannulae were implanted so that NGF, SRIF, or saline could be delivered into the ventricle ipsilateral to the side of the knife cut. Rats receiving knife cuts were analyzed at 4 days, 1 week, 2 weeks, and 1 month following surgery. Solutions of 5μg/5μl of NGF or SRIF dissolved in saline or 5μl of saline were delivered 2 times a week. Additionally, 10μg/5μl and 15μg/5μl concentrations of SRIF were administered to rats surviving 4 days, 1 week, and 2 weeks.

At 1 month, NGF-treated rats demonstrated better healing of knife cuts than did saline-treated controls. Additionally, there appeared to be a greater number of AChE fibers that traversed the cut, and more fibers appeared to have reinnervated cortical regions distal to the knife cuts.

SRIF-treated rats appeared to have an overall reduction in healing and reinnervation. At 1 month, many fewer AChE fibers were found to traverse the scar. Fewer fibers were also found in cortex distal to the knife cut, especially in the deepest cortical layers near the corpus callosum. At 4 days, 1 week, and 2 weeks SRIF-treated rats demonstrated reduced numbers of AChE fibers both proximal and distal to the cut, and this effect was more pronounced at higher concentrations of SRIF (10μg/5μl and 15μg/μl).

Consistent with a neurotrophic role of NGF on central cholinergic neurons, NGF was found to have a positive effect on reinnervation of cerebral cortex by AChE fibers following knife cuts. SRIF demonstrated some negative effects on cortical healing following knife cuts and on the growth of AChE fibers into the damaged area.

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278.9 TRIGEMINAL GANGLION CELLS EXPLANTED WITH GUT PRODUCE VASOACTIVE INTESTINAL PEPTIDE. J.P. Davis* and M.L. Epstein. Department of Anatomy, University of Wisconsin, Madison, WI 53706.

We studied whether embryonic avian gut in coculture could stimulate embryonic peripheral sensory neurons (avian trigeminal ganglion) to produce gut neuropeptides.

Enteric neurons produce a variety of neuropeptides, including vasoactive intestinal peptide (VIP). No vasoactive intestinal peptide-like immunoreactivity (VIP-LIR) was detected immunocytochemically in the native quail trigeminal ganglion.

Embryonic quail trigeminal ganglia were coexplanted with embryonic chick hindgut onto the chorioallantoic membrane (CAM) of host chicks and incubated for 7-8 days. After fixation the explants were sectioned and stained for VIP-LIR.

In sections we observed many viable implanted quail neurons. The majority were large cells with morphology similar to native cells and to trigeminal ganglion cells grown alone on the CAM. A fraction of the coexplanted neurons were small and spindle-shaped and showed VIP-LIR. The percentage of cocultures containing VIP-LIR neurons appeared to decrease with increasing age of the donor neuron.

Explantation of quail trigeminal ganglia alone on the CAM resulted in large numbers of VIP-negative neurons from 6.5 day embryos and newly-hatched donors. This survival of the trigeminal ganglion cells alone on the CAM strikingly contrasts with the survival of the quail ciliary ganglion alone on the CAM.

Our results show that VIP is not expressed by native quail trigeminal ganglion cells, but is expressed by embryonic quail trigeminal ganglion cells grown with embryonic chick gut.

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278.10 **ORNITHINE DECARBOXYLASE ACTIVITY INCREASES DRAMATICALLY IN ADULT HIPPOCAMPUS FOLLOWING RECURRENT LIMBIC SEIZURES.** C.M. Gall[1], M. Baudry[2], and G.Lynch[1]. [1]Department of Anatomy and Neurobiology and [2]Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

The placement of a small electrolytic lesion in the hilus of the dentate gyrus has been shown to induce recurrent limbic seizure activity lasting several hours and, consequently, a large bilateral increase in the synthesis and concentration of the opioid peptide enkephalin in the hippocampal mossy fiber system. Since polyamines and ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine synthesis, have been proposed to play critical roles in the regulation of nucleic acid and protein synthesis we tested for changes in ODC activity in hippocampus following the hilus lesion.

Adult Sprague Dawley rats were anesthetized with ketamine/xylazine and a small electrolytic lesion was placed in the dentate gyrus hilus. Control animals were similarly anesthetized but no surgery was performed. At various postoperative intervals the animals were sacrificed by decapitation and the hippocampi and cerebellum assayed for ODC activity using a modification of the technique of Russel and Snyder (PNAS, 60: 11420, 1968). ODC activity was found to be elevated in both hippocampi by 3 hrs post-hilus lesion, to increase to a maximal 50 fold increase by 12 hrs postlesion, and to decline to control values by 48 hrs. There was no elevation of ODC activity in the cerebellum of hilus lesion animals. The increase in hippocampal ODC did not require the presence of the adrenal glands in that it was of the same magnitude with lesion placement in adrenalectomized rats. The lesion-induced increase in ODC activity appears to be due to epileptiform activity rather than to other consequences of treatment in that it was almost totally blocked by maintenance of hilus-lesion rats under anesthesia, from lesion to sacrifice, with the anticonvulsant sodium pentobarbital. This conclusion is further supported by the observation that other deafferenting lesions which do not stimulate seizures (e.g. retinal deafferentation of superior colliculus) do not increase ODC activity.

The very rapid postlesion increase in ODC activity observed here, which occurs within 2 hrs of the onset of seizure activity and well before the increase in hippocampal enkephalin content, is consistent with the hypothesis that ODC activation represents an intermediate step through which seizure activity can modulate neuropeptide synthesis. Studies are currently in progress to test this hypothesis.

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OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS III

279.1 EFFECTS OF SPECIFIC MU AND DELTA OPIOID AGONISTS IN THE A10 DOPAMINE REGION OF THE RAT. R.P. Dilts, G. Latimer, P.W. Kalivas. Dept. of VCAPP, Washington State Univ., Pullman, Wash. 99164-6520.

Many lines of evidence suggest that opioids act within the A10 dopamine (DA) region to activate DA neurons projecting to limbic terminal areas. Thus, injection of morphine and other enkephalin agonists into the ventral tegmental area (VTA--a subnucleus of the A10 DA neurons) elicits a dopamine receptor antagonist reversible increase in spontaneous motor activity, and increases dopamine metabolism within the nucleus accumbens.

This study utilizes enkephalin analogues with specificity towards the mu or delta opioid receptor to evaluate which opioid receptor subtype(s) is involved in producing the dopamine mediated responses. Male Sprague-Dawley rats were implanted with cannulae directed towards the VTA. Subsequent injections revealed that the mu agonist, Tyr-D-Ala-Gly-NMe-Phe-Gly-ol (DAGO), was significantly more potent than the delta agonist (D-Pen 2,5)-enkephalin (DPEN) in producing an increased spontaneous motor activity, with an ED50 of 0.002 nmol and 1.559 nmol respectively. Likewise, DAGO was 1000 to 100 times more potent than DPEN in causing increased dopamine metabolism in the nucleus accumbens, septum, prefrontal cortex, and dorsolateral striatum. Differential disposition of the microinjected DAGO and DPEN was not responsible for these observed differences in potency. Furthermore, naloxonazine, a mu 1 antagonist, attenuated the motor stimulant effect produced by intra-VTA injections of DAGO.

Consequently, DAGO has been radiolabeled using iodine-125 in order to visualize, by autoradiographic methods, the opioid receptors within the VTA. Studies are currently underway to determine if the receptors eliciting the DA mediated responses are localized on DA perikaryon or adjacent cells present within the A10 region.

279.2 INTRACEREBROVENTRICULAR ADMINSTRATION OF BETA-FNA SELECTIVELY REDUCES THE NUMBER OF MU-NONCOMPETITIVE DELTA BINDING SITES. R. B. Rothman[1], J. B. Long[2], K. C. Rice[3*], A. E. Jacobson[3*] and J. W. Holaday[2]. [1]Laboratory of Preclinical Pharmacology, NIMH, St. Elizabeths Hospital, Washington DC 20032. [2]Dept. of Neuropharmacology, Walter Reed Army Research Institute, Washington DC 20307. [3]Laboratory of Chemistry, NIADDK, Bethesda MD 20205.

Previous work demonstrated that *in vitro* treatment of membranes with the site directed alkylating agent beta-FNA altered the properties of [^{3}H]DADL binding sites of rat brain membranes (*J. Neurochem.* 43,1197-1200, 1984). Additionally, intracerebroventricular (i.c.v.) administration of 20 nmol FNA attenuated the ability of naloxone to reverse endotoxin-induced hypotension in rats tested 18 hr after the FNA injections (*Proc. Natl. Acad. Sci.* 81, 2898-2901, 1984). The goal of this study was to define the population of [^{3}H]DADL binding sites altered by FNA following *in vitro* treatment of membranes ("in vitro membranes") and 18 hr after the i.c.v. injection of 20 nmol FNA ("in vivo membranes"), and then to see if the interaction of naloxone with the affected site/sites was altered.

Two methods were used to determine the number of mu-competitive (higher affinity) and mu-noncompetitive (lower affinity) delta binding sites. 1. Binding surface analysis using [^{3}H]DADL and peptides selective for the mu-competitive binding site (D-pen-L-pen) and the mu-noncompetitive binding site (LY164929). 2. Pretreatment of membranes with alkylating agents BIT which eliminates the mu-noncompetitive site or FIT which eliminates the mu-competitive binding site.

Binding surface analysis of "in vitro" membranes demonstrated a selective 60% decrease in the number mu-noncompetitive binding sites with no change in the number of mu-competitive binding sites. Using "in vivo membranes", a selective 40% reduction in mu-noncompetitive binding sites was observed. The IC50 of naloxone using FIT-treated "in vitro membranes" shifted from 52±7 nM in control membranes to 102±10 nM (±SD) in FNA-treated membranes. In both cases, naloxone was a noncompetitive inhibitor.

These results reinforce the evidence for interactions between opiate receptor subtypes and demonstrate that the mu-antagonist beta-FNA "antagonizes" naloxone.

279.3 AUTORADIOGRAPHIC EVIDENCE FOR TWO CLASSES OF MU OPIATE BINDING SITES DISTINGUISHED BY BETA-FNA. M. Herkenham[1], K. C. Rice[2]*, A. E. Jacobson[2]* and R. B. Rothman[3]. [1]Laboratory of of Neurophysiology, NIMH, Bethesda, MD 20892. [2]Laboratory of Chemistry, NIADDK, Bethesda, MD 20892. [3]Laboratory of Preclinical Pharmacology, NIMH, St. Elizabeths Hospital, Washington, DC 20032.

Beta-FNA, an alkylating derivative of naltrexone, irreversibly blocks activation of opiate mu receptors (Takemori et al., Eur. J. Pharmacol. 70, 445-451, 1981). Previous work demonstrated that pretreatment of membranes with FNA eliminated only a portion of mu binding sites (Rothman et al., Eur. J. Pharmacol. 95, 147-148, 1983). This study addressed the question: do mu binding sites eliminated by FNA vary in proportion across brain regions? Slide-mounted sections were preincubated and then exposed to either no agent (control), 1 μM FNA, or 1 μM of the mu-selective alkylating agent BIT (2-(p-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidizole). Following 4 sequential washes, opiate receptors were assayed with [^{125}I]FK33824 ([D-Ala^2,MePhe^4,Met(o)-ol^5]enkephalin), [^{3}H]oxymorphone, or [^{3}H]DAGO ([D-Ala^2,MePhe^4,Gly-ol^5]enkephalin). The binding of all 3 ligands was reduced >95% by BIT and between 62 and 70% by FNA. For visualization studies, coronal sections at two levels, mid-thalamus and interpeduncular nucleus, were pretreated with either no agent (control) or 1 μM FNA, and mu binding sites visualized with [^{125}I]FK33824. Densitometric analysis of the autoradiograms demonstrated that the proportion of binding sites eliminated by beta-FNA varied across regions of the brain. The greatest effect was seen in the ventrobasal thalamus. The complete elimination of [^{125}I]FK33824 binding sites by BIT and the lack of labeling of the corpus callosum (a delta-selective region) suggests that the FNA-insensitive [^{125}I]FK33824 binding sites are not delta receptors. Although this study demonstrates a differential anatomical distribution of FNA-sensitive and FNA-insensitive mu binding sites, the pharmacological significance of this finding remains to be elucidated.

279.4 PERTUSSIS TOXIN BLOCKS EFFECTS OF OPIOID, MONOAMINERGIC, AND MUSCARINIC AGONISTS ON DORSAL-HORN NETWORK RESPONSES IN MOUSE SPINAL CORD-GANGLION CULTURES. S.M.Crain[1]*, B.Crain[1]*, and M.H.Makman[1,2]. Depts. of Neuroscience[1], Biochemistry[1] and Molecular Pharmacology[2], Albert Einstein Coll. of Med., Bronx, NY 10461.

After chronic exposure of spinal cord-dorsal root ganglion (DRG) explants to morphine, the acute depressant effects of opioids on sensory-evoked dorsal-horn synaptic-network responses are markedly attenuated, and characteristic cord discharges can then occur even in the presence of >100-fold higher opioid concentrations (Crain et al '79). The present study demonstrates that a remarkably similar degree of tolerance to opioids, e.g. morphine, DADLE, develops in these cord-DRG explants after exposure to the islet-activating protein from pertussis toxin (PTX: 1-10 ug/ml) for >1-2 days. The usual acute depressant effects of serotonin, norepinephrine, and oxotremorine on dorsal-horn discharges are also markedly attenuated in PTX-treated cultures. In contrast, high Mg^{++}, GABA, xylocaine and ethanol were still as effective as in control explants.

PTX is known to interfere with the guanine nucleotide protein G_i that is required for opioid, α_2-adrenergic, and muscarinic receptor-mediated inhibition of adenylate cyclase (AC) in various cells. Agents which elevate intracellular cyclic AMP (cAMP) in cord-DRG explants (e.g. forskolin) also attenuate opioid and monoaminergic depressant effects on dorsal-horn responses (Crain et al '84, '86). Furthermore, these explants contain an opioid-inhibited AC system, and explant AC is also inhibited by muscarinic agonists, norepinephrine, and serotonin. Chronic exposure of the explants to morphine as well as to PTX increases AC activity and attenuates opioid inhibition of AC (Dvorkin et al '85; Crain and Makman '86). These findings together with the present results suggest that the neuromodulatory depressant effects of opioid, monoaminergic, and muscarinic agonists on primary afferent networks in the spinal cord may all be mediated by binding to neuronal receptor subtypes that are negatively coupled via G_i to a common pool of AC. The resulting decrease in AC/cAMP levels may, in turn, regulate phosphorylation-dependent gates in K^+ and/or Ca^{++} channels of DRG neurons, so that Ca^{++} influx in presynaptic terminals as well as in the perikarya is attenuated. Convergence of these diverse receptor subtypes on G_i-mediated second messenger systems in DRG synaptic terminals would thereby provide integrative modulation of transmitter release in nociceptive, and perhaps other, primary afferent networks in the CNS. Our data do not, however, preclude G_i-mediated depressant actions via linkage to other second messengers in addition to AC or directly to some ionic channels. (Supported by grant DA-02031 to S.M.C. and NS-09649 & AG-00374 to M.H.M.)

279.5 OPIOIDS PROLONG RATHER THAN SHORTEN THE ACTION POTENTIAL DURATION OF SENSORY NEURONS AFTER TREATMENT OF MOUSE SPINAL CORD-GANGLION EXPLANTS WITH PERTUSSIS TOXIN OR FORSKOLIN. K-F. Shen*, A. Chalazonitis and S.M. Crain (SPON:D.P.Purpura). Dept. of Neuroscience, Albert Einstein Coll. of Med., Bronx, NY 10461

Intracellular recordings of the action potentials (APs) generated by dorsal root ganglion (DRG) neurons in DRG-cord explants demonstrate that various opioids, e.g. D-ala-D-leu enkephalin (DADLE; 1-10 uM), markedly shorten (by ~50%) the duration of the AP-Ca^{++} component (APD) in many (ca. 50%) of the ganglion cell perikarya (Chalazonitis & Crain,'83,'86). After treatment with forskolin (FSK; 50uM for >15 min) or pertussis toxin (PTX; 10 ug/ml for >1-2 days), there was a marked decrease in the fraction of DRG neurons in which opioids shortened the APD: 7 out of 25 cells after FSK; 4 out of 24 after PTX. (All tests were made in the presence of 5mM Ca^{++} and 5mM Ba^{++}.) Moreover, many of the treated neurons showed an unexpected opioid-induced, reversible prolongation of the APD (often by >200%) in 1-10 uM DADLE: 13 out of 25 after FSK; 17.out of 24 after PTX. Naloxone (3uM) reversed these opioid-prolongations of the APD in 4 out of 5 tests. In 5 cases, these opioid-prolonged APDs were elicited in the same neurons which had shown characteristic opioid-shortening effects when tested prior to introduction of FSK. Other FSK-treated DRG neurons showed an attenuation of the degree of opioid-shortening of the APD, rather than an overt opioid-prolongation. These results are consonant with evidence that FSK- or PTX-treatment markedly attenuates the characteristic depressant effects of opioids on sensory-evoked dorsal-horn network responses in DRG-cord explants (Crain et al '84,'86, & this vol.).

Opioid-shortening of the APD of DRG neurons (and opioid-attenuation of DRG-evoked dorsal-horn network responses) in these DRG-cord explants appears to be mediated by binding to opiate receptor subtypes that are negatively coupled to the adenylate cyclase (AC)/cyclic AMP system via the guanine nucleotide regulatory protein G_i (Crain et al, this vol.). FSK is known to selectively activate AC activity, and PTX interferes with G_i functions required for opioid and other receptor-mediated inhibition of AC in various cells. AC assays of these explants show that PTX-treatment increases AC activity and attenuates opioid inhibition of AC (Dvorkin et al '85; Crain & Makman '86). These findings together with the present data suggest that the onset of opioid-prolongations of the APD of DRG neurons after PTX- or FSK-treatment may be due to attenuation or neutralization of the shortening effects mediated by inhibitory opiate receptors on these neurons, resulting in unmasking of activity induced by putative excitatory opiate receptor subtypes on the same cells. (Supported by grant DA-02031 to S.M.C.)

279.6 CYCLIC NUCLEOTIDES MEDIATE SOME EFFECTS OF SPECIFIC ENKEPHALIN AND FMRFamide RECEPTORS ON APLYSIA NEURONS. Katalin S.-Rózsa* and D. O. Carpenter. Wadsworth Laboratories, New York State Department of Health, Albany, NY 12201.

Our previous studies have demonstrated specific receptors for the enkephalins on neurons in the cerebral ganglion of Aplysia, and the presence of an enkephalin-like substances in Aplysia nervous tissue (Life Sci. 38: 1529, 1986). Leu enkephalin elicits a variety of different ionic responses, even on a single cell. Common responses include depolarizing and hyperpolarizing conductance increases and a slow depolarizing conductance decrease. When FMRFamide, a potent four amino acid peptide, is compared to enkephalin we have found that both peptides can elicit a similar variety of responses, although the receptors are distinct since frequently the polarity of responses on a particular neuron were opposite for the two peptides. In addition, the enkephalin but not the FMRFamide response is naloxone sensitive.

The slow, K+ conductance decrease responses are particularly common on cerebral B neurons and could be elicited on different neurons both by leu or met enkephalin and FMRFamide. Since in other systems slow conductance decrease responses to serotonin, dopamine (PNAS 79: 7934, 1982), acetylcholine (J. Physiol. 369: 399, 1985), FMRFamide (J. Neurosci. 9: 2533, 1985) and TRH (PNAS 82: 4282, 1985) are mediated through cyclic AMP production we have tested the actions of forskolin ($5x10^{-5}$M), an activator of adenylate cyclase, and IBMX (10^{-4}M) and RO-1724 (10^{-5}M), inhibitors of phosphodiesterase on currents induced by met enkephalin (10^{-6}M) and FMRFamide (10^{-6}M) applied by microperfusion in voltage clamped neurons. The phosphodiesterase inhibitors, applied by bath perfusion, had no obvious effect on membrane potential or resistance although forskolin causes a dramatic increase in spontaneous synaptic activity, often requiring study in high Mg^{2+} sea water. Perfusion of all three drugs caused an augmentation of the peak inward current and an increase in the duration of the currents. The increase in amplitude was particularly dramatic for the phosphodiesterase inhibitors (sometimes greater than 4 fold) while the effect on duration was particularly dramatic for forskolin and often was over 6 fold. Surprisingly, both forskolin and IBMX blocked Na+ conductance increase acetycholine responses on these cells. These results suggest that the slow conductance decrease responses to met enkephalin and FMRFamide on Aplysia neurons, like those to other peptides and transmitters, are a result of activation of adenylate cyclase.

279.7 RELATIONSHIP OF OPIATE RECEPTOR BINDING SITES AND OPIATE-INHIBITED ADENYLATE CYCLASE IN BRAIN MEMBRANES. S.R. Childers, P. Nijssen* and J. Harris*, Dept. of Pharmacology, Univ. of Florida College of Medicine, Gainesville, FL 32610.

Opiate agonists inhibit adenylate cyclase in both brain and neuroblastoma cell membranes. Adenylate cyclase appears to be coupled to delta opiate receptors in NG108-15 cells, but the pharmacological nature of the adenylate cyclase-linked opiate receptor in mammalian brain has yet to be established. To determine the relationship between high affinity multiple opiate receptor binding sites and opiate-inhibited adenylate cyclase in rat brain, we assayed adenylate cyclase in membranes after treatment with agents which are known to block high affinity opiate agonist binding. One such agent is phospholipase A(2), which inactivates opiate receptor binding but does not inhibit basal adenylate cyclase activity at low concentrations of enzyme. In these experiments, brain membranes were pretreated at pH 4.5 to remove stimulatory and increase inhibitory adenylate cyclase, and opiate-inhibited adenylate cyclase was determined in the presence of various concentrations of D-ala(2)-met(5) enkephalinamide (D-Ala enk). Incubation of membranes with phospholipase A (500 ng/ml) inhibited 93-100% of binding of [^{3}H] DAGO, [^{3}H] DSTLE and [^{3}H] EKC to mu, delta, and kappa sites, respectively. However, adenylate cyclase in treated membranes was still inhibited by D-Ala enk (with inhibition blocked by naloxone); moreover, the D-Ala enk agonist dose response curve was not affected by phospholipase treatment. To obtain more specific receptor blockade, membranes were incubated with irreversible opiates, then washed to remove traces of reversibly-acting drugs. Control membranes contained equal concentrations of naloxone to control for washing. Incubation of membranes with several irreversible opiates, including antagonists like naloxone azine and beta-FNA, and agonists like p-nitro phenyl-oxymorphone, did not affect agonist dose response curves in inhibiting adenylate cyclase, but blocked high affinity receptor binding by 75-100%. However, incubation of membranes with beta-CNA (10 μM), which blocked binding by 95-100%, shifted the adenylate cyclase dose response curve for D-Ala enk by approx. ten-fold. The results with beta-CNA suggest that a large population of spare receptors exists in striatal membranes, since agonist dose response curves were shifted to the right. However, since 75-100% of high affinity binding sites were blocked by other agents that had no effect on agonist dose response curves, receptors linked to adenylate cyclase in brain may not correspond to any of the sites labeled with nM concentrations of [^{3}H] agonists.

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279.8 OPIATE EFFECTS ON ADENYLATE CYCLASE IN C-6 GLIOMA CELLS. D.R. Marckel, S.P. Baker and S.R. Childers, Dept. of Pharmacology, Univ. of Florida College of Medicine, Gainesville, FL 32610.

Rat C-6 glioma cells have been useful models to study beta-adrenergic stimulation of adenylate cyclase. Opiate receptors and opiate-inhibited adenylate cyclase have been reported in these cells (Tocque et al. J. Neurochem. [1984] 42:1101), but only after treatment with desmethylimipramine (DMI). Increase in opiate-inhibited adenylate cyclase can also be seen in brain membranes, where pretreatment at pH 4.5 diminishes stimulatory and increases inhibitory activity without affecting basal activity. To determine whether low pH pretreatment of C-6 glioma cells would mimic the effect seen in brain membranes, opiate-inhibited adenylate cyclase was assayed in C-6 cells before and after treatment with DMI. Membranes from C-6 cells were pretreated in sodium acetate buffer at pH 4.5 and opiate-inhibited adenylate cyclase was determined in the presence of various concentrations of D-ala(2)-met(5) enkephalinamide (D-Ala$_2$enk). Opiate receptor binding assays were conducted with both [^{3}H] D-Ala enk and [^{3}H] naloxone (1-10 nM).

Adenylate cyclase assays revealed interesting effects of low pH pretreatment on different cell types. Like brain membranes, low pH pretreatment had no effect on basal activity in C-6 membranes. These results contrast with those in rat reticulocytes and NG108-15 cells, where low pH treatment drastically reduced basal activity. Assay of isoproterenol stimulated adenylate cyclase showed that low pH pretreatment eliminated stimulation in reticulocytes, with no effect on stimulation in C-6 cells.

The opiate receptor binding assays revealed no significant specific binding in membranes either before or after treatment of cells with DMI, using either [^{3}H] naloxone or [^{3}H]-D-Ala enk as ligands. However, D-Ala enk produced a biphasic dose response effect on adenylate cyclase in untreated, DMI-treated, and low pH pretreated C-6 glioma membranes. Inhibition (approx. 20%) of adenylate cyclase occurred at 1-10 μM D-Ala enk. At concentrations greater than 10 μM D-Ala enk, adenylate cyclase returned to basal levels. To examine this biphasic response more closely, cells were treated with pertussis toxin for 24 hrs to eliminate the inhibitory component (Ni) of adenylate cyclase. In these membranes, the inhibitory effect of D-Ala enk was eliminated, while D-Ala enk and isoproterenol stimulated adenylate cyclase to the same level (60%). These results suggest that significant opiate-coupled adenylate cyclase can occur in cells with receptor binding sites that are undetectable by normal binding assays.

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279.9 KAPPA OPIATE RECEPTORS IN RAT SPINAL CORD-DORSAL ROOT GANGLION COCULTURES: REGULATION AND MODULATION OF ADENYLATE CYCLASE B. Attali* and Z. Vogel. Dept of Neurobiology, Weizmann Institute of Science, Rehovot 76100 Israel.

Rat spinal cord-dorsal root ganglion primary cocultures were used as a model system for exploring κ receptor regulation. κ binding sites on cell membranes were labeled by [^{3}H]diprenorphine in the presence of DAGO(0.1μM) and DADL(0.1μM), while μ and δ sites were labeled by [^{3}H]DAGO and [^{3}H]DSTLE, respectively. Non specific binding was determined with 10μM diprenorphine. During the first 10 days in vitro, the number of κ and μ receptors increased markedly, reaching a Bmax of 180 and 50fmoles/mg protein, respectively. In contrast, no detectable δ sites were observed at any time of culture (up to 4 weeks). Binding of [^{3}H]diprenorphine to κ and μ sites was found to be saturable, stereospecific and of high affinity (Kd of 0.85 and 2.4nM, respectively). Chronic agonist treatment (etorphine 1μM, 1 to 3 days or U50488 10μM, 1day) did not down-regulate κ receptors, while it down-regulated more than 50% of μ sites. Following chronic antagonist exposure (naloxone 10μM, 5 days) μ receptors were strongly up-regulated (240% of control), whereas κ sites exhibited a weaker response (160% of control). The affinity of the κ selective opiate agonist U50488 was markedly decreased (5 to 6 fold) in the presence of 100μM GTP and 100mM NaCl, suggesting coupling to adenylate cyclase. Indeed, by using EGTA-free homogenates from 2-3 weeks old cultures, we found that the κ agonist U50488 significantly and dose dependently inhibited the basal as well as the forskolin-stimulated cyclase activity (20% inhibition at 10μM, $p<0.05$). Following chronic exposure of cultured cells to etorphine (1μM, 4 days without wash), the κ agonist U50488 lost its ability to inhibit the basal as well as the forskolin-stimulated cyclase. This desensitization seems to be heterologous since the forskolin-stimulated enzyme was inhibited by carbachol in control but not in chronic etorphine-treated cultures.

In summary, κ receptors are efficiently expressed in rat spinal cord cultures (more than 70% of total opiate receptors) and have properties similar to those displayed by κ sites in adult CNS. Unlike μ receptors, κ sites in spinal cord cultures are less sensitive to modulation by chronic agonist or antagonist treatments. Furthermore, κ receptors are negatively coupled to adenylate cyclase and undergo heterologous desensitization upon chronic opiate treatment, suggesting that tolerance induced by κ ligands is conveyed by post receptor regulatory mechanisms.

279.10 EFFECT OF A KAPPA RECEPTOR AGONIST (U-50,488H) ON STRIATAL DOPAMINE RELEASE AND UPTAKE. D.W. Clow* and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada.

Opioid receptors of the kappa type have been found to exist in the striatum of several species, but their functional significance is not clear. It has been reported that activation of kappa receptors with bremazocine, ketocyclazocine and dynorphin leads to a depression of the spontaneous and K^+-evoked release of ^{3}H-dopamine (^{3}H-DA) from rat striatal slices (Mulder et al., Nature 308: 278, 1984). In the present study, we have investigated the action of a highly selective kappa opioid receptor agonist, U-50,488H on the spontaneous and K^+-evoked (15 mM) release of endogenous DA from slices of rat striatum. Endogenous DA was measured in the presence of nomifensine (10 μM), a DA uptake inhibitor, using a superfusion method. The content of DA in the superfusate was estimated by HPLC coupled to an electrochemical detector after extraction by an alumina/perchloric acid technique. 3,4-Dihydroxybenzylamine was used as the internal standard in this assay. The effect of U-50,488H on ^{3}H-DA uptake was studied in striatal synaptosomes (P_2 fraction).

The spontaneous release of DA was unaffected by 0.4 μM, but was significantly enhanced by 40.0 μM U-50,488H. At concentrations of 0.4 and 40 μM, U-50,488H significantly depressed the K^+-induced release of DA by 17% and 24% respectively. The presynaptic uptake of ^{3}H-DA was unaffected by 0.4 μM U-50,488H, but was significantly inhibited by a 40.0 μM concentration (21.65% of control).

The data suggest that U-50,488H can influence the endogenous release of striatal DA and the uptake of ^{3}H-DA. However, it is not known whether these effects are mediated by kappa receptors. (Supported by the Medical Research Council of Canada and a Fellowship from the Ontario Mental Health Foundation).

279.11 ANTAGONISM OF MORPHINE-INDUCED HYPERACTIVITY IN MICE BY THE KAPPA AGONIST U-50488H. R.G. Browne. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ 07901

Morphine and related opioids have long been known to produce hyperactivity in rodents. Recent work has shown that the diverse pharmacological effects of morphine are mediated by receptor subtypes designated Mu-1 (eg., analgesia) and Mu-2 (eg., respiratory depression). It has yet to be determined, however, whether morphine-induced hyperactivity results from activation of Mu-1 or Mu-2 receptors. Several kappa opiate receptor agonists, including U-50488H, have been shown to have moderate to high affinities for mu and delta sites where they act as mu-2 antagonists. For example, kappa agonists have been shown to antagonize morphine-dependent increases in striatal DOPAC and HVA without effecting morphine-induced changes in acetylcholine turnover or analgesia (Wood, Drug Development Research, 4: 429-435, 1984; Wood and Thakur, Life Sciences, 31:2313-2317, 1982).

The present studies were designed to test the hypothesis that if morphine-induced hyperactivity is mediated by mu-2 receptors, then the kappa agonist U-50488H should block morphine's effects on locomotor activity. Male CD-1 mice (19-22g) were injected i.p. with various doses of the kappa agonist U-50488H, injected s.c. five minutes later with either saline or morphine (10 mg/kg) immediately before placement into individual Omintec Electronic locomotor activity monitors. Locomotor activity, expressed as distance traveled per 2 hrs, was determined for the different treatment groups. The results of these experiments demonstrate that U-50488H antagonized morphine-induced hyperactivity in mice at doses which alone do not differ significantly from vehicle treated mice, indicating that kappa agonists are capable of antagonizing yet another action of morphine. These findings suggest that morphine-induced hyperactivity may be mediated by the mu-2 opiate receptor subtype.

279.12 BREMAZOCINE IS A κ-OPIOID AGONIST AND A μ-OPIOID ANTAGONIST IN THE GUINEA-PIG MYENTERIC PLEXUS. A.D. Corbett* and H.W. Kosterlitz. Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen, AB9 1AS, U.K.

Although widely used as a κ-opioid receptor agonist, the selectivity of bremazocine for the κ-receptor is not high. We have determined the agonist and antagonist activity of bremazocine in naive guinea-pig myenteric plexus preparations and in tissues in which either μ- or κ-opioid receptors were blocked preferentially.

Preparations of the myenteric plexus-longitudinal muscle of the guinea-pig ileum were set up for field stimulation. To block irreversibly the μ-receptors, preparations were incubated in Krebs solution with 100 nM β-funaltrexamine (β-FNA) for 90 min followed by a 90 min wash with drug-free solution. When the opioid alkylating agent β-chlornaltrexamine (β-CNA) was used, preparations were incubated with 30 nM β-CNA for 30 min and then washed; to prevent alkylation of μ-receptors, the selective μ-agonist [D-Ala^2, MePhe^4,Gly-ol^5]enkephalin (10 μM) was added to the organ bath 5 min prior to the addition of β-CNA. Agonist potencies were determined before and after β-FNA or β-CNA treatment.

After pre-incubation of the myenteric plexus with β-FNA, the IC_{50} value of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin was increased 67-fold whereas the IC_{50} values of the selective κ-ligand U-69,593 and of the non-selective ligand bremazocine were not significantly changed. Following pre-incubation with β-FNA the K_e of naloxone against bremazocine was 13 nM. Therefore, in this experimental design bremazocine acted only on κ-receptors.

After pre-incubation with β-CNA and protection of μ-receptors with [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin, the ratio of IC_{50} after treatment to the IC_{50} before treatment was 2 for [D-Ala^2,MePhe^4, Gly-ol^5]enkephalin and was 32 for U-69,593. When bremazocine was the agonist the initial and final IC_{50} values were 0.16 nM and 10 nM, giving a dose-ratio of 62. Under these experimental conditions it was shown that bremazocine is an antagonist against [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin at the μ-receptor (K_e = 1.6 nM). The residual agonist activity of bremazocine (IC_{50}, 10 nM) is at the κ-receptor. In naive myenteric plexus preparations the μ-antagonist activity of bremazocine cannot be demonstrated because it is masked by the potent agonist action at κ-receptors.

279.13 MULTIPLE OPIOID RECEPTOR TYPES IN THE RAT HIPPOCAMPUS. J. F. Neumaier, S. Mallheau*, and C. Chavkin. Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

We have compared the receptor preference of Dynorphin A (1-17) (DYN) to the mu-selective agonists normorphine (NM), and [N-MePhe^3,D-Pro^4]-morphiceptin (PL017), and [D-Ser^2 Leu^5 Thr^6] enkephalin (DSLET), a delta-selective agonist, in the CA1 and dentate regions of the rat hippocampus. Extracellular recordings were made from hippocampal slices, while stimulating either the Schaffer collaterals in the CA1 region or the perforant path in the dentate gyrus. Opioid agonists were applied by superfusion or by pressure micropipette, and opioid antagonists (naloxone or ICI-174864) were applied by superfusion. Dose-response curves were constructed for each opioid in the presence and absence of antagonists. Antagonist K_i was determined by Schild analysis.

All of the opioids increased excitability to orthodromic stimulation and caused afterpotentials in both regions, although in the dentate region, opioids had a greater effect on afterpotentials than on primary population responses. Increased cell excitability induced by NM and PL017 was inhibited by naloxone (10-50 nM) with K_i values ranging from 1-4 nM in both the CA1 and dentate regions; the differences were not statistically significant. These data are consistent with the presence of mu receptors in both regions. In CA1, the DSLET activity was antagonized by naloxone with a K_i value of 14 nM; this is significantly different from the naloxone K_i values for the mu activating drugs NM and PL017 in that region ($p<0.01$). DYN activity was inhibited by naloxone with K_i values of 6 nM in CA1 and 32 nM in the dentate gyrus. Surprisingly, EKC and U50,488h (kappa agonists) were found to be inactive or to have naloxone insensitive effects at concentrations <1 uM in both the CA1 and dentate regions. The naloxone K_i value for DYN in CA1 is not significantly different from that observed for NM activated receptors. The low K_i value suggests that DYN does not act at kappa receptors in the CA1 region. This observation is consistent with our previous determination that DYN acts at mu receptors in CA1 based upon B-funaltrexamine inhibition of DYN and NM but not DADLE activity. In contrast, the naloxone K_i value for DYN in dentate is significantly greater than that for NM in that region ($p<0.03$). The relatively high k_i in the dentate gyrus indicates that DYN acts at non-mu receptors. We found that the delta-specific antagonist ICI-174864 inhibits DSLET but not NM, PL017 or DYN in the dentate region. These data suggest that DYN acts at mu receptors in CA1 and kappa receptors in the dentate gyrus. The presence of kappa receptors in the dentate region would suggest that endogenous DYN synthesized in the dentate gyrus may be released locally to modulate cell excitability.

279.14 OPIOID-INDUCED INHIBITION OF REFLEX URINARY BLADDER CONTRACTIONS IN RATS: SEPARATE CENTRAL MECHANISMS MEDIATE MU AND DELTA LIGAND EFFECTS. Andre Dray, Linda Nunan and Frank Porreca (SPON: M. Wetzel). Sandoz Institute for Medical Research, London, WC1E 6BN, U.K. and Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, U.S.A.

Centrally-administered morphine has been shown to inhibit reflex bladder contractions by actions at separate supraspinal and spinal sites (Dray and Nunan, 1986, J. Physiol. 371, 258P). The present experiments have examined whether selective mu and delta opioid ligands affect reflex bladder activity by similar mechanisms. The ligands chosen for study were highly selective for mu (D-Ala^2, NMPhe^4, Gly-ol)-enkephalin (DAGO) and delta (D-Pen^2, L-Pen^5)-enkephalin (DPLPE) opioid receptors; morphine was studied as a reference mu agonist for comparison with DAGO. We have previously shown that selective kappa opioids such as U50,488H do not affect bladder activity after central administration.

Volume-induced, spontaneous urinary bladder contractions were recorded isometrically in female rats anesthetized with urethane (1.2 g/kg, i.p.). Drugs were administered intracerebroventricularly (i.c.v.) into a lateral ventricle or by spinal intrathecal (i.th.) microinjection between the intravertebral space (L3-L5). Bladder contractions were inhibited in a dose-dependent manner by i.c.v. and i.th. administration of DAGO, DPLPE and morphine. The i.c.v. but not the i.th effects of DPLPE and morphine were attenuated or abolished 4-5 hr after reserpine pretreatment (5 mg/kg, i.p.) and 7 d after pretreatment with 5,7-dihydroxytryptamine (200 ug, i.c.v.). Pretreatment with 6-hydroxydopamine (6-OHDA)(150 ug, i.c.v. at -7 d) did not change the i.c.v. or i.th. effects of DPLPE. In contrast, 6-OHDA pretreatment attenuated the effects of i.c.v., but not i.th., morphine. None of the pretreatments changed the effects of DAGO. Finally, the i.c.v. effect of DPLPE and morphine were attenuated in a dose-related manner by i.th. methysergide (4-10 ug), naloxone (0.5-2 ug) and the selective delta opioid antagonist, ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH, where Aib is alpha-aminoisobutyric acid)(1-3 ug). I.th. phentolamine (5-10 ug) blocked the effects of i.c.v. morphine but failed to affect i.c.v. DPLPE. The effects of i.c.v. DPLPE or morphine were not affected by i.th. propranolol (10 ug), atropine (8 ug), or saline. The i.c.v. effect of DAGO was attenuated by i.th. naloxone and ICI 174,864 but was not affected by the other i.th. antagonists or saline.

The present data suggest that selective mu and delta opioid (DAGO and DPLPE) ligands inhibit bladder contractions by different supraspinal and spinal mechanisms. The i.c.v. effects of morphine are mediated by descending serotonergic and noradrenergic pathways while DPLPE appears to activate a descending serotonergic, but not adrenergic, system. The mechanisms of action of DAGO are unclear, but are clearly and surprisingly different from those of morphine. This discrepancy is difficult to resolve as both ligands are thought to act at mu opioid receptors. Further, these three agonists all appear to activate a spinal, naloxone and ICI 174,864 sensitive system. USPHS Grants NS 21193 and AM 36289.

279.15 CENTRAL EFFECTS OF A CYCLIC SOMATOSTATIN OCTAPEPTIDE ANALOGUE ON REFLEX URINARY BLADDER CONTRACTIONS IN THE ANESTHETIZED RAT. F. Porreca, L. Nunan, W. Kazmierski and V.J. Hruby, Departments of Pharmacology & Chemistry, University of Arizona, Tucson, AZ 85724, U.S.A.

Numerous reports have suggested an interface between somatostatin and the opioid system in mammals. Cyclic analogues of somatostatin have been developed which bind to preferentially to opioid, rather than to somatostatin receptors. Additionally, these analogues have been found to be selective for mu, rather than delta opioid receptors (Pelton et al., Proc. Natl. Acad. Sci. U.S.A. 82: 236-239, 1985). The present work focuses on the pharmacology of one such cyclic analogue of somatostatin octapeptide which has been found to be a mu-selective antagonist in in vitro binding and bioassays. Both agonist, and opioid antagonist, effects of intracerebroventricularly (i.c.v.) given D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2 (termed CTP for the Cys, D-Trp, Pen substitutions) were evaluated using the micturition reflex of anesthetized female rat as the endpoint. Antagonist effects were evaluated against equieffective doses of selective mu (D-Ala^2, NMPhe^4, Gly-ol)-enkephalin (DAGO) and delta (D-Pen^2, D-Pen^5)-enkephalin (DPDPE) opioid agonists; at low i.c.v. doses (0.03 ug), CTP preferentially antagonized DPDPE rather than DAGO. Increasing the dose of i.c.v. CTP further (0.25 ug) effectively antagonized both mu and delta agonists. Selective opioid antagonist doses of CTP (0.03 ug) failed to block the inhibition of the micturition reflex produced by pentobarbital sodium (25 ug, i.c.v.). In order to determine if the observed antagonism was the result of residual somatostatin-like properties of CTP, somatostatin octapeptide was tested as a possible antagonist of equieffective i.c.v. doses of DPDPE and DAGO. Somatostatin (1 & 3 ug, i.c.v.) did not antagonize either the effects of the delta or mu agonist. Increasing the dose of somatostatin octapeptide (10 & 20 ug) resulted in both non-selective opioid antagonist and direct agonist actions of this peptide. Similarly, increases in the dose of CTP (0.25 - 1 ug, i.c.v.) resulted in a direct and dose-related inhibition of the micturition reflex. This agonist action of CTP was not blocked by prior administration of saline, phentolamine, prazosin, yohimbine, idazoxan, propranolol, atropine, naloxone or ICI 174,864. Repeated exposure to CTP resulted in the development of acute tolerance to the agonist effect, and also prevented the inhibition of the reflex by high doses of somatostatin. The converse experiment showed a similar pattern; thus, repeated somatostatin resulted in tolerance and subsequent cross-tolerance to the agonist effects of CTP. In animals tolerant to somatostatin, CTP (0.03 ug) was nevertheless behaved as an opioid antagonist; both DPDPE and DAGO were antagonized in the somatostatin-tolerant rat. These results indicate that while CTP possesses opioid antagonist properties in vivo, it retains residual somatostatin activity at higher doses. Interestingly, CTP shows antagonist selectivity for delta, rather than mu, opioid receptors, demonstrating profile differences in vitro and in vivo. Support: USPHS #NS 21193 & AM 36289.

279.16 **EFFECTS OF 60 HZ ELECTRIC FIELDS ON THE DAILY RHYTHMS OF OPIATE RECEPTORS.** **B.J. Vasquez, L.E. Anderson, C. Lowery*, and W.R. Adey. Loma Linda Univ. and VA Medical Center, Loma Linda, CA 92357, and Battelle Pacific Northwest Laboratories, Richland, WA 99352.**

To detect and quantify the possible effects of electromagnetic fields on the brain circadian rhythms, male albino rats were exposed in a parallel plate rack system for four weeks, 20 hours a day, to a 39 kV/m (effective dose), 60 Hz electric field. Sham animals were housed in a similar but inactivated system. Room lights were on a 12:12 cycle (lights on at 7:00 AM). This was a double-blind experiment with a code to be broken only after all the tissues have been processed. Animals were sacrificed in groups of 6 sham and 6 exposed every four hours throughout the day starting at 6:00 AM. Six brain areas from each animal and four pair of adrenal glands at each time point were dissected. Homogenates of brain stem, posterior and anterior cortices were used to measure opiate receptor binding to [^{3}H]naloxone. Levels of biogenic amines and their acid metabolites were determined in striatum, hypothalamus, hippocampus, and adrenal glands by HPLC-ECD methods . Five to six independent Scatchard plots (one for each animal) were obtained for each time of day, for each region, and then mean binding and dissociation constants were calculated. Since the amount of homogenate from anterior cortex samples was not large enough to do kinetic studies only one concentration of [^{3}H]naloxone was used to assay for specific binding. Two way analysis of variance (ANOVA) tests done on the brain stem data indicated significant changes over time of day for the Kd constant, with higher affinities during the night, but no significant changes in the number (Bmax) of receptors. Opposite effects, circadian changes in Bmax but not in Kd values, were reported (Naber, D. et al., Neurosc. Lett., 21: 45-50, 1981) when using whole rat brain minus cerebellum. No electric field related effects were detected throughout the 24 hour sampling. The receptor binding data from anterior cortex showed significant time of day related differences (ANOVA) in the number of receptors and a borderline ($p=0.056$) effect of electric field exposure. Data from the posterior cortex showed significant time of day by field interaction (ANOVA, $p=0.002$) for both Bmax and Kd values. (Supported by Department of Energy and Southern California Edison).

279.17 **OPIOID-INDUCED NEURONAL FIRING AND SLOW FIELD DEPOLARIZATION IN CA4 AREA DENTATA ARE MEDIATED BY DIFFERENT MECHANISMS AND RECEPTORS.** **A.G. Sadile and A. Cerbone* Inst. of Human Physiology & Med.Physics 1st Med.Sch., Univ.Naples, I-80138, Naples, Italy.**

Endogenous opioid peptides increase single and multiple unitary activity of hippocampal neurons, both in vivo (Zieglgänsberger, W., et al., Science, 205:295, 1979) and in vitro (Gähwiler, B.H., Brain Res., 194:193, 1980), when ionophoretically applied or micropressure injected, by inhibition of inhibitory interneurons (Lee, H.K., et al., Brain Res., 184:331, 1980). Moreover, DC potential shifts have been reported to follow micropressure injection of enkephalins into the hippocampus of acute pentothal anaesthetized preparations (Sprick, U. et al., Brain Res., 210:243, 1981; Sadile, A.G. et al., Behav. Brain Res., 5:116, 1982; Sadile, A.G. and Cerbone, A., In: Front.Neurosci.Series, Raven Press, 1:59, 1984) and freely moving rats (Oitzl, M-S. and Huston, J.P., Brain Res., 308:33, 1984).

In order to investigate whether the opioid-induced short-lasting excitation and longer lasting electrotonic DC potentials are mediated by different mechanisms and receptor types, we studied the slow field depolarization (SFD) monitoring it extracellularly by calomel electrodes from CA_4 and CA_1 hippocampal subregions upon stereotaxic micropressure injections of met-enkephalin or morphine sulphate, as "δ" and "μ" opioid receptor agonists, respectively, (from 1pg up to 1μg in 0.5μl saline) in acute pentothal anaesthetized preparations or after parenteral injection of morphine sulphate (1mg.Kg^{-1}, body wt.) in adult male rats of the Naples.High (NHE) and Low-Excitable (NLE) lines, genetically selected for divergent activity in spatial novelty (Sadile, A.G., et al., Soc.Neurosci.Abstr., 9:643, 1983).

Intra-hippocampal met-enkephalin (1-100pg) elicited slow field depolarization 7.4 and 8.4mV amplitude, 1.2 and 1.0min duration, in CA_4 but not in CA_1 of the NHE and NLE rats, which was naloxone reversible and blocked by 4-aminopyridine. Morphine did not elicit it neither in CA_4 nor in CA_1 in the NLE rats, while it did it only in CA_1 of the NHE rats. Conversely, peripheral morphine elicited SFD both in CA_4 and CA_1 of the NLE but neither in CA_4 nor in CA_1 of NHE rats. Since met-enkephalin elicits both unitary excitation and DC field slow potentials but morphine does not, the latter is unlikely to be an "ephaptic" by-product of the former and the two are proposed to be mediated by different mechanisms. As different seem to be the opioid receptors involved, since activation of both "δ" and "μ" increase unit activity but only the former is associated to DC slow potentials. The prevention of the latter by 4-AP points to a non neuronal involvment in its generation. The differential, genotype-dependent effect of intra-hippocampal and peripheral morphine might be due to the diffusion of extracellular K^+ ion movements to the hippocampus from extra-hippocampal limbic structures.

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280.1 CARBAMAZEPINE AND PHENYTOIN LIMIT RAPID FIRING OF ACTION POTENTIALS OF DORSAL ROOT GANGLION NEURONS IN CELL CULTURE. M. McLean.* (SPON: T. Page). Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212.

Dorsal root ganglion neurons (DRGN) were used to study the mechanism by which anticonvulsant drugs control neuropathic pain. Ganglia were dissected from post-natal and adult mice and were obtained from patients undergoing surgery for chronic pain (with informed consent). After incubation in collagenase (1 µg/ml) containing solution, single DRGN were obtained by trituration and then plated in sterile plastic culture dishes. The neurons were studied using conventional intracellular electrophysiological recording techniques after 3-30 days in vitro.

In phosphate buffer, single action potentials were elicited by long (450 msec) depolarizing current pulses of varied amplitude. The action potentials were Na^+-dependent (abolished by tetrodotoxin). To simulate rapid burst firing observed in human sensory nerves in association with pain (see Törebjork, Phil.Trans.R.Soc. Lond. (Biol.) 308:207,1985), Ca^{++} concentration in the buffer was reduced to 0.1 mM and 0.2 mM Ba^{++} was added. This resulted in sustained firing of action potentials during depolarizations at rates up to 200 Hz. Addition of carbamazepine (CBZ, 2 µg/ml) or phenytoin (PT, 2 µg/ml) limited firing to a few action potentials at the beginning of the pulses as rate increased above 60 Hz. Resting membrane properties were unaffected. Similar limitation was seen in both mouse and human DRGN. The drug concentrations corresponded to therapeutic anticonvulsant levels (free fraction) in plasma. This effect was similar to limitation of firing of action potentials of central neurons in cell culture (see McLean and Macdonald, JPET 227:779,1983). The limitation evolved with the firing of multiple action potentials, hence it was use-dependent. Hyperpolarization restored repetitive firing in some neurons, suggesting voltage-dependence. These effects on DRGN appeared to result from drug effects on voltage-sensitive Na^+ channels. Limitation of rapid firing could explain, at least in part, the success of CBZ and PT in treatment of neuropathic pain.

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280.2 SELECTIVITY OF SEROTONIN AGONISTS (5-HT-1B SUBTYPE) TO ALTER RELEASE OF ^{3}H-5-HT FROM SPINAL CORD SLICES. R.M. Murphy and F.P. Zemlan. Dept. of Physiology, and Division of Geriatrics, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0555.

Our ^{3}H-5-HT binding studies reported at the present meeting indicate the existence of two 5-HT receptor subtypes in spinal cord, i.e. 1A and 1B binding subsites. In the present study we examined the possibility that presynaptic release of ^{3}H-5-HT in spinal tissue might be associated with a particular 5-HT-1 subtype. The compounds tested included the 5-HT-1A selective agonists 8-hydroxy-2 (di-n-propylamino) tetralin (8-OHDPAT) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), and the 5-HT-1B selective agonists 1-(m-chlorophenyl)piperazine (mCPP) and quipazine (QUIP).

Lumbar spinal cord slices (0.4 mm thick) were incubated for 15 min at 37°C in 5 ml Krebs-Ringer buffer containing 200 nM ^{3}H-5-HT. Two spinal cord slices were transferred to each chamber and superfused with oxygenated medium containing 100 nM fluoxetine, at a rate of 0.5 ml/min. Samples were collected at 3 min intervals. After 60 min, the tissue was exposed to elevated potassium (25 mM K^+) for 6 minutes. Drugs (concentrations: 10 pM to 1 uM) were present throughout the last 20 min of basal efflux and during the 6 min exposure to the elevated K^+. The fractional efflux rate was determined as previously described (Eur. J. Pharm. 63: 179, 1982).

None of the drugs tested at any concentration altered basal ^{3}H efflux. K^+ evoked release, with and without drug present, was Ca^{++} dependent. At nanomolar concentrations, the 5-HT-1B selective agonists, mCPP and QUIP, produced a significant 40-70% reduction ($p<.01$) in the K^+ evoked release of ^{3}H-5-HT, similar to the effect on release observed in earlier studies with 5-HT. Picomolar concentrations of mCPP resulted in a dose-dependent decrease in the inhibition of the K^+ evoked ^{3}H-5-HT release; while similar effects of QUIP on release were observed. Neither of the 5-HT-1B selective agonists altered K^+ evoked release at 1 uM. At all concentrations, the 5-HT-1A selective agonists produced no significant ($p>.05$) effect on K^+ evoked release.

The above data suggest that the presynaptic 5-HT autoreceptor is a 5-HT-1B receptor. Comparison of our ^{3}H-5-HT binding data reported at this meeting and the present data indicate similar K_D and IC_{40} values for mCPP and QUIP in binding and release studies. These data suggest that mCPP and QUIP are selective 5-HT-1B agonists which effectively bind to presynaptic 5-HT autoreceptors. (Supported by NINCDS grants NS 07840 and NS 18326).

280.3 SPINAL CORD 5-HT 1A and 1B RECEPTOR SUBTYPES: RELATION TO PAIN TRANSMISSION. G.D. Harris*, F.P. Zemlan, R.M. Murphy and M.M. Behbehani. (SPON: N. Sperelakis) Lab. of Geriatric Research and Dept. of Physiol. and Biophysics, Univ. of Cincinnati College of Medicine, Cinti., OH 45267.

The purpose of the present ^{3}H-5-HT receptor binding studies was to characterize spinal cord 5-HT 1A and 1B receptors and identify subtype specific agonists and antagonists for use in electrophysiological recording and ^{3}H-5-HT release studies.

Preliminary analysis of 8-OH-DPAT and 5-MeODMT competition (10^{-12} to 10^{-3}M) against ^{3}H-5-HT (0.2 to 5 nM, 0.1 mM GTP) suggests that about 35% of spinal cord ^{3}H-5-HT binding sites are of the 1A subtype with a K_D value of 8-OH-DPA of about 1 nM and a 1,000 fold selectivity. Competition studies (10^{-12} to 10^{-3}M) with mCPP and quipazine and subsequent 8-OH-DPAT masking studies indicated the presence of a second ^{3}H-5-HT spinal cord binding site comprising 50% to 70% of 5-HT receptors for which mCPP demonstrated a K_D of about 0.1 nM and quipazine about 1 nM. The K_D values of mCPP and quipazine were similar to the EC_{50} values of these compounds for inhibition of ^{3}H-5-HT release from spinal cord slices also reported at this meeting.

The ability of 1A and 1B selective agonists, identified in the binding studies, to mimic the effect of iontophoretically applied 5-HT on nociceptive dorsal horn neurons was examined in lumbar enlargement. A seven barrel recording electrode was employed which included: 1) 0.5 M NaAc 2% pontamine sky blue, 2) 3M NaCl balancing electrode, 3) NaAc filled pH control electrode, 4) glutamic acid, 5) 5-HT, 6) the 1A selective agonist 5-MeODMT and 7) the 1B selective agonist mCPP. Iontophoretic application of 5-HT (6 to 60 nA) inhibited the response of all dorsal horn units (N=11) to both noxious stimulation and glutamic acid at ejection currents of about 40 nA. Similarly, iontophoretic application of mCPP (6 to 60 nA) inhibited the response of all 11 neurons to both noxious stimulation and glutamic acid at ejection currents of about 10 nA, while 5-MeODMT (6 to 60 nA) was without effect.

These preliminary data suggest that descending 5-HT inhibition of spinal pain transmission may be mediated by 1B 5-HT receptors. Further, the receptor binding, iontophoresis and ^{3}H-5-HT release studies suggest that mCPP is a highly selective 1B 5-HT receptor agonist. (NS18326).

280.4 ANTINOCICEPTION PRODUCED BY ACTIVATION OF CELL BODIES IN THE LATERAL RETICULAR NUCLEUS (LRN) A.J. Janss and G.F. Gebhart, Dept. Pharmacology, University of Iowa, Iowa City, IA 52242.

Spinal α_2-adrenoceptors mediate stimulation-produced antinociception from the LRN (J. Neurosi. 6(1986)107-113). The purpose of this study was to characterize spinal receptors involved in the descending inhibition produced by intracerebral injection of glutamate, which selectively activates cell bodies, in the LRN.

Male Sprague-Dawley rats were surgically prepared (methods described in preceding reference) and lightly anesthetized by i.v. infusion of pentobarbital. Glutamate (100mM) was injected into the LRN and tail flick (TF) latency determined 1, 2, 5 and 10 min after each injection. This procedure was repeated following the intrathecal (i.t.) administration of a pharmacologic antagonist to the level of the lumbar enlargement. The antagonists were injected in a 7.5 or 15 µl volume and cleared by 7.5 µl of vehicle i.t. Glutamate injection in the LRN produced a short-lived (< 5 min) and dose/volume-dependent inhibition of the TF reflex (see table below). Inhibition was attenuated by i.t. yohimbine, phentolamine, and methysergide, but not vehicle (20µl), naloxone (20µg) or prazosin (30µg).

Intrathecal Treatment	TF latency (mean sec ± S.E.M.) before glutamate	after glutamate 100nl	200nl	400nl
Baseline (n=34)	2.5±0.1	3.9±0.3	5.6±0.3	6.5±0.1
Vehicle (n=6)	2.6±0.1	3.3±0.7	6.0±1.0	6.0±0.5
Yohimbine				
15µg (n=11)	2.0±0.1	2.0±0.1*	3.3±0.9*	5.3±0.6
30µg (n=9)	1.7±0.1	1.8±0.4*	2.0±0.3*	1.7±0.1*
Methysergide				
15µg (n=7)	2.1±0.2	2.1±0.3	4.6±0.7	5.6±0.7
30µg (n=7)	1.8±0.1	2.2±0.3	3.0±0.4*	4.0±0.6*
Phentolamine				
30µg (n=5)	2.4±0.2	3.0±0.2	2.7±0.2*	3.5±0.2*

* $p<0.05$ from corresponding baseline TF latency (two-way ANOVA)

Intracerebral glutamate (100mM; 400nl) also elevated TF and hot plate latencies in unanesthetized rats. This inhibition was attenuated by i.t. yohimbine (30µg), and methysergide (30µg), but not naloxone (20µg) or prazosin (30µg).

The results confirm (1) that descending inhibition of nociceptive transmission mediated by cell bodies in the LRN, and (2) involves spinal α_2-adrenoceptors and serotonin receptors. Supported by DA02879 and T32 GM07069.

280.5 POSSIBLE OPIATE, MONOAMINE AND CHOLINERGIC INVOLVEMENT IN MIDBRAIN SUPPRESSION OF A NOCICEPTIVE HINDLIMB FLEXION WITHDRAWAL REFLEX (WR) IN RAT. I.G. Campbell* & E. Carstens (SPON: M.C.L. Wilson). Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Electrical stimulation in the midbrain periaqueductal gray (PAG) or lateral reticular formation (LRF) decreases the force of the WR elicited by noxious hindpaw heating (Soc. Neurosci. Abs. 11:129). To investigate possible transmitters mediating descending WR suppression, we tested whether it was affected following systemic administration of antagonists of opiates (naloxone), serotonin (methysergide), noradrenaline (phentolamine) or acetylcholine (scopolamine).

In rats lightly anesthetized with sodium pentobarbital (5-10 mg/Kg/hr i.v.), a force transducer measured isometric tension during WR evoked by noxious heat stimuli (48-54° C, 10 s/2 min) applied to left glabrous hindpaw skin. When WR was stable, heat trials with PAG or LRF stimulation (100 ms trains at 100 Hz, 3/s, 25-500 uA; 25 s starting 10 s before heat onset) alternated with controls (heat but no brain stimulation). When a constant PAG or LRF current intensity reproducibly suppressed WR by >50%, one of the following was injected via the right femoral vein: naloxone (1-5 mg/kg), methysergide (2-4 mg/kg), phentolamine (0.5-2.5 mg/kg), or scopolamine (0.02-1 mg/kg). Midbrain-evoked suppression of WR was designated to be reduced if the ratio [WR during PAG or LRF stimulation/control WR] increased by >20% post-drug; changes of <20% were designated as no effect.

Naloxone reduced WR suppression evoked from 1/9 PAG and 2/8 LRF sites. Methysergide reduced WR suppression from 4/10 PAG and 5/7 LRF sites. Phentolamine reduced WR suppression from 4/10 PAG and 3/9 LRF sites. Scopolamine reduced WR suppression from 9/10 PAG and 4/6 LRF sites. Effects were apparently not dose-dependent. These variable drug effects are similar to our previous findings with dorsal horn neurons (Soc. Neurosci. Abs. 11:1177). While these data suggest roles for monoamines and particularly acetylcholine in WR modulation, additional studies with intrathecal and intracranial routes of drug administration are needed to specify sites of action.

We additionally began a study of midbrain modulation of noxious heat-evoked responses of single biceps femoris motor units recorded with bipolar single-fiber EMG electrodes. Curiously, responses of 14/19 motor units were suppressed and/or became unstable for prolonged periods (up to 2 hr) following initial trials with PAG or LRF stimulation (25-100 uA). Prolonged suppression of WR force was also observed in 2 of these rats but not in a third. We are continuing these studies to (a) evaluate the incidence of and possible site-specificity for prolonged vs. phasic WR suppression, and (b) parametric analysis of phasic midbrain suppression of single motor units. Supported by NIH grants NS20037 & NS19330.

280.6 EXCITABILITY CHANGES OF PRIMARY AFFERENT TERMINALS OF CUTANEOUS C FIBRES MEDIATED BY α1 ADRENOCEPTORS IN THE FELINE SPINAL CORD. O. Calvillo, M. Ghignone*, J. Madrid*. Dept. of Anesthesiology, Texas Tech University, Lubbock, Tx. 79430 and Instituto Politecnico Nacional, Mexico 14 D.F. Mexico.

We have reported that clonidine selectively caused primary afferent depolarization (PAD) of cutaneous C fibres in the cat spinal cord. This effect was reversed by yohimbine, therefore it probably was mediated by α2 adrenoceptors. (O. Calvillo, M. Ghignone. Neurosci Lett 64: 335-339, 1986). In this investigation we studied the effects of a selective α1 adrenoceptor agonist (ST 587 Boehringer Ingelheim) on the excitability of primary afferent terminals of the sural nerve in the spinal dorsal horn of the cat. Cats were anesthetized with nembutal, the spinal cord was transected at T13-L1. Presynaptic terminal excitability was tested using conventional techniques of intraspinal stimulation (Calvillo, et al. 1982 Neurosci 7, 1389-1400). ST 587 in doses of 4.5 to 22.5 μg/kg i.v. decreased the excitability of A fibre terminals by about 10-15% (n=6), in two other experiments no effect was observed on the excitability of A fibres. In the doses mentioned above ST 587 decreased the excitability of C fibre primary afferent terminals by about 30% in 8 cats, thus signalling primary afferent hyperpolarization (PAH). The effects on both A and C fibre presynaptic fibres were consistently reversed by the α1 antagonist prazozin (10-30 μg/kg i.v.). These results suggest that the excitability of primary afferent terminals of both A and C fibres of cutaneous nerves is susceptible to presynaptic control. The observation that prazozin, a highly selective α1 adrenoceptor antagonist reversed the effects of ST 587, suggests α1 adrenoceptor mediation. It is generally accepted that PAH leads to presynaptic facilitation, and PAD results in presynaptic inhibition. Our previous observations propose that clonidine an α2 adrenoceptor agonist causes PAD of cutaneous C fibres and therefore presynaptic inhibition. It is possible that the noradrenergic innervation of the spinal cord plays a significant role in modulating synaptic input to second order neurones in the dorsal horn, and that the balance between PAD and PAH is an important determinant of synaptic efficacy in spinal nociceptive pathways.

Supported in part by a grant from T.T.U.H.S.C.

280.7 INTERACTIONS BETWEEN INTRATHECALLY-ADMINISTERED ALPHA$_2$-ADRENERGIC AND OPIOID AGONISTS IN RATS. R.E. Solomon and G.F. Gebhart, Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

The α_2-adrenoceptor agonist, clonidine, and the opioid receptor agonist, morphine, share a number of pharmacological actions, including antinociceptive effects following either peripheral or central administration. While clonidine and morphine are believed to exert antinociceptive actions via different receptor systems, reports of cross-tolerance between the two drugs (e.g., Paalzow, N.-S. Arch. Pharmacol., 1, 1978; Yamazaki and Kaneto, Japan. J. Pharmacol., 461, 1985) have suggested that opioid and α_2-adrenergic systems may interact in producing antinociceptive effects. The present study investigated these interactions in rats chronically treated with intrathecal (i.t.) clonidine.

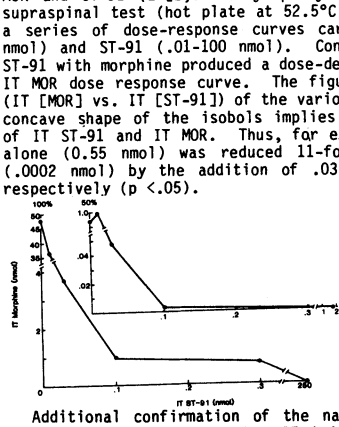
One group of male Sprague-Dawley rats was surgically prepared with femoral arterial and venous catheters and i.t. catheters under deep pentobarbital anesthesia (50.0 mg/kg). A second group was initially prepared with i.t. catheters, and, following daily i.t. administration of clonidine (32.0 μg) for 7 days, had femoral vascular catheters implanted on the eighth day. I.t. catheters were inserted via a slit in the atlanto-occipital membrane and extended to a point just rostral to the lumbar enlargement. Under light pentobarbital anesthesia (3.0 - 6.0 mg/kg/hr; corneal and flexion reflexes present), inhibition of the heat-evoked nociceptive tail-flick (TF) reflex by i.t. clonidine or morphine was studied. Drugs were administered in increasing cumulative doses every six minutes, and TF latencies were determined at one, three and five minutes after drug infusion. All drug injections were in a volume of 7.5 μl, followed by an 8.0 μl saline flush.

Clonidine and morphine produced a dose-dependent inhibition of the TF reflex. In clonidine-naive animals, inhibition of the TF reflex was produced by doses of clonidine between 1.0 and 3.2 μg, whereas a dose of 32.0 μg of clonidine was required to inhibit the TF reflex in chronically clonidine-treated animals. Similarly, whereas doses of morphine from 10.0 - 32.0 μg were effective in inhibiting the TF reflex in clonidine-naive animals, doses of morphine up to 100.0 μg failed to inhibit the TF reflex in animals that had been chronically treated with i.t. clonidine. These results indicate that tolerance develops to the antinociceptive effects of i.t. clonidine, and that chronic i.t. administration of clonidine confers cross-tolerance to i.t. morphine. The additional observation that doses of clonidine required to inhibit the TF reflex in chronically clonidine-treated animals also produced marked elevations in blood pressure suggest, however, that the antinociceptive effects of intrathecally-administered clonidine may not be exerted at spinal sites alone. Supported by T32 GM 07069 and DA 02879.

280.8 SYNERGISTIC INTERACTION OF INTRATHECAL MORPHINE AND AN α_2-AGONIST (ST-91) ON ANTINOCICEPTION IN THE RAT. M.S. Monasky* and T.L. Yaksh* (SPON: J.D. Grabow). Neurosurgical Research Unit, Mayo Clinic, Rochester, MN 55905.

Intrathecal opiates and α_2-adrenergic agonists both exhibit potent antinociceptive (AN) activity which is mediated by separate receptors. There exist data that spinal α-agonists potentiate the effects of morphine (MOR) (Yaksh & Reddy, Anesthesiology 54:451, 1981), but a detailed analysis of this interaction has not been accomplished.

This study examined the AN activity of various combinations of MOR and ST-91 (2-[2,6-diethyl-phenylamino]-2-imidazoline) using a supraspinal test (hot plate at 52.5°C). The analysis was based on a series of dose-response curves carried out with MOR (.001-100 nmol) and ST-91 (.01-100 nmol). Concurrent IT administration of ST-91 with morphine produced a dose-dependent leftward shift of the IT MOR dose response curve. The figure shows the isobol analysis (IT [MOR] vs. IT [ST-91]) of the various dose response curves. The concave shape of the isobols implies a supra-additive interaction of IT ST-91 and IT MOR. Thus, for example, the ED_{50} for morphine alone (0.55 nmol) was reduced 11-fold (.05 nmol) and 2500-fold (.0002 nmol) by the addition of .03 nmol and .3 nmol of ST-91, respectively ($p < .05$).



The isobols represent the various combinations of ST-91 and morphine needed to produce an effect equal to 50% or 100% of the MPE. The isobols are derived from the linear regression plots of the individual dose-response curves of MOR and MOR/ST-91 and represent data from 146 rats.

Additional confirmation of the nature of this interaction was demonstrated by the fact that IT injection of naloxone (30 μg) or phentolamine (30 μg) after IT injection of various combinations of MOR and ST-91 completely abolished the synergistic effect. Equi-analgesic (i.e. MPE) doses of MOR, ST-91, and various combinations of MOR/ST-91 exhibited a similar time of effect (i.e. area under the dose-response curve), suggesting that drug clearance in the spinal space was unaltered.

This functional interaction between receptors which are post-synaptic to local (opioid) and bulbospinal (noradrenergic) systems is compatible with the concept of a supra-additive interaction between spinal and supraspinal modulatory systems (Yaksh & Rudy, Pain 4:299, 1978). (Supported by grant DA02110.)

280.9 CLONIDINE INDUCED ANALGESIA INVOLVES AN OPIOID MECHANISM. L.C.Rovati*, M.Bianchi*, P.Sacerdote* and A.E.Panerai*. Dept. of Pharmacol., Chemother. and Medical Toxicol., School of Medicine, Univ. of Milano, 20129 Milano, Italy.

Clonidine has been shown in several studies to possess an analgesic activity 5-10 times more potent than morphine both in the experimental animal (Skingle et al., Life Sci., 31: 1123, 1982) and in humans (Coombs et al., Anesthesiology, 63: 358, 1985). This analgesic effect of clonidine has been considered to be related to the interaction of the drug with alfa-adrenoceptors, without involvement of the central opioid receptors. Naloxone in fact was not effective in reversing clonidine induced analgesia (Yaksh, Pharm.Bioch. and Behav., 22: 845, 1985). Nevertheless, the pattern of activity shown in the different animals analgesic assays have prompted us to study the possible involvement of kappa opioid receptors in clonidine antinociceptive activity.

In order to verify this hypothesis we explored the ability of the specific kappa antagonist, MR2266 (Boehringer Ingelheim, West Germany), in reversing clonidine induced analgesia in rats. Our results, while confirming the ineffectiveness of naloxone, showed that MR2266 administered prior, concomitantly or after clonidine, significantly inhibited the analgesic effect of clonidine.

These observations suggest the possibility of an involvement of the opioid system, acting through the kappa receptor, in the antinociceptive activity of clonidine.

In order to better investigate this interaction, CNS concentrations of opioid peptides with different specificity for the opioid receptors will be evaluated following clonidine administration in rats.

280.10 INDUCTION OF ANALGESIA AGAINST A MECHANICAL STIMULUS BY INTRATHECAL CLONIDINE IN THE INFANT RAT. Harry E. Hughes* and Gordon A. Barr Biopsychology Program, Hunter College, City University of New York, and Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, New York, 10461.

Convergent lines of evidence suggest that inhibition of dorsal horn afferents and consequent analgesia are in part under the control of descending noradrenergic (NE) pathways in the spinal cord of adult animals. Pharmacological studies indicate that the alpha NE receptor, rather than the beta NE receptor is involved. Recent developmental studies have shown that NE-stimulated cyclic AMP activity, the number and size of spinal NE terminals, and the concentration of NE in the rat spinal cord increase postnatally, to peak near the end of the second week of life. It might therefore be expected that spinally mediated NE induced analgesia would appear at that time. Our lab has demonstrated that intrathecally injected clonidine induced a weak but significant, dose dependent increase in tail-flick latencies against a thermal stimulus in 10 day old rats. That spinal NE induced analgesia occurred more reliably against a mechanical stimulus than a thermal stimulus has been convincingly demonstrated in adult rats (Kuraishi et al., Brain Research, 1985). The present study addressed the contribution of the developing spinal alpha NE receptor in producing analgesia against a mechanical noxious stimulus in the rat.

Long-Evans hooded rats were implanted with intrathecal catheters at 9 days of age. The laminectomised area extended from level T8 to T11 with the catheter tip inserted in a caudal direction within the sub-arachnoid space. Twenty-four hours later, at 10 days of age, pups were injected spinally with 0, 25, 50, or 100 μg in 4 μl of diluent of the alpha NE agonist clonidine HCl. This volume of fluid perfused the cord from the lumbar/sacral segments rostral to the caudal cervical region. Following a 15 minute postinjection period, a 63.75 gram weight with a .2 cm radius was applied sequentially to the forepaw, hindpaw and tail and the latency to withdraw each appendage was recorded. The order of testing of each limb and tail was random.

Clonidine produced significant, dose dependent analgesia as evidenced by increased latencies to withdraw appendages from a mechanical stimulus. Furthermore, the response latencies against a mechanical stimulus were longer than those observed against a thermal stimulus in animals matched for age, drug dose, and baseline levels of responding. Clonidine had a more enhanced effect on withdrawal of the tail than on forepaw and hindpaw. This is consistent with other data that demonstrate developmental differences in the ability of opiates to produce analgesia in the paws compared to the tail. These data suggest that spinal NE circuitry associated with antinociception is functional by at least 10 days of age.

280.11 ANTINOCICEPTION INDUCED BY INTRATHECAL INJECTION OF SUB-EFFECTIVE DOSES OF CLONIDINE AND 5'-N-ETHYLCARBOXAMIDE ADENOSINE. Aran*, S. and Proudfit, H.K. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Intrathecal administration of norepinephrine (NE) or the adenosine analog 5'-N-ethylcarboxamide adenosine (NECA) into the lumbar subarachnoid space of the spinal cord produces dose-dependent antinociception. We recently demonstrated that intrathecal injection of sub-effective doses of NE and NECA interact synergistically to produce potent antinociception. NECA appears to act at adenosine A2 receptors, since 1-phenyl-isopropyl adenosine, a selective adenosine A1 receptor agonist, did not produce antinociception when injected with NE. The noradrenergic receptor at which NE acts to produce antinociception is not known. Therefore, the present studies were undertaken to determine whether alpha-1 or alpha-2 noradrenergic receptors are involved in producing the antinociception induced by NECA and NE. In the first experiment, the capacity of sub-effective doses of clonidine, an alpha-2 noradrenergic agonist, and NECA to induce antinociception was examined. Male Sprague-Dawley derived rats (400-450 gm) were implanted with intrathecal catheters. One week after surgery, animals were tested using the tail flick (TF) and hot plate (HP) tests. Animals were divided into three groups and given the following intrathecal injections: clonidine, 3 ug; NECA, 1 ug; and clonidine and NECA together. These doses were chosen using dose-response curves which were generated previously. Following drug injection, response latencies were determined at fixed intervals for 60-90 min. Neither NE nor NECA produced significant changes in response latencies. However, significant increases in both TF and HP response latencies were observed following the injection of both clonidine and NECA.

Possible synergistic interactions between NECA and the alpha-1 noradrenergic agonist phenylephrine were examined in the second experiment. Each of three groups of animals received one of the following drugs: phenylephrine, 15 ug; NECA, 1 ug; phenylephrine and NECA together. In contrast to the effects of clonidine, phenylephrine produced a significant reduction in mean TF latency and did not produce antinociception when injected with NECA.

The results of these studies suggest that the noradrenergic component of the synergistic interaction between adenosine A2 receptor agonists and adrenergic agonists is mediated by the noradrenergic alpha-2 receptor subtypes. (This work was supported by USPHS Grant DA 03980).

280.12 ROLE OF ASCENDING AND DESCENDING NORADRENALINE PATHWAYS IN THE ANALGESIC ACTION OF BACLOFEN AND CLONIDINE. J. Sawynok and A. Reid*, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Baclofen and clonidine interact with central noradrenergic pathways in a number of ways. Both inhibit firing of neurons in the locus coeruleus (LC) and the release of noradrenaline (NA) from central terminals. Clonidine subsequently decreases NA turnover but baclofen increases NA turnover suggesting an indirect action. In this study, the role of ascending and descending NA pathways was examined by microinjecting 6-hydroxydopamine (6-OHDA) into the dorsal bundle (DB) (ascending), medullary A1 regions or intraspinally (descending) and the LC (ascending and descending). Analgesia was tested 7-14 days post-lesion using the rat tail flick test. The extent of depletion of NA in brain and spinal cord was monitored using HPLC. The analgesic effect of baclofen was inhibited by DB lesions on both day 7 and 14, but analgesia produced by clonidine was only transiently decreased on day 7, returning to normal on day 14. DB lesions decreased NA levels in cortex, hippocampus and hypothalamus but did not alter spinal cord levels. Medullary A1 lesions and intraspinal 6-OHDA inhibited analgesia produced by baclofen but potentiated analgesia produced by clonidine. Spinal cord NA was markedly depleted by both manipulations, but NA in the pons/medulla was only decreased by A1 lesions. LC lesions potentiated baclofen analgesia, did not affect clonidine analgesia, and depleted NA in all brain regions as well as spinal cord.

	DB Lesions	A1 Lesions	i.s.	LC Lesions
Baclofen	↓	↓	↓	↑
Clonidine	↓d 7/↔d 14	↑	↑	↔

These results suggest interactions with centrol NA pathways are important for the analgesic action of baclofen, but the nature of the interactions appears to be complex. Endogenous NA pathways appear less critical for the analgesic action of clonidine. The enhanced effect of clonidine following depletion of spinal cord NA is probably due to supersensitivity of spinal alpha-2 receptors. An important observation from the present study is that there appears to be a significant interaction between ascending and descending NA pathways in relation to analgesic mechanisms, because the effect of depleting NA in spinal pathways can be reversed by the simultaneous depletion of ascending NA pathways. (Supported by MRC Canada).

280.13 BLOCKADE OF MORPHINE ANALGESIA BY YOHIMBINE, AN α_2-NORADRENERGIC ANTAGONIST. S.E. Izenwasser and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Alterations in escape thresholds from aversive electrical stimulation to the mesencephalic reticular formation (MRF) of the rat have been used as indicators of the analgesic efficacy of centrally acting drugs. It has been shown that morphine as well as other opioid analgesics will raise the escape threshold in this pain model. Although, it has been suggested that norepinephrine is one of the transmitter substances which play a role in central analgesic action, its function is unclear. In order to study the contribution of the noradrenergic system to analgesia, the present study was conducted to determine whether yohimbine hydrochloride (YOH), a predominantly α_2-noradrenergic antagonist would change the escape threshold and if it would alter the analgesic effect of morphine.

Bipolar stainless steel electrodes were stereotaxically implanted into the MRF of male CDF rats (Charles River Laboratories). The animals were then trained to turn a wheel manipulandum to escape from electrical stimulation delivered to this brain site. Escape thresholds were determined using a modification of the psychophysical method of limits. YOH by itself, at doses from 0.75-8.0 mg/kg, i.p., increased sensitivity to the stimulation, as evidenced by significantly lowered escape thresholds. Morphine (1-8 mg/kg, s.c.), as in previous studies, caused significant threshold elevations. When administered concomitantly, YOH blocked the effect of morphine even at doses of YOH which by themselves failed to have an effect on threshold. Despite the change in threshold neither YOH alone nor in combination with morphine had any effect on latency to respond, suggesting that the threshold changes were not due to non-specific motor effects but to specific actions on this pain system. These results support the hypothesis that the noradrenergic system maybe involved in the mediation of central analgesia.

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280.14 INVOLVEMENT OF ADENOSINE IN THE ANALGESIC EFFECT OF MORPHINE AND NORADRENALINE IN THE SPINAL CORD. M.I. Sweeney*, T. White, and J. Sawynok, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Morphine and noradrenaline (NA) produce marked analgesia following intrathecal (i.t.) injection. There is evidence for both pre- and post-synaptic effects of both agents within the spinal cord, but endogenous mediators of their actions have not been fully characterized. Intrathecal injection of adenosine receptor agonists also produces analgesia which is blocked by i.t. pretreatment with the adenosine receptor antagonists theophylline (TH) and 8-phenyltheophylline (8PT) (Sawynok et al., Br. J. Pharmacol. 1986, in press). Theophylline has also been shown to reduce the analgesic effect of i.t. morphine in the tail flick test (N.S. Arch. Pharmacol. 1984, 327:23) suggesting a potential role for adenosine in morphine analgesia in the spinal cord. In the present study, we set out to examine directly whether adenosine or its receptors were involved in the spinal analgesic effect of morphine and NA. Initially, the effect of i.t. morphine in the tail flick test and hot plate tests, but did not alter the effect of NA in either test. 8PT was approximately 10 times more potent than TH in reducing the effect of morphine which is comparable to their relative potency as adenosine receptor antagonists. Subsequently, we determined the effect of morphine and NA on the release of endogenous adenosine from synaptosomes prepared from the dorsal half of the spinal cord. Extrasynaptosomal adenosine accumulation (release) was measured using HPLC and was detected as fluorescence of the etheno derivative (J. Neurochem. 1985, 45:791). Both morphine and NA increased the release of adenosine from dorsal spinal cord synaptosomes in a calcium-dependent manner. The increase was dose-dependent following 1, 10 and 100 μM morphine and 5, 50 and 500 μM NA, and the extent of the increase was comparable to that produced by a maximally effective dose of K^+ (25 mM). Morphine-evoked adenosine release was blocked by 10 μM naloxone and 1 μM naltrexone, while NA-evoked accumulation was blocked by 10 μM phentolamine implying that the effects by both agents were receptor-mediated. The nature of adenosine released by morphine and NA was characterized using a 5'-nucleotidase inhibitor cocktail (50 μM α,β-methylene ADP with 5 mM GMP). Morphine-evoked adenosine release was not affected by the inhibitors suggesting adenosine as such was being released, but more than 90% of the NA-evoked release was blocked by the inhibitors, suggesting that in this case, apparent adenosine release was due to the extrasynaptosomal breakdown of a nucleotide (possibly ATP). These results suggest that the release of adenosine in the dorsal spinal cord may play a role in the expression of spinal analgesia by morphine but not NA. (Supported by MRC Canada grants to J.S. and T.D.W.)

280.15 McN-5195: A NOVEL, CENTRALLY ACTING, NON-OPIATE LIKE, NON-NSAID-LIKE ANALGESIC. J.L. Vaught[1]*, R. Scott[1]*, J. Carson[2]*, R. Carmosin[2]*, R. Shank[1], D. End[1]*, H.I. Jacoby[1]*, and J. Gardocki[1]*. (SPON: P. Setler). Depts. Biol.[1] and Chem.[2] Research, McNeil Pharmaceutical, Spring House, PA 19477.

McN-5195 [(+)-trans-3-(2-bromophenyl)octahydroindolizine] exhibits analgesic activity in mouse, rat and monkey. In the mouse, McN-5195 has analgesic ED_{50} values in writhing (p.o.), tail flick (i.p.), tail pinch (i.p.) and hot plate (i.p., 48°C) tests of 20.1 (14.3-27.9), 33.9 (20.9-49.2), 38.2 (24.9-111.1) and 30.9 (20.8-64.7) mg/kg respectively. In the rat, McN-5195 has analgesic ED_{50} values in writhing (p.o.), tail flick (i.p.) and hot plate (i.p.) tests of 33.2 (14.8-127.8), 33.2 (14.7-127.3) and 33.3 (14.4-73.4) mg/kg, respectively. Analgesic activity is rapid in onset and of long duration. McN-5195 is equipotent to codeine, propoxyphene, pentazocine and slightly more potent than nefopam (a novel non-NSAID-, non-opiate-like analgesic). McN-5195 was also orally active in the monkey tooth pulp test (ED_{50}~30 mg/kg). In mouse and rat, McN-5195-induced analgesia is naloxone-insensitive and is not diminished in morphine tolerant animals. McN-5195 does not bind to opioid receptors nor does it inhibit the synthesis (in vitro) of PGE_2, TxB_2 or inhibit ^3H- arachidonate metabolism via the lipoxygenase pathway. Chronic (120 mg/kg/3x per day/10 days) administration does not produce tolerance to the analgesic effects of McN-5195. These data indicate that McN-5195 is not acting via opioid or inhibition of arachidonate systems, and is unlikely to produce tolerance following prolonged administration. It does not bind to $5\text{-}HT_1$, $5\text{-}HT_2$, D_2, D_1, α_1, α_2, β or GABA A receptors and does not inhibit the synaptosomal uptake of NE, 5-HT, DA or GABA. Consistent with these neurochemical data, McN-5195 analgesia is not affected by reserpine, haloperidol or yohimbine pretreatment. McN-5195 produces analgesia following its intracerebroventricular administration to mice or rats (i.p./i.c.v. ratio ≅ 3) but is inactive intrathecally. McN-5195 is devoid of acute gastric irritating properties in rats (consistent with its non-NSAID-like pharmacological profile) and does not effect mouse colonic propulsive motility (indicating a lack of potential constipative activity). Thus, McN-5195 has mechanistically unique and potentially broad analgesic activity. The analgesic profile suggests that its therapeutic usefulness would extend to clinical situations insufficiently managed by non-narcotics and in which there is concern about the use of narcotics.

280.16 THE EFFECT OF McN-5195 ON ACTIVITY IN THE VENTRAL POSTEROLATERAL NUCLEUS (VPL) OF THE RAT IN RESPONSE TO NOXIOUS AND NON-NOXIOUS SOMATIC STIMULATION. P.S. Blum. Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477.

McN-5195 [(+)-trans-3-(2-bromophenyl)octahydroindolizine] exhibits naloxone-insensitive analgesic activity using classical behavioral tests. Activity in these tests resembles the activity of the centrally acting narcotic analgesics and suggests that McN-5195 produces analgesia by inhibiting activity in the central pain-transmission pathway (albeit not via an opioid process). This hypothesis was tested by recording any changes in the response of thalamic neurons to somatic stimuli following administration of McN-5195 and other analgesics. Adult rats were anesthetized with urethane (1400 mg/kg, i.p.), and prepared for multiunit neuronal recording in VPL using standard techniques. Three types of natural stimuli were applied to the contralateral hindpaw (CHP). These stimuli included a) brushing the CHP to activate rapidly adapting mechanoreceptors (brush stimulus); b) resting a brass rod (temperature=21-24°C) on the CHP to activate slowly adapting mechanoreceptors (room temperature probe, RTP); and c) resting a brass rod (temperature=55-57°C) on the CHP to activate slowly adapting mechanoreceptors and nociceptors (heated probe; HP). Stimuli were applied every 4 minutes and baseline activity and activity evoked by brush, RTP, and HP stimuli were calculated. Nociceptor-evoked activity was defined as HP-evoked activity minus RTP-evoked activity. After a predrug period of 14-26 minutes, animals were given either: a) saline; b) McN-5195-11 (75 mg/kg, i.p.); c) morphine sulfate (MS;1 mg/kg, i.v.); or d) zomepirac sodium (ZOM;10 mg/kg, i.v.). ZOM is a peripherally acting NSAID analgesic. There were no significant changes in the activity produced by brush and RTP stimuli during the predrug period and for 140 minutes following administration of any drug. In addition, there were no significant changes in nociceptor-evoked activity following administration of saline or ZOM. There was, however, a significant reduction in nociceptor-evoked activity following administration of MS or McN-5195. These effects were reversible and had a time of onset and duration of action of 5 minutes and 20 minutes for MS and 25 minutes and 60 minutes for McN-5195. The time course for the effects of these drugs on sensory transmission is similar to that seen following their administration in unanesthetized animals during behavioral testing. The fact that the effect of McN-5195 on sensory transmission was qualitatively similar to that produced by MS is consistent with its in vivo analgesic profile. Thus, these data support our hypothesis that McN-5195 inhibits activity in the central pain transmission pathway.

280.17 STABILITY AND EFFECTS OF SYSTEMIC NALOXONE AND SCOPOLAMINE ON RAT MIDBRAIN STIMULATION PRODUCED ANALGESIA (SPA). D.S. Klatt*, M.J. Guinan*, E. Carstens and L.R. Watkins (SPON: P.A. Pappone). Dept. of Animal Physiol., U. of CA, Davis, CA, 95616. U.S.A.

The role of acetylcholine (Ach) and opiates in analgesia produced from midbrain sites is controversial. The purpose of the present study was to determine the relative involvement of these 2 neurotransmitters in SPA elicited from the dorsal raphe region. Since stability of the threshold current (TC) from such midbrain sites in SPA is in question (Neuro. Sci. Abs. 11:638, 85), constancy of TC was also examined.

To date, SPA has been tested in 34 adult male Sprague-Dawley rats implanted with bipolar electrodes in the midbrain near or in the dorsal raphe nucleus. Before each weekly test session the rats were habituated to the restrainers in 3, daily 1.5 hr periods. At the begining of each session and again prior to each TC test 3 baseline tail flick latencies (without stimulation) were recorded. Stimulation consisted of 20 Hz biphasic 50us square waves separated by 0.1ms. TCs were determined using an ascending method of limits (0.2-16mA peak-to-peak). Stimulation began 10 seconds prior to and continued through each tail flick trial. One half hour after the initial TC was found, it was retested. If the TC was ineffective, the current was increased in 0.2mA increments until a new TC was found. Once the effective TC was determined, naloxone (10mg/kg) counter balanced by saline (the first 2 wks) or scopolamine (10mg/kg) counter balanced by saline (the second two weeks) was administered i.p. on a double blind basis. At 26 min, rats were tested for analgesia at the predrug TC. After the four weeks of testing, electrode placements were determined histologically.

Of the 34 rats tested, 28 showed SPA on week 1, but only 20 showed stable analgesia across the 4 week test regimen. The attrition occured due to increased motor activity (n=4), loss of analgesia (n=2), and death (n=2).

Effects of naloxone and scopolamine have been tested at 24 and 21 sites, respectively. Evaluation of present naloxone data is inconclusive, leaving the role of the dorsal raphe and subjacent tegmentum in opiate SPA unclear. However, scopolamine significantly reduced baseline tail flick latencies but had no significant effect on SPA. From these results we conclude that Ach is not a component of SPA in the dorsal raphe and the subjacent tegmentum.

Combining the results of 4 wks of testing, TCs of 73/94 (78%) trials were stable 1/2 hour later. These results indicate that the SPA threshold is inherently a labile phenomenon and this variability must be considered when interpreting the results of such studies.

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280.18 EFFECT OF VASOPRESSIN ON THE RESPONSE OF SPINAL CORD DORSAL HORN CELLS TO NOXIOUS HEAT. C.L. Thurston, E.S. Culhane*, E. Carstens, and L.R. Watkins. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616 USA

Intrathecal (IT) injection of vasopressin (VAS) at the lumbosacral enlargement produces a prolonged decrease in responsiveness to noxious radiant heat (tail flick test) in the awake rat and in the awake spinalized rat suggesting that VAS might be blocking nociceptive transmission at the spinal level. The purpose of this experiment was to examine the effects of VAS on the responsiveness of spinal cord dorsal horn cells to noxious heat.

The lumbosacral enlargement was exposed by laminectomy in pentobarbital anesthetized rats (65 mg/Kg IP, supplemented with 10 mg/Kg/min IV). Each rat was placed in a stereotaxic frame with vertebral clamps & an agar pool was constructed around the enlargement so drug could be topically applied, mimicking the IT injection protocol used in prior behavioral studies. Femoral arterial blood pressure (BP) was monitored because IT injection of VAS can increase BP. Tungsten microelectrodes were used to record dorsal horn neuronal responses (total # of impulses/10 sec) to noxious heat stimuli (48-54°C, 10 sec/2min) applied to the ventral surface of the hind paw. Stable heat trials (+/-10%) performed with saline in the pool were obtained prior to application of drug. Saline was then removed & VAS (0, 0.0025, 0.25, or 25 ng) dissolved in 100 ul saline was placed in the pool for a minimum of 30 min. VAS was then removed, the cord rinsed with saline, & saline replaced into the pool.

To date, the data indicate that VAS usually (n=12) has a prolonged inhibitory effect (<=50% baseline) on dorsal horn cells' responses to heat, but occasionally (n=3) has an excitatory effect (>=150% baseline). Application of 0.0025 ng VAS (n=10) inhibited 4 cells within 15 min after application, inhibited 1 cell after 35 min, and excited 1 cell within 5 minutes. With 0.25 ng VAS (n=5), 3 cells were inhibited starting 30 to 60 min after application and 1 was immediately excited. With 25 ng VAS (n=6), 4 cells were inhibited starting 30 to 60 min after application and 1 was excited after 40 min. In the control group (n=8), response variability generally increased, but only 1 cell showed a significant decrease in response. In cells that were inhibited by application of VAS, the responses to non-noxious stimuli were usually still present suggesting a specific action for VAS. VAS had no effect on BP.

These preliminary data suggest that VAS may be a modulator of spinal cord nociceptive transmission. Studies are currently under way looking both at identified spinothalamic tract cells and testing if there is a dose response effect of VAS.

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280.19 HYPOTHALAMIC STIMULATION (HS) INHIBITS SPINAL NOCICEPTIVE TRANSMISSION BUT NOT THE TAIL FLICK REFLEX IN RAT. E. S. Culhane* and E. Carstens (SPON: L. R. Watkins). Dept. of Animal Physiol., UC Davis, Davis, CA 95616 USA.

Electrical stimulation of the rat hypothalamus inhibits spinal dorsal horn neuronal responses to noxious heating of the skin (Neurosci. Abs. 11:635, 1985). The present study compared behavioral and electrophysiological effects of HS in the same animals to determine whether HS effects on a behavioral measure of pain (tail flick test = TF) were strongly correlated with HS effects on an electrophysiological measure of pain (evoked response to noxious heat).

Male Sprague Dawley rats (N=52) were implanted with a bipolar electrode in histologically verified medial (periventricular) hypothalamic sites. At 2 & 3 wk after implantation, a method of ascending limits was used to determine the threshold current for TF suppression from baseline (≈3 s) to a cutoff of 8 s. HS (100 ms trains at 100 Hz, 3/s, 100-1100 uA) began 10 s before the TF trial and terminated at the end of the trial. Any rat showing replicable TF suppression at 2 & 3 wk was tested again at 4 & 5 wk to test reliability of TF suppression. Some of the rats (24/52) were then used to electrophysiologically test responses of 27 lumbosacral dorsal horn units to skin heating (50-54°C, 10 s/2 min) of the hindfoot pad. Tungsten microelectrodes were used to record heat responses of single units in pentobarbital anesthetized (10mg/Kg/hr) rats both during and in the absence of HS. Stimulation parameters were identical to those used behaviorally except HS lasted a total of 25 s beginning 10 s before heat application.

Behaviorally, only 8/52 rats showed HS induced TF suppression in at least one test session, and only 3/8 showed reproducible TF suppression across test sessions. The other 44 rats had either no TF suppression at the highest HS intensity (N=23, 1100uA) or intense motor activity which disallowed reliable TF testing (N=21). To date, 19/27 dorsal horn units tested were reliably inhibited by HS to at least 50% of their control response. In 20 animals showing no TF suppression, 17/22 dorsal horn units were inhibited by HS. In 4 animals showing TF suppression, 2/5 units were inhibited by HS. There was no correlation between the severity of motor activity during behavioral testing and the degree of unit inhibition.

These data suggest that although HS causes inhibition of single unit nociceptive transmission in the majority of cases, it does not cause reliable TF suppression in unanesthetized rats. These data bring into question the often-held assumption that stimulation-induced suppression of nociceptive units implies behavioral analgesia.

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280.20 DIFFERENTIAL EFFECTS OF LATERAL HYPOTHALAMIC INJECTIONS OF LIDOCAINE ON DIFFERENT PAIN TESTS. R.A.R. Tasker*, M. Choinière*, and R. Melzack*. (SPON: N.M. White) Dept of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1

Several investigations have shown that diencephalic brain regions play a role in pain perception. Hypothalamic involvement is not surprising because this region (1) is known to contain many endogeneous compounds which may be involved in pain perception, (2) receives sensory inputs from both spinal cord and brainstems regions, and (3) contains neurons that project to other brain areas involved in pain and analgesia. The precise role of the hypothalamus in pain mechanisms is, however, unclear. Furthermore, many reports from both our own and other laboratories have shown that pain mechanisms in tests of continuous, moderate pain are both anatomically and pharmacologically dissociable from those involved in tests of brief, rapidly-rising pain. The objective of the current study was therefore to use injections of local anesthetic into the lateral hypothalamus (LH) to determine the role of this structure in the perception of both continuous and brief pain.

Male Long-Evans rats (250-350g) were implanted unilaterally with guide cannulae in various regions of the LH. Infusions (1 μl) of 4% lidocaine in sterile saline or of vehicle alone were made over 2 minutes via inner cannulae connected to a microlitre syringe. Animals were tested for foot-flick latencies and formalin-induced pain scores at 5 and 10 minute intervals for 60 minutes, timed from the end of the injection. Foot-flick latencies for both hindpaws were determined in hand-held rats by immersing each paw in water at 49°C for a maximum of 10 sec. Formalin pain scores were assessed following s.c. injection of 0.05 ml of 2.5% formalin into either hindpaw of rats habituated to the test box. Testing began 30 minutes after formalin injection and used criteria as described by Dubuisson and Dennis (Pain, 4, 1977).

Infusion of lidocaine into histologically-verified sites within the LH resulted in reductions of between 50% and 100% in mean formalin pain scores relative to saline controls in all animals tested, but had no effect in the foot-flick test. No difference between the ipsilateral and contralateral hindpaw was observed in either test and no behavioral effects indicating aversiveness were noted. Similarly, animals receiving lidocaine in the LH showed no signs of motor impairment. Rats whose cannulae were found to be either medial or posterior to the LH failed to show analgesia in either pain test.

These results suggest an involvement of the LH in pain perception and further support the hypothesis that different mechanisms subserve continuous as opposed to brief, rapidly-rising pain. Investigations of the effects of lidocaine infusion into other diencephalic sites and further characterizations of LH involvement in formalin-induced pain are currently in progress. Supported by NSERC Grant A7891.

281.1 RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE IS SELECTIVELY IMPAIRED IN BULBOSPINAL SEROTONIN AXONS DURING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS-INDUCED PARAPLEGIA. S.R. White and R.M. Bowker. (SPON: D.M. Fragaszy) Dept. of Vet. Comp. Anat., Pharmacol., Physiol., Wash. State Univ., Pullman, WA 99164.

Bulbospinal axons stained for serotonin-like immunoreactivity (SIR) appear to become grossly swollen and distorted as they course near inflammatory foci in the ventral and lateral funiculi of the spinal cord of rats in the acute paraplegic stage of experimental allergic encephalomyelitis (EAE) (White et al., Neurosci., 16: 701-709, 1985). In addition, terminal plexuses in the spinal cord gray matter appear to become depleted of SIR during this stage of the disease, suggesting that axoplasmic transport of transmitter to the terminals may be impaired. The present study examined the ability of bulbospinal serotonin-containing axons to retrogradely transport horseradish peroxidase (HRP) from terminals in the lumbar spinal cord to cell bodies in the medulla oblongata.

Lewis rats, paraplegic with EAE, and control rats were anesthetized with methoxyflurane, and HRP was injected bilaterally into the ventral lumbar spinal cord. Two days later, the animals were perfused with saline followed by fixative and the brainstems were removed, frozen and sectioned (25 μm thick sections). Brainstem sections were reacted for HRP (black granules) and then processed for SIR (pale golden brown stain) according to the methods of Bowker et al. (Brain Res. 1983, 288: 33-48). Cell bodies positive for SIR were numerous in the medulla of EAE rats and did not differ in number or morphology from those of control rats. The locations of SIR perikarya were also similar in EAE and control rats with one exception. A ventrolateral group of cells near the nucleus ambiguus region which did not stain for SIR in the control rats was positive for SIR in the EAE group.

There was a striking decrease in medullary cells which were double-labeled for both SIR and HRP in EAE rats compared to controls. Most (>80%) SIR cells were double labeled in the control rats whereas very few (<12%) were double labeled in the EAE rats (the aberrant ventrolateral group of SIR cells in the EAE rats was not included in the calculations). Although most SIR axons did not retrogradely transport HRP in the EAE rats, many other cells that did not contain serotonin were retrogradely labeled in these animals. HRP granules were found in nonserotonin cells, both in the raphe region and in other regions of sections from EAE rats. This finding suggests that retrograde transport is impaired in bulbospinal serotonin axons during EAE but is not impaired in many nonserotonergic bulbospinal axons. Supported by the WSU Research and Arts Committee and NS 22321).

281.2 RADIOIMMUNOCYTOCHEMICAL ASSESSMENT OF REGIONAL CHANGES IN SUBSTANCE P OF RAT SUBSTANTIA NIGRA IN RESPONSE TO CHRONIC HALOPERIDOL OR SCH-23390. C.J. Cruz* and R.M. Beckstead. Dept. Neuroscience, Univ. of Virginia, Charlottesville, VA 22908.

Substance P is a putative transmitter in striatifugal pathways to substantia nigra pars reticulata (SNR) and entopeduncular nucleus. Radioimmunoassays of microdissected tissue have shown declines of 20-40% in whole nigral SP immunoreactivity (SPI) following chronic treatment of rats with the dopamine antagonist haloperidol. The small size of the rodent SNR, however, makes it difficult to resolve regional immunoreactivity changes within SNR using these techniques. We have combined radioimmunocytochemistry and quantitative autoradiography in a method which reliably detects drug induced SPI changes in discrete nigral regions as small as 100 um^2.

Twelve rats received daily injections of either haldol (2mg/kg ip; n=6), the D1 antagonist SCH-23390 (1mg/kg sc; n=5), or vehicle (n=11) for 21 days. Animals were perfused 24h after the final treatment, and the brains were processed for SP radioimmunocytochemistry using [^{3}H]-biotin (NEN, 35 Ci/mol). Preliminary work suggests that the amount of biotin bound in this procedure correlates well (r=.96) with SP concentrations measured by radioimmunoassay. LKB Ultrofilm was apposed to slide-mounted sections in film cassettes and the resulting images were analyzed with a Leitz microdensitometer. Optical densities were collected from medial, middle dorsal, middle ventral, and lateral nigral regions in coronal sections at regular intervals through the rostrocaudal extent of SN. Comparison of drug-treated to control rats revealed: 1)SPI (pmol biotin/mg tissue equivalent) was distributed heterogeneously and the SP concentration measured depended on both the region and the rostrocaudal level examined; 2)SPI averaged across all 4 regions increased linearly from the rostral pole to the caudal pole of SN in both groups; 3)whole nigral SPI was significantly lower (23%) in the haldol group and significantly greater in the SCH-23390 group than in controls (neither drug affected the SP content of the interpeduncular nucleus, superior colliculus, central gray, or arcuate nucleus of the hypothalamus); 4)the change in SPI measured after chronic haldol or SCH-23390 was not homogeneous but depended on the region of SN examined (haldol-induced loss of nigral SP was greater in the middle SN than at either the medial or lateral extremes). Regional heterogeneity of haldol-induced loss of nigral SP may be significant in that the regions displaying the smallest decreases (medial and lateral) appear to correspond to those areas where SPI is preserved following an extensive ipsilateral ibotenic acid lesion of the striatum. We conclude that the haldol and SCH-23390 induced changes in nigral SP are nonuniform and that quantitative radioimmunocytochemistry is an appropriate tool for measuring regional differences in the response of antigen levels to drugs.

Supported by NSF grant BNS-8504438.

281.3 IN SITU HYBRIDIZATION HISTOCHEMISTRY IN THE RAT BRAIN AND ADRENAL MEDULLA USING A PROBE FOR TYROSINE HYDROXYLASE mRNA. M. Schalling*[1], T. Hokfelt[1], M. Goldstein[2], D. Filer[2], C. Yamin[2], D.H. Schlesinger[2] and J. Mallet.[3] (SPON: R. Margolis). [1]Department of Histology, Karolinska Institute, Stockholm, Sweden, [2]Department of Psychiatry, New York University Medical Center, New York, USA, and [3]Laboratory of Cellular Neurobiology, Gif-sur-Yvette, France.

We have examined the content of tyrosine hydroxylase (TH)-mRNA and its products in cells in the rat brain and adrenal medulla. Retrograde tracing with Fast Blue was used together with hybridization histochemistry and immunohistochemistry to establish projections. Single stranded RNA probes were produced by in vitro transcription using the plasmid pGEM-1 from Promega Biotec and polymerases SP6 and T7. Sense mRNA for TH was used as a negative control. Strong labelling was seen over cell bodies in the zona compacta of the substantia nigra and the ventral tegmental area. Staining of such sections for indirect immunofluorescence with TH antiserum revealed a distinct labeling of cell bodies in these areas with high correlation between grain labeled cells and TH-positive cells. After injection of Fast Blue into the corpus of the caudate nucleus, numerous blue fluorescent cells were observed in the ventral mesencephalon. These cells also contained TH-immunoreactivity and were covered by grains indicating presence of TH-mRNA. Thus, projections of cell bodies identified with hybridization histochemistry can be mapped by simultaneous application of retrograde tracing methods. These studies were supported by grants NINCDS 06801, MH 35976 and Swedish Research Council 04X 2887.

281.4 LOCALIZATION OF CHROMOGRANIN A mRNA IN NEUROENDOCRINE CELLS BY *IN SITU* HYBRIDIZATION HISTOCHEMISTRY. R. E. Siegel.* A.L. Iacangelo.* and L.E. Eiden* (SPON: S. C. Landis). Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

Chromogranin A is a soluble protein located within secretory vesicles of many neuronal and neuroendocrine cells. This protein is highly acidic and migrates with an apparent molecular weight of 75kD on polyacrylamide gels. Chromogranin A-like immunoreactivity is widely distributed in neuroendocrine tissues, but in many cases the identity of the cells synthesizing this protein is unknown. In recent work from this laboratory, a cDNA encoding bovine chromogranin A has been isolated and sequenced (Iacangelo et al., these Abstracts). Using this information, we have performed in situ hybridization histochemistry to locate cells containing chromogranin A mRNA.

In situ hybridization histochemistry was performed on sections of bovine tissue. The tissues were rapidly removed, cut into 2-3mm slices, and then frozen at -70°C until use. Prior to hybridization, 12μ frozen sections were cut and fixed in 4% formaldehyde in 0.1M phosphate buffer. Hybridizations were performed using four different ^{35}S-labeled complementary RNA (cRNA) probes. These probes were complementary to internal or terminal sequences of the bovine chromogranin A coding region and ranged in length from 300-700 bases. Following hybridization, the tissues were washed, dipped into NTB 3 liquid emulsion, and exposed 2-14 days for autoradiography.

The distribution of chromogranin A mRNA was examined in several bovine tissues, including the adrenal and parathyroid glands, pituitary, and brain. These regions have all been shown to contain the chromogranin A mRNA by Northern blot analysis. In the adrenal gland, grains were observed over the medulla but were absent from the cortex. The distribution of grains over the medulla was uneven. Most cells located at the periphery of the medulla were heavily labeled while a lower density of autoradiographic grains was detected over cells in the central region. A similar pattern of hybridization was observed with all four cRNA probes and only backround levels of grains were found when a message-sense probe was used. In sections of the parathyroid gland, a tissue rich in chromogranin A mRNA, signal was detected in most of the parenchymal cells. In the caudate region of the brain, where chromogranin A mRNA is less abundant, only scattered labeled cells were observed.

Studies using both in situ hybridization and immunohistochemistry are being performed to examine the colocalization of chromogranin A with the catecholamines and enkephalin. By determining which neurotransmitters and neuropeptides are contained in chromogranin A-synthesizing cells, we hope to shed light on the function of this protein.

281.5 SIMULTANEOUS DEMONSTRATION OF PEPTIDERGIC OR CATECHOLAMINERGIC IMMUNOCYTOCHEMISTRY WITH ACETYLCHOLINESTERASE HISTOCHEMISTRY IN THE RODENT BRAIN. F.F. Marciano*, J.H. Kordower, and D.M. Gash. (Spon. Y. Chen.) Dept. of Neurobiology and Anatomy, Univ. of Rochester ., Rochester N.Y., 14642.

Acetylcholinesterase (ACHE) containing neurons and fibers have been shown to be located within or near some catecholaminergic and peptidergic brain regions and may in some way modulate their activity. It would be clearly advantageous to visualize both substances simultaneously in order to gain a better understanding of the morphological interactions of these neurons. The present study was designed to develop a technique which combines both immunocytochemical and histochemical methods for the simultaneous visualization of peptidergic or catecholaminergic neurons with acetylcholinesterase containing neurons. The regions investigated were the hypothalamic neurosecretory system and hypothalamic, midbrain and brainstem catecholaminergic loci.

Adult male Long Evans rats (n=10) were anesthetized and perfused with 0.9% saline followed by a 4% paraformaldehyde, .025% glutaraldehyde in picric acid fixative. The brains were removed and post-fixed in a 30% sucrose solution until sunk. Frozen fifty micron sections were collected into cryoprotective solution and stored in the freezer until processed. Alternate sections were reacted immunocytochemically with antibodies directed against vasopressin (AVP; 1:2000), neurophysin (NP; 1:12000) and tyrosine hydroxylase (TH; 1:1000). Following the appropriate incubation, the tissue was reacted using the Avidin-Biotin-Peroxidase complex. The chromagens used were either 3,3-diaminobenzidine (DAB; 05%) or a DAB solution containing cobalt chloride and nickel ammonium sulfate with .01% or .005% hydrogen peroxide respectively. Immediately thereafter, the sections processed for ACHE according to the procedure of Genser-Jensen and Blacksted (1971). The tissue was incubated for 3h prior to reaction in potassium ferricyanide for 3.5 min. To assess the specificity of these reactions, control procedures were carried out with the following modifications for each transmitter/ACHE combination: 1) substitution of phosphate buffer for the primary antiserum; 2) substitution of PBS for the secondary antiserum; or 3) incubation of the tissue in a control ACHE medium which did not contain acetylcholine thioiodide.

Clearly discernable individual reaction products for the TH/ACHE, AVP/ACHE, and NP/ACHE combinations were observed. Dark brown or black perikarya and fibers of immunocytochemically stained neurons were evident and in certain regions were found in association with light brown/orange ACHE containing cell bodies and/or fibers. For instance, ACHE containing cell bodies were visualized in perinuclear regions of the supraoptic nucleus and locus coeruleus sending processes toward VP and NP containing cells or TH containing neurons respectively.

These results provide a novel approach for the visualization of different biologically active compounds with widely distributed ACHE containing elements and can serve to enhance our understanding of dynamic and static interactions between neural systems.

(JHK is a John Douglas French Fellow; supported by NIH grant NS 15109)

281.6 SEROTONIN-IMMUNOREACTIVE VARICOSITIES LOCALIZED ON MEDULLARY PNMT-IMMUNOREACTIVE BULBOSPINAL NEURONS. A.P. Nicholas* and M.B. Hancock*. (SPON: J.E. Blankenship). Department of Anatomy and Neurosciences, The University of Texas Medical Branch, Galveston, TX 77550.

Two-color immunoperoxidase staining was used to visualize black-stained, serotonin-immunoreactive (5HTI) fibers and varicosities on amber-stained phenylethanolamine N-methyl transferase-immunoreactive (PNMTI) cells in the medulla of adult rats in which the thoracic spinal cord was injected with HRP. Animals were deeply anesthetized with Nembutal and multiple, bilateral injections of 50% HRP (Sigma; Type VI) in phosphate buffered saline (0.1 M; pH 7.4) were made into the rostral thoracic spinal cord (4-7 µl total) through a micropipette inserted between spinal laminae. After 24-27 hours, the rats were again anesthetized and perfused transcardially with ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). The brainstem was removed and postfixed overnight in 4% paraformaldehyde in 0.1 M PB. Vibratome sections (25-30 µm) were reacted with 0.035% diaminobenzidine (DAB), 2.5% nickel ammonium sulfate, and 0.01% H_2O_2 in 0.1 M acetate buffer (pH 6.0) for 30 minutes. HRP-labeled cells showed fine, black, cytoplasmic granules. Sections containing HRP-labeled cells were washed and stored in PB for 24-48 hours. The tissue was then incubated overnight at room temperature in rabbit anti-5HT (Immuno Nuclear; 1:20,000), 0.2% Triton X-100, and 1% normal goat serum in PB. The sections were then incubated in goat anti-rabbit IgG (1:100), rabbit PAP (1:640), and finally in the nickel-DAB solution used above. 5HTI processes and cells were stained black. The tissue sections were then incubated overnight at room temperature in rabbit anti-PNMT (Eugene Tech; 1:300-500), 0.2% Triton X-100, and 1% normal goat serum in PB. This was followed by incubations in goat anti-rabbit IgG (1:100), rabbit PAP (1:640), and finally in 0.05% DAB and 0.01% H_2O_2 in PB. Amber-stained PNMTI cells enmeshed in 5HTI processes were observed in the ventral medullary tegmentum, in the medullary raphe and in the solitary nucleus. Tissue sections were flat-embedded in Spurr plastic and blocks of selected regions were cut from these sections and cemented on plastic rods. Semi-thin (1-2 µm) sections of this material were cut using an ultramicrotome and glass knives. Black-stained 5HTI processes could be visualized along the borders of individual, amber-stained, PNMTI cells. Many of the PNMTI cells were labeled with HRP, indicating that they projected to the spinal cord. The results suggest that 5HT released from fibers in the medulla may influence activity in brainstem adrenaline neurons that project to the spinal cord.

281.7 THE SIMULTANEOUS ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL LOCALIZATION OF PNMT-CONTAINING TERMINALS AND CORTICOTROPIN RELEASING FACTOR NEURONS IN RAT PARAVENTRICULAR NUCLEUS. J.A. Olschowka. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

In electron microscopic studies of the nervous system it would be advantageous to be able to simultaneously label two or more antigens in order to determine the synaptic relationships of the identified neurons. We recently developed a new method for the electron microscopic immunocytochemical (EM ICC) localization of antigen-antibody reactive sites using a fluorine labeled antibody and electron spectroscopic imaging (ESI). ESI is an EM technique that permits the detection of the spatial distribution of atomic elements at high resolution. Briefly, an antiserum against rat corticotropin releasing factor (CRF) is conjugated, using carbodimide coupling, with nonadecafluorodecanoic acid in order to label it with fluorine atoms. The antibody is incubated with the tissue and the fluorine-labeled antibody is then localized using a Zeiss EM902 electron microscope equipped with an electron spectrometer.

Previous studies have demonstrated a major projection of PNMT containing axons to the paraventricular nucleus (PVN; Hokfelt et al, Brain Res 66:235-251, 1974; Swanson et al, J Comp Neurol 196:271-285, 1981). Following depletion of hypothalamic epinephrine, CRF has been reported to increase in the PVN (Mezey et al, Nature 310:140-141, 1984). This suggests that central epinephrine neurons have an inhibitory role in the physiological response to stress. To determine if this role was due to a direct influence of epinephrine terminals on CRF neurons, we combined the avidin-biotin-peroxidase method (ABC) for the localization of PNMT with the ESI method for localizing our fluorine-labeled anti-CRF. Briefly, male Sprague-Dawley rats were perfused with an acrolein-paraformaldehyde fixative. The PVN was vibratome sectioned, incubated in rabbit anti-PNMT (Eugene Tech), and stained using the ABC method (Vector Labs). Following the diaminobenzidine reaction, the sections were incubated in rabbit anti-CRF labeled with fluorine, osmicated, and embedded in resin. Unstained 30 nm thick thin sections were then examined in the Zeiss EM902.

Numerous PNMT positive terminals were observed within the PVN forming asymmetric synapses with the soma and dendrites of both parvo- and magnocellular neurons. A number of the parvocellular neurons were observed to contain the fluorine-labeled anti-CRF. This demonstrates a direct action of epinephrine axon terminals on CRF neurons of the rat PVN. Supported by PHS grant NS02799.

281.8 DEFINITION OF SUBSETS OF ENTERIC NEURONS BY THE HISTOCHEMICAL DEMONSTRATION OF THE ACTIVITIES OF ENDOGENOUS ALKALINE PHOSPHATASE AND NADPH-DIAPHORASE: CO-LOCALIZATION WITH NEUROPEPTIDES. M.D. Gershon and A. L. Kirchgessner.* Dept. of Anat. and Cell Biol. , Columbia Univ. P&S, New York , N.Y. 10032

The enteric nervous system (ENS) is distinguished by the extreme phenotypic diversity of its component neurons. More than 20 established or putative neurotransmitters have been so far identified in the two enteric plexuses. These include, neuropeptides, ATP, and small molecules, such as acetylcholine (ACh) and serotonin (5-HT). For the most part the different types of enteric neuron have been identified immunocytochemically. We now report that subtypes of enteric neuron are also revealed by the histochemical demonstration of enzymatic markers and we have begun to relate this method of categorization to putative transmitter content. The markers studied were alkaline phosphatase (AP) and nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH; found in cholinergic neurons in the striatum and reticular formation). AP was demonstrated with Vector's™ red or blue substrate reaction kit and NADPH was shown with nitro blue tetrazolium. AP was found to mark a subset of neurons (about 10%) and nerve processes in the myenteric but not the submucosal plexus of the guinea pig. The AP-containing neurons all had many short stubby dendrites (Dogiel type I morphology). In the region around the myenteric plexus, and in the deep muscular plexus, AP was also found in cells that appeared to be interstitial cells of Cajal (ICC). The enzymatic activity of the neurons and ICC cells was different from that of the mucosal epithelium because the neuronal AP was inhibited by levamisole while the mucosal form of the enzyme was not. This difference permits neuronal AP to be localized simultaneously with neurotransmitters when these are shown immunocytochemically using a species specific biotinylated secondary antiserum and avidin coupled to the mucosal type of AP. Endogenous AP did not co-localize in neurons with neuropeptide Y (NPY), substance P, vasoactive intestinal polypeptide, or with 5-HT immunoreactivity; however, most of the neurons with endogenous AP also contained NADPH, although both histochemical markers also occurred singly. NADPH activity marked about a third of the neurons of the myenteric plexus and 100% of the neurons in the submucosal plexus. Some myenteric NADPH neurons also had NPY immunoreactivity and represented a clear subgroup of small neurons; this dually marked group represented less than 10% of the NADPH and about 25% of the NPY neurons. Myenteric neurons demonstrated by NADPH did not contain substance P or 5-HT, and they did not take up quinacrine. These observations indicate that subtypes of enteric neuron, even with the same putative neurotransmitter, can be distinguished when histochemical as well as immunocytochemical markers are used. Moreover, submucosal neurons are different from those of the myenteric plexus even if the same neuropeptide is found in both. Supported by NIH grants NS 12969, and NS07062.

281.9 EVALUATION OF THE METABOLIC HISTORY OF IDENTIFIED ENTERIC NEURONS: SIMULTANEOUS DEMONSTRATION OF PUTATIVE NEUROTRANSMITTER BY IMMUNOCYTOCHEMISTRY WITH THE HISTOCHEMICAL DEMONSTRATION OF CYTOCHROME OXIDASE ACTIVITY. A. L. Kirchgessner*, G. M. Mawe, and M.D. Gershon. (SPON: T. Branchek). Dept. of Anat. and Cell Biol., Columbia Univ. P&S, New York, N.Y. 10032

Many types of neuron have been recognized in the enteric nervous system (ENS). These have been distinguished on the basis of differences in putative or established neurotransmitter content, membrane properties, or cell shape. More recently, we have found that the histochemical demonstration of cytochrome oxidase (CO) activity could be used to demonstrate which enteric neurons are chronically active. We now report the use of the CO technique to assess the level of activity of different enteric neurons identified simultaneously by immunocytochemistry or by the histochemical demonstration of endogenous alkaline phosphatase (AP). Types of neuron studied by this approach included neurons containing serotonin (5-HT), neuropeptide Y (NPY), and calcitonin gene related peptide (CGRP). The immunoreactivity of putative transmitters was demonstrated using biotinylated species-specific secondary antisera and avidin coupled to the enteric mucosal form of AP (Vector red substrate reaction kit). Levamisole, which inhibited the neuronal but not the mucosal form of AP, was used to distinguish sites of immunoreactivity from endogenous neuronal AP. Diaminobenzidine was employed to demonstrate CO activity. Density of the CO reaction product was intensified with cobalt and assessed using computer-assisted video microdensitometry. In the submucosal plexus neurons with CGRP and NPY immunoreactivities were both found to be less active, with respect to their level of CO activity, than the average of neurons that do not have these substances. In the myenteric plexus neurons containing 5-HT, CGRP, NPY, and endogenous AP again were all less active with respect to CO than the remaining neurons. The observation that the level of CO activity is the same in CGRP- and NPY-containing neurons was predicted because they have been reported by Furness et al., 1985 to be co-localized in the same neurons along with cholecystokinin, choline acetyl transferase, and somatostatin. All of the neurons examined in the current study belong to minority subsets of neurons of the ENS. Their below average level of metabolic activity suggests that they are probably phasically rather than tonically active. The neurons are therefore of considerable interest because of the insight into the control of gastrointestinal activity that will be ascertained by determining what kind of perturbation in vivo will increase their activity. Supported by NIH grant # NS 12969, NS07062, and the P.M.A.F.

281.10 PURIFICATION AND CHARACTERIZATION OF ARPP-21, A SUBSTRATE FOR cAMP-DEPENDENT PROTEIN KINASE ENRICHED IN DOPAMINE-INNERVATED BRAIN REGIONS. H. C. Hemmings, Jr.*, C. C. Ouimet and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10021

ARPP-21 (cAMP-regulated phosphoprotein, M_r 21,000) is a major cytosolic substrate for cAMP-stimulated protein phosphorylation in dopamine-innervated regions of rat brain (Walaas et al., J. Neurosci. 3:302, 1983). This phosphoprotein has now been identified in bovine caudate nucleus cytosol and purified to homogeneity from this source. The purification procedure involved diethylaminoethyl-cellulose chromatography, ammonium sulfate fractionation, phenyl-Sepharose 4B chromatography and fast protein liquid chromatography using Mono Q anion exchange resin. Two isoforms of ARPP-21 (ARPP-21A and ARPP-21B) were obtained, which were present in approximately equal amounts in the starting material. ARPP-21A was purified ~2600-fold with a final yield of 20% and ARPP-21B was purified ~2900-fold with a final yield of 21%. The purified preparations of both isoforms were judged to be homogeneous by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. ARPP-21A and ARPP-21B yielded identical two-dimensional thin-layer tryptic phosphopeptide maps, identical amino acid compositions and closely related tryptic peptide HPLC maps. The amino acid compositions of ARPP-21A and ARPP-21B showed a high content of glutamic acid/glutamine, and no methionine, tryptophan, tyrosine, phenylalanine or histidine. The purified catalytic subunit of cAMP-dependent protein kinase catalyzed the incorporation of ~1 mol of phosphate/mol of purified ARPP-21. Phosphorylation occurred exclusively on serine. ARPP-21 remained phosphorylatable and soluble upon heat denaturation or treatment with 50% ethanol, and was partially soluble at pH 2. The isoelectric point of phosphorylated ARPP-21 was 4.5. The M_r determined for ARPP-21 by sodium dodecyl sulfate/polyacrylamide gel electrophoresis was 21,000. The Stokes radius of ARPP-21 was 26.3 Å, and the sedimentation coefficient was 1.3 S; these values yield a calculated molecular mass of 13,700 daltons, a frictional ratio of 1.7 and an axial ratio of 13.5, indicative of an elongated tertiary structure. ARPP-21 is an excellent substrate for cAMP-dependent protein kinase, and is only poorly phosphorylated by cGMP-dependent protein kinase, calcium/calmodulin-dependent protein kinases I and II and protein kinase C. Immunocytochemical data indicate that ARPP-21 is a cAMP-regulated phosphoprotein highly enriched in dopamine-innervated brain regions (Ouimet et al., see accompanying abstract), and may therefore play a role in mediating some of the physiological effects of dopamine acting at D-1 dopamine receptors.

281.11 IMMUNOLOGICAL LOCALIZATION OF NEUROTRANSMITTER-RECEPTORS ON NEURONS AND GLIA IN DISSOCIATED HYPOTHALAMIC CULTURES. R. Ventimiglia, J. Grierson* and H. M. Geller. Dept. of Pharmacology, U.M.D.N.J.-R.W. Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854.

The expression of neurotransmitter receptors is heterogeneous among neuronal populations. Traditionally, electrophysiological methods have been necessary to investigate the expression of neurotransmitter receptors by single neurons. The availability of antibodies directed against neurotransmitter receptors, coupled with dissociated tissue culture preparations, enables the direct visualization of neurotransmitter receptor expression at the single cell level. We have used antibodies directed against purified GABA receptors and beta-adrenergic receptors to characterize the expression of these receptors by hypothalamic neurons in dissociated culture. Moreover, immunologically identical receptors are found on cultured astrocytes.

Dissociated cultures of rat embryonic hypothalamus were prepared and immunocytochemical experiments were conducted as previously described (Neurosci. Abst. 11: 1065, 1985).

Antibody 6CH10 prepared against purified GABA receptors was obtained from J. Tallman (Yale Univ.). Approximately 80% of hypothalamic neurons displayed GABA receptor immunoreactivity. Staining was non-uniform; somata typically displayed a prominent area of intense fluorescence with the remaining area showing little or no reactivity. Similarly, immunoreactivity was limited to discrete segments of neuronal processes. Staining was most prominent on proximal processes; segments of distal processes, including growth cones, often displayed immunoreactivity. Virtually all astrocytes displayed GABA-receptor immunoreactivity. The pattern of staining on astrocytes was quite different from that on neurons. Immunoreactivity was generally limited to 5 μM "patches" on the surface of the astrocyte.

A polyclonal anti-idiotypic antibody (anti-id3) which recognizes the beta-adrenergic receptor epitope was obtained from M. Greene (Univ. of Pa.). Approximately one third of cultured hypothalamic neurons express beta-receptor-like immunoreactivity. In contrast to GABA-receptor immunoreactivity, beta-receptor immunoreactivity is found uniformly on the soma and proximal processes. Astrocytes displayed a uniform low-level of immunoreactivity.

These results demonstrate that immunological methods can be used to delineate the expression of pharmacological receptors by cultured neurons. The heterogeneity of receptor expression by hypothalamic neurons may thus reflect their functional diversity. Additionally, the expression of the same epitope on astrocytes suggests that astrocytes and neurons express closely related receptors. (Supported by grants from N.I.H. and N.S.F. to H. G. and a Merck Fellowship to R. V.)

281.12 **AN IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC ANALYSIS OF PUTATIVE GABAERGIC NEURONS IN THE NEMATODE ASCARIS.** J.Guastella*, C.D.Johnson, and A.O.W.Stretton. (SPON:A.W.Clark). Neurosciences Training Program and The Department of Zoology, University of Wisconsin, Madison, WI 53706.

A number of different approaches have traditionally been used to determine the neurotransmitter phenotype of nerve cells. These methods have included techniques for the direct visualization of the neurotransmitter or its synthetic enzyme, as well as more indirect methods, such as neurotransmitter uptake. The latter technique in particular has been widely used in the localization of cells which utilize amino acid neurotransmitters. However, with the advent of immunocytochemical methods for the direct visualization of amino acids, a controversy has developed over whether all nerve cells which contain a particular amino acid transmitter also possess an uptake system for that amino acid.

We are investigating this problem in the nematode Ascaris with an emphasis on the neurotransmitter gamma-aminobutyric acid (GABA). Both GABA immunocytochemistry and an autoradiographic study of ^{3}H-GABA uptake have been performed. We have found that identified inhibitory motorneurons in the major nerve cords stain intensely with an antiserum to GABA, but that these same neurons do not take up ^{3}H-GABA under the conditions tested. On the other hand, a group of neurons of unknown physiological sign, located in the anterior ganglia, both contain GABA-like immunoreactivity and take up ^{3}H-GABA.

Thus, there appear to be at least two classes of nematode neurons with GABA-related markers: one class, which possesses a GABA-like immunoreactive substance as well as an uptake system for GABA; and a second class which possesses a GABA-like immunoreactive substance, but which lacks an uptake system for GABA. It is of particular interest that GABA uptake, a marker traditionally used by vertebrate neuroanatomists to visualize neurons thought to use GABA as a neurotransmitter, does not result in the labeling of a class of physiologically identified, GABA-immunoreactive inhibitory neurons in the nematode.

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281.13 ULTRASTRUCTURE OF IMMUNOREACTIVE SUBSTANCE P IN HUMAN AND MARMOSET ROSTRAL SUBSTANTIA INNOMINATA. E. T. Hedley-Whyte, R.H. Perry,* J.M. Candy,* and J.E. Edvardson.* Massachusetts General Hospital, Harvard University, Boston, MA 02114 and MRC Neuroendocrinology Unit, Newcastle upon Tyne, UK.

Neuropeptide immunoreactivity (IR) in human rostral substantia innominata (SI) is ribbon-like and broader and denser than nerve terminal IR (Candy et al, J Anat, 140:309, 1985). The ultrastructural location of substance P IR in the rostral SI of 2 marmoset and three human brains was examined. Anesthetised marmosets were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde. The human SI were fixed by immersion. After 3-4d tissues were placed in 0.1M PO_4 buffer with 5% sucrose for 6-10 d. 40 um vibratome sections were exposed to monoclonal anti substance P 1:500 (1-3d). After immersion in 2.5% glutaraldehyde slices were exposed to goat antirat IgG (1:50), rat PAP (1:80) and DAB. IR areas of globus pallidus and medial and subcommissural SI were examined in an electron microscope after fixation in 2% OsO_4. Controls included absorbed substance P and omission of primary antiserum.

Light microscopic distribution of IR resembles prior reports (Beach & McGeer, Neuroscience 13:29, 1984). The intense IR in smooth fine bundles within the ventral internal capsule and anterior commissure is in the unmyelinated axons. The ribbon-like IR in the posterior nucleus accumbens at its junction with medial SI is groups of IR and non-IR unmyelinated axons up to 4 to 4.7um across. In addition some synapses onto large dendrites are IR. In both medial and subcommissural SI, IR axons have varicosities (0.13-3um) along them. The "woolly fibers" in the internal pallidal segments and subcommissural SI are IR terminals on large dendrites as previously suggested. Occasional myelinated fibers are also IR. IR varicosities 1.5um in diameter connected by IR unmyelinated axons run parallel to and around dendrites in subcommissural and rostral SI. Subcommissural SI IR resembles that seen in globus pallidus. This region thus corresponds to ventral globus pallidus (Beach & Geer, Haber & Nauta, Neuroscience 9:245,1983, Ljundahl et al Neuroscience 3:861,1978). In some boutons IR is limited to large dense core granules whereas other boutons have uniform IR.

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281.14 TOPOGRAPHY AND MORPHOLOGY OF SEROTONERGIC NEURONS IN THE HUMAN BRAINSTEM. I. Tork* and J.P. Hornung. Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

The study of serotonergic neurons in the human is hardly possible with the use of antibodies against serotonin, as this monoamine dissipates soon after death, well before fixation of the postmortem tissue is possible. We employed a monoclonal antibody raised against phenylalanine hydroxylase (Jennings et al., Biochem. J. 235:133, 1986) which recognizes tryptophan hydroxylase, the synthetic enzyme of serotonin. Although in vitro this antibody also reacts with tyrosine hydroxylase, in formalin-fixed human brain it demonstrates the serotonergic cells preferentially (Hahn et al., Proc. Natl. Acad. Sci., in press).

Brainstems were fixed in 10% buffered formalin and frozen sections were reacted using the biotin-avidin technique. The DAB reaction was intensified by nickel ions yielding a black reaction product, allowing distinction of the serotonergic cells from the pigmented catecholaminergic cells which appeared brown. The immunostained neurons were plotted and using a computer program the serotonergic cell groups were reconstructed.

The dorsal raphe nucleus (nucleus supratrochlearis of Olszewski and Baxter, Cytoarchitecture of the human brain stem, 1954), is 4.5-5 mm long. The rostral end of the nucleus is broad but in the caudal half the cells are concentrated bilaterally in two rod-shaped regions, located far from the midline. Most cells have large, fusiform bodies with extensive bitufted dendritic fields. By contrast, the immunoreactive cells in the superior central nucleus are small and have small dendritic fields. The cluster of labelled cells extends dorsolaterally as far as the locus coeruleus and ventrolaterally along the medial lemniscus. The distinct, but small raphe pontis nucleus is formed by two rows of cells parallel to the midline. The cells of the raphe magnus nucleus extend far laterally and caudally, dorsal to the inferior olive, where they are small and multipolar. The raphe obscurus nucleus is at least as long as the open medulla and its cells are particularly numerous caudally. In the paragigantocellular reticular nucleus, the immunoreactive cells form a distinct cluster and intermingle with pigmented, presumably catecholaminergic, cells.

The results indicate that the serotonergic system is strongly developed in the human brain. Although the distribution of the serotonergic cells is similar to that found in other mammals, an additional development of lateral cell clusters is evident, particularly in the reticular formation.

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NEUROENDOCRINE CONTROL: PITUITARY IV

282.1 MONOCLONAL ANTIBODY SPECIFIC TO RAT ANTERIOR PITUITARY SOMATOTROPHS. H.T.Chen*and C.S.Pittman* (SPON:R.E.Blackwell).Dept. of Med.,Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The cell surface differentiation antigens of lymhocytes and neural tissues have been studied intensively. However, few studies are available concerning cell surface antigens of endocrine cells. We have applied the hybridoma technology of Kohler and Milstein (Nature , 256:495, 1975) to generate monoclonal antibodies (Mabs) against rat anterior pituitary cells. In this abstract, we report the generation and partial characterization of a Mab WHC-1 which is specific to the cell surface antigen of rat anterior pituitary somatotrophs.

BALB/c mice were immunized with enzymatically dispersed anterior pituitary cells from adult female Sprague-Dawley rats. Spleen cells of immunized mice were fused with murine myeloma Ag8.653. Hybridomas were initially screened for antibodies to cell surface antigens by ELISA using rat anterior pituitary cells and aortic smooth muscle cells as positive and negative controls, respectively. Positive clones were further checked by immunfluorescence (IF) and immunocytochemistry (ICC) using NIADDK anti-rPRL-IC-1 and NIADDK anti-hGH-IC-3 to identify mammotrophs and somatotrophs, respectively.

Mab WHC-1 was found to be an IgM with k light chains and cytotoxic in the presence of complement. Based on double IF staining, this Mab stained ~21% of the anterior pituitary cells of adult female rats. Among them, over 95% stained cells were somatotrophs and less than 3% were mammotrophs. Approximately, 2/3 of somatotrophs were Mab WHC-1 positive. This Mab did not stain gonadotrophs or thyrotrophs. In general, results from IF were in good agreement with those from ICC. No rat tissues surveyed so far except anterior pituitary glands were stained by Mab WHC-1 in ICC. The cell surface antigen defined by Mab WHC-1 was resistant to trypsin digestion. It was soluble in chloroform/methanol (2:1) and partitioned into the methanol/water phase, and thus exhibited the solubility property of a ganglioside.

cell types	IF	ICC
WHC-1 positive	21.1% ± 3.4%[a](3)	26.1% ± 4.1% (3)
mammotrophs	43.6% ± 2.7% (3)	48.6% ± 2.5% (4)
somatotrophs	34.6% ± 2.3% (3)	28.6% ± 2.8% (4)

a; Mean ± SEM

These results provided an immunological evidence for the heterogeneity among somatotrophs and demonstrated the feasibility of making pituitary cell-type specific Mabs. The availability of such Mabs could provide a means to study the heterogeneity of cell populations and to purify cells by affinity purification techniques.
(Supported by NIH Grant HD-18355)

282.2 **ANTERIOR PITUITARY LACTOTROPHS AND SOMATOTROPHS IDENTIFIED BY CELL-SURFACE LABELING AND ISOLATED BY FLUORESCENCE-ACTIVATED CELL SORTING.** **P.A. St. John[1], L. Dufy-Barbe*[2], and J.L. Barker[1]; [1]Lab. of Neurophysiology, NINCDS-NIH, Bethesda, MD. 20892 and [2]Lab. de Neurophysiologie, Univ. de Bordeaux II, Bordeaux, France.**

The anterior pituitary (AP) contains several different cell types, which secrete different peptide hormones, and suspensions of cells dissociated from the anterior pituitary include each of the cell types. Although different classes of cells can be identified by intracellular immunocytochemistry (ICC) following fixation, it has been difficult to identify and isolate the different types while the cells are alive. We now report that live cells from two of the five classes of AP cells, those containing prolactin (PRL) and those containing growth hormone (GH), can be identified with two different cell-surface labels. In each case, the labeled cells have been isolated with a fluorescence-activated cell sorter (FACS) and maintained in vitro.

Antibodies against each of the five pituitary hormones examined (prolactin, growth hormone, adrenocorticotropic hormone, luteinizing hormone, and thyroid-stimulating hormone) bound to subpopulations of live AP cells dissociated from non-pregnant adult female rats. The largest subpopulation was that labeled by anti-PRL antibodies, which bound to 29 +/- 5% of the cells. At least 85-98% of the cells labeled by anti-PRL on the surface were found by ICC to contain immunoreactive PRL inside the cell, although only approx. 50% of all PRL-containing cells were labeled on the cell surface. Surface-labeled cells were isolated with a FACS and placed in culture, where over 85% were found to contain intracellular PRL. Sorted cells maintained detectable levels of PRL for at least 2 weeks in culture.

AP cells that contained GH were found to be fortuitously identifiable by the binding of cholera toxin subunit B. A fluorescent conjugate of this toxin (ChTxB-FITC) bound to most of the cells in AP cell suspensions, but quantitative analysis on a FACS revealed a discrete group of cells, comprising 35-40% of the population, with very intense labeling. Double-label ICC of unfractionated cell suspensions and intracellular ICC of ChTxB-FITC-labeled AP cells isolated with a FACS showed that over 95% of the intensely labeled cells contained intracellular GH and that over 97% of GH-containing cells were intensely labeled.

Thus, live PRL-containing and GH-containing cells from the anterior pituitary can be identified by cell-surface labeling, and highly enriched preparations of these cells can be isolated by cell sorting. The sorted cells are being used to examine responses of identified pituitary cells to transmitters and hormones in vitro (G.G. Chen et al., this volume).

282.3 ADENOSINE AND ADENOSINE ANALOGS STIMULATE THE RELEASE AND SYNTHESIS OF BETA ENDORPHIN IN CULTURED ANTERIOR PITUITARY CELLS. J.C. Bisserbe*, J.R. Dave*, J. Patel* and R.L. Eskay* (SPON: D.B. Lozovsky). Neurochemistry Section, Laboratory of Clinical Studies, DICBR, National Institute of Alcohol Abuse and Alcoholism and Biological Psychiatry Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892.

Adenosine has been shown to play a regulatory role in the release of several neurotransmitters and hormones (e.g. glucagon, prolactin). As a consequence, we have investigated the ability of adenosine and such stable adenosine analogs as 5'-N-ethylcarboxamidoadenosine (NECA) and N^6-cyclohexyl-adenosine (CHA) to alter the synthesis and secretion of beta endorphin (BE) from primary cultured anterior pituitary (AP) cells. Immunoreactive (ir) BE levels from 2 hr incubation media were determined by radiommunoassay, whereas, pro-opiomelanocortin (POMC) messenger (m) RNA levels, following 24 hr drug treatment were quantified by Northern-blot and slot-blot techniques. Adenosine (10^{-6}M) increased the release of ir BE from AP cells 2 fold over basal levels. The addition of 10^{-6}M nitrobenzylthionosine, an adenosine uptake blocker, did not alter the adenosine (10^{-6}M)-induced release of ir BE from AP cells, suggesting a plasma membrane site of action for adenosine. The addition of 10^{-9} to 10^{-4} NECA to cultured AP cells resulted in a dramatic 2- to 10- fold increase in the secretion of ir BE as compared to non drug-treated cells. CHA was also a potent stimulator of corticotrophs with 10^{-9} to 10^{-4}M CHA enhancing ir BE secretion from 0.6 to 4-fold, respectively. The ir BE stimulatory effect of adenosine, NECA or CHA was abolished in calcium free media. The addition of 8-sulfo-phenyl-theophylline, a specific adenosine receptor antagonist, over the range of 10^{-6} to 10^{-4}M, resulted in a dose-related inhibition of NECA (10^{-8}M)-induced release of ir BE. In addition, 24 hr treatment of AP cells with NECA or CHA, over the range of 10^{-6} to 10^{-8}M, resulted in a dose-related increase in POMC mRNA levels with a maximal 2-fold increase in mRNA levels at 10^{-6}M NECA or CHA. These results indicate that adenosine or adenosine analogs stimulate both BE secretion and biosynthesis in cultured corticotrophs and do so presumeably via an adenosine plasma membrane receptor of the A_2 type.

282.4 DIFFERENTIAL EFFECT OF CALCIUM ON SYNTHESIS AND SECRETION OF PRO-OPIOMELANOCORTIN-DERIVED PEPTIDES AND PROLACTIN IN CULTURED RAT ANTERIOR PITUITARY CELLS. J.R. Dave*, L.E. Eiden*, D. Lozovsky, J.A. Waschek* and R.L. Eskay* (SPON: G.B. Ellis). LCS, NIAAA, DICBR, and LCB AND LCS, NIMH, Bethesda, MD 20892.

Stimulus-secretion-synthesis are continuous events occurring in hormone secreting cells; however, the nature of coupling between these events in most anterior pituitary (AP) hormone secreting cells is not well established. Our recent findings suggest that secretogogues, which are known activators of the adenylate cyclase-cyclic AMP system (CRF, forskolin, isoproterenol) in corticotrophs, stimulate both beta-endorphin (BE) release and pro-opiomelanocortin (POMC) mRNA levels, whereas, cyclic AMP-independent activators (phorbol ester, K^+ and calcium ionophores) enhance only BE release. In primary cultured AP cells, we have evaluated the effect of calcium on basal and stimulated release of BE and prolactin (PRL) and their respective mRNAs. Treatment of AP cells with a calcium channel blocker (D600; 10 uM) had no effect on BE-release and POMC mRNA levels. However, this treatment produced a 70% and 25% decrease in PRL release and mRNA levels, respectively. Treatment of AP cells with calcium-free media had no effect on BE release, but decreased PRL release and POMC and PRL mRNA levels by approximately 50%. Treatment of AP cells with the calcium agonist barium (1 mM) produced a one-fold increase in BE relase without affecting POMC mRNA levels, and a two-fold increase in both PRL mRNA and PRL release. The stimulation of BE and PRL release by barium was several-fold higher in the absence of calcium and was blocked by D600. Both calcium-free media and D600 partially blocked FN (10^{-5}M)-stimulated BE release, POMC mRNA levels, and PRL mRNA levels and completely blocked FN-stimulated PRL release. These results document that i) in the absence of calcium, basal or secretogogue-stimulated levels of POMC mRNA, PRL mRNA, and PRL secretion are reduced and ii) the effect of calcium deficiency on PRL mRNA, but not on POMC mRNA levels, can be reversed by barium. These findings suggest that both calcium and cyclic AMP act as intracellular second messengers in lactotrophs, whereas, cyclic AMP is the intracellular second messenger coupling stimulus-secretion-synthesis events in corticotrophs of the anterior pituitary gland.

282.5 INDIVIDUAL MELANOTROPHS OF THE INTERMEDIATE LOBE OF THE RAT PITUITARY MAINTAIN DIFFERENT RATES OF BIOSYNTHETIC ACTIVITY: AN EM MORPHOMETRY AND IN SITU HYBRIDIZATION STUDY. Chronwall B.M., Millington W.R., Unnerstall J.R., Hook G.R., Sartor C.E. and O'Donohue T.L. Experimental Theraputics Branch and Surgical Neurology Branch, NINCDS, NIH, Bethesda MD 20892; Dept. of Physiology, USUHS, Bethesda, MD 20814.

The melanotroph, a polygonal cell with an ovoid smooth nucleus, is the primary cell type of the intermediate lobe (IL) of the rat pituitary. The melanotrophs were not uniform but differed in the tinctorial properties of their cytoplasm: some cells appeared distinctly dark, others light; cells staining in intermediate shades were also found. These features were especially prominent in toluidine blue stained semithin plastic sections and on electron micrographs. There were no obvious differences in the cell size or properties of the nuclei of the differentially stained melanotrophs. On electron micrographs the cytoplasm stained dark or light, mainly due to different densities of flocculent material in the cytosol. In addition, the rough endoplasmic reticulum appeared to differ in morphology and amount between light and dark staining cells. The number of secretory vesicles and mitochondria and the amount of Golgi apparatus are being statistically evaluated. Furthermore, in situ hybridization using a pro-opiomelanocortin (POMC) anti-sense RNA probe on thin paraffin sections showed an uneven distribution of POMC mRNA (range 13-30 grains per 100 μm^2 cytoplasm), which confirms differences in biosynthetic activity between melanotrophs. Low grain counts most likely coincides with light staining cells and high grain counts with dark staining.

Short term haloperidol treatment (2mg/kg/d;2d) significantly increased the POMC mRNA level (181 ± 11 % of control levels; range 28-55 grains). Following long term haloperidol treatment (17 d) the grain count was elevated to 300 % of control. Under the same conditions the ratio of dark staining to light staining cells greatly increased. Bromocriptine (4mg/kg/d; 2d or 17d) had the opposite effect: a dramatic reduction in grain counts (10 % of control levels, range 0-5) and an increased proportion of light staining cells.

In summary, the normal IL seems to contain melanotrophs maintaining different rates of biosynthetic activity as seen with EM morphometry and in situ hybridization. Drug treatments known to alter the secretion of POMC related peptides from the IL produced parallel changes in the amount of metabolically active organelles and POMC mRNA per cell.

282.6 PHENCYCLIDINE-INDUCED SECRETION OF IMMUNOREACTIVE BETA-ENDORPHIN. Patricia C. Contreras, John M. Farah, Jr., Kenner C. Rice*, Arthur E. Jacobson* and Thomas L. O'Donohue. Exp Ther Branch, NINCDS, and Lab Chemistry, NIDDK, NIH, Bethesda, MD 20892.

There is increasing evidence that an endogenous phencyclidine-like substance exists in brain which may participate in normal as well as pathophysiological brain functions. In addition to its effects on mood, behavior and motor control, phencyclidine (PCP) has been reported to influence pituitary secretion of prolactin in rodents. The purpose of the present study was to determine if PCP also participates in the control of the pro-opiomelanocortin (POMC) hormones, β-endorphin and α-melanotropin. Plasma concentrations of immunoreactive β-endorphin (iβ-E) and α-melanotropin (iα-MSH) were measured by RIA. Plasma concentrations of iβ-E, but not iα-MSH, were significantly increased at 5, 15, and 60 min after i.c.v. administration of 500 nmole of PCP/rat. Thus, the increase in plasma iβ-E was due to increased secretion of iβ-E from anterior pituitary, not the intermediate lobe of the pituitary. Additionally, 1-(1-(2-thienyl)-cyclohexyl)piperidine (TCP), (+) 1-(1-phenylcyclohexyl)-3-methylpiperidine (PCMP), and (+) and (-) SKF 10,047, but not (-) PCMP, significantly elevated plasma iβ-E. PCP-stimulated release of iβ-E in rats was probably not due to stress related to ataxia since (-) PCMP, which induced ataxia, did not increase iβ-E. The observation that both (+)- and (-) SKF 10,047 increased plasma iβ-E indicate that kappa or mu opioid receptor-mediated mechanisms may also be involved in release of pituitary β-E. In addition to stimulating release of iβ-E *in vivo*, PCP also increased iβ-E release from anterior pituitary explants *in vitro* in a dose-dependent manner. Additionally, PCP increased secretion of iβ-E from mouse corticotrophic tumor cells both acutely and 24 hr after initial exposure. Furthermore, PCP elevated intracellular content of iβ-E suggesting modulatory effects of PCP on synthesis of POMC peptides. Stimulatory effects of PCP on release and content of iβ-E from corticotrophs may be due to specific interactions of the compounds on PCP receptors. Binding of ^{3}H-TCP to membranes from rat anterior pituitary and AtT-20 corticotrophic tumor cells was found to be specific with a K_d similar to that determined using membrane preparation from whole rat brain. The rank order of potency of PCP analogs and sigma opioid compounds for inhibiting the binding of ^{3}H-TCP to pituitary membranes was similar to that determined for whole brain homogenates. These results demonstrate that PCP stimulates pituitary secretion of POMC-derived peptides *in vivo*, in part, through direct actions on anterior pituitary corticotrophs which appear to specifically bind PCP and its active analogs. These findings indicate that endogenous PCP-like substances may normally participate in the neuroendocrine control of β-E and other hormones derived from POMC in the anterior pituitary.

282.7 COMPUTER-CONTROLLED PERIFUSION: CHARACTERIZATION FOR STUDIES OF PULSATILE GONADOTROPIN SECRETION. M.D. Culler*, M.M. Valenca*, F.Romanelli* and A. Negro-Vilar. Reprod. Neuroendo. Sec., Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

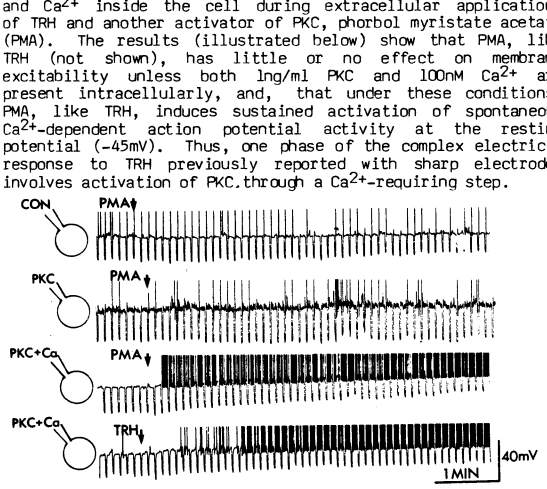
In vitro studies to determine the contribution of the various parameters of a pulsatile hormone signal (pulse shape, pattern, frequency, amplitude, etc.) to target tissue response have been hampered by the limitations of conventional perifusion systems. Recently, however, a computer assisted perifusion system was developed (Endotronics Corp.) which can be programed to regulate any and all parameters of a pulsatile signal. To evaluate this system for use in determining how each component of a pulsatile LHRH signal contributes to the pulsatile gonadotropin response, LHRH pulses were designed to rise from 0 to peak concentration in 30 sec and then decay exponentially according to the 0.5 ml/min perifusion flow rate. Ten such pulses, with a total mass of 10 ng and pulse frequency of 40 min, were sequentially delivered to 3 perifusion chambers, each containing 1 rat hemipituitary. Mean total LHRH delivered/pulse, as measured by RIA in 2 min perifusate fractions, was 10.15 + 0.23 ng. No significant differences were observed between total pulse mass or shape among the 10 pulses within a chamber or between the 3 chambers. Each LHRH pulse resulted in a single pulse of LH release which, similar to the shape of the LHRH pulse and of LH pulses in vivo, rose quickly and then gradually decayed to near basal levels. The first two pulses of LHRH induced a smaller LH response than the subsequent pulses, possibly illustrating a "priming" action of LHRH. Concentration-dependent LH responses were demonstrated with similarly designed pulses of 0.5, 5, and 50 ng LHRH total mass. To more closely examine the LH response, a pulse of 50 ng LHRH total mass was generated after three 10 ng priming pulses of LHRH. LH and LHRH were measured in 0.5 min perifusate fractions. The rapid rise in LH, which would reach 300% baseline within 10 min, was evident within the first fraction in which the LHRH signal was detected. During the approx. 60 min decay of the LH response to baseline levels, LH appeared to be released in episodic waves. Generation of a similarly designed 25 µg pulse of the LHRH antagonist, [D-pGlu^1, D-Phe^2, D-Trp^{3,6}]-LHRH, 30 sec after the initiation of the 50 ng LHRH pulse, quickly reversed the rapid LH response to LHRH and, within 6 min, returned LH to baseline levels. This observation demonstrates the total dependence of the pituitary on LHRH for sustained LH release. In conclusion, the results of the described experiments illustrate the utility of computer-assisted perifusion in executing complex perifusion experiments. Because of the myriad of intricate parameters which comprise a pulsatile signal, computer-assisted perifusion may be the only in vitro model capable of dissecting the contribution that each pulse parameter makes to the final target cell response.

282.8 EVALUATION OF THE INTRACELLULAR EVENTS LEADING TO PULSATILE LH SECRETION USING COMPUTER-DESIGNED INPUT SIGNAL AND CONTROLLED PERIFUSION. M.M. Valenca* M.D. Culler*, F. Romanelli* and A. Negro-Vilar (SPON: O.H. Viveros). Reproductive Neuroendocrinology Section, Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

LH secretion from the pituitary gland is known to follow a characteristic pulsatile pattern, which is believed to be the result of similar fluctuations in the release of LHRH into the hypophyseal portal blood. Little is known, however, about the role that different intracellular messengers play in the series of events leading to the generation of an LH pulse. Using a computerized perifusion system which allows the design of input signals of any desired configuration, we have analyzed the role of several putative intracellular messengers in mediating the LH response to a single pulse of LHRH. Hemipituitaries from intact male rats, perifused with a flow rate of 0.5 ml/min., were stimulated with 3 "priming" pulses of LHRH (10 ng total mass) at 40-minute intervals, to sensitize the pituitary and insure a consistent response to subsequent LHRH pulses. A test pulse of LHRH (50 ng total mass), designed to produce a LH secretory response similar to an in vivo LH pulse, was then applied and the dynamics of the stimulated LH pulse were analyzed in 30 sec. perifusate fractions over the next 70-90 min. A similar pulse of phospholipase C (PL-C) was generated in order to stimulate LH release by inducing membrane phospholipid breakdown, Ca^{2+} mobilization and protein kinase C activation. Both the LHRH and PL-C pulses resulted in a sharp and immediate increase in LH secretion, with several waves of episodic secretion superimposed on the decaying secretory response. In the presence of EGTA (5mM), pulses of either LHRH or PL-C failed to induce an LH secretory response, with the exception of a minor increase during the first few minutes. Addition of the lipoxyenase blocker nordihydroguaiaretic acid (NDGA, 20 µM) did not substantially change the pattern of LH secretion in response to the LHRH pulse, but it did reduce the magnitude of the overall secretory response by about 50%. The results indicate that PL-C can mimick the LH releasing activity of LHRH and that both secretagogues require extracellular Ca^{2+} to exert their stimulatory action. Arachidonic acid metabolized through the lipoxygenase pathway seems to play a role in mediating LH secretion in response to an LHRH pulse, primarily by amplifying the stimulatory action of the peptide. As demonstrated by these studies, computer-assisted perifusion is a powerful approach to advance our understanding of the intracellular events leading to pulsatile hormone secretion.

282.9 PROTEIN KINASE C ACTIVATORS TRIGGER ACTION POTENTIALS IN GH3 CELLS. B. Dufy[1], S. Jaken[2]* and J.L. Barker[3], [1]Lab. Neurophysiologie, Université de Bordeaux II, France; [2]Center for Drugs and Biologics, Division of Virology, FDA and [3]Lab. Neurophysiology, NINCDS, NIH, Bethesda, MD.

Thyrotropin releasing hormone (TRH) triggers a rapid hydrolysis of plasma membrane phosphoinositides followed by quick mobilization of intracellular Ca^{2+} that is temporally coincident with a transient translocation of cytosolic protein kinase C (PKC) to the plasma membrane. We have used the whole-cell patch-clamp recording technique to study the electrical properties of GH3 cells and to impose levels of PKC and Ca^{2+} inside the cell during extracellular applications of TRH and another activator of PKC, phorbol myristate acetate (PMA). The results (illustrated below) show that PMA, like TRH (not shown), has little or no effect on membrane excitability unless both 1ng/ml PKC and 100nM Ca^{2+} are present intracellularly, and, that under these conditions, PMA, like TRH, induces sustained activation of spontaneous Ca^{2+}-dependent action potential activity at the resting potential (-45mV). Thus, one phase of the complex electrical response to TRH previously reported with sharp electrodes involves activation of PKC.through a Ca^{2+}-requiring step.



Legend: Whole-cell intracellular recordings of GH3 cells with either PKC or PKC and Ca^{2+} added to the control (CON) patch-pipette solution (K^+ gluconate, $MgCl_2$, EGTA, HEPES). Resting potentials are all about -45mV. Brief, extracellular applications of PMA induce sustained spontaneous action potential activity only when the patch-pipette contains PKC and Ca and the effects are mimicked by TRH.

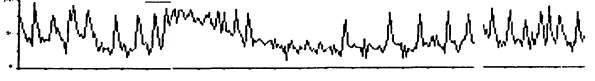
282.10 CALCIUM MEASUREMENTS IN SINGLE CLONAL PITUITARY (GH3) CELLS USING FURA-2. F. Wodajo[1]*, P. A. St John[2], J.L. Barker[2], Dept. of Biology, Princeton University[1]; Laboratory of Neurophysiology[2], N.I.N.C.D.S., N.I.H., Bethesda MD 20892 (Sponsored by J. Krikorian)

Intracellular calcium concentrations in individual, plated GH3 cells were measured using the calcium sensitive fluorescent dye fura-2. Responses of single cells to Thyrotropin Releasing Hormone (TRH) were assayed.

A fluorescence microscope fitted for epifluorescence and equipped with a photomultiplier tube and computer controlled shutters was used to detect fura-2 signals. A mercury lamp was used for excitation light. Two interference filters, 340nm and 380nm, were alternately placed in the excitation light path by a specially constructed, computer controlled filter changer. Fluorescence data at the two excitation wavelengths were gathered and the ratio determined by online computer. Cells were loaded with 0.5 to 1.5 µM of the membrane-permeant ester for 20 minutes and given up to 1 hour to de-esterify the compound before recording. Reagents were added with a perfusion pump.

Unstimulated GH3 cells exhibited periodic and large increases in intracellular Ca^{2+}, which rose rapidly and lasted 8 - 15 seconds. These fluctuations can be approximated as rising to 500 - 1000 nM Ca^{2+}; flourimeter studies with cell suspensions have shown that the resting Ca^{2+} level in GH3 cells to be approximately 100 nM.The fluctuations were reversibly blocked by 5 mM Co^{2+}, 0 mM Ca^{2+} /1.1 mM EGTA or 7 mM Ca^{2+}. Potassium produced an immediate and large increase in Ca^{2+}. After a 7 minute treatment with 10 mM K^+, fluctuations were absent for a period of 3 minutes.

Shown below is the cell response to 50 nM TRH, a hormone known to cause secretion of prolactin by GH3 cells. The bar indicates the presence of TRH in the bath. The y-axis is the ratio of fluorescence at 340nm and 380nm. It is not calibrated with respect to intracelluar Ca^{2+}. It can be seen that prior to the application of TRH, the cell is undergoing large changes in Ca^{2+}. Application of TRH induces a large, transient increase in Ca^{2+} followed by a longer period where the frequency of fluctuations is reduced. After 12 minutes, it is seen that cell behavior is similar to resting conditions.



Fluorimeter and electrophysiological studies of GH3 cells have determined that TRH causes a rapid, large increase in intracellular Ca^{2+}, lasting 1 - 2 minutes, by a release of intracellular Ca^{2+} stores followed by a long period, 5 - 60 minutes, of elevated Ca^{2+} conductance where intracellular Ca^{2+}levels remain elevated.

This data should complement studies done on populations of GH3 cells. Working with single cells, however, it has been possible to investigate Ca^{2+} behavior with unprecedented resolution and illuminate unsuspected Ca^{2+} fluctuations.

282.11 ADENOSINE 3', 5'-MONOPHOSPHATE-MEDIATED ENHANCEMENT OF CALCIUM-EVOKED PROLACTIN SECRETION FROM PERMEABILISED 7315c TUMOR CELLS. E.A. Frey, S.L. Pocotte, & S. Guild*. Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20892.

A previous study in intact 7315c tumor cells (Mol. Pharmacol., 1986, in press) showed that cAMP doubled the amount of prolactin secreted in response to either 60 mM potassium or the calcium ionophore, ionomycin; cAMP had little effect of its own on hormone secretion. The Quin 2 method indicated that the enhancing effect of cAMP did not involve an increase in the cytosolic calcium concentration. Because the Quin 2 method may not be sensitive enough to detect changes in the calcium concentration near the secretory apparatus, in the present study the electrical permeabilization technique of Baker and Knight (J. Membrane Biol. 68: 107-140, 1982) was used to control the calcium concentration to which the secretory apparatus was exposed. 7315c cells were permeabilized by subjecting the cells to an intense electric field (2 discharges of 2kV). The calcium concentration was controlled by calcium-EGTA buffers; the concentration of calcium in the buffers was verified with a calcium-sensitive electrode. Cyclic AMP, as well as calcium, freely equilibrate across the electrically permeabilized cell membrane. In the presence of no calcium and 1 mM EGTA, permeabilized cells secreted prolactin at a rate of 0.4 ng/min. When EGTA was eliminated and 1.5 mM calcium was added to the medium bathing permeabilized cells, the rate of prolactin secretion during the first 5 min was increased to 2.2 ng/min. The amount of prolactin secreted in 5 min from permeabilized cells depended on the calcium concentration between 10^{-7} and 10^{-4} M (half maximal = 30 μM). Permeabilized cells were exposed to cAMP (0.1 mM) for 5 min prior to and during a 5 min challenge with various concentrations of calcium. Cyclic AMP had no effect on prolactin secretion in the absence of added calcium nor did it alter the potency of calcium in stimulating prolactin secretion. Rather, cAMP increased the amount of prolactin secreted at each effective concentration of calcium. The results support the hypothesis that in the 7315c cell, cAMP enhances calcium-dependent secretion without increasing the calcium concentration in a compartment critical for hormone secretion.

282.12 PERSISTENCE OF AGONIST-INDUCED POLYPHOSPHOINOSITIDE HYDROLYSIS IN THE PRESENCE OF DOPAMINE AND BROMOCRIPTINE. W.D. Jarvis*, P.L. Canonico, A.M. Judd* and R.M. MacLeod. Department of Internal Medicine, University of Virginia, Charlottesville, VA 22908.

Dopamine (DA) produces a rapid, concentration-related attenuation of both basal and agonist-stimulated prolactin release which is at least in part effected through an inhibitory coupling of the D-2 receptor and adenylate cyclase. Conversely, several hypothalamic peptides are now known to promote prolactin release through the phospholipase C (PhC)-mediated hydrolysis of membranal phosphatidylinositol bisphosphate (PIP_2) to yield diacylglycerol (DAG) and inositol trisphosphate (IP_3); these include thyrotropin-releasing hormone (TRH), angiotensin II (AII), neurotensin (NT) and bombesin (BBS).

Previously, we described a potent dopaminergic reduction in the basal and agonist-stimulated rates of [^{32}P]-orthophosphate incorporation into anterior pituitary membranal phosphoinositides. However, phosphate radiolabeling studies are only able to provide an index of changes in the rates of phosphorylation of the inositol moiety of the phosphoinositides; we have therefore examined the effects of DA on phosphoinositide catabolism by monitoring changes in the basal and stimulated rates of production of total inositol phosphates (IP_x).

Normal female anterior pituitary cells in primary culture were prelabeled with 1,2-[^{3}H]L-myoinositol before 15- to 30-min exposures to varying concentrations of TRH, AII, NT or BBS in the absence or presence of 100 nM DA or bromocriptine (Br). Labeled inositol phosphate metabolites were then extracted and quantitated by anion-exchange chromatographic assay.

While significantly reducing prolactin release ($p<.01$), 100 nM DA was ineffective at blunting the enhancements of IP_x characteristically elicited by TRH, AII, NT and BBS. Further, neither a 15-min preincubation with 100 nM DA nor a 24-hr preincubation with 100 nM Br during the [^{3}H]inositol labeling period were capable of altering the parameters of IP_x concentration-response curves produced by TRH; that the magnitude of [^{3}H]inositol incorporation into membranal phosphoinositides was unimpaired by Br treatment demonstrates that neither the phosphoinositide biosynthetic pathway nor the dynamic resynthesis cycle are significantly altered by DA. Finally, a 48-hr pretreatment with bromocriptine was unable to impair TRH-stimulated enhancements of IP_3, IP_2 or IP_1 values, suggesting that PhC substrate specificity is also insensitive to control by DA.

We therefore conclude that DA inhibits stimulated prolactin secretion by regulating elements other than PhC or the biosynthetic enzymes involved in phosphoinositide synthesis or resynthesis.

282.13 CRF STIMULATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE IN RAT ANTERIOR PITUITARY CELLS. G.S. Dhillon*, M.A. Oleshansky, T. Maxwell*, C.B. Wormley*, C.C. Kenion*, and G.J. Kant. (SPON= C.F. Tyner) Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307.

Corticotropin releasing factor (CRF), a 41-amino acid hypothalamic peptide characterized by Vale and colleagues, appears to be the primary physiological regulator of pituitary ACTH release. The hypothalamic peptide, somatostatin (SRIF), has been reported to inhibit ACTH release. It has been suggested that the actions of CRF and SRIF upon ACTH release are mediated by stimulation and inhibition respectively of pituitary adenylate cyclase. The intracellular actions of cAMP are thought to be mediated via activation of cAMP-dependent protein kinase (A-kinase). The present study was conducted to determine the effects of CRF and SRIF on pituitary A-kinase activity.

Anterior pituitary cells were prepared by collagenous dispersion of freshly dissected pituitaries from male Sprague-Dawley rats. One ml aliquots of cell suspensions containing 10^6 cells were first preincubated at 37^0 C and then further incubated in the presence or absence of CRF and/or SRIF. Varying preincubation times of pituitary cells from 0 to 4 hours had no effect on the basal A-kinase activity. Incubation with CRF for 0 to 30 min resulted in identical stimulation of A-kinase activity. Standard conditions chosen were 30 min preincubation and 10 min incubation with CRF and/or SRIF. Following the 10 min incubation, cells were either lysed and supernatants were assayed for A-kinase activity or heat inactivated and assayed for cAMP by RIA. The standard assay mix for A-kinase contained 20 mM MOPS, pH 7.0, 16 mM Magnesium acetate, 0.1 mM ATP, 0.4 mg/ml histone H1, 4 mM dithiothreitol and ^{32}P ATP (1uCi/assay tube). Some assay tubes also contained 16 uM cAMP (to maximally stimulate A-kinase) or 0.6 mg/ml cAMP dependent-protein kinase inhibitor from bovine heart (to completely inhibit A-kinase). Corrections were made for non-cAMP dependent protein kinases (Honnor, R.C., Dhillon, G.S., Londos, C., JBC 260, 28:15122, 1985). A-kinase activity was linear for 30 min and for up to 50,000 cells. Standard conditions chosen were 20,000 cells per assay incubated for 15min.

CRF stimulated cAMP accumulation in intact pituitary cells and increased A-kinase activity in a dose dependent manner with an ED_{50} of 10 nM. SRIF (10 to 100nM) had no effect on either basal or CRF-stimulated A-kinase activity. In experiments using anterior pituitary homogenates, CRF stimulated adenylate cyclase activity 4-5 fold with a ED_{50} of 10 nM.

Studies are underway to further elucidate the role of CRF stimulation of A-kinase in the control of ACTH synthesis and release.

282.14 THYMOSIN FRACTION 5 STIMULATES PITUITARY HORMONE RELEASE THROUGH A CALCIUM AND ARACHIDONIC ACID MEDIATED PATHWAY. B.L. Spangelo*, A.M. Judd*, G. Oppizzi*, W.D. Jarvis* and R.M. MacLeod (SPON: T.R. Johns). Department of Internal Medicine, University of Virginia, Charlottesville, VA 22908.

Thymosin fraction 5 (TF5) is a partially purified extract of the bovine thymus consisting of 40 to 50 peptides. TF5 and some of its component peptides have been shown to be potent immunopotentiating agents in assays designed to measure T-cell function. Recent observations suggesting a link between the neuroendocrine and immune systems have prompted investigations designed to determine the effects of TF5 treatment upon dispersed cultures of female rat pituitary cells.

Incubation of normal, dispersed anterior pituitary cells with TF5 (0.1 to 100.0 ug/ml) for 30 minutes resulted in 4- to 5-fold increases ($P < 0.01$) of both prolactin (PRL) and growth hormone release. In contrast, the secretion of luteinizing hormone was not affected by TF5 in either a 30-minute or 3-hour incubation. Initial studies designed to determine the mechanism of the stimulus-secretion coupling of TF5 and PRL release showed no effect on cAMP accumulation or on polyphosphoinositide turnover. Co-incubation of the calcium channel blocker D-600 (25 uM) with TF5 (10 and 100 ug/ml) resulted in attenuated PRL release compared to that achieved with $TF5_3$ alone. Pituitary cells prelabeled with [^{3}H]arachidonic acid ([^{3}H]-AA) responded to TF5 treatment with an increased release of [^{3}H]-AA that was dose-dependent. The involvement of a metabolite of arachidonic acid was suggested by the finding that BW755c (250 uM) caused a significant attenuation of TF5-stimulated PRL release. Treatment of normal anterior pituitary cells with either 10 or 100 ug/ml TF5 and TRH (50 nM) resulted in a potentiation of PRL release greater than that achieved with either TF5 or TRH alone. The results of these studies suggest that TF5 stimulates PRL release from normal anterior pituitary cells through a mechanism dependent on a flux of Ca^{2+} and the generation of arachidonic acid metabolites. In addition, co-incubation studies using TF5 and a maximally stimulative dose of TRH suggest that the TF5 mechanism of action is different from that ascribed to TRH. The modulation of pituitary hormone release by an immunopotentiating product of the thymus gland provides further evidence for a link between the neuroendocrine and immune systems.

282.15 CYTOSOLIC FREE CALCIUM IN RAT ANTERIOR PITUITARY CELLS MONITORED BY FURA-2. J.M. Anderson*, T. Yasumoto* and M.J. Cronin (SPON: C. Desjardins). Dept. Physiology, Univ. Virginia, Charlottesville, VA 22908 & Tohoku Univ., Sendai, Japan.

Our knowledge of stimulus-secretion coupling in normal anterior pituitary cells (AP) promises to be notably extended by monitoring the fluorescence of cation indicators sequestered in the cytoplasm. AP were dispersed, cultured, resuspended and then loaded with the calcium indicator dye fura-2 (5-10 uM). The cells were studied in suspension in a spectrofluorometer at 37°C for their response to several agents, after which the preparation was calibrated by measuring the maximal and minimal fluorescence with digitonin and EGTA, respectively. The results are the mean ± SEM (n independent studies) The calculated intracellular free calcium concentration $[Ca^{++}]_i$ in the resting state was 203 ± 15 nM. The peptide hormones thyrotropin releasing hormone (10 & 100 nM) and angiotensin$_{II}$ (10 & 100 nM) induced a rapid burst of $[Ca^{++}]_i$, peaking at 15 sec. This 30% elevation achieved with both peptides was followed by a prolonged plateau phase during which $[Ca^{++}]_i$ was maintained at 14% above baseline for 6 min, the longest time tested. In contrast, both somatostatin and dopamine (DA) induced an immediate reduction in the $[Ca^{++}]_i$, reaching a minimal level between 10-20 sec and then slowly returning toward baseline. This inhibition was concentration dependent from 1-100 nM somatostatin, with a maximal reduction of 16 ± 2% (7) of baseline. DA also induced a step-wise inhibition between 10 nM and 1 uM, with a maximal attenuation of 21 ± 4% (6) of baseline when 100 nM DA was exposed to naive cells. The antagonist spiperone (10 nM) reversed an established DA inhibition. The sequential addition of increasing levels of either DA or somatostatin induced further reductions in the minimal $[Ca^{++}]_i$ until the highest hormone concentrations produced essentially no further change. In continuing to validate the preparation, the calcium channel activator maitotoxin (10 ng/ml) markedly elevated $[Ca^{++}]_i$ at 3 min to 1.40 ± 0.12 uM (3), which was maintained for at least 15 min. This was a graded response that was initiated around 1 ng/ml maitotoxin. We conclude that the fura-2 technique ought to promote a more refined appreciation of the calcium dynamics contributing to hormone-initiated exocytosis from AP cells. (Supported by RCDA 1KO4NS11601 and FIDIA, Italy)

282.16 A POSSIBLE ROLE FOR ARACHIDONATE RELEASE IN THE PROLACTIN-STIMULATING EFFECT OF ANGIOTENSIN IN VITRO. P.L. Canonico. Department of Pharmacology, University of Catania School of Medicine, 95125 Catania, Italy,

Angiotensin II is one of the most powerful prolactin-stimulating factors in vitro. We recently reported that this peptide rapidly increases the hydrolysis of polyphosphoinositides in anterior pituitary cells in culture. In various systems, the diacylglycerol formed during phosphoinositide breakdown is rich in arachidonate; because of this, we decided to explore the possible role of the fatty acid in the mechanism of action of angiotensin II and of its precursor, angiotensin I, at the pituitary. Both angiotensin I and angiotensin II significantly increased the release of free [^{3}H]-arachidonate from prelabeled anterior pituitary cells in culture in a concentration-dependent manner. Thyrotropin-releasing hormone and neurotensin, other stimulators of prolactin release, produced similar effects, but at concentrations greater than those required for angiotensin II. The effect of 10 nM angiotensin II was apparent within 1 min, maximal after 2 min, and disappeared almost completely after 5 min. The stimulation of both arachidonate and prolactin release by angiotensin peptides was abolished by a pretreatment (5 min) of the cells with angiotensin II-receptor antagonists (saralasin, sar^{1}ile^{8}angiotensin) or by pretreatments (30 min) with the phospholipase A_2 inhibitor, quinacrine (30 uM), or the diacylglycerol lipase inhibitor, RHC 80267 (50 uM). BW755c (250 uM), an inhibitor of both cyclooxygenase and lipoxygenase pathways, inhibited angiotensin II-stimulated prolactin release, but further increased the release of [^{3}H]-arachidonate produced by the peptide. The effect of these drugs was reversible.

To determine the source of free arachidonate, [^{3}H]-arachidonic acid-labeled cells were stimulated by 10 nM angiotensin II, and radioactivity in various phospholipids was measured. Two min after stimulation, radioactivity in phosphatidylinositol was about 86% of unstimulated control, in phosphatidylcholine 94%; in other phospholipids, it had not been modified significantly.

These results indicate that arachidonate metabolism may play a significant role in the prolactin-stimulating effect of angiotensin II and that phosphatidylinositol and, to a lesser extent, phosphatidylcholine are the primary sources of the free fatty acid in the action of the peptide at the pituitary,

282.17 ANTERIOR PITUITARY HORMONE RESPONSE TO ATRIAL NATRIURETIC FACTOR. W.K. Samson, M.C. Aguila, M. Norris and R. Bianchi (SPON: W. Clark). Physiology, Southwestern Medical School, Dallas, TX 75235.

The presence of atrial natriuretic factor (ANF)-like immunoreactivity in hypothalamic extracts and in nerve terminals in the median eminence, together with the presence of ANF binding sites in the anterior pituitary, suggests an action of these peptides on hormone release from the gland. In particular, the possibility that luteinizing hormone (LH) secretion is modulated by ANF was implied by the report of ANF-staining in adenohypophyseal gonadotrophs (Fed. Proc. 44: 1731, 1985). Therefore, we have examined the possibility that ANF exposure in vivo and in vitro might alter basal LH release. Additionally we have examined the possibilities that ANF might alter the gonadotroph's response to luteinizing hormone releasing hormone (LHRH) or that ANF might affect LHRH release from the median eminence. ANF (atriopeptin III, Peninsula Labs) in log doses ranging from 10^{-11} to 10^{-6}M failed to significantly alter LH release from cultured, anterior pituitary cells of intact male or ovariectomized female rats. ANF (10^{-7}M) also failed to significantly alter LHRH stimulated LH release. Similarly, the releases of prolactin, growth hormone and thyrotropin were not significantly altered by ANF. Intravenous infusion (bolus) of 10 micrograms ANF into concious, freely moving, ovariectomized rats (n=8) failed to significantly (F prob. = 0.369) alter plasma LH concentrations (preinfusion 6.8 ± 1.7 ng LH/ml plasma; +5 min, 8.2 ± 1.5; + 15 min, 7.1 ± 1.0; +30 min, 5.6 ± 1.0; +60 min, 4.7 ± 1) even though a diuretic response was observed. These results were in contrast to those recently reported (Fed. Proc. 45: 174, 1986) which indicated that a 30 min infusion of ANF inhibited LH release in the anesthetized rat by an action above the level of the pituitary, possibly in the hypothalamus. However, in our in vitro studies ANF (10^{-10}-10^{-7}M) failed to significantly alter basal LHRH release from median eminence explants in vitro. Additionally, the release of somatostatin was unaffected. Experiments examining the possible interactive effects of ANF with agents known to stimulate LHRH release are being conducted as well as further in vivo protocols involving constant infusion of ANF and a study of LH levels during volume loading, a known stimulus for the release of endogenous ANF; but our current results fail to indicate a role for ANF in the neuroendocrine control of LH secretion. Furthermore, no significant action of ANF on basal GH, prolactin or TSH release from cultured pituitary cells could be demonstrated. (Supported by NIH Grant HD 09988 - Project II and A.H.A. Grant 8SG-087).

282.18

WITHDRAWN

282.19 DOUBLE LABELED IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC STUDIES OF RENIN-ANGIOTENSIN SYSTEM COMPONENTS IN RAT GONADOTROPHS AND LACTOTROPHS. M.S. Brownfield, B. Poff*, and D.H. Harkness. Dept. of Comparative Biosciences, Univ. Wisconsin, Madison, WI 53706.

Immunoreactive angiotensin II (ANG) and angiotensin receptors are present in rat pituitary and ANG administration can affect LH and PRL secretion.

In this study we: (1) Identified pituitary cells containing renin and/or angiotensin together with their major pituitary hormone by using dual label ultrastructural ICC with peroxidase and colloidal gold methods on opposite sides of the grid. (2) Localized ANG binding sites on immunocytochemically identified cells in one micron plastic sections at the light microscopic level.

Ultrastructural studies demonstrated the presence of strongly staining peroxidase labeled renin or angiotensin together with gold labeled LH in type I gonadotrophs. A small subpopulation of lactotrophs exibited weak to moderate staining for ANG and their gold labeled PRL vesicles were of the small granule type described by Nogami and Yoshimura (Anat. Rec. 202: 261, 1982).

Autoradiography of presumptive ANG binding sites showed that nearly all PRL stained cells had grains concentrated along the cell periphery as early as two minutes after injection of pure ANG tracer. By ten minutes post administration numerous PRL cells showed grains over their cytoplasm, indicating likely internalization. Numerous cells that were immunopositive for LH and ANG were also labeled with autoradiographic grains following a similar time course. Most of the cells that were immunopositive for ANG also exhibited ANG binding sites. However, only about a fourth of the LH stained cells were labeled by radioactive ANG.

These studies suggest that gonadotrophs may have the capacity to generate angiotensin II as well as to bind and internalize the peptide or some product of the peptide. The co-localization of immunoreactive ANG and LH in type I gonadotroph vesicles suggests that the two hormones are released together. ANG could therefore act in an autoregulatory capacity or in a paracrine role on numerous nearby lactotrophs which seem to be able to bind the peptide to a much higher degree and to internalize some form of it.
(Supported by NIH HL33563)

282.20 PRESENCE OF HIGH-AFFINITY BINDING-SITES FOR OXYTOCIN IN RAT ADENOHYPOPHYSIS. F.A.Antoni (SPON: J.S.Kelly), Dept. of Human Anatomy, Oxford, OX1 3QX, U.K.

Oxytocin (OT) is a hypothalamic neurohormone, which, apart from its well-defined actions in peripheral tissues, stimulates the secretion of adrenocorticotropin and prolactin by the adenohypophysis. The aim of the present work was to examine the characteristics of purported oxytocin receptors in the anterior pituitary gland.

All experiments were carried out on particulate membrane fractions obtained from the adenohypophysis of female Wistar rats which were pretreated with diethylstilboestrol for 2 days (20 µg/rat s.c.). The radioligand was tritium-labeled OT (^{3}H-OT), bound and free hormone were separated by filtration over GF/C filters, non-specific binding in the presence of 1 µM unlabeled OT and 2.5 nM of radioligand was 30-40% of total binding. The association of ^{3}H-OT to the membrane fraction was time-dependent (steady-state at 60 min) and reversible. Analysis of the on- and off-rates of the reaction gave a dissociation constant (K_d) of 1.4 nM. Saturation of the specific component of binding could be achieved by increasing the concentration of ^{3}H-OT, Scatchard plots indicated the presence of a single site, K_d = 1.5 ± 0.1 nM, B_{max} = 33 ± 4 fmol/mg protein, (mean ± S.E.M., n=3). In ligand displacement experiments oxytocin and Arg^{8}vasopressin (AVP) were virtually equipotent: inhibitory constants were 1.5 and 1.4 nM, respectively. The specific OT agonist OH-Thr^{4}Gly^{7}OT was 2x as potent as OT, but it did not fully displace the radioligand, the maximal displacement was 65 % of specific binding. The potent V_1 antagonist d(CH$_2$)$_5$Tyr(Me)AVP, the selective V_2 agonist dDAVP, and the behaviorally active analog desGlyNH$_2$AVP all had low affinities for the binding site.

The pharmacology of the adenohypophysial binding of OT resembles that recently reported for the hippocampus (EMBO J. 4:1407, 1985) in that OT and AVP were equally efficient in displacing ^{3}H-OT. This suggests that the hypophysial OT binding site and the hippocampal site may be identical. Alternatively, it cannot be ruled out at present, that ^{3}H-OT labels an uterine-type OT receptor, as well as the pituitary-type AVP receptor (Neuroendocrinology 39:186, 1984) in hypophysial membranes.

In summary, the present results clearly show that the rat adenohypophysis contains sites, which bind OT with high affinity and distinct pharmacological specificity, and hence, reinforce the notion that OT may function as a hypohysiotropic hormone.

SUBCORTICAL VISUAL PATHWAYS II

283.1 ELECTRON MICROSCOPY OF CROSSED ISTHMOTECTAL AXONS IN XENOPUS LAEVIS FROGS. S.B. Udin and M.D. Fisher*. Div. of Neurobiology, State Univ. of N.Y., Buffalo, NY 14214.

The nucleus isthmi (NI) of frogs is a relay for ipsilateral eye input to the tectum; each NI receives retinotopic input from one tectum and sends retinotopic output to the other tectum (1). The crossed isthmotectal projection in Xenopus is particularly interesting because it displays tremendous plasticity during development. For example, crossed isthmotectal axons can be rerouted to anomalous terminal sites in the tectum if one eye is rotated prior to metamorphosis (2). Moreover, normal development of matching ipsilateral (isthmotectal) and contralateral (retinotectal maps) fails to occur in dark-reared Xenopus (3).

These results suggest that the location at which a developing isthmotectal axon will terminate is determined by the correlation of its visually-evoked activity with the activity of nearby retinotectal terminals. What structures could mediate such communication? We have used electron microscopy to determine whether retinotectal axons communicate directly with isthmotectal axons via axo-axonic synapses or whether the communication is indirect, e.g., via common postsynaptic dendrites. We have labelled isthmotectal axons in 6 adult Xenopus by injecting horseradish peroxidase into the right NI and have examined approximately 1000 filled profiles in the left tectal lobe. Only three profiles were observed which could possibly be construed as synapses onto isthmotectal axons. Therefore, we conclude that the influence of retinotectal axons on isthmotectal axons is overwhelmingly indirect.

The indirect interaction is probably mediated by postsynaptic dendrites. This preliminary conclusion is based on the observation that all of the 54 isthmotectal synapses in the stratum zonale and 58 isthmotectal synapses in layer 8-deep layer 9 in our sample involved pale postsynaptic profiles which contained vesicles, microtubules, and/or mitochondria. The synapses were unremarkable in general morphology, with asymmetric thickenings of 0.27µm median length (not corrected for shrinkage) and predominantly round clear presynaptic vesicles. En passant synapses were observed.

In conclusion, crossed isthmotectal axons make conventional asymmetric synapses, probably onto apical dendrites of tectal cells. Thus, it is likely that the influence of retinotectal activity on isthmotectal axons is mediated by contacts on common dendritic targets.

This work was supported by USPHS grant EY-03470.

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283.2 DUAL TECTAL EFFERENT ROUTES FOR VISUAL AVOIDANCE BY FROGS D. Ingle, Eye Research Institute, Boston, MA 02114.

Earlier studies showed that the frog's ability to turn towards prey stimuli depends upon the integrity of the contralateral tectal projection to the brainstem, while the ability to turn away from visual threat depends upon the integrity of the ipsilateral tectobulbar projection. However, the situation is complicated by the fact that frogs with cuts through the ipsilateral tectobulbar tract still respond with wrong-way jumps towards the approaching disks, often colliding with them. How can one account for the adaptive value of a mechanism mediating such ipsiversive avoidance jumps?

One natural situation in which ipsiversive escape is elicited is that where a disk approaches obliquely from the rostral field and crosses the midline in front of the frog. Frogs typically respond to these noncollision trajectories by leaping into the same hemifield from which the threat originated. Single-frame analysis of film records from 6 normal and 6 monocular frogs revealed that ipsiversive jumps are elicited on most trials with crossing stimuli before the disks actually cross the midline, while collision stimuli elicit mainly contraversive escape directions. This discrimination is based upon the trajectory of motion and not upon stimulus location just before the response.

Frogs with the tegmentum split (severing the crossed tectobulbospinal tract) can still make ipsiversive avoidance jumps, although they fail to turn towards prey. However, a more caudal midline transection of the isthmus (below the cerebellum) abolishes the tendency to make ipsiversive jumps to crossing stimuli. Split-isthmus frogs now jump into the crossing stimulus trajectory and often collide with these stimuli. Since tectal fibers do not cross the isthmus, we conclude that ipsiversive jumps involve at least one relay station between the tectum and the medulla of the opposite side, although contraversive jumps may be mediated by direct tectomedullary connections.

283.3 TOPOGRAPHIC ORGANIZATION OF RETINAL, TECTAL AND PRETECTAL PROJECTIONS TO THE THALAMUS IN RANA PIPIENS. Neil M. Montgomery, Katherine V. Fite and Lynn Bengston. Division of Neuroscience and Behavior, University of Massachusetts, Amherst, MA 01003

An ascending pathway linking the tectum with thalamic visual nuclei is a consistent feature of vertebrate neural organization. Whether this projection is topographically organized and whether retinotopic coordinates characterize both first- and second-order optic afferents across species is unknown. Optic axons originating predominantly from a single retinal quadrant were transected and 30% HRP was applied for a 5-day survival period. In the optic tectum, HRP-positive terminals indicated a restricted uptake of HRP, in accordance with the known retinotectal map. Likewise, HRP-positive axons in thalamic and pretectal retinorecipient areas confirmed that these projections are *mirror-image* reversals of the tectal representation (Scalia and Fite, 1984). Using these first-order projections as reference material, a second group of frogs received discrete injections of HRP such that the entire tectal lobe and pretecal region were explored across animals. The distribution of anterograde-labelled HRP axons was charted throughout the thalamus.

The tectal projection to the caudal thalamic neuropil is topographically organized, with the caudal tectum represented in the anterior portion, while the anterior tectum projects to the caudal region. Thus, the tectal projection shows a reversal of the retinotectal representationand is in correspondence with the direct retinal projection. The corpus geniculatum neuropil receives afferents only from the anterior and middle portions of the tectum (binocular region) with a topographic organization. The anterior tectum innervates the caudal portion, while the middle tectum innervates the central portion of c. geniculatum. Again, the second-order tectal projection appears to be in register with the direct retinal projections to geniculatum.

The pretectal thalamic projection originates primarily from the mesencephalic pretectal gray (which does not receive direct retinal input), and parallels the tectothalamic pathway. It reaches both caudal and anterior-thalamic optic neuropils, and terminates medially, near the central gray. A topographic organization appears to exist within this projection, but its relationship to retinal coordinates could not be determined (Supported by NSF BNS-8510381).

283.4 3(H) 2-DEOXYGLUCOSE ANALYSIS OF THE NEURAL SUBSTRATES OF HORIZONTAL OPTOKINETIC NYSTAGMUS IN RANA PIPIENS Katherine V. Fite, Gregory Taggart, Lynn Bengston, and Neil M. Montgomery. University of Massachusetts/Amherst

Recent studies based upon lesion-behavioral analysis have demonstrated that the pretectal nucleus, lentiformis mesencephali (nLM) is critical for horizontal optokinetic nystagmus (hOKN) in Rana pipiens. In the present study, the functional organization of hOKN was further studied with the 3(H) 2-deoxyglucose (2-DG) method, to further clarify its neural substrates.

Animals were injected with 2-DG (1 uCi/gm body weight) in the dorsal lymph sac, and were exposed to hOKN for a period of 2½ hours at a pattern velocity of 1.5°/sec. Animals were immediately perfused and brains sectioned on a cryostat at -14° C. Sections were mounted on coverslips, placed in x-ray cassettes with LKB ultrafilm for 40 days exposure. Exposed negatives were analysed densitometrically using a Leitz microscope and MVD-DADS system for autoradiographic image-enhancement. Tissue sections were counterstained for comparative chartings.

With binocular stimulation, the highest levels of 2-DG uptake were observedbilaterally in the pretectal nucleus, nLM. 2-DG uptake appeared to be equivalent for both right and left nLM (even though only one eye had received the prefered, temporal-to-nasal direction). In addition, bilateral uptake of 2-DG was also observed in the ventral motor nuclei, nucleus interstitialis and the posterior tuberculum. Monocular stimulation in the temporal-to-nasal direction correlated with 2-DG uptake in the contralateral nLM and the distribution of label appeared to match that of the primary retinal projection to nLM, i.e. a dense-core central region surrounded by a region of lesser uptake.

No uptake of 2-DG was observed in the nucleus of the basal optic tract with low pattern velocities. Further studies should clarify whether higher velocities will reveal involvement of the accessory optic system in hOKN, as previous behavioral investigations have suggested (Fite, et al, 1979), and which has been reported in avian species as well (McKenna and Wallman, 1981). (Supported by the Whitehall Foundation)

283.5 TECTAL AFFERENTS IN A GYMNOTID TELEOST FISH, WITH SPECIAL REFERENCE TO THE RAPHE AND HYPOTHALAMIC INPUTS. E. Sas and L. Maler. Dept. of Anatomy, Fac. of Health Sciences, Ottawa K1H 8M5 Canada.

The tectal afferents of Gymnotid fish have been examined by iontophoretic delivery of WGA-HRP. In previous studies we have shown some chief sources of tectal afferents such as: retinal (Sas & Maler, '86), electrosensory (Carr et al., '81; Sas & Maler, '86), and isthmic (Sas & Maler, submitted). Our present investigation focuses on the following important additional sites of tectal afferents: telencephalic, hypothalamic, raphe, tuberal, pretectal, and various mesencephalic and medullary inputs.

1) In the telencephalon, area dorsalis centralis neurons were bilaterally labeled, with a stronger ipsilateral component.
2) In the diencephalon, labeled cells were localized in: a) the n. dorsolateralis thalami, b) four tuberal cell groups located in a paraventricular position, c) various hypothalamic nuclei, including a cell group located at the dorsal boundary of the inferior hypothalamic lobe, and oblique strand of large cells within the inferior lobe, and the small celled lateral hypothalamic area, d) a neuronal group located dorsal to the n. glomerulosum.
3) In the pretectal region, the n. of the posterior commissure and pretectal area showed a denser labeling ipsilaterally, whereas the dorsal large celled part of n. pretectalis was only labeled ipsilaterally.
4) At mesencephalic levels, sources of afferents included: the n. isthmi and n. raphe dorsalis bilaterally, the ipsilateral mesencephalic tegmentum, torus semicircularis dorsalis and ventralis, mesencephalic n. of V, and the contralateral optic tectum.
5) At medullary levels, neurons of the ipsilateral octaval nucleus and paramedian reticular formation, appeared moderately labeled.

As a result of this study we may conclude that in Gymnotid fish, as in other vertebrates the optic tectum is the target of multisensory inputs (visual, electrosensory, trigeminal, octavolateral). In addition, the input of n. raphe (d) suggests that a serotonergic input is also present. Hypothalamic projections to the tectum of teleosts are controversial although such connections are found in mammals. The hypothalamo-tectal projection may allow for correlation of electrosensory, visual, orienting behavior and emotional (visceral) states.

283.6 STRUCTURE AND FUNCTION OF TECTOSPINAL NEURONS IN THE HAMSTER. R.D. Mooney, B.G. Klein, M.F. Jacquin, A.M. Szczepanik*, N.L. Chiaia and R.W. Rhoades. Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine & Rutgers Medical School, Piscataway, NJ 08854.

Intracellular recording and horseradish peroxidase (HRP) injection techniques were used to characterize the structure and function of tectospinal (TS) cells in the superior colliculus (SC) of the hamster. Thirty one neurons, which could be antidromically activated from the first cervical segment of the spinal cord, were recorded, filled with HRP and recovered. Anatomical data from another 21 TS cells was only sufficient to mark the laminar position of the cell body. Of the total sample of 52 cells, 7.7% had their somata in the stratum griseum intermediale (SGI), 50% were in the stratum album intermedium (SAI), 36.5% were in the stratum griseum profundum (SGP) and 5.8% were in the stratum album profundum (SAP). The TS cells were fairly uniform morphologically. They had large (27.7 ± 5.5 µm diameter) cell bodies which gave rise to an average of 6.7 ± 1.2 primary dendrites. These were generally smooth and extended up to 500 µm away from the cell body. In many cases, they ascended out of the deep laminae into the stratum opticum (SO), and some reached the stratum griseum superficiale (SGS). The axons of TS cells averaged 3.4 ± 0.8 µm in diameter and they generally coursed radially to the SAP where they curved around the periaqueductal gray (PAG) and entered the predorsal bundle (PB). These axons often gave rise to collaterals which arborized in the deep laminae of the ipsilateral SC and subjacent reticular formation.

The TS cells were also fairly uniform physiologically. Their average conduction latency was 2.0 ± 2.3 ms and this variable had a strong negative correlation (-.81) with axon diameter for the recovered cells. Most (53.5%) of the TS cells were exclusively somatosensory and gave rapidly adapting responses to deflection of vibrissae and/or guard hairs: 7.7% were bimodal (visual-somatosensory); 11.5% had complex (Rhoades et al., J. Neurosci., 7: 1342, '83) somatosensory receptive fields; 1.9% were discharged only by a noxious pinch and 15.4% were unresponsive. The only clear-cut correlation between the structural characteristics and receptive field properties of TS neurons was that all bimodal cells extended dendrites at least as far dorsally as the SO. We also noted that all of the cells with complex somatosensory receptive fields received inhibitory input from axons that either originated from, or passed through, the contralateral SC.

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283.7 EFFECTS OF NOREPINEPHRINE UPON THE RECEPTIVE FIELD PROPERTIES OF SUPERIOR COLLICULAR NEURONS IN THE HAMSTER. N. Sahibzada*, R.D. Mooney and R.W. Rhoades (SPON: A.M. Golub). Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine & Rutgers Medical School, Piscataway, NJ 08854.

Both histofluorescence and immunocytochemical studies have demonstrated that the superior colliculus (SC) in rodents is innervated by numerous norepinephrine (NE) containing axons which probably arise from the locus ceruleus. There is, however, very little information regarding the effects of this catecholamine upon the receptive field properties of SC neurons. We have therefore begun to examine the effects of NE upon the responses of SC cells in the hamster. This abstract is based upon data from 55 SC neurons recorded from 30 hamsters that were anesthetized with sodium pentobarbital, paralyzed with gallamine triethiodide and artificially respirated. Iontophoretic currents (no higher than +50 nA) were used to apply NE (0.1 M; pH 4.5) and these were typically balanced by ejection of opposite polarity current through a NaCl-filled pipette. Responses of SC neurons were evaluated with handheld stimuli and computer controlled visual and somatosensory stimulators. Twenty of the cells in our sample were recorded in the superficial SC laminae and they were all exclusively visual. Sixty percent (N=12) of these neurons had their spontaneous activity and/or evoked discharges suppressed by iontophoresis of NE. Similar effects of NE were observed for neurons that responded best to stationary, flashed stimuli, movement sensitive cells and directionally selective neurons. Eighty one percent (N=25) of the 31 somatosensory neurons isolated in the deep SC laminae had their spontaneous activity and/or evoked discharges suppressed by NE iontophoresis. The effects of NE were similar for cells characterized as low threshold, wide dynamic range, noxious and complex (Rhoades et al., J. Neurosci., 7: 1342, '83).). We also tested 4 unresponsive cells and 3 of these had their spontaneous discharges suppressed by NE. In all of the neurons in which it was tested, iontophoresis of the beta adrenergic antagonist propranolol blocked the effects of NE. Finally, no excitatory effects of NE were observed for any of the SC cells that we recorded.

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283.8 HEAD MOVEMENTS PRODUCED BY MICROINJECTION OF GLUTAMATE INTO SUPERIOR COLLICULUS OF RATS: EVIDENCE FOR INVOLVEMENT OF MULTIPLE OUTPUT PATHWAYS. P. Dean, I.J. Mitchell* and P. Redgrave*. Department of Psychology, University of Sheffield, Sheffield, S10 2TN, UK.

The crossed tectoreticulospinal pathway (TRSP), which runs from the superior colliculus in the contralateral predorsal bundle to the brainstem and spinal cord, is generally considered to mediate the orienting-like movements produced by tectal stimulation. It is unclear, however, whether the TRSP alone mediates these movements, or whether additional tectal output pathways are involved. In rats, the cells of origin of the TRSP are largely segregated within the lateral part of the stratum album intermediale (SAI) (Redgrave et al., Exp. Brain Res. in press). It is therefore possible to investigate in rats whether orienting head movements can be produced by appropriate stimulation of tectal areas that contain few cells of origin of the TRSP.

Hooded rats were filmed during microinjections of L-glutamate (50 mM, 200 nl in 24 sec) into the superior colliculus and surrounding areas. Contralateral movements of the head, in some cases also involving the body, were observed after injections in sites distant from lateral SAI. These sites were mainly in the caudal half of the superior colliculus, in the strata profunda and in the medial portion of the intermediate layers. The majority of cells in these areas project into the ipsilateral descending pathway. There was some suggestion that, whereas injections close to lateral SAI produced continuous, circling movements, the other effective injections produced either sustained postures, or repeated discontinuous movements, that more resembled natural orienting.

This evidence suggests that tectal efferent pathways besides the TRSP may be concerned in the control of contralateral head movement. It is consistent with previous findings that section of the TRSP fails to abolish head movement, either to sensory stimuli, or to electrical stimulation of the superior colliculus (Dean et al., Neurosci. in press). It is possible that the different output pathways mediate different functional classes of head movement: whereas the TRSP may emphasise pursuit of a moving target, the other pathways may be involved in open-loop orienting to particular positions in space.

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283.9 TECTO-TECTAL AND CORTICO-TECTAL EFFECTS ON HYPEREXCITABILITY IN THE SUPERIOR COLLICULUS OF 2,5-HEXANEDIONE TREATED RATS. R.D. Wilson*, D.Impelman*, D.Ruan* and D.A.Fox. University of Houston, College of Optometry, Houston, TX 77004.

Previously, we presented data from Long-Evans hooded rats with 2,5-hexanedione (2,5-HD) axonopathy showing (1) selective decreases in optic tract (OT) t2 conduction velocity (medium diameter group), and (2) hyperexcitability in OT t2 and superior colliculus (SC C2) amplitude recovery functions (RFs) following optic chiasm electrical stimulation. The hyperexcitability in SC C2 preceded OT t2 RF changes (Neurosci. Abs., 1985). More recent analysis of 2,5-HD SC C2 data shows: (1) excitability increases in rheobase thresholds: mean control rheobase was 32 microamps while 2,5-HD rheobase was 14 microamps, (2) compression and increased sensitivity of amplitude-intensity (A-I) functions, and (3) 2,5-HD amplitude RFs superimpose on control RFs with a translation of coordinates on inter-response interval (IRI) plots. The differences in 2,5-HD RFs may be accounted for by slowed afferent OT conduction, increased synaptic delay and increased tonic excitation. Slowed afferent OT conduction (-30%) and increased synaptic delay (+80%) recently have been documented in these 2,5-HD rats. To determine the possible role of increased tonic excitation to SC hyperexcitability in 2,5-HD rats, ablation or KCl studies examining tecto-tectal inhibition and cortico- cortical facilitation were performed. In tecto-tectal experiments simultaneous field potential depth recordings of OT/SC response functions were obtained 20-80 hours following contralateral SC ablation, the degeneration time established for OT t2 axons (Eysel and Grusser, 1974). Results show: (1) the sensitivity of SC luminance intensity functions (LIFs) are decreased approximately 50% with no change in OT LIFs and (2) OT/SC electrical A-I and RFs are unaffected. Thus, the acute excitatory effects of tectal ablation on SC LIFs (Goodale, 1973) are not found at longer time points. The absence of OT LIF post-lesion changes indicates that ablation effects are localized to the ipsilateral SC. The cortico-tectal effects, following the application of KCl to the VC, consist of a depression of VC and long latency SC postsynaptic responses. These effects are consistent with the acute effects of ipsilateral cortical ablation (Goodale, 1973). The results of tectal and cortico-tectal lesions suggest that SC hyperexcitability may be produced by acute lesion effects. Further experiments are in progress to investigate the acute effects of tectal ablation on SC excitability. Supported by NIEHS Grant ES 03183 (DAF).

283.10 CHOLINERGIC PROJECTIONS TO THE INTERMEDIATE LAYER OF THE RAT SUPERIOR COLLICULUS DEMONSTRATED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE AND CHOLINE ACETYLTRANSFERASE IMMUNOHISTOCHEMISTRY. M. Beninato* and R.F. Spencer. Dept. Anat., Med. Coll. of Virginia, Richmond, VA 23298.

Acetylcholinesterase (AChE) has been localized by histochemistry in the superior colliculus and in the tegmentum of the caudal midbrain and rostral pons of the rat. The pattern of AChE localization in the superior colliculus was characterized by homogeneous staining in the superficial layers and patch-like staining in the intermediate gray layer. In the tegmentum, AChE was localized in the pedunculopontine tegmental nucleus (PPN), beginning rostrally at the caudal pole of the substantia nigra and extending caudally to the level of the parabrachial nuclei, and in the lateral dorsal tegmental nucleus (LDTN) of the central gray. The localization of AChE in these nuclei overlapped the distribution of neurones stained by immunohistochemistry using an antibody to choline acetyltransferase (ChAT). The ChAT-immunoreactive neurones at these locations comprise cholinergic cell groups Ch5 (PPN) and Ch6 (LDTN).

Neurones in the PPN and the LDTN were determined to be potential sources of the cholinergic projection to the intermediate gray layer of the rat superior colliculus by double labelling with retrograde transport of horseradish peroxidase (HRP) combined with the immunohistochemical localization of ChAT. Three populations of neurones were identified. A predominantly ipsilateral ChAT-immunoreactive population was located in the pars compacta subdivision of PPN. Retrograde HRP-labelled neurones in the pars dissipata subdivision of the PPN, located ventral to the superior cerebellar peduncle at the level of the inferior colliculus, comprised a second population that was predominantly contralateral but was not ChAT-immunoreactive. A third population of retrogradely-labelled neurones was predominantly ipsilateral and ChAT-immunoreactive and was located in the LDTN. These findings compared favorably with the full extent of the projection from this tegmental region revealed by retrograde transport of HRP from the superior colliculus using more compatible fixation and chromogen procedures.

The results suggest that the PPN and the LDTN are two sources of the cholinergic input to the intermediate gray layer of the superior colliculus. Since the PPN also has extensive efferent, and afferent, connections with basal ganglia-related structures, this cholinergic excitatory input to the superior colliculus, like the GABAergic inhibitory input from the substantia nigra pars reticulata, may provide the basis for an additional influence of the basal ganglia on visuomotor behavior.

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283.11 TEMPORAL STRUCTURE OF SPIKE RESPONSES IN MULTIMODAL UNITS OF THE DEEPER LAYERS OF THE MOUSE SUPERIOR COLLICULUS. S.I. Wiener and P.H. Hartline. Eye Research Institute of the Retina Foundation, 20 Staniford St., Boston, MA 02114.

In order to better understand the nature of multimodal interactions in neurons of the superior colliculus (SC), we studied the time courses of spike responses to brief sensory stimuli by recording extracellularly from single units in the deeper layers of the SC of anesthetized mice.

Fifteen multimodal neurons had multi-phase responses, that is spikes occurred within more than one discrete interval, or phase, following brief stimuli. In most such cells, the first phase of the response consisted of one or two spikes at a fairly constant post-stimulus latency which ranged from 15-35 msec. The other phase was more extended, occurring in a 70-200 msec latency range and could contain from zero to eight spikes per response. In different cells, the multi-phased responses were evoked by visual, auditory or somatosensory stimuli.

In multi-phase multimodal cells responsive to single-modality stimulation of more than one modality (OR units), only one of the effective modalities evoked a multi-phase response. In these OR units, when the time interval between the bimodal stimuli was adjusted so the response to the second stimulus overlapped with one of the two phases of the first response, the combined spike count within that phase could be greater than additive, additive or less than additive in different units. If the response to the second stimulus preceded the early phase, it could interact to cause both phases to be enhanced or depressed. Alternatively the two phases could be affected independently; in two units, the late phase was enhanced (in one, by over 800%) and the early phase was unaffected by bimodal stimulation. In two other units, the first phase was depressed and the second phase was enhanced. These interactions were dependent on interstimulus timing.

Previous investigations in other laboratories suggest that the early response phase may correspond to the sensory input signals from primary ascending pathways and the late phase may result from excitation relayed through other pathways, perhaps including the cerebral cortex. The alteration of the multi-phase response by multimodal stimuli may have functional significance if the "downstream" premotor neurons that receive these signals are sensitive to temporal pattern, as has been suggested by other authors.

(This work was supported by grants from NIH and the Peter Murray Fund.)

283.12 TWO TECHNIQUES, ONE RETINOTOPY: METABOLIC AND ANATOMICAL MAPPING TECHNIQUES REVEAL SIMILAR ORGANIZATION OF A PRETECTAL NUCLEUS. Ximena Rojas*, Stefan R. Bodnarenko and Olivia C. McKenna, Biology Department, City College of New York, N. Y., N. Y. 10031.

In a previous HRP study of an avian pretectal nucleus, the lentiform nucleus of the mesencephalon (LM), we have shown that the LM receives a projection from a population of retinal ganglion cells that forms a broad oblique band extending from the superior temporal to the inferior nasal retina. In addition, this retinal projection appears to map topographically within the LM. These results, however, must be interpreted cautiously, since the complex C-shape of the nucleus and its location adjacent to the optic tract and to other retinorecipient nuclei give rise to the possibility that not all the HRP labeled cells seen in the retina actually project to the LM.

Therefore, we have taken advantage of the previous finding that the LM is labeled with (^{14}C)-deoxy-D-glucose (2-DG) when an animal is exposed to large field horizontal visual motion. On the assumption that differential stimulation of the retina will result in differential metabolic labeling within the LM, in twenty 3 week old birds we immobilized the eyes and then covered them with translucent hemi-occluders that exposed only half of the retina. After injecting an animal with 2-DG, we stimulated either the superior, nasal, temporal or inferior retina with horizontal visual motion.

When the results from these 4 different experimental conditions are combined, we find that stimulation of the superior nasal retina results in no discernible label of the LM. When the superior temporal retina is stimulated, the label is found throughout the nucleus in its rostral portion, but in the caudal portion the label becomes located progressively more ventral, leaving the dorsal limb unlabeled. In contrast, inferior temporal and inferior nasal stimulation of the retina labels the dorsal LM along its entire rostrocaudal extent. The label due to stimulation of the two inferior quadrants appears to overlap in the center of the nucleus; however, the label resulting from stimulation of only the temporal quadrant extends more rostrally within the nucleus, whereas label due to stimulation of the nasal quadrant extends more caudally.

The results of the topographical organization of the retinal projection to the LM as revealed by the 2-DG and by the HRP techniques are in basic agreement, despite the fact that the retinotopy suggested by the 2-DG method reflects the functional retinotopic organization of the nucleus, whereas the HRP technique demonstrates the anatomical retinotopy of the actual projections from the retina to the LM.

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SUBCORTICAL VISUAL PATHWAYS III

284.1 **TRANSNEURONAL ANTEROGRADE TRANSPORT OF FRAGMENT C OF TETANUS TOXIN IN THE VISUAL SYSTEM.** K.A. Manning, J.T. Erichsen and C. Evinger. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, N.Y. 11794

Until now, tetanus toxin has been used exclusively for retrograde transsynaptic transport studies. We report for the first time that the atoxic C fragment of tetanus toxin exhibits excellent anterograde transneuronal transport. We demonstrate transneuronal labeling in the visual system of both mammalian and avian species.

Intravitreal injections of 20-40 ul of concentrated fragment C of tetanus toxin (TTC) were made into the eye of rats, rabbits and pigeons. Eight days later, the animals were sacrificed and the brains processed for immunohistochemistry. A monoclonal antibody we have raised to TTC was used in conjunction with the standard Avidin-Biotin procedure to localize TTC in the brain. The pattern of labeling was compared with the results of HRP injections into the eye.

In each species, strong immunostaining occurred in all retinorecipient areas, including the superior colliculus (or tectum), dorsal and ventral geniculate nuclei, posterior pretectal nucleus, pretectal olivary nucleus (or area pretectalis), nucleus of the optic tract, suprachiasmatic nucleus and accessory optic nuclei (e.g., nucleus of the basal optic root). In the pigeon, the isthmooptic nucleus, which is the source of the centrifugal pathway to the retina, labeled retrogradely. Examination with the light microscope suggested that in the primary retinorecipient areas (i.e., after direct anterograde transport) TTC was largely localized to terminal fields. Interestingly, fibers such as the optic tract exhibited little or no staining.

In the mammals, several regions contained label from anterograde transneuronal transport. These areas include visual cortex, thalamic reticular nucleus, parabigeminal nucleus, and dorsal cap of Kooy and Beta nucleus of the inferior olive. In transneuronal labeling, the pattern of staining around individual neurons suggested localization of TTC in either the soma or presynaptic membranes directly apposed to the cell. In the pigeon, label occurred in the visual Wulst, which receives a projection from a thalamic retinorecipient area.

The data presented here demonstrate rapid anterograde transneuronal transport of TTC in visual pathways. However, because of the existence of reciprocal connections between a few of these visual areas, some of the transneuronal staining may also include retrograde label from retinorecipient areas.

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284.2 NIGROTECTAL PROJECTIONS IN THE CAT: ANTEROGRADE AND RETROGRADE TRANSPORT STUDIES. T. Hashikawa*, J.T. Weber, R. Hartwich-Young, D.P. Van Lieshout* and J.K. Harting*, Departments of Anatomy, Iwate Medical University, Morioka, JAPAN, Tulane University, New Orleans, LA, and University of Wisconsin, Madison, WI.

The nigrotectal projection in the cat has been described as terminating in a thin tier just ventral to the stratum opticum (SO), within the most dorsal portion of the stratum griseum intermediale (SGI). Moreover, this tier of nigrotectal input ends in patches which form longitudinal bands (Graybiel, '78).

The present anterograde data support and considerably extend the findings described above. Most importantly, our data reveal that nigrotectal axons distribute to numerous collicular laminae and sublaminae. Thus, while a patchy tier of label is present within the SO-SGI region in many but not all of our experimental cases, it is always accompanied by either a deeper tier within the intermediate portion of the SGI, by a sparse input to the striatum griseum profundum (SGP), or by a diffuse yet substantial input to the superficial layers.

Preliminary retrograde data suggest that the three primary tiers of input (superficial gray layer, SO-SGI tier, intermediate SGI tier) arise from different regions of the substantia nigra. Berman ('68) has divided the substantia nigra of the cat into three portions, i.e., the pars reticularis (SNpr), the pars compacta (SNpc) and the pars lateralis (SNpl). Our HRP data demonstrate that the nigrotectal projection to the superficial layers arises from neurons located within the SNpl. The tier of nigrotectal input to the SO-SGI interface region appears to arise from neurons located most laterally within the SNpr, while the projection to the intermediate tier within the SGI takes origin from cells located more medially within the SNpr.

Certainly more data are needed to confirm the cellular origins of the three primary tiers of nigrotectal input. However, it is important to emphasize the extensive laminar and sublaminar distribution of nigrotectal axons in the cat and the apparent connectional relationships of specific regions of the substantia nigra with particular collicular sublaminae.

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284.3 ULTRASTRUCTURAL ANALYSIS OF RETINAL AND VISUAL CORTICAL PROJECTIONS WITHIN THE CAT'S PRETECTAL NUCLEUS OF THE OPTIC TRACT. B. Hutchins and J.T. Weber. Baylor College of Dentistry, Dallas, Tx. 75246 and Tulane Medical School, New Orleans, La. 70112

Numerous studies have shown both retinal and visual cortical projections to the cat's pretectal nucleus of the optic tract (NTO), however, there have been little data describing the ultrastructure or possible interaction of these two important visual projections within the NTO. Therefore, the present study was undertaken to analyze these projections at the electron microscopic level. In order to systematically sample the NTO, the nuclear dimensions of the NTO were measured in four normal adult cats. The approximate dimensions of the NTO were determined to be 5.5 mm (rostral/caudal length) X 4.0 mm (medial/lateral width) X 2.2 mm (dorsal/ventral depth).

Sixteen animals were used to study the ultrastructure of retinal or visual cortical terminals in the NTO using either anterograde degeneration or anterograde HRP histochemistry. The classification of synaptic profiles is based on the criteria as described by Guillery, Z. Zellforsch.,96 ('69). In all cases, retinal terminals in the NTO are identified as RLP profiles. Labelled RLP profiles are occasionally found as the central process in synaptic glomeruli although the majority of labelled RLP terminals are extraglomerular and in contact with a single medium to large diameter dendrite. Supporting our earlier observations, visual cortical terminals are of two types, i.e., the RSD and RLD profiles (Hutchins and Weber, Neurosci. Abstr. '82). Labelled RSD profiles typically are found in contact with dendrites of small to medium diameter, while RLD profiles are found presynaptic along various regions of dendrites. Additionally, RLD axon terminals are occasionally found presynaptic to cell somata, although only one HRP labelled RLD profile has been observed in association with perikarya. There are no obvious regional differences within the NTO with regard to the types of labelled synaptic terminals or differences in the postsynaptic elements of labelled axon terminals. However, both the retinal and visual cortical inputs do appear to terminate in clusters.

To test the hypothesis that there is direct retinal and visual cortical projections to single NTO neurons, four additional experiments were performed which combined Wallerian degeneration and the anterograde transport of HRP. Preliminary data indicate that axons from both retinal and cortical neurons do indeed synapse on single NTO cells. Thus, these data suggest that within the NTO, there is at least some direct modulation of retinal information by the visual cortex. Supported by NIH Grant EY03731 (J.T.W.) and Baylor College of Dentistry Research Funds.

284.4 SPRAGUE EFFECT MEDIATED BY REMOVAL OF NON TECTO-TECTAL FIBERS IN THE COMMISSURE OF THE SUPERIOR COLLICULUS. J.M. Sprague, S.F. Wallace* and A.C. Rosenquist. Dept. of Anat., Sch. of Med., Univ. of Penn., Phila., PA 19104-6058.

Total contralateral hemianopia follows unilateral removal of all visual areas in the parieto-occipital cortex of the cat. In 1966 Sprague showed that orienting responses, as measured by visual perimetry, can be restored to the "blind" hemifield by ablation of the superior colliculus (SC) opposite the cortical lesion or by transection of the commissure of superior colliculus (CSC). The original explanation for this recovery phenomenon (the "Sprague Effect") proposed that the SC ipsilateral to the cortical lesion, which lacks the normal corticotectal input, is also suppressed by inhibition from the opposite SC. Removal of the inhibition by ablation of the contralateral SC or tectal commissurotomy restores orienting behavior to the blind field. Tecto-tectal connections occur only between cells in the rostral 1/2 of the two SC and the fibers lie in the rostral 1/2 of the CSC (Edwards, 1977). If the loss of the tecto-tectal component of the CSC is responsible for the Sprague Effect, then cutting only the rostral 1/2 of the CSC should produce the effect. This is not the case. We found that orientation was restored after transection of the caudal 1/2 of the CSC (N = 3), but not after transection of rostral 1/2 (N = 3). Therefore the Sprague Effect appears to be due to interruption of CSC fibers whose cell bodies lie outside the SC. This conclusion is also supported by experiments in which the cortical lesion is preceded by injection of ibotenic acid in the contralateral SC, destroying the tecto-tectal system but sparing other commissural fibers. No return of vision occurred to the field opposite the cortical lesion (N = 3).

The original Sprague hypothesis also proposed that the recovered vision is mediated by cells in the SC ipsilateral to the cortical lesion. This is supported by an experiment in which cortical hemianopia is first abolished by commissurotomy and then re-instituted by ibotenic acid injection into the SC ipsilateral to the cortical lesion.

Thus we conclude that the Sprague Effect is mediated by removal of non tecto-tectal fibers in the CSC which influence cells mediating visual orientation in the SC ipsilateral to the cortical lesion. (Supported by NIH grants 2R01EY02654; 5T32GM07170; 2P01EY01583).

284.5 THE ROLE OF THE SUBSTANTIA NIGRA (PARS RETICULATA) IN THE MEDIATION OF THE SPRAGUE EFFECT. S.F. Wallace*, A.C. Rosenquist (SPON: L. Palmer) Dept. of Anat., Sch. of Med., Univ. of Penn, Phila., PA 19104-6058.

Transection of the commissure of the superior colliculus (CSC) in a cortically "blind" cat restores visual orientation in the previously hemianopic field. Further studies of this phenomenon (the Sprague Effect) indicate that the fibers of interest do not arise from the contralateral superior colliculus (SC) but rather, from an extra-collicular site and pass through the caudal 1/2 of the CSC. (Sprague et al, this volume).

In order to identify this site, we undertook an anatomical study to determine which SC afferents pass through the caudal 1/2 of the CSC. Unilateral injections of WGA-HRP were made into the SC at identical loci in pairs of cats. The caudal 1/2 of the CSC had been previously transected in 1 cat while the other served as a control. Quantitative counts of retrogradely labelled cells were then performed and the results within each pair compared. Two areas were found to contain many more labelled cells when the CSC was intact: the nucleus cuneiformis and the substantia nigra pars reticulata (SN_r). Both of these regions have been reported to project to the SC via the CSC but the topography of these connections and their distribution with the CSC is not known. We are currently examining this with orthograde tracers.

To determine whether these nuclei are involved in the Sprague Effect, behavioral experiments were conducted. Cats were trained in a visual perimetry paradigm that permits accurate assessment of their visual fields. All of the known visual cortical areas were then unilaterally removed and the cats allowed 6-8 weeks recovery. Re-assessment at this time revealed the persistence of a contralateral hemianopia which has previously been reported to be permanent (Sprague 1966). Subsequent lesion of the nucleus cuneiformis contralateral to the ablated cortex was performed by injection of ibotenic acid. This did not result in any recovery of visual orientation. By contrast, destruction of the rostro-lateral aspect of the contralateral SN_r gave a complete and robust return of visual orienting within three weeks (Sprague Effect). Large lesions of the caudo-lateral SN_r or injections into the cerebral peduncle gave no recovery.

When the order of the lesions was reversed, a contralateral hemianopia initially followed the cortical lesion but it resolved in less than 4 weeks. The behavioral consequences of the rostro-lateral nigral lesion alone could also be observed in these cats. These animals exhibited a transient compulsive contraversive circling, decreased contralateral eye movements, and perioral dyskinesia. A conceptual framework for these findings involving cortico-striato-nigro-tectal interactions will be presented. (Supported by NIH grants 2R01YE02654; 5T32GM07170; 2P01EY01583.)

284.6 PROLONGED HEMIANOPIA PRODUCED BY IBOTENIC ACID LESIONS OF THE CAT SUPERIOR COLLICULUS. A.C. Rosenquist and S.F. Wallace*. Dept. of Anat., Sch. of Med., Univ. of Penn., Phila., PA 19104-6058

Large unilateral injections of the excitotoxin ibotenic acid into the cat superior colliculus (SC) produce a complete contralateral hemianopia of 8 to 11 weeks duration when measured in a standard visual perimetry test. By contrast, removal of one SC (by subpial aspiration) results in a contralateral hemianopia of less than 2 weeks duration. This reduction in the severity of visual deficits in the aspiration lesions (when compared to the ibotenic acid lesions) may be due to severing of fibers of pasage coursing through the SC and/or its commissure. It is likely that the loss of these fibers may reduce the adverse behavioral consequences of collicular cell loss. Removal of fibers in this same midbrain region has also been shown to reduce adverse behavioral effects (hemianopia) of large visual cortical lesions (i.e. the Sprague Effect).

Anatomical reconstruction of the ibotenic acid lesions indicates that the most effective lesion site for producing a long standing hemianopia is located within the superficial and intermediate layers of the SC (SZ,SGS,SO,SGI).

If a cat that is hemianopic due to an ibotenic acid lesion of one SC is then subjected to removal of all visual cortical areas opposite the SC injection, an immediate and enduring hemianopia is produced opposite the cortical lesion and an immediate and complete recovery of vision is produced in the previously blind hemifield (opposite the collicular lesion). The recovery implies that ibotenic acid lesions in the SC produce a severe neglect of the opposite hemifield which can be overcome when the competing "good" hemifield is rendered blind by the cortical lesion. (Supported by NIH grants 2R01EY012654; 5T326M07170; 2P01EY01583).

284.7 POSTNATAL DEVELOPMENT OF ACETYLCHOLINESTERASE HISTOCHEMICAL STAINING PATTERN IN THE CAT SUPERIOR COLLICULUS. J.G. McHaffie*, R.F. Spencer, M. Beninato*, and B.E. Stein (SPON: A.L. Berman). Depts. Physiol. & Biophys. and Anat., Med. Col. of Virginia, Richmond, VA 23298.

The superior colliculus (SC) in the adult cat displays laminar-specific patterns of AChE staining. AChE is distributed homogeneously throughout the entire rostral-caudal and medial-lateral extent of the stratum griseum superficiale (SGS). The stratum griseum intermediale (SGI) displays the characteristic patch-like distribution of AChE in the caudal half of the SC with a medial-lateral gradient. AChE staining also is observed in the stratum griseum profundum (SGP) and the dorsolateral periaqueductal gray (PAG) but to a lesser extent than the SGS and SGI.

At birth, the homogeneous pattern of AChE staining in the SGS is similar to the adult. AChE staining associated with the neuropil in the SGI and SGP, however, is absent. At 7 dpn, the adult patch-like staining pattern in the SGI is apparent. By 28 dpn, the complete laminar-specific pattern of AChE staining is observed including that associated with the SGP and the PAG.

The cholinergic projection to the intermediate layer of the SC arises from the pedunculopontine tegmental and lateral dorsal tegmental nuclei, or cholinergic cell groups CH5 and CH6 in the caudal midbrain and rostral pons. The immunohistochemical localization of choline acetyltransferase (ChAT) reveals intense immunoreactive staining of neurones in the Ch5 cell group early in the postnatal period. Neurones in the Ch6 cell group, however, are only weakly immunoreactive, similar to neurones in the parabigeminal nucleus. Correlated at least in part with the absence of AChE in the SGI in neonatal animals, injections of horseradish peroxidase into the SC retrogradely label few, if any, neurones in these cell groups. By contrast, an extensive projection from these cell groups is demonstrated by similar injections in adult animals. At all stages, however, retrogradely labelled neurones are observed in the substantia nigra pars reticulata, albeit fewer in neonatal animals when compared to the adult.

The findings collectively indicate that the cholinergic projection to the intermediate and deep layers of the cat SC undergoes considerable postnatal development. The superficial to deep developmental gradient of AChE staining may be related to functional differences in the afferent and efferent relationships of the different layers. These results suggest that the influence of basal ganglia-related structures on motor behaviors associated with the deeper layers of the SC may develop after the major sensory afferent and tectal efferent connections are formed.

Supported by NSF Grant BNS-8209857 and NIH Grant EY02191.

284.8 COLOCALIZATION OF GAMMA-AMINOBUTYRIC ACID AND LEUCINE ENKEPHALIN IN SINGLE NEURONS OF THE CAT SUPERIOR COLLICULUS: AN IMMUNOCYTOCHEMICAL STUDY. R. Ranney Mize, Department of Anatomy and Neurobiology, University of Tennessee, The Health Science Center, Memphis, TN 38163

Both gamma-aminobutyric acid (GABA) (R.R. Mize et al., J. Comp. Neurol., 206:180, 1982) and leucine enkephalin (R.R. Mize, Invest. Ophthal. Vis. Sci., 27:205, 1986) are found in the cat superior colliculus (SC). The distributions of the two transmitters partially overlap, and some cells stained by antisera to these two substances have similar morphologies. These results suggest that some SC neurons may contain both GABA and leucine enkephalin. To study this directly, we used a double-label technique to mark both transmitters in the same tissue.

Aldehyde-fixed sections were incubated in varying dilutions of antisera to GABA and leucine enkephalin and then reacted with the avidin-biotin (ABC) technique. GABA antiserum reactivity was stained with a green chromagen (o-dianisidine) and leucine-enkephalin antiserum reactivity with a brown chromagen (diaminobenzidine).

GABA immunoreactivity was found throughout the SC, although immunoreactive neurons were most heavily concentrated within the superficial gray layer (SGS). Anti-leucine-enkephalin staining was far more restricted. Neuropil labeling was largely confined to a thin band within the upper SGS. Most stained cells were contained within this band, although a few were seen deeper. Both GABA and leucine enkephalin stained cells were small, with a mean average diameter of 13 um.

Some cells in SC contained both green and brown reaction product. These double-labeled cells represented 18% of the total population of immunoreactive neurons (42 of 232 cells). 46% were labeled only by GABA and 36% only by leucine enkephalin. Nearly two-thirds of these cells were confined to the upper 200 um of SGS. Only 6 were located below this lamina. They ranged in size from 9.6 to 18.7 um in average diameter with a mean of 12.7 um. They were thus identical in size to single labeled neurons. The double labeled cells had varying morphologies and included (1) neurons with horizontal fusiform cell bodies and horizontally oriented dendrites and (2) neurons with small round cell bodies.

These results show that a small population of neurons in the cat SC contains both GABA and leucine enkephalin. These neurons include both horizontal and granule morphologies. Because not all horizontal cells were double-labeled, cells with identical morphologies may prove to be chemically heterogeneous.

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284.9 A SLOWLY CONDUCTING SUBCLASS OF W-CELLS INNERVATES THE UPPER SUPERFICIAL GRAY OF CAT SUPERIOR COLLICULUS. D.M. Berson. Sect. Neurobiol., Div. Biol. & Med., Brown Univ., Providence, RI 02912

Retinal input to the cat's superior colliculus (SC) exhibits laminar heterogeneity. Y-cells project to the deeper part of the superficial layers, and W-cells more superficially. The W-cell input to the SC is itself heterogeneous, but little is known of the laminar distribution of inputs from particular morphological or functional W-cell subclasses. Here, retinal input to the upper third of the superficial gray layer ("the upper tier") is shown to originate from a slowly conducting subclass of W-cells.

Juxtazonal potentials (JZPs) are unitary extracellular potentials that can be recorded only in the upper tier (McIlwain, J. Neurophys. 41:1343,'78). A single JZP reflects the local current sink produced when postsynaptic elements in the upper tier are synchronously depolarized by a single W-cell's axonal arbor, following its invasion by a spike. A JZP is recorded in isolation (and at a fixed latency) when a shock to retinofugal fibers is suprathreshold for the W-cell axon generating that JZP but subthreshold for other axons terminating nearby.

In this study, a collision technique was used to estimate, in Nembutal- or ketamine-anesthetized cats, the conduction velocities of W-cell axons generating JZPs. A shock to the optic disk (OD; 50 μsec, <1 mA) that, alone, always elicited a single JZP was preceded at various intervals by an intense conditioning shock to the optic chiasm or tract. At (or below) a critical intershock interval, the OD stimulus failed to evoke the JZP; this indicated that an antidromic spike, evoked in the axon generating the JZP by the conditioning shock, had collided with the orthodromic spike which normally produced that JZP. The spike's interelectrode conduction time was taken to be the collision interval minus the axon's refractory period (which was estimated as the minimum intershock interval at which two OD shocks produced two JZPs). Dividing interelectrode conduction distance (obtained from dissection) by this conduction time yielded an estimate of the conduction velocity of the JZP-generating axon.

Conduction-velocity estimates for retinal axons generating JZPs ranged from 2.9 - 6.8 m/sec ($\bar{X}$= 4.5 ± 1.0; n=28). These axons thus have among the slowest conduction velocities of any in the W-cell spectrum (2-18 m/s; $\bar{X}$= 8.7; Stone & Fukuda, J. Neurophys. 37:722,'74). They are markedly slower than axons of tonic, concentric W-cells ($\bar{X}$= 11.7 m/s), but are comparable to those of phasic W-cells, particularly the ON-OFF type (2-9 m/s; $\bar{X}$= 5.9; ibid). The implication that phasic W-cells may innervate the upper tier is consistent with the fact that JZPs can be evoked only from the contralateral eye (McIlwain, '78), since the axons of almost all phasic W-cells decussate (Kirk, et al., Vision Res. 16:433,'76).

284.10 CODING FOR OPTICAL FLOW BY SINGLE CELLS IN CAT SUPERIOR COLLICULUS (SC). G. Mandl, Aerospace Med. Res. Unit, Dept. of Physiol., McGill University, Montreal, Canada H3G 1Y6.

For an observer in linear motion, instantaneous position and (apparent) velocity of a fixed point in visual space are defined with reference to a system of meridians on the surface of an observer-centered imaginary sphere, with all meridians passing through the vanishing point (instantaneous position and velocity fields; Gordon, 1965). The direction of each fixed point's instantaneous velocity vector is tangential to its meridian, and vector magnitude is a sine function of the point's angular distance from the vanishing point. The present results suggest that single cells in superficial and intermediate layers of cat SC signal both, instantaneous position and velocity, of moving targets, and thus contribute to the normal encoding of dynamic spatial relations within patterns of optical flow. METHODS Trains of extracellularly recorded unit spike discharges, from the SC of pretrigeminal paralyzed cat preparations, were evaluated statistically. Visual stimuli consisted of small luminous slits (2.5x0.4°), either flashed or moving with uniform velocities (1-160°/s), across a CRT display screen. RESULTS (1) While the response to a single stationary briefly flashed (5ms) slit outlasted stimulus duration by some 600 ms, the response to a moving (either smoothly or sequentially flashed) slit was consistently limited in time to the duration of stimulus presence in the receptive field. This motion-contingent spatio-temporal "gating" process constitutes a necessary neural basis for the normally distortion-free appearance of moving patterns during their (even quite rapid) sequential displacement in visual space (instantaneous position field). (2) Spike trains were characterized by abrupt alternations between fast ("bursting") and slow ("resting") discharge states. The temporal structure, and rate of alternation, of consecutive bursting and resting episodes was systematically related to stimulus velocity. This non-linear switching of temporal patterning provides a most plausible candidate code for the detection of instantaneous velocity vectors, especially since discharge frequency, in response to a 160-fold change in stimulus velocity, typically changed no more than 3-5 fold. (3) Linear receptive field dimensions were shown to increase as a (approximate) sine function of their angular distance from the area centralis. In view of a similar relation between the magnitude of a point's velocity vector and its distance from the vanishing point (cf. above), all points within a moving observer's instantaneous velocity field, regardless of their angular distance from the vanishing point, traverse equal receptive field fractions in equal times. Thus, the dynamic structure and coherence of global optical flow patterns (relative positions and velocities of individual loci) are preserved . Ref: D.A. Gordon, J. opt. Soc. Amer., 55: 1296 (1965). Supported by the Canadian Medical Research Council.

284.11 MULTISENSORY INTERACTIONS DEPEND ON ALIGNMENT OF RECEPTIVE FIELDS IN CAT SUPERIOR COLLICULUS NEURONS M. A. Meredith and B.E. Stein, Dept. of Anat. & Dept. of Physiol. & Biophys., Med. Col. Va., Richmond, VA 23298

Receptive fields (RFs) of the different modalities of multisensory cells are in register with one another when their respective sensory organs are aligned. This topographic relationship facilitates the integration of multisensory cues from the same point in space. However, during normal behavior sensory organs can be out of alignment. The present study examines the effect of RF misalignment on responses of visual-auditory neurons in the cat superior colliculus to multisensory stimli.

Cats (n=3), chronically prepared for extracellular recording, were anesthetized with ketamine hydrochloride, paralyzed and artificially respired with a mixture of 75% N2O and 25% O2. Each animal was positioned so that its visual axis was perpendicular to the horizontal interaural axis. Visual RFs were mapped onto a translucent plexiglass hemisphere and visual stimuli were electronically-controlled moving bars of light. Auditory RFs were mapped using near threshold broad-band noise bursts from a hoop-mounted speaker whose elevation and azimuth could be changed. Weighted hooks were placed on the margins of the pinnae and changes in pinnae position were effected by pulling both pinnae contralaterally. Auditory as well as visual RFs were remapped in this new position. Single- and combined-modality stimuli were presented at predetermined points in space during normal and pinnae-adjusted conditions.

As expected, the locations of auditory RFs were shifted by changes in pinnae position. However, no compensatory changes in the visual RFs occurred. Thus the combined visual and auditory stimulation that evoked response enhancement before pinnae movement, now elicited the response depression that was previously evoked only when one stimulus was located outside its RF borders. Repositioning the auditory stimulus to be at the center of the new auditory RF position reproduced the original response enhancement. Therefore, realignment of multisensory maps in cats (necessary for maximizing effects of multisensory cues from the same locus) appears not to be an immutable consequence of the displacement of a single sensory organ. During normal behavior, realignment of sensory maps may be accomplished by either compensatory shifts in the sensory maps (Jay and Sparks, Nature 309:345, 1984) or by realigning of the sensory organs themselves (Harris, Donaghy and Blakemore, Nature, 288:56, 1980).

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284.12 CONTRIBUTION OF THE CORTICOTECTAL TRACT TO GLOBAL VISUAL PROCESSING IN THE MONKEY SUPERIOR COLLICULUS. R.M. Davidson, J.E. Albano, and D.B. Bender* (SPON: P.G. Funch). Div Neurobiology, Dept. Physiology, SUNYAB, Buffalo, NY 14226.

We previously showed that most superficial layer colliculus cells are sensitive to differential movement between a local target and the global visual scene. These cells respond well to a target whose direction and speed differ from that of a textured moving background; when target direction and speed are similar to that of the background, the response is suppressed (Davidson and Bender, Soc. Neurosci. Abstr., 1985,11: 1006). Similar phenomena have been described in visual cortex, suggesting that local-global interactions in the colliculus might depend on descending input from cortex. We therefore destroyed the corticotectal tract by means of unilateral heat lesions of the pulvinar (Bender and Baizer, J. Comp. Neurol., 1984, 228: 284), and then estimated motion-specific suppression in the colliculus. To estimate suppression, we paired movement of the target through a cell's receptive field in one direction with movement of a large textured background in either the same or opposite direction, and then divided one response by the other.

Interruption of the corticotectal tract greatly reduced motion-specific suppression ipsilateral to the lesion. In normal animals, suppression ranges from 0% to 90% among cells in the superficial 350 microns of the colliculus; suppression is more potent in deeper cells, ranging from 50% to 100%. By contrast, suppression did not exceed 50% at any depth ipsilateral to the lesion. Contralateral to the lesion, suppression was little affected.

Other changes were also seen following the lesion. In normal animals, most cells have spontaneous firing rates less than 5 spikes/sec. Ipsilateral to the lesion, however, many cells had rates of 5-15 spikes/sec. In normal animals, most cells respond with a transient burst of spikes to the onset and offset of a flash. Ipsilateral to the lesion, however, many cells gave a strong sustained response to a flash.

These results suggest that motion-specific suppression in monkey superior colliculus depends on descending projections from visual cortex. However, some of the observed effects may have resulted from retrograde changes in those tectal cells that project to the pulvinar; we are currently using kainic acid pulvinar lesions to control for this possibility.

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284.13 VISUAL MOTION PROCESSING IN THE DORSOLATERAL PONTINE NUCLEUS, A RECIPIENT OF MT, MST, AND PARIETAL INPUTS IN THE ALERT MONKEY. D.A. Suzuki and R.D. Yee*, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The dorsolateral pontine nucleus (DLPN) is a major brain stem terminus for converging pathways originating in cerebral cortical areas that are known to process visual motion-related information. The responses of cells in DLPN to moving visual stimuli were investigated to clarify the neuronal processing of visual motion occurring in this brain stem structure. Extracellular activity was recorded in alert monkeys that were trained to fixate a stationary, 0.5 deg red spot during the presentation of moving visual stimuli. All images were back projected onto a 90- by 90-deg square tangent screen. Eye position was monitored with the scleral search coil technique.

Out of 217 DLPN cells recorded in three monkeys, 196 (90%) were responsive to moving visual stimuli. The remaining 21 (10%) cells were responsive during smooth-pursuit eye movements, but were not responsive to moving visual stimuli. Of the visually responsive DLPN cells, 139 (71%) exhibited direction selective changes in firing rates that were related to retinal image velocity. All horizontal and vertical directions were represented in the population. DLPN firing rates increased with increases in retinal image velocities up to 30-40 deg/sec. Either a decline or a saturation in firing rate were observed in response to visual motion greater than 40 deg/sec, In cells without an eye movement component, the sensitivity to visual motion was enhanced during pursuit, when retinal image motion was behaviorally significant.

Receptive field (rf) types: 1) Two components: a central, retinal image velocity-related component that included the fovea, plus a large, non-velocity component that typically extended out to the limits of the tangent screen. 2) Paracentral, velocity related: small rf that was eccentric to the foveal region. 3) Large, velocity-related: rf covering one-half to all of tangent screen. Also observed were DLPN cells that had 4) direction selective, but non-velocity related responses to moving random dot patterns that filled the entire screen, and 5) eccentric rf that were responsive to the presence of stationary visual stimuli.

The results are consistent with afferent input to DLPN from the superior temporal sulcus (areas "MT" and "MST") and the inferior parietal cortex, two cortical areas that process visual motion. The behavior-dependent, enhanced visual response, together with pharmacological lesion and stimulation results implicate DLPN roles in the regulation of smooth-pursuit eye movements. The pursuit system is just one example of a motor system that utilizes visual motion signals. Subsequent DLPN efferent projections to the cerebellum raises the possibility that the DLPN contributes to a more global utilization of visual motion information in the coordination of motor behavior. (Supported by NIH Grant EY05167.)

284.14 EXTRAGENICULAR PROJECTIONS TO VISUAL CORTEX IN THE TREE SHREW: EVIDENCE FOR AN EXCLUSIVE PRETECTAL PATH TO AREAS 17 AND 18. R.G. Carey, D. Fitzpatrick[1] and K.M. Horn: Div. Neurobiol., Barrow Neurological Institue, Phoenix, AZ 85013 and [1]Dept. of Anat., Duke University, Durham, N.C. 27706.

Past studies in tree shrew have indicated that the strip of pulvinar (Pulcap) adjacent to the LGN, the lateral intermediate nucleus (Li), and the central intralaminar nucleus (CIN) of the visual thalamus are reciprocally connected with visual cortex including areas 17 and 18. More recent studies seeking a reevaluation of the tectopulvinar pathways have indicated a paucity of connections with these thalamic areas following injections of the superficial layers of the tectum. However, injections that included the pretectal complex often led to terminal activity within these thalamic areas. The central question of the present study is whether tectal or pretectal neurons provide the major ascending input to the thalamic nuclei that relay to areas 17 and 18.

Small electrophoretic injections of WGA/HRP or other tracers were made into either visual cortex, visual thalamus, tectum or pretectum in adult tree shrews. Following injections in areas 17 or 18 labeled activity was restricted to the Pulcap portion of the pulvinar and was continuous with activity in Li. Injections in area 18 resulted in a further focus of activity along the lateral extent of CIN that often extended dorsomedially to join the ventromedial Li at more rostral levels. Following injections in Pulcap or CIN labeled terminals were present in layers IV, VI and I of area 18, with labeled cells principally in layer VI of area 18. Additionally, Pulcap injections resulted in scant terminal activity in layer I and labeled cells in layer V of area 17. Results following injections of Li appeared more complex, but generally indicated diffuse terminations in layer I and widely dispersed cells in layer V in both areas. After these thalamic injections no labeled cells were found within the superficial layers of the superior colliculus. However, dense labeled activity was present in the anterior (Pta) and/or posterior (Ptp) pretectal nuclei. Injections of Ptp or Pta verified these projections and further indicated that at least the projection to Pulcap is topographically organized. Injections placed either in caudal Ptp or rostral Pta resulted in labeled terminals in the caudal levels of Pulcap, whereas injections placed in rostral Ptp or caudal Pta resulted in labeled activity at rostral levels of the Pulcap.

We conclude that the neurons in Pulcap, Li and CIN relay impulses from the anterior and posterior pretectal complex to areas 17 and 18 in the tree shrew. This path is distinct from the tectopulvinar projection that eschews areas 17 and 18 and terminates in more remote areas of visual cortex.

Supported by NIH EY04641, NSF BNS8411964 and NRSA EY05801.

284.15 THE DORSAL CLAUSTRUM OF TREE SHREW AND FERRET: VISUAL RESPONSE PROPERTIES AND RETINOTOPIC ORGANIZATION. K. M. Horn and R. G. Carey. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Connections between the dorsal claustrum and portions of visual cortex and thalamus have been documented in many species, however the cat has been the only model used in physiological studies. It was the purpose of our study to determine the organization and visual receptive field properties of neurons within the dorsal claustrum in two other species (tree shrew and ferret).

Neurons in the caudal portion of the dorsal claustrum in both species are responsive to visual stimulation. The majority of these neurons in the tree shrew have receptive fields that are situated within the central 20° of the visual field (86%) and are binocularly activated, while the ferret has fewer centrally located units (36%). Two major classes of visual claustral units have been identified in these species. The first and major class in both species (tree shrew 72%; ferret 81%) had well-defined, elongated receptive fields that shared some of the response characteristics reported by Sherk and LeVay (1981) in their study of the cat claustrum. This class had very little spontaneous activity, did not adapt to repeated presentation of stimulus, and had a wide range of contrast sensitivities. The majority of these units (tree shrew 53%; ferret 67%) had a preferred axial orientation with some cells also showing directional selectivity (tree shrew 17%; ferret 29%). Few units expressed appreciable length summation, and none demonstrated end-stop inhibition. A major subgroup of this first class (tree shrew 75%; ferret 55%) were extremely sensitive to the movement of small stimuli across or within the receptive fields (spots < .5°; slits < .25° wide). The second class (tree shrew 28%; ferret 10%) had diffuse, poorly-defined receptive fields that often extended for more than 30° and rapidly habituated to repeated stimulation. In the tree shrew these neurons were found near the borders and in the hilum of the claustrum, while in the ferret they were admixed with the units from the first class. A third class of cells (10%) was found in the ferret rostral and ventral to the traditional visual claustrum. This area contained units that responded to global changes in light intensity, stroboscopic stimulation, or to complex visual stimuli located near the horizontal meridian. These neurons were characterized by the appearance of spontaneous background activity and habituated with repeated stimulation.

While the area of the claustrum that is devoted to central vision in these two species differs, the overall response properties of many of these neurons to specific features of moving stimuli indicates a possible role in spatial-temporal processing. [Supported by NRSA fellowship EY05801 (KMH), NIH grant EY04641 (RGC) and BRSG RR0572.]

284.PO AERIAL PRIMATES: ADVANCED VISUAL PATHWAYS IN MEGABATS AND GLIDING LEMURS.
John D.Pettigrew and Howard M. Cooper*, Neuroscience Lab., University of Queensland, St.Lucia 4067 Australia.

A number of features characterise the visual pathways of all living primates and distinguish them from other mammals, including tree-shrews, often thought to be primates' closest living relatives. These features include:-
i. a reduced, but binocularly-balanced, retinotectal pathway subserving only the contralateral hemifield of visual space
ii. ipsilateral retinal input to the rostral-most tectum
iii. ipsilateral bias in the retino-hypothalamic pathway
iv. reduced accessory optic input to the medial terminal nucleus
v. a complex of characters in the organisation of the lateral geniculate nucleus , with segregation of ocular input to different laminae and with the magnocellular laminae adjacent to the optic tract.

Using both HRP tracing and visual recording techniques, we have found these "primate" features to be present in megachiropteran bats (Pteropus ,Rousettus, Cynopterus, Syconycteris) but not in microchiropteran bats (Macroderma, Taphozous, Nyctophilus). Convergent evolution of so many different details in primates and megabats is less likely than independent evolution of flight in two groups of mammals. This becomes particularly evident on examination of the few derived features linking the two kinds of bats, all of which are associated with flight, and the morphological differences between the two kinds of bat wing which are consistent with separate derivations.

We propose that an early branch of the primates gave rise to the dermopterans (gliding lemurs) some of which developed true flight to give rise to the megachiropterans (flying foxes). Dermopterans share with megachiropterans a number of derived features in the patagium, as well as serological and genital affinities with primates. We now find that the gliding lemur, Cynocephalus variegatus, shares with primates and megabats the derived visual pathway features listed above. We cannot explain the ecological pressures which gave rise to the derived "primate" visual features since they are not found in other gliding, flying and arboreal mammals such as phalangers, microbats and flying squirrels. Whatever their origins, these features must have been present early in the evolution of the "archontan" branch of the mammalian radiation leading to the primates.

SUBCORTICAL VISUAL PATHWAYS IV

285.1 GABAERGIC CELLS IN THE CHICK OPTIC TRACT: LIGHT MICROSCOPIC OBSERVATIONS. R. H. Granda*, W. J. Crossland, and G. R. Ten Eyck*, Dept. Anatomy and Cell Biology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

There are cells in the chick optic tract and chiasm which stain intensely using an antibody to gamma aminobutyric acid (GABA+ cells) (W.J. Crossland, R.H. Granda, Soc. Neurosci Abstr, 1985, 11: 864). Our light microscopic observations now suggest that at least some of the cells are neurons.

Chicks between the ages of one day and 10 weeks posthatch were anesthetized, perfused with a mixed aldehyde fixative, the brains cut on a freezing microtome at 40 µm, and the free-floating sections incubated in rabbit anti-GABA antibody (Immuno Nuclear Corp.). A biotin-avidin system (ABC kit, Vector Labs) was used to visualize the antibody. The GABA+ cells were not stained if the primary antiserum was omitted or if normal rabbit serum was substituted for it.

There are three principal findings: 1) Morphology The GABA+ cells had a wide range of cross-sectional areas, from approximately the same size as glia to larger somas than found for most GABA+ cells in the neuropil lamina of the ventral lateral geniculate nucleus. The cells were usually round or elongated in the plane of the optic tract fibers. Some cells were stained sufficiently to observe one or two stout dendrite-like processes issuing from the soma which branched extensively in the optic tract or chiasm. In other cases small fine caliber axon-like processes could be seen leaving the cell body. 2) Distribution The cells were found throughout the thickness and length of the optic tract but were not associated with a particular nuclear group. Some cells were also found in the stump of the optic nerve adjacent to the chiasm. 3) Stability The GABA+ cells were not a transient population since some could be identified at least through 70 days posthatch.

Thus many of the GABA+ cells have neuronal characterisitics although the possibility that some, especially the smaller cells, may be glial cannot be ruled out.

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285.2 IMMUNOCYTOCHEMICAL IDENTIFICATION OF GABAERGIC NEURONS IN THE CHICK OPTIC TRACT AND CHIASM. G.R.Ten Eyck*, R.H.Granda*, and W.J.Crossland, Dept. Anatomy and Cell Biology, Wayne State Univ. Sch. Med., Detroit, MI 48201. (SPON: W. Bartlett)

Because many of the GABA+ cells (cells stained with an antibody to gamma aminobutyric acid) in the chick optic tract and chiasm appeared to be neuronal at the light microscopic level, an electron microscopic study of the ultrastructural morphology of these cells was performed in an attempt to further clarify their identity.

Hatchling chicks were anesthetized then perfused with a mixed aldehyde solution. The brains were sectioned on a vibratome, the sections incubated in 1/24,000 rabbit anti-GABA (Immuno Nuclear Corp), and visualized with an avidin-biotin system (ABC kit, Vector Labs) using diaminobenzidine as a chromagen. Labeled cells in the optic tract were identified in 1 µm sections, then the block was retrimmed for thin sections. To eliminate misidentification of an unlabeled cell adjacent to a labeled process only cells which had been sectioned through the nucleus were selected.

The ultrastructural features of the cells studied thus far are neuronal: The nuclei of the labeled cells had clearly identifiable nucleoli, dispersed coarse chromatin but no signs of marginated heterochromatin. The cytoplasmic matrix of the GABA+ cells was electron lucent and formed a thin annulus around the nucleus. The perikaryon contained small patches of rough endoplasmic reticulum, free ribosomes, and well-defined Golgi apparatus. The contours of the cell body were smooth and separated from the majority of the myelinated and unmyelinated axons of the optic tract by electron lucent (presumably astrocytic) processes.

Although the function of these neurons is unclear as yet, two possibile roles are: 1) an ectopic population of neurons associated with one or more of the diencephalic or mesencephalic nuclei or 2) an interstitial cell group as yet uncharacterized in the visual pathway.

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285.3 VISUAL RESPONSES OF NEURONS IN THE INTERGENICULATE LEAFLET AND VENTRAL LATERAL GENICULATE NUCLEUS OF THE HAMSTER. M.E. Harrington and B. Rusak, Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The intergeniculate leaflet (IGL) is located between the dorsal and the ventral lateral geniculate nuclei of the thalamus. IGL neurons receive a direct binocular retinal projection. Many neurons in the IGL and some in the ventral lateral geniculate nucleus (vLGN) project to the suprachiasmatic nucleus (SCN) of the hypothalamus. The IGL may play a role in mediating photic responses of the circadian system. In this study we have compared the extracellularly recorded responses of IGL and vLGN neurons to diffuse whole eye illumination.

Single-unit recordings were taken from forty-one male golden hamsters anesthetized with 20% urethane (2 g/kg). Conventional recording techniques were used. In some cases a concentric stimulating electrode was lowered to the area of the SCN to provide antidromic activation of geniculate neurons projecting to this area. Locations of all visually responsive units were determined histologically.

A total of 73 units in the IGL and 196 units in the vLGN were tested for visual responses. Most IGL units (86%) gave sustained responses to illumination of the retina. The majority showed "sustained on" responses to illumination of the contralateral eye. Units tested over a range of illumination levels responded with monotonic increases in firing rate as illumination intensity increased. Many IGL units showed binocular responses; most of these were inhibited by illumination of the ipsilateral eye. Photic responses of units activated antidromically from the SCN were typical of the total sample of IGL units.

Sustained responses to illumination were less common in units in the vLGN. 37% of vLGN units gave sustained responses to photic stimulation. Most units were driven only by illumination of the contralateral eye. Two "sustained on" units were tested for responses as light intensity was first decreased and then increased. Responses saturated at lower intensity levels when intensities were increased then when they were decreased.

IGL neurons differ from vLGN neurons in their responses to retinal illumination. The majority of geniculate neurons that project to the SCN appear to have "sustained on" responses to photic stimulation in the hamster. These neurons have luminance coding properties with thresholds dependent upon previously experienced light intensities. These characteristics resemble those of SCN units and they are consistent with a role for the IGL in entrainment of circadian rhythms.

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285.4 CENTRAL PROJECTIONS OF SUBSTANCE P-CONTAINING GANGLION CELLS IN THE RABBIT RETINA. N. Brecha, D. Johnson*, A.R. Lieberman* and J. Parnavelas*. Departments of Medicine and Anatomy, UCLA School of Medicine, LA, CA, 90024 and Department of Anatomy and Embryology, University College London, London, England, WC1E6BT.

Numerous substance P-like immunoreactive (SPI) ganglion cells are present in all regions of the rabbit retina. These cells are characterized by dendrites which are distributed to the proximal inner plexiform layer. The present studies have examined their projections upon the central nervous system. Normal and unilateral enucleated rabbits were processed by standard light and electron microscopic immunohistochemical methods using a rat SP monoclonal antibody. No staining is observed in sections incubated with 10 uM synthetic substance P. SPI is present in the optic nerve and tract as well as the suprachiasmatic nucleus, dorsal and ventral lateral geniculate nuclei (LGd and LGv), intergeniculate leaflet, pretectal region, superior colliculus (SC) and the accessory optic nuclei (AON). A prominent plexus of varicose axons is present in the superficial portion of the LGd and extends to a depth of about 150 um immediately below the optic tract within the external division of the alpha sector of the LGd. A few scattered axons are found in the rest of the LGd. SPI axons, some of which can be traced from the optic tract, are predominantly large in diameter and give rise to terminal arborizations characterized by numerous varicosities, many of which have a large size (up to 10 um in diameter) and an irregular surface contour. By electron microscopy, SPI varicosities in the LGd are identifiable as large axonal boutons within well defined synaptic glomeruli. These profiles are intimately apposed to and commonly deeply invaginated by other non-immunoreactive constituents of the glomeruli. Coarse, varicose SPI axons are also present in the stratum griseum superficiale (SGS) of the SC, but they are less clearly visualized due to the presence of a large number of intrinsic SPI cells. Similarly, a coarse plexus of SPI axons and cells is present in the AON. The appearance of the SPI plexus in the LGd is strikingly different from the SPI plexus in adjacent areas including the LGv and pulvinar. In these areas SPI plexus is characterized by fine axons and smaller, smooth varicosities. Following enucleation, the prominent plexus of SPI in the contralateral LGd is eliminated, in addition the SPI plexus in the SGS of the contralateral SC and the AON is reduced. The present studies demonstrate SPI in retinal terminals and provide strong evidence that SP and/or related tachykinins are transmitters of a selected population of ganglion cells in the rabbit retina.

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285.5 DIFFERENTIAL EFFECTS ON dLGN AND SC SYNAPTIC FUNCTION IN THE 2,5-HEXANEDIONE T2/X-LIKE DISTAL AXONOPATHY IN HOODED RAT. D.Impelman*, R.D.Wilson* and D.A.Fox(SPON:B.Brooks). University of Houston, College of Optometry, Houston, TX 77004.

Our previous electrophysiological and morphological work demonstrates that 2,5-hexanedione (2,5-HD) distal axonopathy of optic tract (OT) fibers preferentially affects medium diameter, X-like fibers in the t2 middle conduction group (Neurosci Abs., 1984; ARVO, 1986). In addition, SC C2 postsynaptic recovery functions (RFs) are supernormal (>100% recovery) and temporally correlated with the presynaptic t2 supernormal periods (Neurosci. Abs., 1985). In hooded rat, most t2 axons branch and project to dLGN and SC. The presynaptic response properties of t2 terminals in dLGN and SC are similar, however, t2 postsynaptic RFs are characteristically different for dLGN and SC responses (Sefton, 1965; 1969). In 2,5-HD rats, OT terminals in SC have morphological (i.e., neurofilament accumulation, axonal swelling and paranodal demyelination) changes and decreased glucose utilization prior to such changes occurring in the dLGN (Griffiths et al., 1981). To compare and contrast the changes in dLGN and SC synaptic function in 2,5-HD rats, depth recordings of presynaptic t2 and postsynaptic dLGN R2 and SC C2 field potential responses were recorded. Synaptic delays, absolute and relative refractory periods, response amplitudes and half-widths, frequency following, tnd pre/postsynaptic amplitude and RF measurements of synaptic function were obtained. The main effects show: (1) the onset of changes in the SC C2 RFs temporally precede the dLGN RF effects, (2) SC RFs remain hyperexcitable while dLGN RFs show a progressive decrease in excitability during the demyelinating and degenerative stages of the t2 distal axonopathy, (3) dLGN RFs do not exhibit the early supernormal period characteristic of 2,5-HD SC RFs, (4) in the absence of presynaptic t2 excitability, the postsynaptic depression of SC is decreased from 40% to 10% (Neurosci. Abs., 1985). During peak t2 supernormality, the dLGN postsynaptic depression, mediated by I cell feedback inhibition, is also decreased from 60% to 10% and shows periods of undamped cyclic burst activity, and (5) in the later degenerative stages of 2,5-HD axonopathy, the early phase of the dLGN RF is depressed 40-50% and there is an absence of normal I cell burst activity during the postsynaptic depression period. The relative increased sensitivity of dLGN R2 responses to the distal axonopathy of t2 terminals and its later onset may be related to the synaptic glomerular specializations of retinal afferents in the dLGN compared to the unencapsulated free endings in the SC and/or the functional differences underlying the higher spatial resolving capacity of the dLGN compared to the SC (Dean, 1981). Supported by NIEHS Grant ES 03183 (DAF).

285.6 POSTSYNAPTIC POTENTIALS RECORDED IN NEURONS IN THE CAT'S LATERAL GENICULATE NUCLEUS. S.A. Bloomfield, J.E. Hamos, and S.M. Sherman, Dept. Neurobiology & Behavior, SUNY at Stony Brook, N.Y. 11794

We are currently studying the postsynaptic potentials (PSPs) generated in geniculate neurons and examining differences between X- and Y-cells in terms of their postsynaptic responses. Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) generated spontaneously, following the presentation of a light stimulus, or after electrical stimulation of the optic chiasm were recorded intracellularly from geniculate neurons in vivo. Because PSPs are only 0.3-5.8 mV when recorded at the soma, we used computer averaging techniques to study them. To date, we have recorded from 41 neurons (24 X- and 17 Y-cells); 28 cells have been identified as projection neurons as they could be driven antidromically with cortical stimulation, 2 cells could not be driven from visual cortex and were tentatively identified as interneurons, and the remainder of the cells were not tested for this.

All projection cells show a variety of spontaneously evoked PSPs. For a given cell, EPSPs are uniform in duration and amplitude, whereas IPSPs are of various duration and amplitude. Following chiasm stimulation, all projection cells display an initial EPSP followed immediately by an IPSP. However, the 2 cells classified as interneurons showed EPSPs but no IPSPs following chiasm shock, suggesting that they lack an inhibitory pathway clearly present in the other cells.

For all neurons, if the chiasm is stimulated adequately, the summed EPSP reaches threshold for the generation of an action potential; the difference between the latency of the EPSP and the spike varies across cells, with a range of 0.2-1.1 msec.

Although the IPSPs generated in projection cells following chiasm stimulation all show similar latencies from the onset of the corresponding EPSPs, they can be divided into 3 groups based on their durations. The majority of both X- and Y-cells display short-duration IPSPs (3-25 msec), whereas the remainder of the Y-cells show medium-duration IPSPs (35-60 msec), and the remainder of the X-cells show only long-duration IPSPs (75-100 msec). The reversal potentials for the short-duration IPSPs, displayed by 69% of the cells, are similar with a mean of -80 mV; we have not as yet determined the reversal potentials for the other types of inhibition. Occasionally, X- or Y-cells display IPSPs which appear to consist of 2 components; it is presently unclear whether this response reflects a mixture of the 3 types of IPSPs or inhibition from other sources.

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285.7 BRAINSTEM INNERVATION TO THE CAT'S LATERAL GENICULATE NUCLEUS: AN EM-IMMUNOHISTOCHEMICAL STUDY USING THE TRACER PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). J.B. Cucchiaro, D.J. Uhlrich and S.M. Sherman. Dept. Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Electrical stimulation of certain brainstem centers facilitates the transmission of visual information through the cat's dorsal lateral geniculate nucleus (LGN) to the cortex. This suggests that the brainstem plays an important role in the gating of visual information. We have studied the synaptic circuitry involved in this brainstem control.

A small, iontophoretic injection ($\tilde{=}$ 1 mm^3) of the plant lectin, PHA-L, was made in an adult cat (Gerfen and Sawchenko, Brain Res. 290: 219, 1984). The injection was limited to the parabrachial region of the brainstem just dorsal to the brachium conjunctivum. This region was chosen because it has been shown to be rich in retrogradely labelled cells following tracer injections to the LGN complex. After a 7 day postoperative survival, the ipsilateral LGN was processed for immunohistochemistry to show the distribution of PHA-L labelled fibers, and those sections showing label were prepared for EM analysis (Eldred et al., J.Histochem.Cytochem. 31: 285, 1983). Because penetration of the antibody to PHA-L is often incomplete through the thickness of each 50 μm section, fibers could not be reconstructed at the light microscopic level.

Labelled fibers are found throughout the thalamus including all laminae of the LGN and the perigeniculate nucleus. Thus far, our EM analysis is limited to the binocular portion of the A laminae, where the labelled fibers are fine and beaded.

Serial EM analysis of the labelled terminals shows that the parabrachial afferents are unmyelinated within the LGN and make en passant synaptic contacts at swellings 0.5-1.3 μm in diameter. The parabrachial terminals make symmetrical contacts, showing little or no postsynaptic density, and are exclusively presynaptic in relation to other structures. The synaptic contacts are made largely within synapse-rich glomerular areas, onto large caliber dendrites and dendritic appendages of geniculate cells, presumed to be projection neurons. Labelled terminals also make contacts onto flat vesicle-containing F2 profiles. These are the dendritic terminals of geniculate local circuit neurons and are thought to subserve the local inhibition of geniculate projection neurons. Therefore, one possible role of the parabrachial contacts onto the local circuit neurons may be to block local inhibition, thereby facilitating the transmission of visual information to the cortex. However, contacts onto geniculate projection neurons are also seen and must be considered in attempts to model brainstem control.

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285.8 SYNAPTIC CIRCUITRY OF AN INDIVIDUAL RETINOGENICULATE AXON FROM A RETINAL Y-CELL. J.E. Hamos, S.C. Van Horn* and S.M. Sherman. Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

We investigated the synaptic relationships of terminals from the axon of a retinal Y-cell to determine its circuitry in the lateral geniculate nucleus (LGN) and to compare this with our earlier findings for X-cell axons. Intracellular recordings were obtained in the optic tract from an axon that we had identified as being from a retinal Y-cell using a battery of physiological tests. We subsequently iontophoresed horseradish peroxidase (HRP) into the axon, perfused the animal and processed the LGN for electron microscopy. After serially sectioning tissue containing the axon's terminal field in the LGN (which was wholly located in lamina A1), we examined the sections with the electron microscope and recon-structed neurons that were postsynaptic to HRP-labeled terminals from the injected axon.

Labeled terminals from the Y-cell axon repeatedly contact dendrites of some geniculate neurons while totally avoiding those of others. Our reconstructions of the cells postsynaptic to the Y-cell axon suggest that this axon provides at least two different patterns of input. One pattern of input is characterized by numerous contacts from labeled terminals onto the shafts of dendrites from a large (soma diameter >30um) geniculate neuron. These synapses are all located within 100um of the soma of the neuron and, for the most part, they do not participate in synaptic triads. A second pattern of input from labeled terminals is characterized by labeled terminals making synapses onto dendritic shafts that themselves are presynaptic to other geniculate neurons. Morphological features of these presynaptic dendrites include arrays of microtubules, ribosomes and small clusters of vesicles; they make symmetrical synaptic contacts. At present, we have not reconstructed these dendrites to their somatic origins.

Retinogeniculate axons from both X- and Y-cells are selective in their circuitry in that a retinal axon synapses onto a limited postsynaptic population of geniculate neurons. However, these two pathways utilize retinal information in different manners. Geniculate X-cells receive excitatory retinal input together with synapses from inhibitory terminals in synaptic triads and other complex relationships, much of which occurs on the dendritic appendages that typify X-cells. A major source for this inhibition is the dendritic terminals of local circuit neurons. In contrast, geniculate Y-cells receive much of their retinal input onto dendritic shafts and these are spatially segregated from inhibitory inputs. Moreover, presynaptic dendrites that are directly contacted by the Y-cell axon may be part of a variety of local circuit neuron that is unique to the Y-cell pathway.

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285.9 ULTRASTRUCTURE OF THE INTRAGENICULATE AXON COLLATERAL OF A PROJECTION NEURON IN THE CAT. Susan C. Van Horn*, James E. Hamos, and S. Murray Sherman. Dept. Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

Geniculate neurons whose axons project to the visual cortex also display axon collaterals that terminate in the perigeniculate nucleus (PGN) and/or laminae A and A1 of the dorsal lateral geniculate nucleus (dLGN). We have initiated a study of the synaptic circuitry of intrageniculate axon collaterals from geniculate cells. For the present analysis, we recorded the responses of a geniculate neuron to a battery of physiological tests and subsequently labelled the cell with horseradish peroxidase (HRP). Despite recording a number of its response properties, we could not classify the labelled cell purely as an X- or Y-cell. After the animal was perfused, the dLGN was sectioned at 50 μm, reacted for HRP histochemistry, and processed for electron microscopy (EM). The labelled cell is located in lamina A of the dLGN with a few distal dendrites extending into lamina A1. The cell has axon collaterals both in lamina A and in the PGN, while the axon itself extends into the optic radiation, suggesting that it is a geniculocortical projection neuron.

Serial reconstruction and EM analysis of the HRP-labelled intrageniculate collateral, confirmed the origin of the collateral to be the axon hillock of the labelled cell. Labelled terminals found along the intrageniculate collateral are small, generally being 1.0 μm or less in diameter. These terminals are filled with vesicles and are involved in a variety of synaptic arrangements. Thus far, all of the labelled terminals that we have reconstructed make symmetrical synaptic contacts. These synapses are formed primarily onto dendritic appendages from unlabelled geniculate neurons that are involved in complex glomeruli, in that the same postsynaptic appendages receive numerous other synapses, including those from the retina. Labelled terminals also occasionally contact other unlabelled terminals that participate in complex glomeruli. The participation of labelled terminals in glomeruli suggest that their postsynaptic targets are X-cells. Therefore, in addition to local circuit neurons, the collaterals of some geniculate projection neurons provide an additional intrageniculate circuit to influence complex synaptic arrangements related to the X-cell pathway.

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285.10 DIFFERENT INHIBITORY MECHANISMS IN SHIFT RESPONSES OF CAT LATERAL GENICULATE X AND Y CELLS. H.-Chr.Pape* and U.Th. Eysel (SPON:ENA). Institute of Physiology, University of Essen, D-4300 Essen 1, West Germany.

A different excitability from outside the antagonistic receptive field (RF) area ("shift-effect") has been reported for the X and Y cell population in the cat dorsal lateral geniculate nucleus (dLGN; Derrington, A.M. and Fuchs, A.F. J.Physiol.,231:347, 1979). This difference might originate in the specific inhibitory networks in the dLGN. The purpose of the present study was to discriminate influences of different inhibitory geniculate processes on the X and Y cell shift effects. In the anaesthetized cat (nitrous oxide/halothane) phase reversing gratings (0.5-0.25 c/°, contrast 0.92, stimulus period 1 s, 40° central RF sparing) elicited transient excitatory shift responses in 77 out of 79 units, that were classified as X (31) or Y (48), according to a battery of optical tests and electrical stimulation. The Y population tended to phasic shift responses with significantly higher amplitudes (mean: 52 imp/s) in comparison to the weak, tonic effects in X cells (mean: 25 imp/s). The differences in shift amplitudes of the two cell classes were abolished by microiontophoretic application of the GABA antagonist bicuculline, which increased the early peak of the shift effect towards values of 100 imp/s, irrespective of the cell type and strength of the response under control conditions. Acetylcholine raised the discharge rate of X and Y units and characteristically elevated the late part of the Y cell shift response, thus abolishing its phasic time course. The latter effect was particularly evident under barbiturate anaesthesia, which is known to block cholinergic excitation, but not cholinergic disinhibition of long range inhibitory processes in the dLGN (Eysel, U.T., Pape, H.-C. and van Schayck, R., J.Physiol.,370:233,1986). L-glutamate increased the neuronal activity, leaving the differences in shift amplitudes and time courses between X and Y unchanged.

In summary, the results indicate that inhibitory processes specifically shape geniculate X and Y shift effects. Early inhibition is blocked by bicuculline and seems to stronger influence X neurons. Long lasting inhibitory mechanisms, cholinergically disinhibited, prevail in the Y cell population and possibly reflect a preponderance of long range lateral inhibition via the perigeniculate nucleus in this cell class.

285.11 WGA-HRP AND GABA-IMMUNOCYTOCHEMICAL STUDIES OF THE LATERALIS MEDIALIS-SUPRAGENICULATE NUCLEAR COMPLEX IN THE CAT. M.Norita, Y.Katoh* and T.Hashikawa*. (MN and YK) Dept. of Anatomy, Sch. of Med., Fujita-Gakuen Health Univ., Toyoake 470-11, and (TH) Dept. of Anatomy, Sch. of Med., Iwate Med. Univ., Morioka 020, Japan.

The projections from the thalamic reticular nucleus (TRN) to the lateralis medialis-suprageniculate nuclear (LM-Sg) complex in the cat were studied using WGA-HRP. Following injection of WGA-HRP into the LM-Sg complex, a number of retrogradely labeled cells showing a topographical arrangement were observed in the TRN; the dorsal TRN was seen to be connected mainly with the dorsomedial portion of the LM-Sg complex, while the ventrolateral TRN was connected chiefly with the ventrolateral portion of the LM-Sg complex. Following injection of the tracer into the TRN, ultrastructural examination of anterograde labeling in the LM-Sg complex revealed labeled synaptic terminals containing pleomorphic vesicles and making symmetrical contacts with mainly small- to medium-sized dendrites in the extraglomerular neuropil.

The GABA-immunoreactive constituents of the LM-Sg complex were then examined. Small immunoreactive cells were found throughout this nuclear complex, in addition to a conspicuous granular immunoreactivity in the neuropil. Ultrastructurally, immunoreactive presynaptic profiles could be detected both in the synaptic glomeruli and in the extraglomerular neuropil; the former may correspond to the presynaptic dendrites of intrinsic neurons, while the latter appear to correspond to axon terminals from the TRN as well as those of intrinsic neurons.

The present results anatomically support the possibility that the cells projecting from the LM-Sg complex may be inhibited by both the TRN axons and the presynaptic dendrites, probably through the mediation of GABA.

(The authors wish to thank Drs. R.J. Wenthold and J. Zempel for providing the anti-GABA serum used in this study.)

285.12 MORPHOLOGIC DIVERSITY IN THE GANGLION CELL PROJECTION TO DIFFERENT ZONES WITHIN THE CAT LATERAL GENICULATE NUCLEUS. R.W. Rodieck and M. Watanabe*, Department of Ophthalmology, University of Washington, Seattle WA 98195

By means of a Hamilton syringe, a number of small (0.1µl) injections of rhodamine labeled latex microspheres were made along a single needle penetration of one LGNd. Two days later, the retinas were dissected and placed in a chamber where they were superfused with oxygenated Ames medium. Labeled ganglion cells were visualized in an epifluorescence microscope, injected with rhodamine-labeled horseradish peroxidase (HRP), and subsequently fixed and reacted with diaminobenzidine. This method yields what appears to be complete filling of even the smallest ganglion cells having the thinnest axons. In general there proved to be a good correlation between the regions of concentration of labeled ganglion cells in the two retinas, and the retinotopic projections of each of the eyes to the different zones or laminae traversed by the needle penetration through the LGNd, as determined histologically.

Injections to the Geniculate Wing (GW), anterior to the main laminae of the LGNd, labeled a homogeneous group of cells with large and circular dendritic fields, and a characteristic morphology. Their dendrites pass directly away from the soma, and appear to ramify in the inner most portion of the inner plexiform layer (IPL). They have medium-sized somas (ca 22µm), and large dendritic fields (1,000 to 1,300µm), that exceed those of alpha cells at the same eccentricity. These cells are almost certainly equivalent to the *epsilon* cells previously described, on the basis of retrograde transport of HRP iontophoretically injected to the GW, although only a small portion of their dendritic fields proved to be revealed by this less direct method of filling the cells with HRP.

Injections of microspheres to the medial interlaminar nucleus (MIN), possibly including GW as well, labeled alpha cells, as well as ganglion cells with a wide range of somal diameters, dendritic-field diameters, and morphologies. These include cells with medium-to-small somas (ca 20µm) and very large dendritic fields with diameters of 1,500µm or more across, as well as others in the same somal size range, but with compact (ca 250µm) and highly branched dendritic fields.

For injections to the C laminae, the needle penetration lay in the sagittal plane, and was directed anteroventrally at an angle of 35° with respect to the vertical, so as to pass through the curved anterior portions of the A and C laminae. A typical penetration entered the A laminae near the projection of the horizontal midline, about 5° from the area centralis, grazed the different C laminae to varying degrees, and exited via the projection of the upper retina to the A laminae.

Cells projecting contralaterally to lamina C, in addition to alpha cells, include a group with medium somas (ca 23µm), and large (1,200µm) dendritic fields. No 'gamma' cells were observed to project to any portion of the LGNd.

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285.13 CLASSES OF RETINAL GANGLION CELLS PROJECTING TO DORSAL LATERAL GENIGULATE NUCLEUS IN THE OPOSSUM. T.M.Fischer*, P.D.Wilson, and J.N.Parker* (SPON:M.Nachman). Dept. Psychology, Univ. California, Riverside, CA 92521.

In anesthetized opossums, the dorsal lateral geniculate nucleus (DLGN) was identified physiologically with micropippette electrode recordings of cell activity and horseradish peroxidase (30% HRP in 2% DMSO and 0.1M Tris) was then injected iontophoretically with 2-7 µA pulses. Animals were given antibiotics, recovered from anesthesia, maintained 5 days, then given a lethal dose of Nembutal and perfused transcardially with phosphate buffer and mixed aldehydes. Retinae were dissected and reacted with the heavy-metal intensified diaminobenzidine method (Adams, 1977), flat mounted on slides and counterstained with cresyl violet. Coronal sections through the DLGN were also reacted for HRP, mounted on slides and Nissl stained. Data were obtained from 4 animals in which the HRP injection site was restricted to the DLGN.

Nissl stained retinal ganglion cells ranged 8-32 microns in soma diameter. Ganglion cells labeled with HRP from DLGN injections were predominantly 15 to 32 microns in dia. Only rarely were cells <14 micron dia. labeled, and the smallest sized ganglion cells (<12 micron dia.) were unlabeled. The HRP-labeled cells with the largest soma sizes and relatively thick primary dendrites (Type-1 cells of Wilson and Condo, 1983) resembled alpha cells in cat retina. Many medium sized cells were comparable to the Type-2 cells previously reported (Wilson and Condo, 1983) in having dendritic patterns similar to beta-like cells in cat retina. Other medium sized cells with a variety of soma shapes had dendritic patterns similar to epsilon, or possibly gamma, cells in cat retina.

In the opossum as in the cat, the ganglion cells projecting to DLGN include cells with soma sizes and dendritic morphology similar to alpha, beta, and epsilon cells, as previously inferred (Rapaport and Wilson, 1985) from study of soma sizes alone, and these cells presumably provide the afferents for the cells with Y, X, and W-like properties found in the opossum DLGN (Kirby and Wilson, in press). Either the smallest ganglion cells rarely project to the opossum DLGN, or, if they project to DLGN in substantial numbers, were unlabeled in this experiment.

285.14 COMPARISON OF RETINAL GANGLION CELLS PROJECTING TO VENTRAL LATERAL GENICULATE NUCLEUS AND SUPERIOR COLLICULUS IN THE OPOSSUM. P.D.Wilson, M.A.Kirby, and J.N.Parker*. Dept. Psychology, Univ. California, Riverside and Davis, Riverside, CA, 92521, and Davis, CA, 95616.

The ventral lateral geniculate nucleus (VLGN) in the opossum was identified physiologically and horseradish peroxidase (30% HRP in 2% DMSO and 0.1M Tris) was injected iontophoretically in the VLGN of 5 anesthetized opossums. In 4 other animals, HRP was pressure injected into the superior colliculus (SC). Animals were given antibiotics, recovered from anesthesia, maintained 4-5 days, and then given a lethal dose of Nembutal and perfused transcardially with phosphate buffer and mixed aldehydes. Retinae were dissected and reacted with the heavy-metal intensified diaminobenzidine method (Adams, 1977), flat mounted on slides, and counterstained with cresyl violet. Coronal sections were taken through midbrain and dorsal thalamus and the brain sections were also reacted for HRP and then mounted on slides and Nissl stained.

As previously (Rapaport et al., 1981) Nissl stained ganglion cells in superior retina varied in some size from 8-35 microns dia. Ganglion cells labeled with HRP from VLGN or from SC included all soma sizes except the smallest (8-9 micron dia.). On the basis of soma size and dendritic arbors, ganglion cells projecting to VLGN could be classified into three groups: 1) Cells with large, round or polygonal somata (<18 um dia.) and thick primary dendrites, with dendritic branching patterns similar to alpha cells in cat retina. 2) Cells with 3-4 primary dendrites arising from medium size somata, often triangular in profile, which were reminiscent of epsilon cells in cat retina. 3) Cells with small somata (10-14 micron mean dia.), usually oval with long to short diameter ratio <1.5:1, and with relatively thin, sparsely branched dendrites, similar to gamma cells in cat.

The ganglion cells labeled from SC included cells morphologically similar to those labeled from VLGN, and additionally included small to medium cells with various shaped somata.

It is concluded that with the exception of beta-like ganglion cells all major classes of retinal ganglion cells project to the VLGN and the retinal projection to VLGN resembles that to the superior colliculus in the opossum.

285.15 AN ELECTRON MICROSCOPIC ANALYSIS OF THE LOCALIZATION OF CYTOCHROME OXIDASE IN THE LATERAL GENICULATE NUCLEUS OF MACAQUE MONKEYS. S.Y. Liu* and M. Wong-Riley . (SPON: F.D. Anderson). Dept. of Anat. & Cellular Biology, Med. Coll. of Wis., Milwaukee, WI 53226.

Our previous light microscopic studies have shown that in the lateral geniculate nucleus (LGN) of normal macaque monkeys, the two magnocellular layers (ML) and parvocellular lamina (PL) 6 showed the highest level of cytochrome oxidase (C.O.) activity. After tetrodotoxin (TTX) treatment, a decrease in C.O. activity was found in the affected LGN lam. (Nature , 307:262,'84; J. Neurosci., 4:2445, '84). The present study extended these findings and incorporated quantitative EM morphometry. Most of the reactive neurons were medium to large-sized, whereas small neurons tended to be less reactive. The neurons in lam. 1, 2, and 6 had the highest level of C.O. activity. They contained a large number of moderate to darkly (M-D) reactive mitochondria (mito.), which comprised 40.4%, 35.5%, and 34.3% of the total mito. population within neurons of lam. 1, 2, and 6 respectively. There were fewer M-D reactive mito. in lam. 5 (31.9%), and even less so in lam. 3 and 4 (20.7% and 23% respectively). Both the mito. packing density and the percentage of reactive mito. were significantly higher in ML than in PL. Distinct components of the neuropil had specific levels of C.O. reactivity. Quantitative analysis of the total mito. population and the level of C.O. reactivity showed that, in all six laminae, darkly reactive mito. were localized primarily within dendrites; F profiles (F1, F2, Fd) with flattened or pleomorphic vesicles were more reactive than RS or RL terminals with round vesicles; while RL's were the least reactive. In comparing the neuropil of ML with PL, we found that the total mito. number and the number of darkly reactive mito. were significantly higher in the former than in the latter. These differences were most prominent within dendrites and F profiles, while the RL terminals were only slightly higher. In PL, layers 5 and 6 were significantly more reactive than layers 3 and 4. Contralateral lam. (1, 4, and 6) had greater C.O. reactivity than the corresponding ipsilateral lam. (2, 3, and 5). Moreover, most of the reactive postsynaptic profiles tended to contact RL or RS rather than F profiles. After TTX treatment, a prominent decrease in the level of C.O. reactivity within both cell bodies and neuropil were noted. The RL terminals and their postsynaptic dendrites were the most severely affected. Other changes are still under investigation. Our results indicated that ML were metabolically more active than PL, implying that the predominantly Y-like pathway may be more highly active than the X-like pathway. Among PL, ON-center ones (5 and 6) appeared to be metabolically more active than OFF-center ones (3 and 4). Finally, the contralateral pathway may sustain a higher level of activity than the ipsilateral pathway. (Supported by NIH EY05439).

285.16 CONNECTIONS OF TWO SUBDIVISIONS OF INFERIOR TEMPORAL CORTEX OF OWL MONKEYS WITH THE PULVINAR COMPLEX. R. E. Weller and J. H. Kaas. Dept. of Psychology, Univ. of Alabama at Birmingham, AL 35294 and Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240.

Previously we defined in owl monkeys a caudal division of inferior temporal cortex, ITc, on the basis of inputs from extrastriate visual cortex (primarily the Dorsolateral Area, DL). Projections from ITc in turn defined a more rostral subdivision of inferior temporal cortex, ITr. Both ITc and ITr are within the traditional architectonic field TE. Patterns of connections with the pulvinar complex were determined from injections of ^{3}H-proline and/or horseradish peroxidase in ITc or ITr of 11 adult owl monkeys (Aotus trivirgatus). The pulvinar complex of owl monkeys has been divided into inferior and superior divisions. The inferior pulvinar includes central (IPc), medial (IPm), and posterior (IPp) nuclei, while the superior pulvinar has been divided into lateral (SPl), central (SPc), and medial (SPm) divisions. SPm, previously identified in owl monkeys as the dorsomedial part of the superior pulvinar, does not have connections with previously studied subdivisions of extrastriate cortex, and its cortical connections, in fact, were unknown.

Injections in either ITc or ITr label a large region of the superior pulvinar that includes much of the caudal pole and at more rostral levels extends dorsomedially. This result suggests that both the caudal pole and the dorsomedial portion of the superior pulvinar constitute the medial division of the superior pulvinar in owl monkeys. Other label in the superior pulvinar was located more laterally, above the inferior pulvinar complex, in the region considered to be SPc. In addition, the injections demonstrated that both ITc and ITr have connections with IPp. The results also indicate that ITc and ITr have connections with different parts of the superior pulvinar. ITc is interconnected with the dorsolateral half, and ITr with the ventromedial half, of the caudal pole of the superior pulvinar.

We conclude that the main pulvinar connections of ITc and ITr are with the medial division of the superior pulvinar, SPm. Furthermore, ITc and ITr have connections with different portions of SPm. This difference in connections is consistent with our previous evidence that ITc and ITr are distinct subdivisions of IT (TE) cortex in owl monkeys.
Supported by NIH Grant EY-02686 and UAB Faculty Research Grant 212822.

285.17 EFFECTS OF KAINIC ACID LESIONS OF THE PULVINAR ON SACCADIC EYE MOVEMENTS IN MONKEYS. D.B. Bender and J.S. Baizer. Division of Neurobiology, Dept. of Physiology, SUNYAB, Buffalo, NY 14226.

The pulvinar receives projections from the superior colliculus and projects to extrastriate cortex. It has been suggested that this pathway may be involved in the control of visually-guided eye movements. Thus, Ungerleider and Christensen (1979) reported that pulvinar lesioned rhesus monkeys were "visually captured," showing longer fixations than normal monkeys during scanning of a complex visual scene. However, they used heat lesions of the pulvinar, which are now known to damage the corticotectal tract as well as pulvinar neurons (Bender and Baizer, 1984). Thus, the abnormally long fixations could have resulted from corticotectal damage. To evaluate this possibility, we tried to replicate Ungerleider and Christensen's results using animals with kainic acid (KA) lesions of the pulvinar.

Subjects were two monkeys with bilateral KA lesions, and normal controls. The lesions destroyed most of the inferior and lateral pulvinar, and part of the medial pulvinar. Following Ungerleider and Christensen, eye movements were recorded as the animal spontaneously scanned a 36 x 36 deg array of 16 geometrical shapes. We also wished to evaluate the effects of pulvinar lesions on saccades to known targets. Thus, the animals were trained to make saccades from a central point to small target lights, and to respond to dimming of the targets.

The KA pulvinar lesions did not prolong fixations during scanning. For both lesioned and intact animals, the average duration of fixations was between 450-550 msec. Similarly, on the saccade task, the KA lesions did not consistently alter oculomotor function. The lesions did impair task performance, but this was probably due to slight spread of KA into the LGN, with consequent production of a foveal scotoma and increased difficulty in detection of target dimming. Different animals adopted different strategies, and patterns of eye movements, to solve the detection task. On those trials where animals did make saccades to the targets, however, saccade latency and velocity were comparable to preoperative levels.

These results suggest that the pulvinar does not play a critical role in the initiation and control of eye movements, either during spontaneous scanning or in response to flashed targets. Previously reported deficits in eye movement control are thus likely to have resulted from interruption of the corticotectal tract.

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285.18 PROJECTIONS OF THE PULVINAR TO THE ANTERIOR BANK OF THE LUNATE SULCUS AND DORSAL SUPERIOR TEMPORAL SULCUS. G.P. Standage. Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Our previous investigations have described the projections of the inferior pulvinar (PI) and lateral pulvinar (PL) to visual areas V1, V2, V4 and MT (Soc. Neurosci. Abstr. 6,1979; Brain Res. 262. 1983) in the macaque monkey. It was of interest to further refine our understanding of pulvino-cortical projections by including in our analysis, the anterior bank of the lunate sulcus, and the posterior bank and fundus of the superior temporal sulcus (sts) dorsal to visual area MT (V5). Subpial asperation along with bipolar electrocoagulation techniques were use to surgically remove the posterior bank of the lunate sulcus, or the anterior bank of sts above the sts-lateral sulcus junction . Injections of combined wheat germ conjugated HRP (5% in saline) and HRP (60% in saline) were injected into surgically exposed cortex using a 1.0 µl Hamilton syringe. Injection volumes never exceeded .05 µl per injection site.

It was found that the medial region of PI projects preferentially to the posterior bank and floor of dorsal sts. The remaining portion of PI and PL contained scattered labelled cells but not terminal grains. This region of PI lies medial to the MT-crescent area of PI previously defined (Standage and Benevento, Brain Res 262, '82) as projecting heavily to visual area MT. Injections placed along the anterior bank of the lunate sulcus (region of V3) resulted in preferential labelling in PL. Retrograde and anterograde labelling was very dense in PL, especially along the lateral border. PI contained only a few labelled cells. It should be pointed out that the regions of PI and PL labelled receive direct projections from the superficial retinorecipient layers of the superior colliculus (Benevento and Standage, JCN 217, '83). In summary, although pulvinar projections overlap, there may exist within the pulvinar complex, regions that receive unique input from the superficial layers of the superior colliculus which, inturn, project preferentially to specific regions of visual responsive cortex (Supported by NIH grant NS-21935).

285.19 VISUAL PROPERTIES OF PULVINAR CELLS IN RABBITS.
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The pulvinar may be considered as a major interface between the superior colliculus and the visual cortical areas. This link seems to be present in all mammals. As a prelude to studying the influences of the superior colliculus on the pulvinar cells, we investigated the visual properties of the latter in rabbits. Animals were urethan-anesthetized and paralyzed. Single cells from pulvinar were recorded from, using a glass micropipette. Each cell was stimulated with an electrical shock applied to the optic chiasm and with circumscribed stimuli presented in the receptive field. Two hundred and thirty-nine cells were studied. Amongst them, 200 were visually responsive. One hundred and seventy-six cells responded to stationary on-off pulses and 129 to moving targets. Thirty-three cells were directionnaly selective. Finally, nine cells were shown to be selective to the velocity of the visual target. Generally, the receptive fields were large although small size fields were also recorded. The salient feature is the fact that most fields encroached upon the vertical meridian and their centers were located within 45° of the optic axis. Although a retinotopic organization seems to exist, it is not very rigourous. This may be contrasted with the dorsal lateral geniculate organization. As a general rule, as the electrode progressed from the dorsal to ventral side of the pulvinar the fields moved along the same axis. However, very often, one cell had its fields far from this general axis. Further more, the overlap between RF was quite extensive. Finally, the distribution of response latencies to optic chiasm shock was bimodal (mean: 10.9 ± 3.2 ms, N=24). By comparison in the LGN, the mean is considerably shorter: 3.9 ± 2.9 ms, suggesting that the pulvinar is innervated by slow conducting fibers or indirectly through the cortex or the colliculi. Many of the characteristics suggest that the properties of the rabbit's pulvinar resemble those reported for cats and to a lesser degree for monkeys. Experiments currently in progress are aimed at showing how these properties are modified by local inactivation of the superior colliculus.
(Supp. NSERC and FCAR.)

PEPTIDE BIOSYNTHESIS AND METABOLISM II

286.1 DISTRIBUTION OF IMMUNOREACTIVE PEPTIDE B IN THE RAT BRAIN.
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Peptide B, the carboxyl terminal 3700 dalton fragment of proenkephalin, represents one of the most highly conserved sequences in this opioid peptide precursor; the rat and human sequences differ in only two positions (one of which represents a a conservative substitution) from the bovine peptide. Peptide B, first purified from adrenal medullary tissue by Udenfriend and coworkers, is one of the major enkephalin-containing peptides in the bovine adrenal medulla. In order to investigate the potential presence of Peptide B in the mammalian nervous system, an antiserum raised to the bovine form of this peptide was used to measure the distribution and molecular weight forms of Peptide B-immunoreactive peptides in the rat brain. Peptide B-immunoreactivity (ir) was found to be most concentrated in the hypothalamus and the striatum, with lower concentrations in the midbrain and medulla-pons. Little Peptide B-ir was present in the cortex, hippocampus, and cerebellum. Characterization of Peptide B-ir by gel filtration demonstrated that the major immunoreactive species in the hypothalamus and the striatum corresponded to a peptide with the approximate molecular weight of Peptide B. Analysis by HPLC of the major Peptide B-immunoreactive peak obtained from gel filtration of hypothalamic and striatal extracts revealed the presence of two minor and one major immunoreactive species. The major immunoreactive peptide exhibited a retention time indicative of a more hydrophilic peptide than bovine Peptide B. These results suggest that one of the processing products of proenkephalin in the rat brain is a peptide closely related to bovine Peptide B.
Supported by NIH AM 35199-01.

286.2 **THE CLONING AND SEQUENCING OF VALOSIN, A NOVEL GUT/BRAIN PEPTIDE.**
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Valosin is a novel 25 amino acid residue gastrointestinal peptide that has recently been purified from porcine gut acid extracts. Schmidt and his coworkers (*FEBS Lett.*, **191**, 264, 1985) isolated this peptide from side-fractions obtained from secretin purification and demonstrated in preliminary experiments that the peptide seemed to exert a dose-dependent CCK- or bombesin-like stimulatory action on exocrine pancreatic secretion and to cause a delay in the appearance of the slow migrating myoelectric complexes in different parts of the intestine. In order to begin to study the biosynthesis of this peptide, the DNA complementary to the mRNA precursor was isolated from a porcine adrenal medulla library, and this clone was sequenced. In addition, tissue distribution of the mRNA was studied using northern analysis.

A 36 residue, 16-fold degenerate oligonucleotide probe was designed based on the published amino acid sequence of valosin and synthesized on an Applied Biosystems oligonucleotide synthesizer. The resulting mixture of probes was radiolabeled using ^{32}P-gamma-ATP and T4 DNA kinase and used to screen an Okayama-Berg porcine adrenal medulla cDNA library (provided by Dr. Åke Rokaeus) by the cloning hybridization technique of Grunstein and Hogness. Positive clones were subcloned to homogeneity and analyzed with restriction endonucleases. The largest clone isolated was sequenced using the Sanger enzymatic dideoxy chain termination method. For northern anaylsis, total RNA was isolated from porcine brain and peripheral tissue by guanidium thiocyanate extraction, and radioactive cDNA probes were labeled by nick-translation or oligolabeling.

The largest isolated cDNA clone had an insert size of 3.2 kilobases. Northern blot analysis of RNA isolated form porcine tissue demonstrated one RNA species approximately 3.5 kilobases in size that hybridized to ^{32}P-labeled fragments of cDNA. While this peptide was isolated from gut, northern analysis illustrates that the mRNA coding for valosin is present in a wide spectrum of pig tissue including adrenal medulla, adrenal cortex, gut, spinal cord, brain, and heart. The complete amino acid sequence suggests that a novel processing signal may be involved in the biosynthesis of valosin. The sequence of the isolated peptide is not flanked by two basic amino acids; however, a lysine-arginine pair appears five amino acids before the putative first amino acid, and a double leucine is present eight amino acids after the proposed terminus. Therefore, it is possible that the peptide valsoin may be a fragment of a larger peptide or protein. Antibodies are being generated against valsoin to be used in tissue extraction and *in vitro* translation experiments to help answer these questions. (K.J.K. is supported by NINCDS post-doctoral grant number F32 NS07599).

286.3 LEVELS OF IMMUNOREACTIVE ANGIOTENSIN II IN MICRODISSECTED BRAIN NUCLEI OF THE DEVELOPING SPRAGUE DAWLEY RAT. L. Lark* and J.A. Weyhenmeyer. Neural and Behavioral Biology Program and College of Medicine, University of Illinois, Urbana, IL 61801.

An endogenous renin-angiotensin system (RAS) has been postulated to exist in the central nervous system and function in the regulation of blood pressure, fluid balance, sodium intake, and hormone release. Lind et al. (Neuroendocrinol. 40:20, 1985) have characterized the immunohistochemical distribution of angiotensin II (ANG II), the end product of the RAS, in the brain of the adult male Sprague Dawley rat. The purpose of the present study was to measure levels of ANG II in microdissected brain nuclei of the developing male Sprague Dawley rat.

Four and ten week male Sprague Dawley rats were anesthetized with ketamine/acepromazine and perfused transcardially with ice cold 0.9% saline. Brains were removed and immediately frozen on dry ice. Serial coronal sections (300 μm) were cut at 9°C. Twenty four brain regions were micropunch dissected and stored at -70°C until preparation. Dissected regions were weighed and homogenized in 0.1 N HCl, boiled for 5 min, and centrifuged at 30,000 x g for 30 min at 4°C. The supernatants were collected and the pellets re-extracted with 0.1 N HCl. Pooled supernatants were lyophilized, resuspended in 0.1 M phosphate buffered saline (pH 7.2) containing 0.1% BSA, and analyzed by a competitive inhibition radioimmunoassay using a specific ANG II antibody.

Preliminary results indicate that picogram levels of ANG II (per mg wet tissue weight) are present in various brain nuclei of four and ten week rats. Levels of ANG II decreased with age in several hypothalamic nuclei including paraventricular, lateral, ventromedial, anterior and supraoptic, as well as lateral septum, amygdala, bed nucleus of the stria terminalis, and the subfornical organ. Levels in the suprachiasmatic nucleus remained constant while the posterior hypothalamus and the paraventricular nucleus of the thalamus showed an increase with age.

The quantitative ANG II distribution found in this study parallels previous findings on the immunohistochemical distribution of this neuropeptide. Age related changes in the levels and relative distribution of ANG II suggest that this peptide may have a role in the development of the brain.

This work was supported by NIH HL27757 and Amer. Heart Assoc. (IL) grants to J.A.W.

286.4 NEURONAL LOCALIZATION OF CHOLECYSTOKININ mRNAS IN RAT AND GUINEA PIG BRAIN. M. Réthelyi*, D. McGehee* and P.K. Lund* (SPON: E.R. Perl). Dept. of Physiol., Univ. of North Carolina, Chapel Hill, NC, 27514.

We have used in situ hybridization histochemistry (ISHH) to identify cellular sites of cholecystokinin (CCK) biosynthesis by localization of mRNAs using labeled synthetic oligodeoxyribonucleotide (oligomer) probes. The amino acid sequence of guinea pig CCK is known, but not the sequence of the mRNA encoding it. Guinea pig intestinal CCK has a valine residue substituted for the methionine residue found at position 6 from the carboxyl-terminus of CCK in other mammals. We have predicted the sequences of synthetic oligomers that would be appropriate probes for localization of both rat and guinea pig CCK mRNAs by ISHH.

Two oligomer probes of 30 and 26 bases in length were synthesized complementary to the most homologous regions of rat and porcine CCK mRNAs, the coding sequences for CCK 12 and the flanking carboxy-terminal extension. At points of codon ambiguity in rat and porcine CCK mRNAs, and in the codon for the amino acid residue that differs in rat and guinea pig CCK 12, inosine, which forms stable hybrids with all bases in mRNA was used in the complementary oligomer sequences. The synthetic oligomers were end-labeled with ^{32}P using polynucleotide kinase and hybridized at high stringency (30% formamide, 1M NaCl at 42°C) with perfusion fixed, coronal sections of rat and guinea pig brain. Autoradiography was performed on hybridized sections using Kodak NTB3 photographic emulsion. Sections were counterstained with cresyl violet.

In both rat and guinea pig brains, both of the oligomers specifically localized CCK mRNAs in neurons in the cerebral cortex and hippocampus. In both species, CCK mRNA was localized in medium sized neurons in laminae II and VI of the neocortex, in large neurons of the stratum radiatum and stratum oriens in the CA1 region of the hippocampus and in the hilus of the dentate gyrus. In the rat, CCK mRNA was also found in neurons located between nerve fibers in the corpus callosum. Thus, both rat and guinea pig brain shows a similar distribution of neurons expressing CCK mRNA.

Mapping of CCK mRNAs represents a first step toward investigations of the regulation and significance of neuronal CCK synthesis. Localization of guinea pig CCK mRNAs was achieved using oligomer sequences based on conserved mammalian CCK mRNA sequences and with the use of inosine at ambiguous bases. This strategy in probe design for ISHH may have broad application for the cellular localization of other neuropeptide mRNAs.

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286.5 MEASUREMENT OF ACTH AND CRF IMMUNOREACTIVITY IN ADRENAL GLAND AND LYMPHOCYTES. J.C. Ritchie, M.J. Owens*, L.W. O'Conner*, A.E. Kegelmeyer*, J.T. Walker*, M. Stanley*, G. Bissette and C.B. Nemeroff. Dept. Psychiatry and Pharmacology, Duke Univ. Med. Ctr. Durham, NC 27710

As part of an ongoing research effort to elucidate the physiological mechanisms that regulate adrenal cortical function, the possibility that adrenocorticotrophic hormone (ACTH) and corticotropin releasing factor (CRF) are present in various peripheral tissues was investigated.

To determine the concentration of these peptides in adrenal tissue, adrenal glands were placed in dry ice at time of slaughter (bovine) or at autopsy (human) and transported to the laboratory. The tissue was minced and suspended in 1.0 N hydrochloric acid (20 ml/gm). The tissue/acid suspension was then homogenized and sonicated on ice to ensure cell disruption and the debris was sedimented by centrifugation. ACTH and CRF-like immunoreactivity was measured in the resultant clear supernatant using sensitive and specific radioimmunoassay methods.

Lymphocyte concentrations of these peptides were determined by a similar extraction technique performed on peripheral lymphocytes isolated by Ficoll-Hypaque density gradient centrifugation.

Low detectable quantities of ACTH were found in both the lymphocytes and adrenals. The human adrenals averaged 1.33 pmol ACTH/gm wet weight. Lymphocytes contained approximately 1 pg/10^6 cells. This immunoreactivity produced curves upon dilution parallel to the standard curve for ACTH 1-39. CRF was undetectable (<0.625 pg) in adrenal tissue from either species but was detectable in low quantities in the peripheral lymphocytes. This lymphocyte CRF immunoreactivity showed curves parallel to the standard curve upon dilution.

The possibility that lymphocytes contain high affinity binding sites for CRF was studied by using a high specific activity iodinated CRF analog, ^{125}I-Tyr° CRF. No such binding sites were detected.

These findings raise the interesting possibility that circulating lymphocytes may influence glucocorticoid production by an action on the adrenal cortex. Further studies are underway to fully characterize these immunoreactive compounds and to determine their possible role(s) in hypothalamic-pituitary-adrenal physiology.

286.6 STUDIES ON THE DISTRIBUTION AND RELEASE OF MAMMALIAN BRAIN TACHYKININS. H Arai* F J Diez-Guerra* and P C Emson. MRC Group, Dept. of Neuroendocrinology, AFRC Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT, UK.

Neurokinin A and neurokinin B (NKA and NKB) and neuropeptide K (NPK) have been isolated from porcine brain and spinal cord (Kimura et al., Proc. Japan, Acad. 59 101-104 1983, Tatemoto et al., Biochem. Biophys. Res. Commun. 128 947-953 1985). These tachykinins together with substance P (SP) considerably extend the list of tachykinins present in the mammalian brain. In order to study the metabolism, release and distribution of these three additional tachykinins HPLC separations conditions and sensitive radioimmunoassays have been developed to allow unambiguous identification of NKA, NKB, NPK and SP in rat brain samples.

The distribution of NKA closely parallels that of substance P with high concentrations in the striato-nigral system and in the substantia gelatinosa. NPK is also concentrated in these regions, but is relatively enriched in cerebral cortical regions, where it is the major form of NKA-like immunoreactivity. NKB was most concentrated in the spinal cord. Studies on the electrically evoked release of tachykinins from the substantia nigra show that NKA, NPK, NKB and SP are released in a calcium dependent, tetrodotoxin sensitive manner and that the release of NKA-like immunoreactivity is influenced by GABA-agonists.

H Arai is supported by the UK Mental Health Foundation and F J Diez-Guerra by the British Council.

286.7 ARE ENZYME INHIBITORS BENEFICIAL FOR STUDYING MOLECULAR PROFILES OF IMMUNOREACTIVE ACTH AND β-ENDORPHIN IN RAT BRAIN AND PITUITARY? Leonard P. Kapcala, M.D.* (SPON: N. Pilotte). Depts. of Med., Case Western Reserve Univ., Metro. Gen. Hosp., Cleveland, OH 44109 and Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Processing of pro-opiomelanocortin (POMC) in brain and pituitary results in various proportions of multiple peptides including $ACTH_{1-39}$, β-endorphin and related derivatives appearing as immunoreactive (IR-) ACTH and IR-β-endorphin. Because it is desirable that molecular profiles of POMC-related peptides reflect in vivo processing and not isolation artifact, inhibition of proteolytic activity during extraction is critical. Enzyme inhibitors have been advocated during the extraction of POMC-related peptides, however, the benefit or necessity of using enzyme inhibitors has not been established. A study was performed to determine the benefit of using enzyme inhibitors for studying molecular profiles of IR-ACTH and IR-β-endorphin from rat brain and pituitary. Extracts were obtained from adult hypothalamus, amygdala, anterior pituitary and neurointermediate lobe with 0.2 N HCl and an enzyme inhibitor (phenylmethylsulfonyl fluoride, iodoacetamide, N-ethyl maleimide, pepstatin A, or dithiodipyridine) or no enzyme inhibitor (control) and heat inactivation in a boiling water bath prior to and after homogenization. Molecular profiles using G-75 Sephadex under denaturing conditions (8 M urea) were compared to determine if the enzyme inhibitor altered the molecular profile by inhibiting extraction proteolysis and therefore showing an increase in the proportion of larger molecular forms. Each tissue showed a characteristic molecular distribution of IR-ACTH and IR-β-endorphin on chromatographic separation. Molecular profiles which were qualitatively similar were analyzed to determine the proportion of IR-ACTH and IR-β-endorphin eluting before, with and after synthetic ACTH and β-endorphin, respectively. There was no significant increase in the proportion of larger molecular forms in the extracts obtained in the presence of an enzyme inhibitor vs. the control (no enzyme inhibitor). The addition of these enzyme inhibitors during the extraction of POMC-related peptides from hypothalamus, amygdala, anterior pituitary or neurointermediate lobe did not provide additional inhibition of proteolytic activity over that of the control. Conclusion: Enzyme inhibitors are not beneficial for inhibiting proteolytic activity in the study of molecular profiles of IR-ACTH and IR-β-endorphin from rat brain and pituitary when tissue proteases are inhibited with a strong acid and heat inactivation.

286.8 NON-RIBOSOMAL BIOSYNTHESIS OF N-ACETYLASPARTYLGLUTAMATE BY SPINAL SENSORY GANGLIA, IN VITRO. C.B.Cangro*, M.A.A. Namboodiri, L.A.Sklar* and J.H.Neale, Dept. of Biology, Georgetown University, Washington, D. C. 20057.

N-acetylaspartylglutamate (NAAG) has long been recognized as one of the more prevalent dipeptides in the nervous system. We have participated in studies of NAAG in the spinal sensory axis, using several approaches. NAAG has been found to activate the NMDA class of glutamate receptors when applied to spinal cord neurons, but it is several hundred-fold less effective than glutamate (Westbrook et al., J.Neurosci. in press, 1986). Antisera which we prepared against NAAG have demonstrated the presence of immunoreactivity (IR) in a subpopulation of spinal sensory neurons, suggesting a role for the dipeptide in a discrete primary afferent function.

NAAG biosynthesis by spinal sensory ganglia was studied by following the incorporation(3hr) of ^{3}H-glutamine, ^{3}H-glutamate and ^{3}H-aspartate into the dipeptide during incubation in vitro. Newly synthesized NAAG was extracted from the cells and purified using sequential cation exchange chromatography, anion exchange HPLC and reverse phase HPLC. A significant radiolabel incorporation from ^{3}H-glutamine and ^{3}H-glutamate was detected; glutamine was a more efficient precursor than glutamate, indicating the involvement of the glutamine-glutamate cycle. Acid hydrolysis of the radiolabled NAAG followed by anion exchange HPLC showed the radiolabel to be in glutamate. ^{3}H-Aspartate incorporation into NAAG could not be detected under similar incubation conditions, suggesting a precursor role for the large, cellular N-acetylaspartate pool. Incubation of the ganglia with 200 μM cycloheximide or anisomysin one hour prior to incorporation of the radiolabel, substantially reduced incorporation into protein, but did not inhibit the biosynthesis of NAAG. Demonstration of the nonribosomal synthesis of NAAG expands the possible roles of this molecule to include the metabolic regulation of free glutamate within some neurons.

In another approach to the study of sensory neurons which express NAAG-IR, we have analyzed the size of these cell bodies and have found them generally to be larger than those which we previously identified as possessing elevated glutaminase. Double label immunohistochemical studies of glutamate-related molecules are being pursued in order to obtain further insight into the role of NAAG in the nervous system.

286.9 IN VITRO TRANSLATION OF RNAS ENCODING TWO TYPES OF RAT PREPROTACHYKININS. M.R. MacDonald* & J.E. Krause (SPON: G. Cole). Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

We are interested in processing events that lead to the formation of the rat tachykinin transmitters Substance P (SP) and Substance K (SK) from their common precursor protein. Because the precursor is not present in vivo in large amounts, and because the mRNA is found only in low amounts and in complex tissues, studies utilizing isolated precursor protein or mRNA are difficult. With advances in molecular biological techniques, it is possible to synthesize, in vitro, RNA that corresponds to mRNA found in vivo. This cRNA can be translated in vitro to produce protein, which can then be used for processing studies. Because cDNAs representing several types of alternately spliced messages for the SP precursor have been cloned in our laboratory, we decided to use this technique to study tachykinin production.

The coding regions for rat SP precursor protein from cDNAs of 2 alternately spliced messages, β- and γ-types, have been subcloned into two vectors, pGEM1 and pSP64T, containing viral promotors. By DNA sequence analysis it has been determined that the mRNAs that these clones represent code for proteins of Mr 15,003 and 13,343, respectively, and that both types code for amino acid sequences, which could be processed to form SP and/or SK. Using SP6 RNA polymerase, β- and γ-type cRNAs have been transcribed, followed by translation in a wheat germ cell-free system. Each produces one predominant protein; the β- and γ-type proteins differ in size by ~1,800 D, as determined by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Inclusion of the cap analog methyl^{7}G(5')ppp(5')G in the transcription reaction produces cRNA whose in vitro translation is enhanced 7- to 17-fold.

Having produced labelled precursor protein, early processing events were examined, which may include signal peptide cleavage or other co-translational endoplasmic reticulum-associated steps. Inclusion of canine pancreatic microsomes during translation results in the conversion of the primary product to a trypsin resistant form that migrates in SDS-PAGE with an apparent increase in molecular weight of ~4,000 D, suggestive of glycosylation. However, the deduced precursor sequence contains no consensus sites for N-linked glycosylation. Experiments are in progress to determine the nature of this modification.

Future experiments will address the nature of processing events that occur in other subcellular locations. We intend to delineate the events that lead to the production of SP, SK, and other potentially bioactive peptides from the precursor protein, and determine which of these steps might be regulated in vivo.

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286.10 IN VIVO BIOSYNTHESIS OF VASOPRESSIN AND OXYTOCIN IN INDIVIDUAL SALINE-CHALLENGED RATS. P. Camp* and J.F. McKelvy.(SPON:C Phelps) Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794.

As the major antidiuretic hormone, arginine vasopressin (AVP) release responds to changes in the osmotic balance and blood pressure of the animal. Oxytocin (OT) is also released during osmotic imbalance, however the role of OT in salt and water balance is still undetermined. We measured the in vivo biosynthesis of AVP and OT in response to an oral saline challenge. Control male rats had free access to drinking water, while the experimental rats had 2% saline to drink for 6 days. All rats were bilaterally cannulated to the area just dorsal to the paraventricular nucleus of the hypothalamus (A: 0.2 mm, L: 0.5 mm, V: 7.3 mm with respect to bregma). Twenty-four hours later, each rat received a 2 hour pulse of ^{35}S-cysteine (500uCi) using an Alzet osmotic mini-pump delivery system at a flow rate of 1 ul/hour. After a 2-4 hour chase period, during which time artificial brain extracellular fluid was infused, the rats were decapitated. Trunk blood was collected for radioimmunoassay of AVP and OT. The neural lobe of the pituitary was removed immediately and homogenized in ice-cold extraction buffer (1% NaCl, 1% TFA, 5% HCOOH, 1M HCl). Standard carrier AVP and OT were added at the time of homogenization. ^{35}S-cysteine-labeled AVP and OT were purified by sequential linear HPLC gradient analysis, chemical modification, and exponential HPLC gradient analyses. As described previously, we have identified ^{35}S-labeled material from the neural lobe which co-purifies with carrier AVP and OT. In this study, we have demonstrated that the amount of ^{35}S-labeled AVP and OT increases after 6 days of oral 2% saline. Additionally, proportionately more AVP was labeled than OT.

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286.11 REGULATION OF PRODYNORPHIN SYSTEM IN THE BASAL GANGLIA: INFLUENCE OF LITHIUM AND HALOPERIDOL. S.P. Sivam, K. Takeuchi, S. Li*, O. Civelli, J. Douglass, E. Herbert and J.S. Hong, Neuropharmacology Section, Laboratory of Behavioral and Neurological Toxicology, NIEHS, NIH, Research Triangle Park, NC 27709 and Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201.

Previous reports indicate that the antimanic drug lithium, and the antipsychotic drug haloperidol affect neuropeptide systems such as Met^5-enkephalin and Substance P in the basal ganglia. Recent reports show that there is a distinct striatonigral dynorphinergic pathway and that dynorphin is present in high concentrations in the substantia nigra. The aim of this study was to understand the possible influence of lithium and haloperidol alone or in combination on the striatonigral dynorphinergic pathway. The study was done using male Fisher-344 rats following subchronic administration of lithium chloride or chronic oral administration of a lithium-containing diet. Subchronic administration of lithium (4 mEq/kg, i.p. for 1, 2, 4, or 6 days) increased striatal dynorphin A (1-8)-like immunoreactivity (DYN) in a time-dependent fashion. The increase in striatal DYN returned to control level eight days following the cessation of a four-day regimen of lithium administration. Concurrent administration of lithium and an opiate antagonist, naltrexone or a dopamine receptor blocker haloperidol did not influence the changes induced by lithium. Chronic oral administration of lithium for 7, 14, 21 or 28 days led to a time-related increase in DYN in the striatum. Administration of haloperidol (1 mg/kg for 21 days) with a 21 day oral administration regimen of lithium failed to affect the increase in DYN elicited by lithium. None of the treatments affected the DYN in the substantia nigra. The prodynorphin messenger RNA abundance in the striatum was quanitated by hybridization procedure using a prodynorphin RNA probe generated from the Riboprobe system. Preliminary evidence from Northern blot analysis reveals that the prodynorphin messenger RNA abundance is marginally increased following chronic oral administration of lithium for 28 days. A more detailed analysis of changes in prodynorphin messenger RNA after lithium treatments is underway. The results suggest that lithium affects the dynamics of prodynorphin biosynthesis and metabolism in the striatonigral projection whereas haloperidol does not.

286.12

WITHDRAWN

286.13 ALPHA$_2$-ADRENERGIC MODULATION OF ANGIOTENSIN II RELEASE FROM NEURONAL CULTURES. K. Hermann*, E.M. Richards*, M.I. Phillips, M.K. Raizada* and C. Sumners. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

In previous studies we have shown that catecholamines (CA) can influence angiotensin II receptor (AngII-R) regulation in neuronal cultures in a reciprocal manner, e.g. norepinephrine (NE) elicits downregulation of AngII-R by acting at alpha$_1$-adrenergic receptors. In the present study we wanted to determine whether the influence of CA on the neuronal AngII system was limited to AngII-R, or whether AngII itself could also be altered. We have examined the effects of α_2-adrenergic antagonists on AngII release from neuronal cultures. These were chosen because presynaptic α_2-adrenergic receptors (α_2-R) modulate NE release from CA cells, and we wanted to determine whether similar modulation of AngII release occurred. Neuronal cultures were prepared from whole brains of one day old Sprague-Dawley rats exactly as described previously (Sumners et al., Am. J. Physiol. 250:C236, 1986). Cultures grown for 10-14 days were used in experiments, at which time they contained 75-80% neurons. For the release experiments cultures were washed with PBS and then preincubated for 5 min at 37°C in the presence of PBS containing 0.75 mM $CaCl_2$, 0.75 mM $MgCl_2$ and 30 µM glucose (modified PBS). After 5 min this buffer was replaced with either modified PBS (controls) or with buffer containing yohimbine (5 µM) for 5 min. These incubations were followed by two further incubations (5 min each) in release buffer. The buffer from each incubation period was collected and AngII analyzed by radioimmunoassay (RIA) following purification on Sep Pak C_{18} Cartridges (Ganten et al., Science 221: 869, 1983). Following each experiment the cells were extracted and AngII content determined by RIA. Yohimbine (YOH, 5 µM) caused a significant release of AngII-like material from neuronal cultures (19.1 ± 4.6%, n = 4 experiments, 10 dishes/expt.). Results are expressed as a percentage of the neuronal AngII content. In a second set of experiments, the effects of different concentrations of yohimbine (0.1 - 50 µM) were examined, and results clearly showed that the stimulation of AngII release by this α_2-blocker was dose dependent (e.g. Control, 84.6 pg AngII-like material/0.5 ml incubation buffer; YOH, 0.1 µM, 133.7 pg/0.5 ml; YOH, 1.0 µM, 143.1 pg/0.5 ml; YOH, 10 µM, 215.1 pg/0.5 ml; YOH, 50 µM, 591.7 pg/0.5 ml). Similar results were obtained with another α_2-blocker, rauwolscine (5 µM). Our results demonstrate that inhibition of α_2-adrenergic receptors results in release of AngII-like material from neuronal cultures. These results further demonstrate modulation of the central AngII system by CA.

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286.14 CHOLECYSTOKININ RELEASE FROM RAT CAUDATO-PUTAMEN: EVIDENCE FOR INHIBITORY CONTROL. M.C. Beinfeld*, L.R. Allard* and K. Gysling* (SPON: M.H. Cooper). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Cholecystokinin (CCK) is very abundant in rat caudato-putamen (cp). The CCK neurons innervating the cp probably originate in the claustrum-piriform cortex or the amygdala. Previous studies on the release of CCK from cp have focused on the role of dopamine. CCK release from slices of rat cp elicited by high potassium which was detected with a CCK RIA was entirely Ca^{++}-dependent. Several lines of evidence indicate that CCK release from cp is inhibited by some unknown substance (which we call "X") which is released from cp and cerebral cortex by a Ca^{++}-dependent mechanism. Release media from slices of cp or cortex inhibits the release of CCK from fresh slices of cp but not from cortex, which suggests that CCK release is regulated differently in cortex than in cp.

The identification of "X" is in progress. It is unlikely that "X" is CCK, GABA, acetylcholine, somatostatin, or leucine enkephalin, as CCK, GABA, muscimol, bethanechol, and leucine enkephalin do not inhibit CCK release. The antagonists proglumide (CCK), picrotoxin (GABA), atropine (acetylcholine) or naloxone (enkephalin) also do not alter release. It is unlikely that "X" is dopamine, as pharmacological manipulations which either significantly increased or decreased dopamine release had no effect on CCK release.

Vasoactive intestinal polypeptide (VIP), which is present in both cp and cortex, inhibits the potassium-stimulated release of CCK in a dose-dependent manner. The maximal effect was observed at 10^{-7}M. VIP is known to elevate cAMP levels in rat brain. The inhibitory effect of VIP on CCK release may be independent of the elevation of cAMP because treatment of cp slices with forskolin (2×10^{-6} to 10^{-4}M) did not mimic the inhibitory action of VIP. It is also unlikely that "X" is VIP because trypsin treatment of cp or cortex release media does not block its inhibition of CCK release.

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286.15 REGULATION OF THE METABOLISM OF OPIOID PEPTIDES AND TACHYKININ IN THE STRIATONIGRAL PATHWAY BY THE DOPAMINERGIC SYSTEM. S. Li, S. P. Sivam, J. F. McGinty[1] and J. S. Hong. LBNT, NIEHS, NIH, Research Triangle Park, NC, [1]Dept. Anatomy, East Carolina Univ., Greenville, NC, USA.

It has been reported that the dopaminergic system exerts potent influence on the metabolism of [Met^5]-enkephalin (ME) and Substance P (SP) in the basal ganglia. Recent reports indicate that dynorphin-like immunoreactivity (DN-LI) is present in the striato-nigral pathway and is found in high concentration in substantia nigra. However, the regulation of dynorphin (DN) in this pathway is not known. The purpose of this study was to explore the dopaminergic control of the striatonigral DN, SP and ME systems by measuring the levels of these peptides after repeated injections of a dopaminergic agonist and antagonist. Rats received different doses of apomorphine (0.5 mg-5.0 mg/kg, s.c. twice a day), haloperidol (1 mg/kg i.p. twice a day) or in combination for different periods and were decapitated 16 h after the last injection. Concentrations of DN-LI, ME-like immunoreactivity (ME-LI) and SP-like immunoreactivity (SP-LI) were determined by radioimmunoassay. Seven daily injections of apomorphine (0.5, 1.0, 2.5 and 5.0 mg/kg) caused a dose-related increase of DN-LI and SP-LI, significantly both in the striatum and substantia nigra. However, such treatment failed to alter the level of ME-LI in the striatum. Immunocytochemistry revealed an increase in DN-LI and SP-LI, but not ME-LI in cell bodies located in striatal patches. An increase in fiber staining of DN-LI and SP-LI, both in the striatum and in the substantia nigra was also observed. In another experiment, rats received 5 mg/kg of apomorphine for 1, 3, and 7 days. There was a time-related increase in DN-LI and SP-LI both in the striatum and substantia nigra. In order to examine whether the apomorphine-elicited increase in DN-LI and SP-LI in the basal ganglia is mediated by dopaminergic receptors, haloperidol was injected alone or in combination with apomorphine for 7 days. Haloperidol alone failed to alter the striatal DN-LI and SP-LI, but completely blocked the apomorphine-induced increase in the levels of these two peptides. Consistent with previous reports, haloperidol caused a large increase in the striatal level of ME-LI. However, concurrent administration with apomorphine failed to affect the haloperidol-induced increase in ME-LI. Single injections of various doses of apomorphine caused a decrease at high doses in the striatal levels of DN-LI and SP-LI, but not ME-LI. It is possible that these initial decreases in DN-LI and SP-LI are related to the increase of the levels of these peptides after repeated administration of apomorphine. These results suggest that dopamine exerts a potent influence on the metabolism of DN, SP, and ME in the basal ganglia.

PEPTIDE BIOSYNTHESIS AND METABOLISM III

287.1 ANTISERA AND RADIOIMMUNOASSAY (RIA) FOR THE FREE CARBOXYL TERMINUS OF SYNENKEPHALIN. W.K. Stell, J. Rossier, M. Chaminade*, C. Rougeot* and F. Dray*. Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif-sur-Yvette, France, and U.R.I.A., Institut Pasteur, Paris, France.

Synenkephalin (SYN), the non-opioid N-terminal part of proenkephalin (PRO), is metabolically stable and a promising marker for enkephalinergic systems. Because existing antisera to bovine SYN (=bovine PRO(1-70)) are highly species-specific, we attempted to obtain antisera against the C-terminal octapeptide, SYN(63-70), which is well conserved in mammals. By coupling a synthetic peptide, YEESHLLA (=Tyr^{63}-SYN(63-70) to KLH, we obtained a rabbit antiserum (681) with which we established a RIA. We used this RIA to detect SYN(63-70)-immunoreactive (SYN-IR) peptides in mammalian tissues, extracted in 1M acetic acid (10 ml/g) and separated by Sephadex G100 gel filtration in 1M acetic acid. Sequential digestion by trypsin and carboxypeptidase B was employed to liberate SYN(63-70) from larger precursors.

Using Tyr^{63}-SYN(63-70) as standard and ^{125}I-Tyr^{63}-SYN(63-70) as displaceable radioligand, the RIA gives IC_{50}=30-40 fmole/100µl and sensitivity ≤3 fmole/100µl. Cross-reactivity is 100% with Cys^{63}-SYN(63-70). 8.6 KDa bovine adrenal peptide (PRO(1-77)) gives parallel dilution curves and 50-100% cross-reactivity after complete digestion, but only <10% cross-reactivity after trypsin alone and <1% without digestion. Cross-reactivity is ≤0.0002% for Leu-Leu-Ala (=SYN(68-70)) and <10^{-6} for a wide variety of enkephalins and enkephalin-containing peptides. Therefore the RIA is specific for the free C-terminus of SYN and strongly prefers a longer sequence, e.g. SYN(64-70) or its configuration, to the C-terminal tripeptide. Parallel dilution curves were obtained for extracts of bovine striatum and retina, feline adrenal, human globus pallidus and pheochromocytoma. On gel filtration of the human and bovine extracts, more than 90% of the SYN-IR material coeluted with purified natural bovine PRO(1-77). A minor peak at apparently lower molecular weight (ca. 2-5 KDa) suggested that some SYN is processed to a smaller C-terminal fragment in these tissues.

These results show that antisera to the C-terminal part of SYN should be useful for measuring and purifying synenkephalins from various mammals, including man.

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287.2 OXYTOCIN AND VASOPRESSIN PRECURSORS ARE DIFFERENTIALLY PROCESSED IN DEVELOPING RAT BRAIN. M. Altstein* and H. Gainer, LNN, NICHD, National Institutes of Health, Bethesda, MD 20892.

The peptide hormones arginine vasopressin (AVP), oxytocin (OT), and their neurophysins (NPs) are synthesized as large precursor molecules which are then post-translationally processed to produce the mature biologically active hormones. Recent immunocytochemical studies from our laboratory have suggested that there is differential processing of the oxytocin and vasopressin precursors in the hypothalamic-neurohypophysial system (HNS) during embryonic development (Whitnall et al. J. Neurosci. 5:98,1985). The purpose of the present study was to determine: 1) When are the prohormones and biologically active peptides expressed during embryonic development? 2) What are the in vivo patterns of cleavage which generate the biologically active peptide from their precursors? We have studied the HNS of the developing rat using sensitive and selective immunological procedures. Studies of processing mechanisms have revealed that prohormones are usually cleaved at pairs of basic amino acids by an endopeptidase activity. In the AVP and OT precursors, the AVP and OT peptides are located at the N-termini, separated from their respective NPs by a Gly-Lys-Arg spacer sequence. Therefore, the theoretical peptide intermediate forms of processing by the endopeptidase would be those extended at their C-terminus by either Gly-Lys-Arg, Gly-Lys or a single Gly. Antibodies against fully processed (amidated) and immature extended forms of both hormones were raised and characterized. Sensitive RIAs were developed (3 pg/tube) and used to quantitate the mature and immature forms of the hormones in acid extracts of brain and pituitary at various embryonic (E18-E22) and early postnatal stages (PN7-PN21). Fully processed AVP could be detected in the brain at E18 (0.40 ng/rat). In contrast, no amidated forms of OT could be detected in the brain between E18 to E22. Analysis of immature forms in the brain revealed no extended AVP forms at E18-PN21. Immature forms of OT, however, could be detected as early as E18 (40 pg/rat). This difference in processing of the AVP and OT intermediates was also accompanied by a lower extent of endopeptidase cleavage of the OT precursor (i.e., at E18-22, only about 20% of the OT precursor was cleaved). Analysis of the in vivo patterns of cleavage at these early developmental stages revealed that the major partially processed product was OT-Gly-Lys-Arg. This indicates that the endopeptidase cleavage in vivo must have occurred at the C-terminus of Arg in the tripeptide spacer. The presence of a stable OT-Gly-Lys-Arg intermediate in the rat brain at E18-22 suggests that a carboxypeptidase-B-like enzyme is either absent or inactive in OT-neurons at this stage of development. The early expression of amidated AVP and the OT intermediate in the brain may indicate a trophic function for these peptides in brain development.

287.3 THE PRESENCE OF IMMUNOREACTIVE α-BAG CELL PEPTIDE[1-8] IN BAG CELL NEURONS OF APLYSIA CALIFORNICA SUGGESTS THE EXISTENCE OF NOVEL CARBOXYPEPTIDASE PROCESSING. Stefan M. Pulst*, Barry S. Rothman and Earl Mayeri, Department of Physiology, University of California, San Francisco, CA 94143.

α-bag cell peptide (αBCP), a putative neurotransmitter released from bag cell neurons, has 3 neuroactive forms: αBCP[1-9], αBCP[1-8] which lacks a C-terminal leucine, and αBCP[1-7]. α BCP[1-9] and [1-8] (but not [1-7]) are present in bag cell extracts and are released from bag cells. This suggests that both forms of the peptide serve as neurotransmitters and that αBCP[1-8] is formed before release, after release or both (Sigvardt et al., 1986). In the presence of protease inhibitors, the amount of α BCP[1-8] in the extract is only approximately 10% of αBCP[1-9]. This raises the question whether the presence of αBCP[1-8] is an artifact due to incomplete inhibition of carboxypeptidase activity during extraction, or whether it is due to a naturally occurring intravesicular processing event.

In support of the latter possibility we have found that both forms are stable in extract for at least 1 hr, which suggests that proleolysis has been effectively inhibited. We studied this question further by immunocytochemical means. Antibodies (AB's) to αBCP[1-8] were raised in rabbits, and crossreactivity to α BCP[1-9] and αBCP[1-7] was absorbed out on affinity columns to which these peptides were covalently bound. The remaining AB's were passed through an αBCP[1-8] affinity column and eluted with glycine/HCl pH 2.4. A filter paper assay confirmed that this AB had only 1% crossreactivity with the other αBCP's. Abdominal ganglia were perfusion-fixed with 4% paraformaldehyde, and cryostat cut sections processed for indirect immunofluorescence. Adjacent sections were stained with the AB to αBCP[1-8] and with a previously generated AB that is highly specific to αBCP[1-9] (Pulst et al., 1986). Intense immunoreactivity to both peptides was seen in bag cell somata and fibers. Staining for each peptide was absorbed out by the addition of 1 μM αBCP[1-8] or 10 μM α BCP[1-9] to the respective AB solution.

These results suggest that both αBCP[1-8] and [1-9] are present in bag cell somata and terminals and that a small amount of αBCP[1-9] is processed to αBCP[1-8] in vesicles before release. Although it seems unlikely, we cannot exclude the possibility that αBCP[1-8] is formed during the process of fixation. Since αBCP[1-8] is 30 times as potent as αBCP[1-9] in inhibiting left upper quadrant neurons in the abdominal ganglion, the activity of an intravesicular carboxypeptidase may regulate the amount of inhibitory activity released during a bag cell discharge.

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287.4 PROTEOLYSIS OF FMRFamide: A MODEL OF NEUROPEPTIDE DEGRADATION. S. M. Lambert* and M. J. Greenberg. C. V. Whitney Lab., Univ. of Florida, St. Augustine, FL 32086.

The degradation of neuropeptides at their target tissues is a fundamental aspect of their physiological action. Proteolytic enzymes might serve to terminate peptidergic signals; but are they nonspecific proteases, or do they possess catalytic specificity for a particular peptide or peptide family? The clam ventricle, a well-characterized target tissue for FMRFamide, was used to characterize the enzymes that might be degrading this peptide and its natural analogs.

When FMRFamide (Phe-Met-Arg-Phe-NH_2) is incubated with either isolated, intact ventricles, or ventricular membrane preparations, the peptide disappears with time, and several transient products are formed. The identification of these products by HPLC and amino acid analysis revealed the characteristics of the activities directed toward FMRFamide. Hydrolysis occurs, initially, at the peptide bonds between Phe and Met and between Arg and Phe-amide; but the peptide is ultimately degraded to its constituent amino acids. The hydrolytic activities were solubilized with detergents and purified by combinations of gel filtration, ion exchange and affinity chromatography. Three enzymatic species, all metallopeptidases, were detected in the tissue: a high molecular weight aminopeptidase (900 Kd); an endopeptidase (500 Kd); and a smaller aminopeptidase (90 Kd) with catalytic properties dissimilar to the larger enzyme.

The large aminopeptidase, a glycoprotein, appears to have little specificity for FMRFamide, either in its substrate requirement or binding affinity. The small aminopeptidase, although active against the 2-naphthylamide derivatives of most amino acids, shows a strong preference for peptides with a phenylalanine residue at the N-terminal, and it completely degrades FMRFamide.

The endopeptidase, a chloride-dependent glycoprotein, shares similarities with mammalian neutral endopeptidase (EC 3.4.24.11; enkephalinase A), both in its catalytic specificity (cleaving at the N-terminal side of hydrophobic amino acids), and in its sensitivity to inhibitors. Maximal rates of hydrolysis occur with the FMRFamide sequence; substitutions at any of the four residues or N-terminal extensions, decrease catalytic efficiency. In particular, the replacement of Met^2 by Leu decreases activity significantly. Thus, if this endopeptidase is a characteristic component of FMRFamide-sensitive tissues in all molluscs, then FLRFamide and its heptapeptide analogs would be biologically stable. Most notably, since pQDPFLRFamide has a blocked N-terminal, it would also be resistant to attack by aminopeptidases and would then fit well its proposed role as a cardioregulatory hormone in some pulmonate snails. (Supported by NIH grant HL-28440.)

287.5 LOCALIZATION OF ENDOPEPTIDASE 24.11 IN RAT CNS AT THE LIGHT AND ELECTRON MICROSCOPIC LEVEL. R. S. Lasher, E. M. Lutz* and D. Sanderson*. Dept. of Cellular and Structural Biology, Univ. of Colorado Med. Sch., Denver, CO 80262.

Endopeptidases appear to be important in generating biologically active fragments of peptides. In the case of substance P, the SP(1-7) fragment produced by cleavage of the Phe-Phe bond by the enzyme endopeptidase 24.11 (R. Matsas et al., PNAS 80:3111, 1983) appears to be the centrally active agent for production of antinociception (J. M. Stewart et al., Peptides 3:851, 1982). Localization of endopeptidase appears to be similar to localization of metabolic products (J. Kenny, TIBS 1:40, 1986). We have produced a monoclonal antibody (172-3) of the IgM class against endopeptidase 24.11 isolated from rat kidney (F. Mulholland and C. Bublitz, unpublished results) in order to visualize its distribution in rat CNS. Two types of evidence suggest this antibody is specific for endopeptidase 24.11. First, on Western blots, only the band corresponding to the enzyme (95,000 MW) reacts with the antibody. Second, the antibody appears to markedly potentiate the activity of the enzyme, whereas a monoclonal IgM to calcium/calmodulin-dependent protein kinase does not affect endopeptidase activity.

Frozen-thawed sections (40 μM) of rat brain and spinal cord fixed by perfusion with 5% paraformaldehyde + 0.2% glutaraldehyde were incubated with diluted ascites fluid containing 172-3, rinsed, and processed using the ABC-peroxidase technique. At the light microscopic level, localization of the epitope appeared to be quite specific. For example, in the spinal cord it was found to be localized primarily in the gray matter, being most prominent around the central canal and in the lateral portion of the dorsal horns. In the cerebellum, epitope appeared to be highly enriched in the molecular layer, but very sparse in other areas. In the medulla (just rostral to the obex), the epitope was most abundant lateral and subjacent to the fourth ventricle and in the nucleus of the spinal tract of the trigeminal nerve. However, it was virtually absent from the spinal tract of the trigeminal nerve. The reaction product generally appeared punctate, suggesting a synaptic localization, or it appeared diffuse, but without specific subcellular localization. At the ultrastructural level, reaction product was primarily associated either with membranes of glial processes or with the postsynaptic density and subjacent filamentous material within, for example, dendritic spines of Purkinje cells. Little, if any, reaction product was seen within presynaptic structures. Controls utilizing IgM from MOPC 104E lacked specific staining, and sections incubated with antibodies to calcium/calmodulin-dependent protein kinases gave a totally different pattern of staining. (Supported by NIH grant HL-33534).

287.6 SYNAPTOSOMAL MEMBRANE-BOUND ENDOPEPTIDASE-24.15 AND OPIOID PEPTIDE PROCESSING. G.R. Acker*, C. Molineaux and M. Orlowski*. Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Endopeptidase-24.15, a predominantly soluble metalloendopeptidase (Orlowski, et al., Eur. J. Biochem., 125:81-88, 1983), is found to be tightly bound to synaptosomal membranes isolated from rat brain. Subcellular fractionation of the enzyme in rat brain showed that 20-25% of the activity is associated with membrane fractions. The enzyme could be solubilized from membranes by Triton X-100. Twenty-five percent of the total activity present in purified synaptosomal membranes was rapidly released by incubation with trypsin, but was not removed by repeated washing with isotonic sucrose (0.32 M), hypotonic buffers (0.005 M Tris-HCl, pH 7.4), hypertonic salt solutions (0.5 M NaCl in Tris HCl, pH 7.4) or by sonication. The specific activity of endopeptidase-24.15 in synaptosomal membranes was higher than that of endopeptidase-24.11. Purified synaptosomal membranes rapidly converted dynorphin-(1-8), α- and β-neoendorphin into Leu-enkephalin, and converted Met-enkephalin-Arg-Gly-Leu into Met-enkephalin in the presence of captopril, bestatin and N-[1-(R,S)-carboxy-2-phenylethyl]-Phe-p-aminobenzoate, inhibitors of angiotensin converting enzyme (EC 3.4.15.1), aminopeptidase (EC 3.4.11.2) and membrane-bound metalloendopeptidase (EC 3.4.24.11), respectively. The generation of enkephalins was inhibited by N-[1-(R,S)-carboxy-2-phenylethyl]-Ala-Ala-Phe-p-aminobenzoate, an active-site-directed inhibitor of endopeptidase-24.15, indicating involvement of this enzyme in the observed conversions. The data show that endopeptidase-24.15 is tightly bound to synaptosomal membranes and is capable of generating Leu- and Met-enkephalin from larger peptides. [Supported by a grant from the NIH, AM-25377].

287.7 EFFECTS OF CYSTEAMINE INJECTION ON THE IN VIVO INCORPORATION OF [^{35}S]-CYSTEINE INTO SOMATOSTATIN-14, SOMATOSTATIN-28, ARGININE VASOPRESSIN, AND OXYTOCIN IN RAT HYPOTHALAMUS. J.L. CAMERON and J.D. FERNSTROM, Department of Psychiatry and Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15213.

The effect of cysteamine injection was studied on the in vivo incorporation of [^{35}S]-cysteine into somatostatin-14 (SRIF-14), somatostatin-28 (SRIF-28), arginine vasopressin (AVP), and oxytocin (OXT) in rat hypothalamus. [^{35}S]-cysteine was injected into the third ventricle 1hr, 4hr, or 1 week after cysteamine (300 mg/kg sc) injection. Animals were killed 4 hr later, and hypothalami were removed and homogenized, and the resulting supernatants either subjected to radioimmunoassay, or run over two sequential HPLC separations (Van Itallie and Fernstrom, Brain Research 249: 177, 1982; Fisher and Fernstrom, Life Sciences 28: 1471, 1981). Fractions were collected and counted for ^{35}S radioactivity; labeling of individual peptides was identified as radioactive peaks with the same retention times as the standard peptides. Cysteamine was observed to reduce substantially immunoreactive levels of somatostatin, but not of OXT or AVP 4 hr after its injection. The drug also caused large reductions in label incorporation into SRIF-14, SRIF-28, and OXT 1 hr and 4 hr after its injection (see table). However, [^{35}S]-cysteine incorporation into AVP was increased substantially at these timepoints, while that into acid-precipitable protein was normal. One week after cysteamine injection, label incorporation into all hypothalamic peptides was normal. Cysteine specific activity was also measured after [^{35}S]-cysteine injection, and was found to be comparable in treatment and control groups. These results suggest that cysteamine injection may inhibit the syntheses of SRIF-14, SRIF-28, and OXT, and stimulate that of AVP.

Variable and Group	Time after cysteamine injection 1 hr	4 hr	1 wk
SRIF-14 Incorporation			
Vehicle	1.90 ± 0.58(3)	1.99 ± 0.26(5)	3.24 ± 1.20(3)
Cysteamine	0.15 ± 0.09(3)*	0.41 ± 0.29(5)*	3.31 ± 1.20(3)
SRIF-28 Incorporation			
Vehicle	1.01 ± 0.29(3)	1.16 ± 0.11(5)	1.91 ± 0.60(3)
Cysteamine	0.11 ± 0.10(3)*	0.22 ± 0.10(5)*	1.76 ± 0.70(3)
Oxytocin Incorporation			
Vehicle	4.78 ± 1.90(3)	5.53 ± 0.86(4)	5.55 ± 0.26(3)
Cysteamine	0.06 ± 0.06(3)*	1.17 ± 1.00(3)*	5.28 ± 1.90(3)
Vasopressin Incorporation			
Vehicle	4.10 ± 1.20(3)	4.81 ± 0.98(4)	3.13 ± 1.40(3)
Cysteamine	9.24 ± 2.50(3)*	9.62 ± 1.80(3)*	3.91 ± 1.50(3)

Means ± sem; incorporation rate = dpm[^{35}S]-peptide/protein specific activity. * $P < 0.025$ compared to vehicle values (t-test).

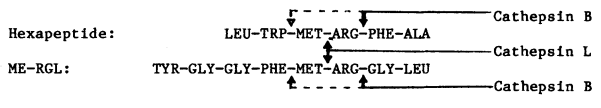
287.8 PRECOLUMN DERIVATIZATION OF PEPTIDES WITH PHENYLISOTHIOCYANATE: APPLICATION TO THE MEASUREMENT OF THE SECRETORY PRODUCTS OF PERFUSED BOVINE ADRENAL MEDULLA. M. Dumont, P. Mercier*, S. Lemaire. Department of Pharmacology, School of Medicine, University of Ottawa, Health Science Center, Ottawa, Ontario, Canada, K1H 8N5.

A rapid and ultra sensitive method for the identification and quantification of peptide hormones has been developed. The method is based on precolumn derivatization of peptides with phenylisothiocyanate to form the phenylthiocarbamoyl derivatives (PTC-peptides). The derivatized peptides are injected on a reverse-phase high performance chromatography (HPLC) column (Zorbax ODS, Dupont), eluted with a gradient of acetonitrile in sodium acetate buffer (0.03M, pH 6.4) and detected at 269 nm with a limit sensitivity of 1 picomole. The method was very effective in separating closely related peptides such as a mixture of synthetic Dynorphin A (1-13), -(1-12), -(1-11), Leu - and Met - enkephalins. It was also applied to the separation and identification of secreted peptides in bovine adrenal perfusates. Bovine adrenal glands were perfused retrogradely at 10 ml per min for sixty min with Krebs buffer pH 7.4. They were then stimulated by perfusion with a solution of carbamylcholine (5×10^{-4}M) for 1.5 min. The effluents were collected 5 min prior to stimulations (controls) and 5 min, starting with the beginning of the stimulations (assays). They were passed through Sep Pak C18 cartridges (Waters) and peptides were eluted with acetonitrile (60% in 0.1% trifluoroacetic acid). The eluted material was treated with phenylisothiocyanate and analyzed on HPLC. Many peptides (up to 15 peptides) were shown to be released in the presence of carbamylcholine. Among these peptides, Leu-enkephalin was clearly identified and its measurement indicated a basal secretion of 0.6 n mole per 5 min of perfusion at 10 ml/min. Stimulation with carbamylcholine induced a 4-fold increase in the release of Leu-enkephalin. It is hoped that the proper application of this method will allow the complete identification and a better quantification of secreted peptides from endocrine glands.

(Supported by the Canadian Heart Foundation and the Medical Research Council of Canada).

287.9 RAT BRAIN CATHEPSIN L: CHARACTERIZATION AND COMPARISON TO CATHEPSIN B USING MODEL PEPTIDES, PROENKEPHALINS, AND BRAIN PROTEINS. M.J. Berg* and N. Marks. Center for Neurochemistry, Nathan S. Kline Institute, Ward's Island, New York, N.Y. 10035

Cathepsin L is a cysteine proteinase known to degrade proteins at significantly higher rates than Cathepsins B or H, but has not been previously studied in the CNS. To examine its potential role in brain protein turnover, we report on the first isolation of a rat brain enzyme conforming in properties to Cathepsin L of other tissues. Cysteine proteinases were extracted and then activated by the method of Suhar and Marks (Eur. J. Biochem., 101:23, 1979) and separated by ion-exchange, affinity, hydrophobic interaction and FPLC (Mono S) chromatography to yield two fractions hydrolysing 1) Z-Phe-Arg- and Z-Arg-Arg-NMec and 2) preferentially Z-Phe-Arg-NMec. Both fractions were devoid of Cathepsin H as judged by absence of activity towards Arg-NNap, were active in presence of cysteine at pH 5.5-6.0, and inhibited by leupeptin, E-64, and the endogenous brain factor, cerebrocystatin, shown previously to suppress cysteine proteinase fragmentation of myelin basic protein (Kopitar et al, Biochem. Biophys. Res. Commun. 112:1000, 1983). Fraction 1 acted like a peptidyl dipeptidase towards a model hexapeptide and several proenkephalins, and differed in specificity as compared to Fraction 2 (Cathepsin L).



Both cysteine proteinases converted the proenkephalin ME-RF by cleavage at the Met^5-Arg^6 bond to liberate Met-enkephalin and Arg-Phe. The kinetics of proenkephalin conversions, and the hydrolysis of fluorogenic substrates are compared. The specific activities, as well as the cleavage products observed by gel-electrophoresis for several cytoskeletal proteins (spectrin, tubulin/filament) are compared to patterns observed for myelin basic protein, and collagen proteins. The presence in rat brain of this potent intracellular proteinase is relevant to protein and neuropeptide processing, and to changes found as a result of maturation and disease states.

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287.10 ENKEPHALIN HYDROLYSIS IN RAT PLASMA. Susan B. Weinberger, Robert C. Walker*, Cara E. Zafuto*, and Joe L. Martinez, Jr. Psychology Dept., Univ. of California, Berkeley, CA 94720.

We previously reported (Martinez, et al., Neurosci Abs., 1985, 11, 982) a very high correlation between the rate of [leu]enkephalin (LE) hydrolysis in plasma and escape latency on the first trial in an active avoidance task. In the present study we characterize the enzymes associated with this hydrolysis.

Metabolism of LE was measured in blood samples collected from rats through indwelling femoral artery cannulae and from mice following decapitation. Plasma was incubated in vitro at 37 deg C with ^{3}H-LE (6 µl, specific activity 43,6 Ci/mmol); the reaction was terminated with two volumes of 0.1% trifluoroacetic acid in methanol. Unmetabolized LE was separated from its combined metabolites by thin layer chromatography (Ziring, et al., Int. J. Pep. Prot. Res., 1983, 22, 32) and quantified by liquid scintillation counting.

The half-life of LE in rat plasma was determined to be 2.4 min, which is in close agreement with the 2.5 min half-life determined by Hambrook, et al. (Nature, 1976, 262, 782) in a vas deferens bioassay. A much longer LE half-life, 9.3 min, was found for mouse plasma.

Approximately 80% of LE hydrolysis can be attributed to aminopeptidase activity, since bestatin (5-1500 µM), an aminopeptidase inhibitor, produced concentration-dependent decreases in the rate of LE metabolism, to a maximum of 80% inhibition. Thiorphan-sensitive dipeptidylcarboxypeptidase ("enkephalinase") activity could not be detected when thiorphan, in concentrations up to 10 µM, was tested alone or in combination with bestatin. When ^{3}H-2-d-ala-l-LE, which is reportedly insensitive to aminopeptidase degradation, replaced ^{3}H-LE as substrate for the reaction, bestatin (50-1500 µM) produced concentration-dependent decreases in hydrolysis, to a maximum of 30-40%, while thiorphan (10 µM) produced only 10-20% inhibition of hydrolysis. The plasma half-life of 2-d-ala-l-LE is approximately 4-6 hr. Thus 2-d-ala-l-LE is metabolized in rat plasma in part by an unidentified bestatin-sensitive enzyme, and only to a small extent by "enkephalinase."

These data indicate that aminopeptidase activity accounts for approximately 80% of enkephalin hydrolysis in plasma, and that "enkephalinase" has a minor role in plasma enkephalin metabolism in the rat.

(Supported by ONR N00014-83-K-0408)

287.11 SUBCELLULAR AND REGIONAL DISTRIBUTION OF THE MEMBRANE BOUND PYROGLUTAMATE AMINOPEPTIDASE DEGRADING TRH IN RAT BRAIN. P. Joseph-Bravo$ M.A.Vargas$ M. Méndez$ M. Cisneros*and J.L.Charli*(SPON: J. Alanís). Dept. of Biochemistry, Centro de Investigaciones sobre Ingeniería Genética y Biotecnología, U.N.A.M. A.P.70-479 México, D.F. México

Thyrotropin Releasing Hormone (TRH) is a candidate neurotransmitter in the Nervous System. The way by which TRH is inactivated once released is still poorly understood. We had shown that TRH is degraded by a membrane bound pyroglutamate amino peptidase (PGAm) distinct from the soluble pyroglutamate aminopeptidase (PGAs). The PGAm is a metalloprotease specific for peptides similar to TRH (Garat et al., *Neuropeptides* 6:27, 1985). In order to understand the role of this enzyme in TRH metabolism, we have studied TRH degradation in slices and subcellular fractions. Extensively washed hypothalamic slices, degrade (^{3}Hpro)-TRH mainly to his-pro-NH_2 by action of the PGAm while the catabolism by PGAs is very low. We developed a specific assay for PGAm using (^{3}H-pro)-TRH as substrate and inhibitors of PGAs and further his-proNH_2 catabolism. With this assay, we showed that the enzyme is localized on synaptosomal membranes and that PGAs is present in low concentrations in nerve endings. The PGAm activity is maximal at neutral pH; the activity is the same whether the synaptosomes are intact or broken. The regional distribution of the PGAm in brain is very different from the homogeneous distribution of the PGAs. There is a 10 fold difference between higher and lower values of different areas: the order is: olfactory bulb>nucleus accumbens> posterior cerebral cortex > hypothalamus > cerebellar cortex >neurohypophysis> mesencephalon >adenohypophysis > pons > cervical area of spinal chord. We have also measured in tissue slices, the regional distribution of TRH release (basal or K^+ stimulated) but found no correlation with PGAm activity. The data show that the PGAm is localized on the external face of the synaptic plasma membrane and may play an important role in TRH inactivation once released. Because of the lack of correlation between the PGAm activity and TRH content, response to release and receptors, it is possible that the enzyme recognizes other as yet unidentified neuropeptides.
(Supported by CONACYT and Fondo R.J. Zevada).

287.12 DEGRADATION OF BRADYKININ BY RAT BRAIN PEPTIDASES. A.T. Orawski and W.H. Simmons (SPON: T. Khan). Dept. of Biochem. & Biophys., Loyola Univ. of Chicago Stritch Sch. of Med., Maywood, IL 60153.

Evidence exists that bradykinin may serve as a neurotransmitter or neuromodulator in the nervous system. We have examined the metabolism of bradykinin by washed membranous fractions derived from lysed synaptosomes from whole rat brain. Metabolites were detected by reversed-phase HPLC. The rate of degradation of bradykinin was similar to that for Met-enkephalin. Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) (BK1-9) was cleaved primarily at three peptide bonds, Phe^5-Ser^6, Pro^7-Phe^8 and Phe^8-Arg^9. All cleavages, though due to different enzymes, were inhibited completely by o-phenanthroline or EDTA and to varying extents by 1 mM p-chloromercuriphenylsulfonate and phenelzine. The enzyme responsible for the Phe^5-Ser^6 cleavage is probably the membrane-bound form of metalloendopeptidase 24.15 (Chu and Orlowski, Biochemistry 23, 3598 (1984)) since it was inhibited by N-[1-(R,S)-carboxy-2-phenylethyl]-Ala-Ala-Phe-p-aminobenzoate but not by N-[1-(R,S)-carboxy-2-phenylethyl]-Phe-p-aminobenzoate, phosphoramidon or captopril. Extraction with Nonidet P-40 followed by gel permeation HPLC gave a M.W. for this enzyme of about 44,000. The pH optimum was in the neutral to basic range. The partially purified enzyme cleaved the Phe^5-Ser^6 bond more than twice as fast in BK2-9 than in BK1-9 or BK1-8. BK1-7 and Met-enkephalin were not hydrolyzed by this enzyme while Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu was cleaved at the Met-Arg bond as expected for endopeptidase 24.15 (Endocrinol. 116, 1418 (1985)). The enzyme activity responsible for hydrolysis of the Phe^8-Arg^9 bond in BK1-9 was maximally active at pH 6.5 and was inhibited by DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid and excited by Co^{++}. The resulting BK1-8 fragment was subject to cleavage by either endopeptidase 24.15 or a DFP-inhibitable exopeptidase which released the C-terminal Phe (pH optimum, 5.8, and activated by 2-mercaptoethanol). Hydrolysis of the Pro^7-Phe^8 bond to release Phe-Arg occurred maximally at pH 7.8 and was only partially inhibited by phosphoramidon, captopril, a combination of these inhibitors, Trp-hydroxamate or Phe-hydroxamate. Nonidet P-40 extraction and gel permeation HPLC of this Phe-Arg-releasing enzyme gave a M.W. of 54,000. (Minor activity cleaving the Pro^7-Phe^8 bond had a pH optimum at 6.5, which corresponded with the pH optimum for captopril-inhibited Hip-His-Leu hydrolysis and the captopril-inhibited release of Ser-Pro from BK1-7, BK2-7 and BK4-7). This work was supported by USPHS grant NS20252).

287.13 CHARACTERIZATION OF AN AMINOPEPTIDASE ACTIVITY IN BOVINE PITUITARY SECRETORY VESICLES. N.P. Birch* and Y.P. Loh (SPON: J. Yu). Lab. of Neurochemistry & Neuroimmunology, NICHD, National Institutes of Health, Bethesda, MD 20892.

Most peptide hormones are synthesized as larger precursor molecules with the biologically active peptide(s) being released upon limited proteolysis in a post-translational processing step. Many of these hormone precursors contain pairs of basic amino acids bracketing the biologically active peptide(s) which have been implicated as cleavage sites for processing enzymes. Recently, an endopeptidase has been characterized in the intermediate lobe of the bovine pituitary which specifically cleaved at Lys-Arg pairs of pro-opiomelanocortin (Loh, Y.P., Parish, D.C. and Tuteja, R., J.Biol.Chem. 260:7194,1985). In addition to hydrolysis on the carboxyl side of these paired basic residues, the pro-opiomelanocortin converting enzyme cleaved between the Lys-Arg amino acids. Together with the reported cleavage of enkaphalin intermediates (Lindberg, I., Yang, H.-Y.T, & Costa, E.,J. Neurochem. 42:1411,1984) and synthetic enkephalin-containing peptides (Mizuno, K. & Matsuo, H., Neuropeptides 5:489,1985) between Lys-Arg residues, these data support a role for an aminopeptidase B-like and/or carboxypeptidase B-like enzyme in the release of biologically active peptides.

We have previously described a cobalt-sensitive aminopeptidase B-like activity associated with the membrane component of neural and intermediate lobe secretory vesicles which specifically removed the N-terminal arginine from Arg-Met-enkephalin and Arg-MCA (Gainer, H., Russell, J.T. & Loh, Y.P., FEBS Lett. 175:135,1984). Further characterization after lysis of purified neural and intermediate lobe secretory vesicles by freezing and thawing indicated an approximate equal distribution of enzyme activity between the membrane and soluble fractions with the fluorometric substrate Arg-MCA, whereas 70-80% of the Arg-Met-enkephalin cleaving activity detected by HPLC was membrane-associated. Intermediate lobe secretory vesicle membranes also cleaved Lys-Arg-Met-enkephalin to free enkephalin with no detectable Arg-Met-enkephalin. The rate of release of Met-enkephalin, however, was approximately 20% of that with Arg-Met-enkephalin as substrate. Two other potential substrates for an aminopeptidase B-like activity, Lys-Bradykinin and Lys-γ-MSH were essentially resistant to cleavage as indicated by the failure to detect free lysine residues. These data suggest the existance of a specific aminopeptidase B-like enzyme within secretory granules which has a high affinity for arginine residues. Work is in progress to purify such a membrane-associated aminopeptidase activity to allow more detailed chacterization studies.

287.14 **ACTIVATION BY DITHIOTHREITOL [DTT] OF THYROTROPIN RELEASING HORMONE [TRH] CATABOLISM IN PRIMATE SPINAL CORD**
J. Turner*, T. Schwartz*, B. R. Brooks, (Spon: HS Schutta) Neurology Service, William S. Middleton VA Hospital and University of Wisconsin ALS Clinical Research Center, Madison, WI 53792

When 3H-TRH was added to buffered homogenates of freshly dissected regions of the primate CNS significant differences in the rates of TRH catabolism were noted:

Percent added 3H-TRH Degraded / minute [x ± SD]

Buffer	Cerebral Cortex	Cerebellar Cortex	Brainstem	Spinal Cord
KP04	7.80 ±0.33	5.40 ±0.42	3.98 ±0.59	1.77 ±0.64
vs CTX	-	$p < 0.04$	$p < 0.006$	$p < 0.0001$
DTT	7.63 ±0.06	6.24 ±0.50	5.18 ±0.29	3.03 ±0.26
vs CTX	-	ns	$p < 0.002$	$p < 0.003$
vs buffer	ns	ns	$p < 0.06$	$p < 0.03$

Further studies in brainstem and spinal cord [SC] were performed to determine whether bacitracin inhibited TRH catabolism activated by DTT:

KP04 / Bacitracin	0.37 ±0.13	0.72 ±0.61
DTT / Bacitracin	0.79 ±0.03	1.14 ±0.37
vs buffer	$p < 0.002$	$p < 0.02$

Product analysis indicated that TRH-OH was formed in the CTX and SC but addition of DTT decreased TRH-OH formation in the SC but not the CTX. Concomitant with the decrease in TRH-OH in SC, there was an increase in cyclo-HisPro, HisPro-OH, and Proline formation. However, in CTX, only cyclo-HisPro and HisPro-OH increased suggesting that while pyroglutamyl aminopeptidase activity is increased by DTT in both CTX and SC, proline dipeptidase activity is increased more in SC by addition of DTT. Bacitracin, which blocks TRH-OH formation, is equally effective in brainstem and SC in both buffers. Differential catabolism of TRH in the CNS may be important in determining the role of TRH during different pathological processes.

288.1 POSTEMBRYONIC GROWTH OF THE LATERAL LINE SYSTEM IN THE MOTTLED SCULPIN, Cottus bairdi. S. Coombs, J. Janssen*, D. Hoekstra*, and C. Platt. Parmly Hearing Institute, Loyola University of Chicago, Illinois 60626

Scanning electron microscopy and standard histological techniques have been used to determine various anatomical changes in the lateral line system of Lake Michigan mottled sculpin, ranging in size from 2 to 9 cm SL. Hair cell number, sensory strip and neuromast area were determined for neuromasts in the mandibular, preopercular, infraorbital, supraorbital and trunk canals and for various lines of superficial neuromasts on the head. We found a statistically significant increase in these parameters for all canal neuromasts as a function of fish standard length. In addition, neuromasts from different canals showed differential growth. Neuromasts from the preopercular, infraorbital and supraorbital canal were relatively homogenous in their size and growth, but as a group grew significantly faster than trunk neuromasts and slower than mandibular neuromasts - the largest neuromasts in the system. In adult fish, superficial neuromasts were significantly smaller than all canal neuromasts and although their rate of growth was almost negligible relative to most canal neuromasts, they showed significant increases in size and hair cell number. Canal and superficial neuromasts also showed differences in the size and shape of the cupula and the neuromast surface, which became more elongate with growth for canal neuromasts, but grew symmetrically for superficial neuromasts. Changes with growth are discussed relative to the functional morphology and behavioral significance of the lateral line system in the mottled sculpin.

288.2 AN EXPERIMENTAL DETERMINATION OF THE TEMPORAL AND SPATIAL PATTERN OF HAIR CELL PRODUCTION IN THE EMBRYONIC COCHLEA OF THE CHICKEN. A. Katayama* and J. T. Corwin. Dept. of Zoology and Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

In the chicken cochlea, structural features of hair cells such as the number, the length, and the width of their surface stereocilia have been shown to vary systematically in precise relation to the position of cells in the basilar papilla sensory epithelium (Tilney and Saunders. J. Cell Biol. 96:807-821, 1983). This indicates that genetic and/or position-related environmental mechanisms precisely control the differentiation of these hair cells. As a first approach to identifying mechanisms that control the determination of the cell-specific ultrastructural phenotypes expressed in this neuroepithelium we have investigated the temporal and spatial pattern of hair cell production.

Hair cell production in the embryonic basilar papilla sensory epithelium in the chicken cochlea was traced using the radioactive DNA precursor ^{3}H-thymidine. This was injected into the yolk sac of chick embryos at times ranging from Embryonic Day 3 to 10 (E.D. 3-10). All embryos were incubated until E.D. 11 by which time the majority of hair cells would have been produced. Then their cochleae were fixed, embedded, sectioned, and mounted on glass slides. They were then coated with NTB2 emulsion, incubated, and developed. This permitted identification of those cells which contained DNA that had been replicated during the period when the ^{3}H-thymidine was available.

Between E.D. 3 and E.D. 5 most cells in the basilar papilla are dividing, and no hair cells are produced. The first hair cells, produced after E.D. 5, form a longitudinal band running the length of the basilar papilla. This band of initially produced hair cells extends along the superior edge of the epithelium in the proximal half of the cochlea and along the center in the distal half. After E.D. 6, other hair cells are added by apposition along the edges of that band. Hair cell production is nearly complete by E.D. 9. Hair cell production is first completed in the proximal end and then in the distal half at the superior edge of the sensory epithelium.

The appositional pattern of hair cell production that has been observed in the chicken cochlea provides certain constraints for speculation as to the mechanisms controlling specific phenotype determination in this neuroepithelium, but does not suggest a particular control mechanism. This pattern of production may allow the cells to utilize both temporal and spatial differences in microenvironments as cues guiding their expression of position-related phenotypes.

(Supported by a grant from NINCDS to JTC)

288.3 DIFFERENTIAL DISTRIBUTION OF CHOLINERGIC ENZYMES IN THE DEVELOPING MOUSE COCHLEA. M.R. Emmerling and H.M. Sobkowicz (SPON: J. Chan). Dept. Of Neurology, Univ. of Wisconsin, Madison, 53706

In order to study the differentiation of cholinergic innervation in the cochlea, the specific activities (enzyme activity/ug protein) of choline acetyltransferase (ChAT), acetylcholinesterase (AChE) and the molecular forms of AChE were determined. Assays were made separately in the base, mid and apex using ICR mice, age 1 to 19 postnatal days (P). Both ChAT and AChE activities were already present in all three turns in the newborn animals.

Fifty percent of total ChAT activity in the adult was present in the cochlea at birth. Initially, the specific activity of ChAT was greater in the mid and apex than it was in the base but became lower due to increasing protein content of the tissue. Thus, by P19 the levels of ChAT in the base and mid became similar, each containing approximately twice the ChAT activity found in the apex.

Twenty percent of adult level AChE activity was detected in the cochlea of newborn mice. From birth to P14 the specific activity of AChE increased in all turns. Throughout the development a constant ratio of about 50:30:20 of AChE specific activity was observed between the base, mid and apex.

Velocity sedimentation analysis of AChE revealed an increase in all three AChE forms studied in each turn of the cochlea. The level of 10S, a globular tetrameric form, and 6/4S AChE, combined peaks of 6S dimeric and 4S monomeric globular forms, began to increase after birth in the base and mid but not until P7 in the apex. An asymmetric collagen-tailed 16S AChE form was not detected in any turn until P7. Absolute levels and proportional distribution of AChE molecular forms differed depending on their localization. The levels of 10S and 16S AChE were highest in the base, lower in the mid and the lowest in the apex, while the level of 6/4S AChE was constant along the entire cochlea. Proportionally, the distribution of the molecular forms revealed that the base and mid contained predominantly 10S AChE while the percent of 6/4S AChE was highest in the apex. The percent of 16S AChE decreased from the base to apex.

In conclusion: Our results indicate that cholinergic nerve fibers are present throughout the cochlea at birth. The absolute levels and proportional distribution of AChE molecular forms (with the exception of 6/4S AChE) differ in each turn. The base of the cochlea contains most of the enzyme activity and the apex the least. Among the molecular forms studied only the distribution of 10S AChE activity along the cochlea parallelled that of ChAT. Variations in the content of AChE molecular forms in the different turns suggest functional differences in activity of cholinergic synapses along the cochlea. (Supported by NIH grant NS 15061)

288.4 BIOCHEMICAL AND MORPHOLOGICAL DIFFERENTIATION OF EFFERENT FIBERS IN THE MOUSE COCHLEA. H.M. Sobkowicz, M.R. Emmerling* and G.L. Scott*, Depts. of Neurology and Anatomy, Univ. of Wisconsin, Madison, WI 53706

During cochlear development, the activity of choline acetyltransferase (ChAT) remains stable during the first week and about doubles by the 15th postnatal day (P). Changes in the activity of acetylcholinesterase (AChE) and its molecular forms appear to occur in three phases. During the first phase between P1 and 5, there is little increase in total AChE and the two molecular forms 10S AChE and 6/4S AChE. The second phase (P7 to P14) shows a four-fold increase in the level of total AChE, 10S AChE and 6/4S AChE and the appearance of a 16S AChE form. In the third phase (P14 to P26) an increase solely in 10S AChE is seen. Surface preparations of organ of Corti were stained for cholinesterase using the method of Karnovsky and Roots. No staining could be detected in the light microscope in animals younger than P4. Intraganglionic and radial bundles were stained in the base and mid on P4 and the apex on P7. Fibers of inner spiral bundle in the base and mid stained between P4 and P6; they were followed by the tunnel fibers stained at P7. The structures did not stain in the apex until P10. Numerous stained nerve endings were found on outer hair cells (OHCs) in the first row and cholinesterase-positive nerve fibers were seen in the 1st outer spiral bundle in the base and mid on P7. No staining was observed in the apex until P10. All three rows of OHCs possessed numerous cholinesterase-positive nerve endings by P10 in the base and mid and by P12 in the apex. All three outer spiral bundles showed the presence of positive nerve fibers. Ultrastructurally, the vesiculated nerve endings within the inner spiral bundle are present on P1 and some form synapses with afferent fibers or inner hair cells during the following days. Electron microscope examination of OHC regions at P9 showed a few vesiculated nerve endings. By P11, endings with an efferent morphology became numerous and by P14, synaptic, albeit immature, contacts with OHCs are present. By P18, the size of efferent endings and numbers of their synaptic vesicles and mitochondrial content increased. Mature synapses showed post-synaptic cisternae in OHCs. In conclusion, our studies indicate that the expression of total AChE and its molecular forms in the developing organ of Corti is correlated with the growth and differentiation of efferent fibers. The greatest increase in the levels of ChAT and AChE occurs during the time of growth of efferent fibers innervating OHCs. Among the AChE forms studied, the initial increase of 10S and 6/4S coincides with the growth of efferent nerves, while the manifestation of 16S relates in time to the innervation of OHCs and the continued increase of 10S is concurrent with the maturation of efferent synapses to OHCs. (NIH Grant NS 15061)

288.5 THE EFFECT OF α-DIFLUOROMETHYLORNITHINE (DFMO) ON POSTNATAL DEVELOPMENT OF THE DORSAL COCHLEAR NUCLEUS (DCN) OF THE HAMSTER. L. Schweitzer, J.M. Bell and T.A. Slotkin. Depts. of Anatomy and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

The DCN is a highly organized, laminated nucleus in the auditory system in which the ramifications of depletion of specific cell types can be studied. The present experiments were done in an attempt to deplete granule cells in the DCN of the hamster by administering DFMO, a drug that inhibits the biosynthesis of polyamines, which play an essential role in proliferation and differentiation of brain cells. Although DFMO does not deplete postmitotic cells, we have shown that postnatal administration markedly reduces the number of granule cells in the cerebellar cortex, presumably by arresting cell division. DFMO also disrupts the migration of many of the granule cells that remain. Like cerebellar granule cells, the granule cells in the DCN undergo mitosis postnatally in an external granule cell layer (Taber Pierce, 1967). We confirmed observations (Ochs and Brunso-Bectold, 1982) that granule cells migrate down from this proliferative zone into the fusiform cell layer and deep layer. In order to deplete granule cells, hamsters were treated twice daily on postnatal days 4-14 with either saline or DFMO (200 or 500 mg/kg sc). Half the animals were sacrificed on day 15 and half on day 60, and sections through the cochlear nucleus were stained with cresylect-violet. DFMO resulted in a reduced number of granule cells in the superficial DCN at 15 days; by 60 days this effect was also apparent in the deep layer, suggesting that cells located superficially that would have migrated into the deep DCN had either failed to develop or did not arrive at their normal location. In the DFMO-treated animals, the fusiform cell layer was thinner than in controls even though the number of fusiform cells was unaffected. Many of the granule cells that are normally intermingled with fusiform cells were missing and therefore, rather than being staggered as in the normal DCN, the fusiform cells were lined up in a discrete row. While these results occurred at either dose of DFMO, the higher dose, which had a general growth-retardant effect (lowered body weight, increased brain/body weight ratio) reduced the size of the somas of the fusiform and giant cells. In contrast, at the lower dose (which specifically impeded brain development and not body growth), only the fusiform cells were affected: this may be secondary to the depletion of granule cells (which are a main input to the fusiform cells but not the giant cells) or alternatively, may reflect direct actions of DFMO on the fusiform cells. Future studies analyzing the morphogenesis of fusiform cells following DFMO administration should clarify the site of action of the drug.

(Supported by USPHS HD-09713 and NS-20162).

288.6 DIFFERENTIAL RESPONSE OF AUDITORY CORTICAL CELL TYPES TO NEONATAL DEAFENING. B.A. Goldberger*, N.T. McMullen and E.M. Glaser. Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Earlier (McMullen, et al., Soc. Neuro., 11, 1985) we reported that unilateral neonatal deafening in rabbits results in a significant increase in the size of the dendrite systems of lamina III/IV spine free nonpyramidal cells contralateral to the deafened ear. The greatest increase was in the direction of the tangential plane. Using the same 300-400 um thick sections, we have now digitized randomly chosen Golgi-Cox impregnated pyramidal cells from III/IV. The dendritic arbor of 50 pyramidal cells (30 deafened, 20 control) have been examined from 2 litters of rabbits subjected to unilateral ossicle removal and kanamycin injection into the oval window as neonates. Control pups received a sham operation. Pyramidal cells contralateral to the deafened ear were not significantly different from those of control in terms of cell area (248 vs. 269 um2), number of dendrites (5.1 vs. 5.2), number of dendritic branches (56.8 vs. 59.3) or total basal dendritic length (3783 vs. 4118 um). Spine counts along basal dendrites revealed a 38% reduction in spine density in deafened rabbits (Control = 6.7 spines/10 um; Deafened = 4.4 spines/10 um). The spine loss was not uniform but was maximal at 2nd order basal dendrites (56% reduction relative to control) and linearly decreased to an 18% reduction at 5th order basal dendrites. The significant loss of spines along the basal dendrites of pyramidal cells suggests a reduced afferent input from the medial geniculate. The greater spine loss on the lower order branches may be evidence for their role as preferential targets of medial geniculate afferents. We conclude that unilateral neonatal deafening results in significant reorganization of the contralateral auditory cortex affecting both pyramidal and nonpyramidal neurons and points to the importance of bilateral acoustic input for normal dendritic development. The significant alteration in nonpyramidal dendrite systems in contrast to the lack of such in pyramidal cell basal dendrites suggests extrinsic afferent control of nonpyramidal dendrite growth and intrinsic control of pyramidal cell basal dendritic trees. One explanation for the expanded dendritic system response of the nonpyramidal cells to deafening may lie in the bilaterality of the acoustic pathway. We hypothesize that a form of dendritic sprouting may occur in nonpyramidal cells in response to the partial deafferentation as a result of unilateral deafening. Dendritic growth may be maximal in the tangential plane since it is the direction which would maximize the possibility of increased thalamocortical synapse formation. (Supported by The Deafness Research Foundation and NIH Grants NS17861 and IR24RR02169)

288.7 TRANSIENT CHANGES IN RETROGRADE LABELING OF DIENCEPHALIC-PROJECTING NEURONS IN THE MONKEY DORSAL COLUMN NUCLEI FOLLOWING DORSAL COLUMN LESIONS. Karen J. Berkley and Charles J. Vierck, Jr. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306-1051 (KJB) and Dept. of Neuroscience and Center for Neurobiological Sciences, Univ. of Florida College of Medicine, Gainesville, FL 32610 (CJV) USA.

As in most cases of injury to the nervous system, lesions of the dorsal columns of the primate spinal cord produce a number of acute behavioral deficits, many of which recover over a relatively short time period (1-3 weeks), while others recover more slowly or incompletely (over a period of many months) and still others fail to recover. In an effort to examine potential anatomical correlates of this recovery pattern, the retrograde transport of horseradish peroxidase (HRP) was used to study diencephalic-projecting neurons in the dorsal column nuclei (DCN) of 7 monkeys (M. mulatta or M. nemestrina) at various intervals following unilateral transections of their dorsal columns at Th 3-Th 8. In each monkey, 4.4 ul of a 50% solution of HRP was injected bilaterally into large areas of the diencephalon 1 week to 10.5 months following the unilateral transection. Thirty six to 48 hrs later, the monkey was sacrificed and sections through DCN processed using tetramethyl benzidine following Mesulam's protocol (1982). In the first two weeks post-transection, few neurons in the deafferented gracile nucleus were labeled---in striking contrast to the dense labeling of neurons in the ipsilateral cuneate and contralateral gracile and cuneate nuclei. By 17 days post-transection, many more neurons in the deafferented region became labeled and, by 7 months, labeling in the shrunken deafferented region was comparable to that in the normal regions. Thus, transient behavioral deficits that result from injury to the dorsal columns appear to be accompanied by a similar transient deficit in some aspect of the uptake and retrograde transport of HRP by deafferented, diencephalic-projecting DCN neurons.

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288.8 MAINTAINED PROJECTIONS OF HETEROTOPIC TRANSPLANTS OF FETAL VISUAL AND SENSORIMOTOR CORTEX ARE CHARACTERISTIC OF NEW CORTICAL LOCALE. Dennis D.M. O'Leary and Brent B. Stanfield. The Salk Institue, La Jolla, CA 92037.

In neonatal rats pyramidal tract (PT) neurons are found in all areas of the neocortex, including the occipital (visual) cortex where none is found in adults. Rather than dying, occipital PT neurons lose their PT axon by P18. Moreover, many of these early occipital PT neurons maintain an axon to the superior colliculus (SC). We have previosuly found that fetal occipital neurons transplanted to rostral (sensorimotor) cortex maintain PT axons until at least P30. We have now extended this work.

Pieces of cortex from E17 rat fetuses (exposed to ^{3}H-TdR on E15) were transplanted to the cortex of newborns; rostral cortex was placed into the occipital region (R-O), or occipital cortex into the rostral region (O-R). In one group, on P34 Fast Blue (FB) was injected into the pyramidal decussation (PD) of both O-R and R-O cases, and Diamidino Yellow (DY) was injected into the SC of R-O cases; the rats were killed on P40. In the O-R cases many FB labeled neurons are found in the transplant; in the R-O cases only DY labeled neurons are seen in the transplant, although large numbers of FB labeled neurons are present in host rostral cortex. Thus the transplants extend and maintain subcortical projections characteristic of their new cortical locale. A second group was also injected on P34 and killed on P40: FB was injected into the PD of the O-R cases, but into the SC of the R-O cases, and in both paradigms DY was also injected into the opposite cortex. Many neurons singly labeled with FB or DY (none doubly labeled) are found within both types of transplants. Thus, as in normal rats, in the transplants the corticotectal and PT neurons do not project callosally suggesting that they behave normally and are the same cells that would extend a PT axon if left in situ. In a third group, FB was injected into the PD of R-O cases on P9 and the rats killed on P13. FB labeled neurons are found in the transplants indicating that some cells in the R-O transplants do initially extend but none maintain a PT axon. In all groups, some dye labeled neurons in the transplants are also labeled with ^{3}H-TdR. These findings suggest that the position of a neuron within the tangential plane of the neocortex is a critical factor in determining which of its initially extended axon collaterals will be eliminated or retained to give rise to its mature set of projections. Further they support the notion that within its tangential plane the early neocortex is a rather homogeneous structure; the areal differentiation characteristic of the projections of the mature cortex is not a feature intrinsic to the cells in these regions, but is imposed on them by factors which operate regionally.

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288.9 SUCCESSIVE WAVES OF DIFFERENTIATION OF CUTANEOUS SENSORY RECEPTORS WITHIN THE SPINAL DERMATOMES. Bryce L. Munger* and Terrell E. Jones* (SPON: W.B. Severs). Department of Anatomy, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033

The early differentiation of spinal nerves and associated cutaneous innervation has been studied in silver-impregnated serial paraffin sections of chicken and rat embryos. Chicken embryos were especially useful for tracing the early events of spinal nerve differentiation. Axons can first be identified as precursors of spinal nerves of the adult in Stage 16 chicken embryos (2 1/2 day old embryos). The axons tightly abut the myotome and some axons pierce the myotome along its inferior and posterior border. Terminal branches of these axons fan out into the dermis but do not contact the epidermis. Axons reach the epidermis in E4 chick embryos. The cutaneous branches of spinal nerves innervate the skin over the majority of the trunk and each has a large ventral and smaller dorsal branch. These cutaneous nerves are equivalent to the posterior, lateral or intermediate and anterior cutaneous nerves of mammals.

Spinal nerves can be identified in E13 rat embryos tightly abutting the myotomes fitting in a groove on the posterior and inferior border of the myotomes. As viewed in sagittal section, the successive myotomes tightly abut one another and the developing spinal nerve is sandwiched between adjacent myotomes. The skin over the entire trunk is innervated by terminal branches of posterior cutaneous nerves by E13.5. The lateral (intermediate) and anterior cutaneous nerves develop after the ribs can be recognized by E16 in the rat. The sensory dermatomes are thus established by E4 in the chick and E13.5 in the rat embryo. In both species the earliest differentiation of dorsal root ganglia can be recognized one half day earlier than the onset of cutaneous innervation, and differentiation of dorsal root ganglia continue for at least 3-4 additional embryonic days. The dermatotopic map once established at E13.5 in rat and E4 in chick embryos subsequently develops by sequential differentiation of additional dorsal root ganglia. We have previously described spatial and temporal gradients in the differentiation of cutaneous sensory receptors in the rat and opossum mystacial pads. On the basis of the present observations we conclude that similar gradients are present within the truncal dermatomes as well. Within the truncal skin of chickens and rats a distinct gradient is present from the middle of the back extending anteriorly along the course of each spinal dermatome. In the chicken this gradient matches exactly the sequence of feather formation in the dorsopelvic pteryla as described by Holmes (1935). Supported in part by USPHS Research Grant NS 19462.

288.10 **ONTOGENY OF CENTRAL PROJECTIONS OF LUMBAR PRIMARY SENSORY NEURONS IN CHICK.** F.A. Johnson*, B.M. Davis, E. Frank, Department of Neurobiology and Physiology, Northwestern University, Evanston IL, 60201.

The chick spinal cord, and in particular the lumbar lateral motor column (LMC), has been the focus of intense study by developmental biologists. Numerous studies have explored the birth and death of sensory neurons and the ontogeny of their peripheral connections. Despite this attention, little is known about the development of afferent connections in the spinal gray matter. In these studies HRP pathway tracing methods were used to examine the ontogeny of central projections of sensory fibers.

White Leghorn chick embryos were dissected and maintained in oxygenated chick Ringer at room temperature. HRP injections were made into lumbar dorsal root ganglia (DRG) and/or spinal nerves were cut and sucked into HRP-filled glass pipettes. Injections into stage 25 (E5) DRGs revealed small bipolar sensory neurons projecting centrally into the dorsal root and ventrally toward the developing peripheral nerve. By stage 28 (E6) injection of HRP into DRG or spinal nerve revealed a large number of sensory fibers in the dorsal root. These fibers formed a small dense aggregation at the periphery of the spinal cord representing the primodium of the dorsal funiculus (or oval bundle of His). The majority of these sensory fibers appeared to be coursing rostrally and caudally over the surface of the developing spinal cord. A few fibers, small and unbranched, penetrated the dorsal horn and occasionally extended into the LMC. By this stage numerous motoneurons were labeled and many of these had branching dendrites. At stage 33 (E8) the sensory fibers projecting into the developing dorsal funiculus had spread over the dorsal surface of the spinal cord and had begun to grow into the superficial layers of the dorsal horn. A few fibers extended into deeper layers. All the sensory fibers at this stage were small and unbranched and no difference could be detected between those fibers in the superficial layers of the dorsal horn and those in deeper layers. By stage 35(E9) all the components of the adult sensory fiber projection were present. Sensory fibers could be seen throughout the upper laminae of the dorsal horn and numerous fibers extended into the zone of the motoneuron dendrites in the LMC. Many of the sensory axons at this stage had branched processes. The sensory fiber projection into the LMC, which presumably represents muscle afferent fibers destined to innervate motoneurons, appeared diffuse with ventrally projecting fibers traversing all regions of the dorsal horn. By stage 39(E13) the projection to the LMC was formed by a dense plexus of sensory fibers originating in the superficial layers of the dorsal horn and coursing ventrally through the center of the dorsal horn, bisectings it. Our observations suggest that cutaneous and muscle fiber projections (those terminating in the dorsal and ventral horn, respectively) develop simultaneously as has been observed in frogs and mammals. Supported by training grant NS07223 and NS20859 to E.F.

288.11 THE S-I HINDPAW REPRESENTATION IN THE RAT AFTER SCIATIC NERVE SECTION AT DIFFERENT POSTNATAL AGES. L.E. Baker and J.T. Wall. Vanderbilt University, Nashville, TN 37240.

The rat hindpaw is innervated by the sciatic and saphenous nerves. Cutaneous mechanoreceptors from each nerve contribute in an orderly way to a topographically organized representation of the hindpaw in the S-I cortex. When the hindpaw of an adult rat is partially denervated by sciatic nerve section and ligation, part of the cortical zone usually representing sciatic inputs becomes responsive to saphenous inputs. As a result, the cortical area representing saphenous hindpaw inputs expands in size. In contrast, in adult rats that were reared after sciatic transection and ligation on postnatal day 1, the cortical representation of saphenous hindpaw inputs does not appear expanded in size. The goal of the present study was to define the age period during which sciatic injury causes an adult versus neonatal pattern of cortical representation.

The hindpaws of 6 and 16 day old rats were partially denervated by transecting and ligating the sciatic nerve and the S-I hindpaw representation was subsequently mapped when the animals reached adulthood. Receptive fields to light tactile stimuli were defined from multiunit responses at 60-110 locations across the hindpaw and adjacent cortical region in ketamine anesthetized animals. Cortical areas representing hindpaw inputs were measured with a planimeter and compared to similar data from adult and day 1 denervates.

There were three main results. (1) The cortical areas representing saphenous inputs from the hindpaws of day 16 denervates ($\bar{x}$ = .62 mm^2, N=5) and day 6 denervates ($\bar{x}$ = .64 mm^2, N=5) were not different (t[8] = 0.13). (2) The cortical areas of day 16 and day 6 denervates were also not different (respectively, t[10] = 1.50 and 1.31) from the areas of adult denervates ($\bar{x}$ = .47 mm^2, N=7). (3) In contrast, the areas of both day 16 and day 6 denervates were significantly different (respectively, t[14] = 8.40 and 4.88, $p < .05$) from areas of day 1 denervates ($\bar{x}$ = .20 mm^2, N=11).

We suggest that removal of sciatic inputs in these experiments allows an evaluation of the maximal cortical projection area of saphenous hindpaw inputs. The similarity in cortical areas for injury on or after day 6, and the differences in areas following injury on days 1 and 6, suggest (1) that developmental events between days 1-6 are important for the normal formation of a maximal saphenous projection area, and (2) that sciatic inputs somehow contribute to these events. (Supported by grants NS21105 and BNS8205745.)

288.12 PROGRESSIVE CHANGE IN SI CORTEX OF KITTENS AFTER SPINAL TRANSECTION. P.A. McKinley and L.Swyter*. Dept. Kinesiology, UCLA, Los Angeles, CA 90024

In a previous communication (Soc. Neurosci. Abst. 11:452, 1985) we reported that reorganization of SI cortex in chronic spinal cats after spinal cord transection at T_{12} at 6 weeks of age was characterized by occupation of the deafferented hindlimb region by trunk afferent receptive fields (RFs). This representation differed from that found in normal cats in two ways: 1) trunk RF representation was extended to the medial border but was narrowed both rostrally and caudally; 2) the trunk map was oriented mediolaterally rather than rostrocaudally with respect to position of dorsal and ventral RFs. We were interested in the process of these changes and therefore mapped the cortex at various times post-cordotomy (PC).

RF characteristics of SI cortex were mapped electrophysiologically from multiunit responses to light tactile stimulation in ketamine anesthetized kittens (n=3/time period) at either 3, 6, 9, 12 and 16 weeks PC. As a control, normal kittens were mapped at 6 weeks of age, and immediately after cord transection. No trunk RFs were found in the 6 week old kitten either before or just PC. By 3 weeks PC, the deafferented hindlimb cortex was fully occupied by trunk RFs along both the rostrocaudal and mediolateral axes. RF size varied from small to large. Typically, RFs representing the axilla were small, while those representing the trunk saddle were large. Many RFs were represented more than once, and there was no topological organization. This pattern was unchanged up to 6 weeks PC, although there was a slight narrowing of the reoccupied area along the rostrocaudal margins. By 9 weeks PC, however, there was a dramatic reduction in representation within the reoccupied cortex. Only a central core of trunk RFs was found in the deafferented hindlimb area that extended to the medial SI border. This core was organized topologically in a pattern similar to that reported initially in the chronic spinal cat, and was surrounded by regions of no cutaneous response both rostrally and caudally.

These results indicate that in cats cordotomized at 6 weeks of age, 1) SI cortical reorganization occurs in 2 stages: initially, response to deafferentation is characterized by dramatic capture of the entire vacated region by trunk RFs that occurs within 3 weeks; this expansion is not stable, however, and is followed by an equally dramatic reduction in trunk representation at 9 weeks PC, that is coupled with an increase in topological organization of the remaining representation. 2) There may be a limit to the amount of cortex that a particular region of the periphery can control. Supported by NS 19864

288.13 ALTERATIONS IN THE SYNAPTIC ARCHITECTURE OF THE SOMATOSENSORY CORTEX OF THE RAT FOLLOWING NEONATAL PERIPHERAL NERVE DAMAGE K. Jensen, J. O'Callaghan and E. Gregory. Neurotoxicology Division, HERL, U. S. Environmental Protection Agency, and Northrop Services, Inc., Research Triangle Park, NC 27711

Previous investigations have demonstrated that thalamocortical projections to the rat somatosensory cortex are discretely organized in a pattern that mimics the pattern of whiskers on the face. These orgnanization of these projections is altered following neonatal damage to the whisker innervation. In the present study, we have investigated the immunohistochemical labeling pattern of an antibody to a synaptic vesicle protein, P-38, in the neocortex following neonatal peripheral nerve damage. P-38 has been identified as an intrinsic protein of the membrane of small synaptic vesicles (Jahn et al., 1985, PNAS 82:4137-4141).

The infraorbital nerve was sectioned in Longs-Evans rat pups on the day of birth. The animals were sacrificed at various times between 5-90 days of age. The animals were sacrificed by perfusion with 4% paraformaldehyde under barbiturate anaesthesia. The brains were removed, postfixed for 1-4 hours, and sectioned on either a vibratome or cyrostat. Sections were incubated in either a serum or monoclonal antibody to P-38 and processed for indirect flourescein and PAP label. In addition animals, the somatosensory neocortex was removed and processed for radioimmunoassay of P-38.

At high magnification, the label appeared as small punctate clusters surrounding cell bodies and dendrites. In the cortex ipsilateral to the nerve cut patches of intense labeling appeared to correspond to the clustering of thalamocortical projections. In the cortex contralateral to the nerve cut, a corresponding pattern of patches were not apparent. These observations suggest that neonatal infraorbital nerve cut alters the overall synaptic architecture of the somatosensory neocortex. One possible interpretation of the present observations is that the thalamocortical projections provide a relatively large proportion of the synapses to the the somatosensory cortex. Alternatively, the altered pattern of synaptic labeling may represent numerous sources of synaptic input, the organization of which is altered as a consequence of changes in the thalamocortical projections.

288.14 THE PATTERN OF TYROSINE HYDROXYLASE-POSITIVE FIBERS PROJECTING TO THE SOMATOSENSORY CORTEX OF THE RAT: ALTERATIONS FOLLOWING NEONATAL INFRAORBITAL NERVE CUT. E. Gregory and K. Jensen. Northrop Services, Inc. and Neurotoxicology Division, HERL, U. S. Environmental Protection Agency, Research Triangle Park, NC 27711

The somatosensory neocortex of the rat is characterized by a clustered projection of thalamocortical afferents to layer IV. The normal clustering of these afferents is dependent upon the intact innervation of the vibrissae during early postnatal development. Catecholamine axons have terminal fields associated with the vibrissae-related in layer IV. In the present investigation we used an antibody against tyrosine hydroxylase to demonstrate the morphology and distribution of these axons animals in which the innervation of the vibrissae was disrupted at birth.

The infraorbital nerve was sectioned in Long-Evans rats on the day of birth. At survival times ranging from 5-30 days following nerve cut the animals were perfused with 4% paraformaldehyde under barbiturate anaesthesia. The brains were removed, postfixed, and sectioned on either a vibratome or cryostat. The sections were incubated with a serum antibody against tyrosine hyrdoxylase (Eugene Tech) and processed for indirect peroxidase or PAP labeling.

In the cortex ipsilateral to the nerve cut there were several strata containing terminal plexuses. The majority of fibers in the upper stratum branched in layer II and ascended to layer I where they bifurcated and ran parallel to the pial surface. Very fine fibers with intricate branches were observed in an intermediate stratum at the level of layer IV. The fibers of the deepest stratum tended to be oriented obliquely to the plane of the layer VI. In the cortex contralateral to the nerve cut, alterations were apparent in the pattern and density of the fine intricate plexus of layer IV. Since the catecholamine fibers are present in the neocortex before the thalamocortical projections, our observations suggest that the terminal plexuses of tyrosine hyrodoxlyase positive fibers may undergo reogranization secondary to the altered development of thalamocortical projections.

288.15 THE SENSORY INNERVATION OF THE OVARY: A HORSERADISH PEROXIDASE STUDY IN THE GUINEA PIG. C.M. Klein* and H.W. Burden* (Spon: R. Ray). Dept. of Anatomy, East Carolina Univ. Sch. of Med., Greenville, NC 27834

Numerous studies have indicated that ovarian autonomic nerves may be involved in the regulation of ovarian activity. Based upon experimental evidence for vagal involvement in ovarian function, Burden et al. (Anat. Rec., 207:623-627, 1983) identified perikarya of sensory nerves innervating the rat ovary using horseradish peroxidase tracing techniques. In the current study, ovarian afferent perikarya in the guinea pig were identified in dorsal root and nodose ganglia. Adult Dunkin-Hartley guinea pigs were anesthetized with ketamine hydrochloride and xylazine, and a polyethylene enclosure was placed around each ovary to isolate it from adjacent viscera. Horseradish peroxidase (HRP) was dissolved in isotonic saline containing 10% dimethylsulfoxide. Twenty-five microliters of a 25% HRP solution was injected into the enclosure onto the surface of the ovary. After a survival period of 4 days, guinea pigs were reanesthetized and perfused transcardially. Nodose and dorsal root ganglia (DRG) were removed bilaterally, serially sectioned at 15 um, and collected directly onto gelatin-treated glass slides. Sections were incubated to demonstrate HRP using a modification of the tetramethylbenzidine method of Mesulam (J. Histochem. Cytochem. 26:106-117, 1978). Sections were examined using dark field microscopy. Labeled cells were identified in the ipsilateral DRG from the T10 to L4 segmental level. Labeled cells were scattered throughout the DRG at all levels. HRP-containing nerve cell bodies in DRG were distributed as follows: T10:2±0.9 cells (N=5 DRG), T11:2±0.8 cells (N=5 DRG), T12:10±4.1 cells (N=9 DRG), T13:36±11.6 cells (N=8 DRG), L1:26±8.5 cells (N=8 DRG), L2:19±5.6 cells (N=9 DRG), L3:22±9.0 cells (N=9 DRG), L4:2±2.2 cells (N=5 DRG). Both the right and left nodose ganglia contained labeled cells in approximately equal numbers (3±0.5 cells per ganglion, N=7 ganglia). The high standard errors at some segmental levels probably reflect the inability to control the degree of access of HRP to ovarian nerve terminals. The identification of specific ovarian afferent neurons in the guinea pig provides an anatomical basis for the participation of viscero-visceral reflexes in control of ovarian function.

288.16 COMPARISON OF INHIBITORY AND EXCITATORY FUNCTION IN THE DEVELOPING LATERAL SUPERIOR OLIVE. D.H. Sanes* and E.W Rubel. Depts. of Otolaryngol. and Physiol., Univ. of Virginia Med. Ctr., Charlottesville, Virginia 22908.

Although the development of excitatory responses has been the focus of much research, comparatively less attention has been paid to inhibitory connections. The lateral superior olivary nucleus (LSO), an auditory nucleus in the ventral brainstem of the Mongolian Gerbil, is a favorable system in which to compare the maturation of an inhibitory and an excitatory input. Neurons in LSO are excited by stimuli delivered to the ipsilateral ear and are inhibited by stimuli to the contralateral ear.

We first quantified the frequency tuning of single units for postnatal animals of 13-14 days, 15-16 days, and 12-16 weeks. Both the 13-14 and 15-16 day groups exhibited broader frequency tuning than the adults ($p<.01$), although there were several units with adult-like selectivity. The frequency tuning of the inhibitory process was nearly identical to that of the excitatory process within each age group. In 11 cases, units of similar characteristic frequency, isolated within the same animal, had markedly different frequency selectivity. We next compared the threshold sound levels required to elicit an inhibitory or excitatory response. Neurons in 13-14 and 15-16 day animals had nearly equivalent thresholds for excitation and inhibition. However, in adult animals, excitatory thresholds tended to be lower, especially below 7 kHz.

The interaction of the two inputs requires that they be well matched in the frequency domain. Therefore, we compared the excitatory characteristic frequency (CF) to the inhibitory CF for all age groups. Whereas adults exhibited a very high correlation ($r=0.99$) between CFs, there was statistically greater variability about the regression line in both the 13-14 and 15-16 day groups. Finally, we characterized the binaural property for which LSO neurons code, interaural intensity difference (IID). The slopes of the IID functions, indicative of resolution (i.e. Δ spike rate/Δ IID), was similar for all age groups, despite a much enhanced dynamic range in adult animals.

We conclude that the functional maturation of the inhibitory and excitatory systems are nearly identical. One possible exception is that the efficacy of the inhibitory input is greater in young than in adult animals. (Supported by NIH Grants NS15478 and NS07462).

288.17 DEVELOPMENT OF MORPHOLOGICAL CHARACTERISTICS OF THE SUPERIOR OLIVARY COMPLEX IN THE FERRET. M. E. Wells*, C. K. Henkel, and J. K. Brunso-Bechtold. (Spon. J. Ryu). Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

The anatomical development of the nuclei involved in mammalian sound localization is not well-understood. In an attempt to investigate this development, we have studied cell distribution and morphology in the medial and lateral superior olivary nuclei (MSO and LSO) at postnatal days 0, 2, 4, 8, 16, and 32 in the ferret, and compared our observations at these ages with similar observations in the adult ferret. Hearing in the ferret begins at about one month postnatal, allowing us to study auditory development for an extended period before the onset of function. Two animals at each age were perfused with Bouin's fixative, embedded in paraffin, sectioned at 10μ, and stained with cresyl violet.

The adult ferret possesses well-defined superior olivary nuclei similar to the cat, although the LSO in the ferret is more convoluted in appearance. As in the cat, the MSO and LSO cells are bipolar with evenly distributed Nissl substance in the cytoplasm. In addition, the cells are oriented perpendicular to the long axes of the nuclei and are loosely packed with numerous, interspersed glial cells. At birth, the MSO and LSO are relatively segregated, although they are clearly less organized than in the adult. The cells at this time, however, are quite immature in appearance; there is scant cytoplasm, the bulk of which forms a "tail" or cap on one side of the nucleus. The most immature cells are loosely packed, but glial cells could not be distinguished between the neurons. During maturation the cells become more densely packed and more clearly oriented. By the second postnatal week, the cells are becoming more mature in appearance with more cytoplasm and evenly distributed Nissl substance. Furthermore, by this time, glial cells can be distinguished among the neurons, and shortly thereafter the cells again appear to be more loosely packed. During the ages we have studied, there appears to be a gradient of maturation from ventral to dorsal in the MSO and from medial to lateral in the LSO. This gradient is consistent with the high frequency to low frequency gradient of anatomical maturation described in other species.

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288.18 SENSITIVE AND CRITICAL PERIODS FOR THE VISUAL CONTROL OF SOUND LOCALIZATION. P.F. Knudsen and E.I. Knudsen, Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305.

In previous studies we found that barn owls use vision to guide the development of sound localization. Here we demonstrate that the ability to align auditory with visual localization is age dependent.

We placed 40 diopter, right deviating Fresnel prisms on 8 barn owls at 10, 21, 35, 42, 49, 63, 84 and 112 days of age (1 diopter = .57°). The owls were trained to orient their heads towards auditory (noise burst) and visual (LED) stimuli, which were presented at random locations in a darkened, sound attenuating chamber. The owls' orientations were measured by a search coil attached to the owls' head. The mean response to the auditory stimulus was compared to the mean response to the visual stimulus, and the sound localization error was defined as the difference between the two means.

We found that the younger the owl was when the prisms were attached, the further sound localization shifted to the right to align with visual localization. The owl that had prisms attached at 10 days of age shifted its sound localization by the full 23°, so that, with the prisms on, it was localizing sound and light stimuli in the same place. In contrast, the owl that had prisms attached at 49 days of age shifted its sound localization by only 12°. Visual localization was tested by having the owls strike at stationary visual targets. The owls consistently struck to the right of the target indicating that visual localization was essentially unaffected by prism rearing. The results demonstrate that there is a sensitive period during which sound localization can be maximally modified by abnormal visual experience.

When the prisms were removed from a young owl that had shifted its sound localization, the owl initially exhibited a sound localization error which gradually disappeared over several weeks. We next looked at the effect of age on the ability to adjust these visually induced auditory errors. The prisms were removed at various ages from 9, prism-reared owls and sound localization errors were followed for several months. The results showed that owls whose prisms were removed before 160 days of age corrected their auditory errors within several weeks. However, when the prisms were removed after 190 days of age, the owls maintained the visually induced sound localization errors for as long as they were tested (3 months). We believe this age (190 days) to be near the end of a critical period during which auditory cues become permanently associated with locations in space.

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288.19 DUCKING AND ROBBING: AN ETHOLOGICAL TEST OF SENSORIMOTOR FUNCTION WITH RESPECT TO ACTIVITY IN CORTICAL AND DOPAMINERGIC SYSTEMS. I.Q. Whishaw and J. Tomie*. Dept. Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4

Rats in social groups will attempt to obtain food by robbing each other. The victim protects the food by ducking. There are two types of ducks: Type I, involves a body turn and rear leg step that carries the rat in a 160° arc contralateral to the robber and in which food in held in the front paws; Type II, a body turn and rear leg step that carries the rat in a similar but longer arc but in which food is transferred to the mouth and then back to the paws. Ducks are triggered by contralateral tactile stimulation, particularly to the ventral area of the snout. Vibrissae removal disrupts ducking.

Hemidecortication initially abolishes ducks to contralateral robs and the robber obtains the food. Recovery occurs over sixty days by which time ducking is again normal. There is no ipsilateral impairment.

Unilateral dopamine depletion by nigrastriatal bundle 6-hydroxydopamine produces different effects. Impairments emerge over the first 10 postoperative days and there is little subsequent recovery. The rats show an apparent contralateral neglect because they are frequently robbed from that side, but when ducks occur they are normal. The rats duck to ipsilateral approaches but the ducks are usually abnormal and the direction of the duck is usually reversed, ipsiversive to the approach rather than contraversive.

Ducking is mediated by different neural systems than those thought to mediate "classical" orienting. Neocortical lesions and dopamine lesion effects can be dissociated with respect to their effects on the behavior. Both sensory and motor system changes can differentially influence ducking. The demonstration provides a new methodology with a new philosophy for studying the neural basis of sensorimotor behavior. Other features of ducking and robbing are described with respect to stimulation, movements, and the contributions of other neural centers.

288.20 USE OF AN ULTRASONIC ECHOLOCATING PROSTHESIS BY VISUALLY DEPRIVED CATS. C. Veraart*, M.-C. Wanet-Defalque* and J. Crémieux* (SPON : M. Meulders). Lab. of Neurophysiology, Univ. of Louvain, UCL-5449, B-1200 Brussels, Belgium.

An ultrasonic echolocating prosthesis has been designed in order to help blind people during locomotion; obstacles could be located, in distance and in direction, thanks to auditorily coded signals. A modified version of this prosthesis was developed in order to test its use in visually deprived cats (Veraart and Wanet, in: "Electronic Spatial Sensing for the Blind", D.H. Warren and E.R. Strelow, Eds, M. Nijhoff Publ. Dordrecht, 1985, pp. 217-238). This device consisted in a small ultrasonic echolocating unit provided with a pair of earphones and connected by wires to remote electronics powered with batteries; the distance of obstacles was coded proportionnally to the pitch of the sounds. Four animals were bilaterally enucleated under deep anaesthesia during the first week after birth; when adult, cats were trained to wear and use the prosthesis in several tasks.

In a first task, three of these cats were conditioned to cross an obstacle field (a corridor provided with up to four barriers presented in various configurations). After several month of training, no significant differences could be recorded between performances obtained with and without the prosthesis. In a second task, animals were located on a platform, 1 m high from the ground, and were required to jump on one of two stools whose height differed. Two animals were trained to choose the highest stool and a third one, the lowest one. Side and height (from 0 to 0.7 m) of the stools were changed at random. All three animals performed at a high level (above 75% of success) with the prosthesis while without it, their performance stayed at chance level. In a last task, again performed in an obstacle field, one animal (the one trained, in the second task, to choose the lowest stool) was rewarded only when he avoided the obstacles. In these conditions, with up to two barriers, this animal performed significantly better with the prosthesis than without.

In conclusion, in jumping behaviour as well as in locomotion, visually deprived cats proved to be able to integrate artificial sensory signals, coding spatial information, in their motor activities.

289.1 CONNECTIONS BETWEEN THE HYPOTHALAMUS AND MEDULLARY CATECHOLAMINERGIC NEURONS: NEW DATA IN CENTRAL AUTONOMIC CONTROL. J.J. Viola*, K. Fuxe, M. Goldstein and M. Kalia (SPON: J.L. Osterholm). Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107

The distribution of catecholaminergic (CA) neurons in the medulla oblongata that project to the hypothalamus was examined in the rat by the immunoperoxidase method for immunocytochemistry combined with retrograde labeling using horseradish peroxidase (HRP) histochemistry. The use of conjugates of HRP with wheat germ agglutinin (WGAHRP) and cholera toxin (CTHRP) combined with tetramethyl benzidine (TMB) histochemistry optimized visualization of peroxidase reaction product in the retrogradely labeled projection neurons. The granular, blue TMB reaction-product could be easily distinguished from the smooth brown labeling of CA-positive immunoreactivity. In 21 male, pathogen-free, Sprague Dawley rats (250-350 gms body weight), anesthetized with chloral hydrate (350 mg/kg, ip), the left hypothalamus was injected with 0.2-0.4 ul of a mixture of WGAHRP (8.3%), CTHRP (4.8%) and FHRP (25%, Type VI Sigma). Following a 48-72 hr. post-injection survival time the animals were perfused and the brain stem was processed for HRP histochemistry and tyrosine hydroxylase (TH) immunocytochemistry. We examined 50 u thick, serial frozen sections of the medulla oblongata from levels 2mm caudal to 3mm rostral to the obex. Sections were initially processed for HRP histochemistry. In selected alternate sections, the TMB reaction product was stabilized with cobalt acetate (Rye et al, '84) followed by incubation with TH antiserum (1:1000) for 24 hours. Analysis of the double-labeled sections revealed the existence of 3 populations of CA bulbo-hypothalamic neurons located in the A1, A2, and C1 cell groups. Some neurons bilaterally in the nor-adrenergic A1 cell groups projected to the hypothalamus. The ipsilateral, nor-adrenergic A2 cell group also demonstrated bulbo-hypothalamic projections located in the following specific sub-nuclei of the nucleus of the tractus solitarius (nTS): dorsal strip (ds), dorsal parasolitary region (dPSR), medial nTS (mnTS), intermediate nucleus (nI) and ventral nTS (vnTS). The rostral, adrenergic C1 cell group contained scattered hypothalamic projection neurons. No non-CA hypothalamic projections were found in these regions. These results demonstrate that prominent hypothalamic projections arise from distinct regions of medullary CA cell groups and lead to the conclusion that autonomic reflexes involving the medulla and hypothalamus are mediated primarily by CA mechanisms. This neuronal system provides the anatomical substrate by which medullary CA neurons could independently influence hypothalamic control of cardiovascular, gastrointestinal and respiratory function. (Supported by USPHS Grants HL 30991, 31997 and HL 33632 to M.K.).

289.2 NEUROTRANSMITTERS IN THE HYPOTHALAMO-SPINAL PROJECTION. D.F.Cechetto and C.B.Saper. Depts. of Pharm. & Physiol. Sci. and Neurol., Univ. of Chicago, Chicago, IL 60637.

Neurons in the paraventricular nucleus of the hypothalamus (PVH) and the adjacent lateral hypothalamic area (LHA) project to the sympathetic preganglionic neurons in the spinal cord. Previous studies have shown that some PVH-spinal neurons contain oxytocin, vasopressin, dopamine, somatostatin or enkephalins, but only α-melanocyte stimulating hormone (α-MSH) has been demonstrated in LHA-spinal neurons. We have used immunohistochemical staining for 13 neurotransmitter substances in combination with fluorescent retrograde tracing to survey the putative neurotransmitters involved in hypothalamic projections to the spinal cord.

Fast blue (200 nl at 3 sites) was injected into the thoracic spinal cord in 15 rats. After 5 days the animals were reanesthetized and treated with colchicine (195 μg intraventricularly) and 30 hours later the brains were processed for immunohistochemistry. The antisera used and the results indicating the distribution of neurons double-labeled for fluorescent dye and specific antibodies are shown in the table:

ANTISERA	SPINAL PROJECTIONS PVH	LHA
atrial natriuretic peptide	-	+
α-melanocyte stimulating hormone...	+	++++
dynorphin (DYN).....	+	++
enkephalin.....	+	+
corticotrophin releasing factor....	-	+
arginine vasopressin (AVP).....	++	-
somatostatin.....	+	-
tyrosine hydroxylase.....	+	-
neurotensin.....	+	+
oxytocin (OXY).....	+++	+
cholecystokinin.....	+	-
substance P.....	-	+
galanin.....	+	+

Our results suggest that the major neurotransmitter in the LHA-spinal projection is an α-MSH-like peptide, and that DYN makes a smaller contribution. No predominant transmitter has yet been found in the PVH-spinal projection, although about 25% of the cells stain for either OXY and AVP.

Supported by NINCDS 22835, Amer. Heart Assoc. 850894, McKnight Fdn. and Cdn. Heart Fdn.

289.3 FETAL ADRENAL SUBSTANCE P - LOCALIZATION AND REGULATION OF MEDULLARY CATECHOLAMINE BIOSYNTHESIS AND RELEASE. C.Y. Cheung and M.A. Holzwarth. Dept. of Reprod. Med., Univ. of Calif. at San Diego, La Jolla, CA 92093; Dept. Anat. Sci. Univ. Illinois, Urbana, IL 61801.

In the fetus, the adrenal medulla (AM) plays an active role in the maintenance of cardiovascular homeostasis under normal and stress conditions. In the adult, the function of the AM is primarily regulated by the sympathetic nervous system. However in the fetus, endocrine factors may play a more important role in this regulation than in the adult. We have previously shown that in the ovine fetus, catecholamine(CA) release from the AM is more sensitive to hormone regulation than in the adult. Furthermore, the fetal adrenal cortex can exert modulatory effects on AM function through the release of peptides. With the identification of substance P in the adult adrenal gland, and its role in modulating ACh-induced CA release in the AM, it is possible that substance P plays a similar role in the fetus. Our present study was designed to investigate the presence of substance P in fetal adrenal gland and to determine its effect on CA release and biosynthesis in the AM. Adrenal glands from ovine fetuses at 130 days gestation were used for either immunocytochemical localization of substance P, or for the determination of substance P effects on CA release and tyrosine hydrosylase (TH) activity. For the immunocytochemical study, substance P immunoreactivity was visualized using the PAP technique. An extensive network of substance P immunoreactive fibers was found in the AM. Fibers were also found within the splanchnic nerve and in the capsule. For the release study, the fetal AM was dispersed into single cells and incubated with substance P in Krebs Henseleit buffer containing 0.5% BSA and 10-6M bacitracin. CA release into the medium was measured at 5 and 10 min, 1 and 3 hours using HPLC methods. Substance P at 10-6M caused a transient 37% increase in total CA (sum of dopamine, norepinephrine and epinephrine concentrations) release at 5 min, followed by significant suppressions of release at 10 min, 1 and 3 hours by 43%, 37% and 15%, respectively. This effect was not modified by 200 μM ACh. For the study of TH activity, the enzyme was prepared from fetal AM homogenate and TH activity assayed using the method of Waymire et al (1971). Substance P at 10-6M significantly inhibited TH activity by 23%, and the effect was augmented by 200 μM ACh ($p<0.05$). Substance P at 10-5 and 10-7M were less effective than 10-6M in suppressing CA release and TH activity. In summary, our study demonstrates that in the ovine fetal adrenal, substance P immunoreactive fibers are present, and that substance P exerts an inhibitory effect on medullary CA release and TH activity.

289.4 CONNECTIONS OF THE A-1 NORADRENERGIC CELL GROUP: A WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE AND IMMUNOHISTOCHEMICAL STUDY. J.J. Woulfe*, A.W. Hrycyshyn* and B.A. Flumerfelt (SPON: J.A. Kiernan). Department of Anatomy, Health Sciences Centre, University of Western Ontario, London, Canada.

Anatomical and physiological evidence has implicated the A-1 noradrenergic cell group of the caudal ventrolateral medulla in various aspects of autonomic function. This study was undertaken to investigate the central projections of the A-1 cell group to establish the neural connections which underlie its role in autonomic regulation. The A-1 cell group was delimited immunohistochemically through the use of antisera to tyrosine hydroxylase (TH), dopamine-B-hydroxylase (DBH) and phenylethanolamine-N-methyl transferase (PNMT). Wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was then selectively placed within the region of A-1 using both dorsal and ventral approaches. WGA-HRP activity was demonstrated using the chromogen tetramethylbenzidine and the reaction product was visualized under polarized light. Adjacent sections were stained immunohistochemically for DBH and PNMT. The distribution of reaction product in A-1 efferents as seen with both techniques was then mapped throughout the neuraxis.

Numerous sites demonstrated terminal labelling for WGA-HRP, and were DBH +ve and PNMT -ve, indicating the presence of noradrenaline. Areas of greatest activity included: 1) the bed nucleus of the stria terminalis, particularly the regions directly ventral and medial to the rostralmost anterior commissure, 2) the paraventricular nucleus of the hypothalamus in which terminal immunoreactivity was present in both the magnocellular and parvocellular divisions, and 3) the pontine tegmentum including the locus coeruleus in which WGA-HRP and DBH immunoreactivity were identified in both the principal and sub-coerulear areas. DBH and WGA-HRP labelling were sparsely distributed in the supraoptic nucleus of the hypothalamus.

Retrogradely labelled cells were consistently observed in both the magnocellular and parvocellular divisions of the paraventricular nucleus, indicating a reciprocal projection between them and A-1. Cell body labelling was also observed in locus coeruleus and, to a lesser degree, in the retrochiasmatic area of the hypothalamus and the nucleus of the solitary tract. These data provide evidence for the existence of ascending A-1 noradrenergic projections to diencephalic and pontine regions, many of which are associated with autonomic function.
(Supported by the Medical Research Council of Canada)

289.5 CYTOARCHITECTURE OF THE PARABRACHIAL NUCLEAR COMPLEX IN THE HUMAN PONS. C.H. Block, M.L. Estes, K.L. Barnes and C.M. Ferrario. Departments of Brain and Vascular Research and Pathology, Cleveland Clinic Foundation, Cleveland, OH 44106.

The parabrachial nuclear complex in the rat, rabbit, cat, and monkey has been implicated in autonomic integration. Lying in the dorsolateral pontine tegmentum, surrounding the superior cerebellar peduncle (SCP), this complex is interconnected with autonomic centers in the nucleus tractus solitarius, area postrema, hypothalamus, thalamus, amygdala, and cortex. In addition, this region is highly organized functionally, cyto- and chemoarchitecturally, in the rat. However, a similar organization of this complex in human has not been described. These studies were designed to examine the cytoarchitectural features of this region in the infant and adult human brain.

Postmortem human brains were obtained from infant and adult. Paraffin embedded sections were cut at 10 μm, in coronal or sagittal planes, and stained for Nissl substance and fiber tracts using Luxol Fast Blue, cresyl violet, and neutral red. Examination of the infant rostral pons, from the level of the trochlear nerve decussation through to the level of the abducens nucleus, revealed distinct medial and lateral subdivisions, similar to the locations described in other mammals. Medium sized cells (20-25 μm) were observed in the medial subdivision and the ventral aspect of the lateral subdivision. Many small (10-15 μm) and medium sized cells were found within the SCP. At the level of abducens nerve, round, medium sized cells were seen in the lateral subdivision, adjacent to the SCP. Groups of cells were also found within the SCP and these appeared to be arranged in cellular bridges between the medial and lateral subdivisions. At the level where the SCP exits the cerebellum, there is a reduction in the size of the lateral subdivision, but a group of medium sized cells remain within the SCP. In the adult, there is less gray matter associated with the very large SCP and only at the more rostral levels are there distinct medial and lateral subdivisions. At the decussation of the SCP, the cells are primarily found in the ventral aspect of the lateral subdivision. Medium sized cells are found adjacent to and within the SCP. In general, these cells are round, with centrally located nuclei and nucleoli. Scattered pigmented cells of the locus coeruleus (30-35 μm) are found within the SCP. These data suggest that the parabrachial nucleus in the human infant may be cytoarchitecturally suborganized, however this organization is obscured with increasing myelination in adulthood. (Supported by NIH Physician Scientist Award to M.E. HL-01454).

289.6 DISTRIBUTION OF FOREBRAIN AFFERENTS TO THE PARABRACHIAL NUCLEUS IN THE RAT. M.M. Moga, K.M. Hurley-Gius, D.F. Cechetto, T.S. Gray and C.B. Saper. Dept. of Anatomy, Loyola Univ., Maywood, IL 60153 and Depts. of Pharm. & Physiol. Sci. and Neurol., Univ. of Chicago, Chicago, IL 60637.

The parabrachial nucleus (PB) receives heavy input from subregions of the hypothalamus, amygdala and cortex involved in central autonomic regulation. A previous study (Fulwiler and Saper, '84) demonstrated that PB efferent projections arise from characteristic groups of subnuclei. In this study, we examine whether PB forebrain afferents also display a subnuclear topography.

Injections of 1% WGA-HRP were made into previously identified forebrain sources of PB input: infralimbic and insular cortex, lateral and paraventricular hypothalamus, bed nucleus of the stria terminalis (BST), central nucleus of the amygdala (CNA), substantia innominata and zona incerta. The pattern of anterograde label within PB varied with the injection site. In general, forebrain areas tend to innervate the same PB subnuclei from which they receive their afferents. Hypothalamic afferents innervate predominantly rostral portions of PB, particularly the central lateral and dorsal lateral subnuclei. The amygdaloid projection to the PB ends heavily in the external lateral and 'waist' subnuclei. Cortical afferents terminate most heavily in the caudal half of PB, primarily within the medial subnuclei. The primary afferent zones for the hypothalamus, amygdala and cortex are depicted schematically below:



The BST, possibly reflecting its dual nature, projects to both 'hypothalamic' and 'amygdaloid' areas of PB.

In addition, considerable topography was found within the individual projections. For example, 'cardiovascular' regions within the insular cortex project most heavily to 'cardiovascular' regions within PB. And, peptide cell-rich areas within lateral CNA and lateral BST project primarily to lateral PB subnuclei. These topographical differences provide a substrate for the diversity of visceral functions associated with the PB.

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289.7 PEPTIDERGIC EFFERENTS FROM THE PARABRACHIAL NUCLEUS TO THE CENTRAL NUCLEUS OF THE AMYGDALA IN THE RAT. A.M. Zardetto-Smith and T.S. Gray. Dept. Anat., Loyola Univ., Maywood, IL 60153.

The central nucleus of the rat amygdala (CNA) is a major target of the parabrachial nucleus (PBN) and represents one route by which feedback from visceral receptors is relayed to the CNA. Previous studies have localized calcitonin gene-related peptide (CGRP)-, corticotropin releasing factor (CRF)-, enkephalin (ENK)-, bombesin (BOM)-, and somatostatin (SS)-immunoreactive cell bodies within the PBN. In this study, we examined the possibility that CGRP-, CRF-, BOM-, ENK-, and SS-immunoreactive neurons in the PBN project to the CNA. Long-Evans rats were anesthetized and unilateral 50-100 nl of the retrograde tracer Fluoro-Gold was injected into the CNA. After 11 days, animals received intracerebroventricular injections of colchicine. The animals were deeply anesthetized and perfused with 3% paraformaldehyde 24 h later. Adjacent sections were processed using the avidin-biotin-Texas red immunofluorescence technique with antibodies generated against CGRP, CRF, BOM, ENK or SS. The sections were examined for cells that contained both Fluoro-Gold and Texas red fluorescence. Retrograde labeling (in relative order of magnitude) was distributed within the external lateral, ventral lateral, central lateral and medial subnuclei as defined previously by Fulwiler and Saper ('84). CGRP-immunoreactive cells were most densely distributed in the external lateral subnucleus of the PBN, capping the superior cerebellar peduncle. Retrogradely labeled CGRP cells were observed in the external lateral subnucleus. Retrogradely labeled ENK-immunoreactive cells were distributed within the ventral lateral and the external lateral subnuclei. In the external and dorsal lateral subnuclei CRF cells formed distinct groups, but none contained Fluoro-Gold. A few BOM-immunoreactive cells were observed in the medial subnucleus, but they were not retrogradely labeled. SS-immunoreactive cells were found scattered in the dorsal portions of the ventral, central and dorsal lateral subnuclei. Retrogradely labeled SS-immunoreactive neurons were not detected. The results demonstrate that peptides in the PBN exhibit a characteristic distribution which are related to their connectional specificity. CGRP neurons, in the external lateral subnucleus, and ENK neurons, primarily in the ventrolateral subnucleus, project to the CNA. CRF-, BOM-, and SS-immunoreactive neurons were distributed primarily throughout subnuclei of the PBN that do not have the CNA as their major terminal field. CRF and SS neurons in the CNA have been shown to participate in the descending projection to the PBN, while ENK neurons do not. Thus, although afferent and efferent connections exist between these two nuclei, their peptidergic projections are not necessarily reciprocated. (Supported by NS 20041 and BRSG 447-42-LUMC).

289.8 SUBSTANCE P (SP) AND MONOAMINE CONTAINING AFFERENTS TO THE NUCLEUS TRACTUS SOLITARIUS (NTS) OF THE RAT: A DOUBLE FLUORESCENCE STUDY USING RHODAMINE-FILLED LATEX MICROSPHERES ("rhodamine beads", RB) AS A RETROGRADE MARKER. K. B. Thor, M. Dailey, and C. J. Helke, Dept. of Pharmacol., Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814

The NTS is an area of the medulla which integrates peripheral and central autonomic inputs. Nerve terminals in the NTS have been shown to contain SP, serotonin (5HT), and dopamine beta hydroxylase (DBH, i.e. catecholamines). The present study was conducted to localize central anatomical sources for these immunoreactive (IR) terminals.

Afferent neurons to the NTS were labelled by injecting rats with 25-100 nl of undiluted RB from micropipette tips positioned in the intermediate NTS and allowing 3-7 days for retrograde transport. The rats were then injected with colchicine and perfused the next day. Serial sections were cut, mounted, and processed for 5HT, DBH, or SP immunoreactivity using fluorescein (FITC) conjugated secondary antibodies. Counts of RB-, FITC-, and doubly -labelled neurons were made with Leitz N2 and I2 filters.

At the injection site, RB were remarkably well contained within the confines of the NTS in 4 of 6 rats. Longitudinal diffusion of the RB from the injection site (600-900 μm) was more extensive than transverse diffusion (100-500 μm). Transverse diffusion of the RB appeared restricted by the architectonics of the NTS.

Hindbrain sections processed for DBH-IR contained FITC labelled neurons in the A1/C1, A2/C2, A3, A4, A5, locus coeruleus (LC), subcoeruleus (SC), and Kollicke-Fuse (KF) regions of the hindbrain. Among these regions, the A1/C1 and A2/C2 areas contained the greatest number of RB labelled neurons with 30-50% of the RB labelled A1/C1 cells exhibitting DBH-IR while less than 10% of the A2/C2 cells were double labelled. KF also contained a large number of RB labelled cells, but none was DBH-IR. The A3 and A5 regions contained an intermediate number of the RB labelled cells, and high percentages of these cells exhibitted DBH-IR (30-50% and 50-70%, respectively). The A4, LC, and SC areas contained few RB labelled cells, but these few cells were often DBH-IR.

5HT-IR cells were found in nucleus raphe pallidus (NRP), obscurus (NRO), magnus (NRM), pontis (NRPo), dorsalis (NRD), and in the ventrolateral medulla (VLM). Of these regions, NRM contained the greatest number of RB labelled cells; 20-30% of these also exhibitted 5HT-IR. NRP, NRO, and VLM also contained a large number of RB labelled cells; 20-40% of these were also 5HT-IR. NRD and NRPo rarely contained RB labelled cells, but these few cells were usually 5HT-IR.

SP-IR cells were found in many of the above areas which contained DBH or 5HT-IR cells. Of these areas, RB labelled cells in each nucleus which were also SP-IR (expressed as %) were seen in A1/C1 (5-10%), A3/VLM (5-20%), A5 (0-10%), NRP (30-50%), NRO (20-30%), and NRM (0-10%).

These experiments indicate, firstly, that for each transmitter system examined; SP, DBH, and 5HT; there is no single projection which could be described as providing "the" major transmitter-specific afferent input to the NTS as a whole. Rather, SP, DBH, and 5HT containing terminals in the NTS each arise from multiple, often overlapping, areas of the hindbrain. Possibly, greater specificity will be found when afferent inputs to individual subnuclei of the NTS are examined. Secondly, since all of the nuclei examined in these experiments contained RB labelled cells which were not IR for a given transmitter substance, it appears that multiple transmitters are contained within each NTS afferent nucleus. Thirdly, the anatomical overlap of monoamine and SP containing NTS afferents in certain areas of the hindbrain allows for the possibility of colocalization of the monoamines and/or SP within single NTS afferent neurons. Finally, since most of the areas which contain NTS afferents also project to other autonomic centers in the hindbrain, forebrain, or spinal cord, it is tempting to speculate that some individual neurons in these areas may provide diffuse collateral networks to various autonomic structures in the central nervous system. Supported by NIH grant # NS20991.

289.9 LOCAL PROJECTIONS FROM THE MEDIAL PART OF THE NUCLEUS OF THE SOLITARY TRACT IN RAT. E.T. Cunningham, Jr. and P.E. Sawchenko. The Salk Institute and Dept. Neurosci., Univ. Cal. San Diego, La Jolla, CA 92037.

Axonal transport and immunohistochemical methods were used to investigate the anatomical organization of local projections from the medial and commissural parts of the nucleus of the solitary tract (NTS) in rat. Discrete iontophoretic deposits of the anterogradely transported plant lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) were placed in the medial part of the NTS at various rostrocaudal levels. The projections were then visualized using indirect immunofluorescence or avidin-biotin immunoperoxidase protocols. The most widespread projections were seen after placements at about the level of the area postrema. Such deposits labeled heavy projections within the medial part of the nucleus itself and within the adjacent dorsal motor nucleus of the vagus throughout their rostrocaudal extent. Ventrolaterally directed projections ramified within the regions of the C1 and A1 catecholaminergic cell groups (as demonstrated by concurrent co-staining for both PHA-L and dopamine beta-hydroxylase), while the nucleus ambiguus was sparsely innervated. By contrast, deposits placed 1.0 to 2.0 mm rostral to the level of the area postrema labeled an extremely heavy and discrete projection to the nucleus ambiguus, with relatively few labeled fibers being found in the NTS itself, in the dorsal motor nucleus, or in the C1 or A1 regions. Finally, deposits placed 0.5 to 1.0 mm caudal to the area postrema, within the so-called commissural part of the NTS, labeled fibers predominantly within the commissural part of the nucleus itself, as well as projections to the area postrema and the adjoining "subpostrema" region of the NTS. More rostral portions of the NTS, nearby vagal and catacholaminergic cell groups were only very sparsely innervated. Regardless of the placement of the injections, the projections from the medial part of the NTS are bilaterally symmetrical with ipsilateral predominance. These results suggest the existence of topographically organized projections from the medial part of the NTS to vagal and catecholaminergic effector neuron populations in the caudal brainstem. A discrete rostral zone of the medial NTS projects preferentially to the nucleus ambiguus, while the more caudally situated commissural region projects predominantly within the commissural part of the nucleus itself. Mid-level neurons at about the level of the area postrema project most expansively within the medial NTS, to the dorsal motor nucleus, and to the regions of the C1 and A1 cell groups.

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289.10 GASTRIC AND DUODENAL CONTROL BY THE MEDULLA OBLONGATA IN THE RAT: AFFERENT AND EFFERENT PROJECTIONS. B. Nathan*, D.J. Gelb* and M. Kalia. (SPON: G.B. Grunwald). Department of Pharmacolgy, Thomas Jefferson University, Philadelphia, PA 19107

The sensory and motor connections between the stomach (fundus and antrum) and the duodenum were studied using transganglionic and retrograde transport of horseradish peroxidase (HRP). Conjugates of HRP with wheat germ agglutinin (WGAHRP) and cholera toxin (CTHRP) were used to increase the sensitivity of the labeling. Twenty-one male, pathogen-free, Sprague-Dawley rats (175-350 g) anesthetized with chloral hydrate (350 mg/kg, i.p.) were used. Ten to 15 ul of a mixture of WGAHRP (8.3%), CTHRP (4.8%) and free HRP (25% Type VI, Sigma) was injected into: a) the fundus (anterior and posterior aspects) - 5 rats; b) the anterior aspect of the antrum - 6 rats; c) the duodenum 1 cm caudal to the pylorus (anterior and posterior aspects) - 6 rats. Following a 72-96 hour post-injection survival time, the animals were perfused and the brainstem and cervical spinal cord were removed and processed for HRP histochemistry using tetramethyl benzidine (TMB) as the chromogen. We examined 60 u thick, serial, frozen sections from levels 4.5 mm caudal to 2.0 mm rostral to the obex. Representative series from each case were counterstained with neutral red to delineate nuclear boundaries in this region. The nucleus of the tractus solitarius (nTS) received sensory projections from each of the enteric viscera examined. Numerous duodenal afferents terminated in the nTS from -1.08 to +0.90 and were distributed primarily in the ncom and PVR subnuclei of the nTS. A prominent feature of this projection was a distinct band 50 u wide in the PVR at the junction of the area postrema and the nTS at levels -0.72 to -0.18. Afferents from the antrum were sparse and could be identified in the nTS at levels -2.1 to 0.36, located in the ds, mnTS, ncom, PVR subnuclei of the nTS. The fundus (anterior and posterior aspects) resulted in labeling of a few scattered sensory terminals in the ds, mnTS, ncom subnuclei of the nTS at levels -0.36 to 0. Efferents to the duodenum, antrum and fundus arose exclusively from the dorsal motor nucleus of the vagus (dmnX). Duodenal innervation originated from levels -1.80 to +0.54 with heavier labeling on the left side. The antrum and fundus were innervated bilaterally by the dmnX at levels -3.90 to +1.26 and -3.40 to +0.78 respectively. In control animals 10-15 ul of the mixture of HRP conjugates was injected into the peritoneal cavity resulting in 3 to 5 labeled neurons in the dmnX. These results demonstrate that the brainstem control of enteric viscera is selectively organized. Regional and subnuclear differences in the sites of termination of afferents and origin of efferents as revealed by this study provide the anatomical substrate for focused physiological effects. (Supported by USPHS Grants HL 30991, HL 31997 and HL 33632.)

289.11 TRACING OF SPINAL AND SUPRAPONTINE PATHWAYS TO THE PONTINE MICTURITION CENTER OF THE CAT. A. Ragoowansi*, J.R. Roppolo and W.C. de Groat. (SPON: J.R. Boston) Dept. of Pharmacol., Ctr. for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15261.

Previous studies suggest that the micturition reflex pathway is a supraspinal pathway, organized with fibers ascending from the sacral spinal cord to an area in the dorsolateral pons known as the "pontine micturiton center" (PMC) and a descending fiber pathway back to the sacral cord. Studies of areas rostral to the PMC suggest that they may modulate bladder reflexes, but are not necessary for reflex activity. In the present study we have used tracing techniques to examine the connections between sacral spinal cord and PMC and the projections from suprapontine structures to the PMC in the cat.

Two types of experiments were performed: First, under α-chloralose anesthesia the PMC was identified by electrical stimulation through a stereotaxically positioned bipolar/concentric electrode (coordinates P=2, ML=2, H=-3). The area which gave a short latency ($<$2 sec) bladder contraction to electrical stimulation was then shown to be activated by a excitatory amino acid L-glutamate (0.1-1 μl; 100 mM). This area was then injected with 20% HRP (0.2-0.5 μl) or WGA-HRP 5% (0.7 μl). Short survival times (20-28 hrs) were used to examine rostral sites which project to the PMC. These areas include the hypothalamus and the cortex deep within the suprasylvian gyrus. Long survival times (4-6 days) labelled neurons in the sacral cord. These neurons were located in lamina V, in lamina I on the lateral edge of the dorsal horn, and in the intermediolateral gray. In the second series of experiments cats were anesthetized with halothane and the second sacral segment (S_2) was exposed. Three injections of a tracer substance (0.5 to 1 μl of either 20% HRP, 5% HRP-WGA, 2% fast blue, or rhodamine beads) was made along its rostrocaudal length at the level of the sacral parasympathetic nucleus. Following appropriate times for transport of the tracers the animals were perfused and the brainstem examined for labeled neurons. A variety of brainstem structures contained labeled cells including the lateral vestibular nucleus, red nucleus, medullary raphe nuclei, and the reticular formation. In addition, a group of neurons in the area of the PMC was labeled consistently. This area corresponds to coordinates mentioned above and was medial to the superior cerebellar peduncle, ventral to the periaqueductal gray and was adjacent to and partially overlapped with the locus coeruleus.

These data support the idea that the S_2 segment of the spinal cord, which is the principal source of parasympathetic efferent outflow to the bladder and the site of afferent input from the bladder has direct interconnections with the region of the brainstem (the PMC) which controls micturition. These data also suggest that the PMC receives direct suprapontine input.

289.12 AN ANALYSIS OF THE AFFERENT AND EFFERENT PATHWAYS TO THE UTERUS OF THE CAT USING AXONAL TRACING TECHNIQUES. M. Kawatani, C. Takeshige*, S. Narasimhan*, and W.C. de Groat, Dept. Physiol., Sch. Med., Showa Univ., Tokyo, and Dept. Pharmacol. Sch. Med., and Ctr. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15261.

Clinical studies indicate that sensory input from the female reproductive organs is relayed to various segments of the spinal cord. In the present experiments, axonal tracing techniques were used to examine the segmental distribution and central terminations of afferent pathways to the uterine cervix and uterine horn of the cat. The origin of autonomic efferent input to these organs was also studied.

HRP, WGA-HRP or fast blue (FB) were injected into uterine cervix or uterine horns using an intravaginal or intraabdominal approach. After 3-5 days transport time for HRP or 14 days transport time for FB the animals were anesthetized, perfused with fixative solution and the tissues were processed by standard procedures to identify HRP or FB.

The afferent neurons projecting to the uterus were located in the L1-L5 and S1-S3 dorsal root ganglia. The neurons were small to medium size averaging 33 μm and 27.5 μm in the lumbar and sacral DRG, respectively. When tracers were injected into the uterine cervix, an average of 780 cells (range, 590-1454) were identified in the dorsal root ganglia, with more than 90% of cells in the S1-S3 ganglia. On the other hand when tracer was applied to the uterine horns an average of 461 DRG cells (range, 350-700) were labeled with 93% in the L2-L4 ganglia. In the uterine cervix experiments, the number of labeled cells was not significantly altered by transection of the pudendal nerve. Afferent projections in the sacral spinal cord were labeled following injection of HRP into the cervix. Labeled afferents were most prominent in Lissauer's tract and in lamina I on the lateral edge of the dorsal horn. A few neurons were also identified in the S2 segment in the region of the dorsal band of the sacral parasympathetic nucleus.

Following injection of tracer substances into the uterine horn a large number of labeled neurons (1500-2500) were identified in the inferior mesenteric ganglia and a few neurons were identified in the intermediolateral nucleus of the lumbar spinal cord. In the uterine cervix experiments the sacral sympathetic chain ganglia exhibited a considerable number of labeled cells.

In summary the results indicate that the uterine cervix is innervated by sacral afferent neurons traveling in the pelvic nerve whereas the uterine horn is innervated by lumbar afferents presumably carried by sympathetic nerves. The central projections of uterine cervix afferents to Lissauer's tract and to lateral lamina I are similar to the projections of afferents from other pelvic viscera.

289.13 DISTRIBUTION OF CELLS AND FIBERS PROJECTING TO THE PENIS OF THE CAT. A.M. Booth, J.R. Roppolo, and W.C. de Groat. Department of Pharmacolology and Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

The penis receives an innervation from somatic nerves as well as from autonomic nerves of both sympathetic and parasympathetic origin. In the present experiments HRP was employed to identifying various components of the penile innervation including: (1) autonomic ganglion cells, (2) spinal motor neurons projecting to the striated penile musculature, (3) dorsal root ganglion cells and afferent projections within the lumbosacral spinal cord. HRP solution (25% HRP in 2% DMSO and H_2O) was either injected into the glans and/or shaft of the penis or applied to the proximal stump of the cut dorsal nerve of the penis (DNP) midway along its length. Following fixation and processing the pelvic ganglion (PG), sympathetic chain (SC), inferior mesenteric ganglia (IMG) and the spinal cord and dorsal root ganglia caudal to T_{13} were examined.

After injection into the shaft of the penis, ganglion cells were found in the IMG, PG at the level of the prostate and SC (S_1-S_3). Motor neurons were found in the vicinity of Onuf's nucleus in S_2. These neurons had extensive rostral-caudal and dorsal dendritic projections some of which extended to lamina VI, although most were confined to lamina VII. There were also modest lateral dendritic projections to the border of the ventral horn. HRP applied to the DNP did not label motor neurons but did label afferent cell bodies which were concentrated in the S_1-S_3 DRGs. In these animals central afferent projections coursed medially through laminae I-VI of the dorsal horn, with many fibers extending into lamina X bilaterally. Densely labeled bundles of afferent fibers could be seen entering the dorsal column at intervals along the medial course of the afferent pathway. When the shaft of the penis was injected a similar medial distribution of fibers was noted and there was also present a compact bundle of fibers which followed the lateral edge of the dorsal horn in lamina I ventrally to the level of lamina IV where it abruptly ended. Labeling was also present in Lissauer's tract, however, densely labeled bundles of fibers were not seen entering the dorsal columns.

In summary, the present results have revealed the distribution of various populations of neurons innervating the penis of the cat. The identification of postganglionic neurons in the PG should allow in future experiments a more detailed analysis of the efferent pathways to the penis. In addition, HRP labeling of the central projections of penile afferent neurons indicates that the medial dorsal horn and the dorsal commissure region are important areas for processing afferent input from the penis and presumably for coordinating sexual reflexes.

289.14 SPECIFICITY OF SYMPATHETIC PREGANGLIONIC PROJECTIONS: RAT PREGANGLIONIC NEURONS PROJECT TO EITHER ROSTRAL OR CAUDAL GANGLIA ALONG THE SYMPATHETIC CHAIN. C.J. Forehand and E. Rubin. Dept. of Anat. and Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Adjacent sympathetic chain ganglia are innervated by overlapping sets of spinal cord segments; the peripheral targets innervated by these ganglia overlap to some extent, but are largely separate (Langley, J. Physiol. 15:176, 1894; Lichtman et al., J. Physiol. 298:285, 1980). Accordingly, the preganglionic motor neurons must be organized in ways that enable specific activation of particular targets. To explore one aspect of this organization, we have mapped the rostro-caudal projection patterns of preganglionic axons from individual spinal segments.

The experiments employed two fluorescent dyes, fast blue (FB) and 2-diamidino yellow (2DY), that are transported retrogradely along axons; these dyes can be visualized simultaneously in a single neuronal cell body. One or both dyes were placed on the cut sympathetic trunk at different sites to determine the direction and branching pattern of axons arising from neurons in different spinal segments.

We first studied the general pattern of preganglionic outflow from the thoracic spinal cord using a single dye. Nearly all preganglionic axons from segments T1 and T2 course rostrally along the sympathetic chain, whereas axons originating in segment T10 and below are virtually all directed caudally. Preganglionic axons from intermediate segments project both rostrally and caudally.

To ask whether the bidirectional outflow from these intermediate segments reflects rostro-caudal branching of individual preganglionic axons, FB and 2DY were concurrently placed on the sympathetic trunk. One dye was placed just rostral and the other just caudal to a sympathetic chain ganglion marking the junction of an identified communicating ramus with the trunk. About half of the labeled preganglionic neurons from a given segment contained only FB while the other half contained only 2DY. Less than 2% of the labeled neurons contained both dyes; appropriate controls showed that all neurons could be doubly-labeled by applying both dyes to the same site.

We conclude that while midthoracic segments provide preganglionic innervation to both rostral and caudal ganglia, individual preganglionic neurons in these segments generally send their axons either rostrally or caudally. Thus the unique rostro-caudal outflow patterns of different spinal segments simply reflect the relative number of rostral or caudal projecting cells contained within those segments. Whether neurons with different projection patterns are segregated within the spinal cord is presently under investigation.

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289.15 VIP DISTRIBUTION AND COEXISTENCE WITH DOPAMINE-β-HYDROXYLASE IN SYMPATHETIC CHAIN GANGLIA OF THE PIG. E. L. Hill* and R. Elde (SPON:G. Giesler). Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Mpls., MN 55455

Vasoactive intestinal peptide (VIP)-immunoreactive nerve fibers have been demonstrated in bone and periosteum of several species. VIP increases bone resorption in vitro and VIP receptors have been characterized in an osteoblastic cell line, suggesting a possible role for VIP in neuronal regulation of bone metabolism. In pig VIP-immunoreactive fibers innervating rib bone and periosteum originate from cell bodies in sympathetic thoracic chain ganglia (Hohmann, et. al., Science, in press).

The extent to which VIP occurs in postganglionic cells of the sympathetic chain and whether these cells are noradrenergic, cholinergic, or neither is unknown. We have attempted to 1) establish the extent of VIP-containing cell bodies and fibers in all levels of the sympathetic chain and 2) determine the possible coexistence of VIP and dopamine-β-hydroxylase (DBH) using immunofluorescence.

4-8 week old pigs were perfused intracardially with Zamboni's fixative. Serial 4-5μ cryostat sections were taken of cervical, thoracic and lumbar paravertebral ganglia and alternate sections incubated with primary antisera directed against VIP or DBH. Sections were incubated with FITC-labelled goat anti-rabbit IgG and counterstained with ethidium bromide. Areas with VIP-immunoreactive cells and fibers and the equivalent areas in adjacent DBH-stained sections were photographed on a fluorescence microscope and examined for coexistence.

VIP-immunoreactive cells were most evident in the thoracic chain ganglia, with fibers present in cervical and lumbar ganglia. While the cells were distributed throughout individual ganglia, intensely stained cells consistently occurred just proximal to exiting fiber bundles and sent long processes into these bundles. Varicose fibers with VIP staining were found to be especially evident from the caudal cervicothoracic ganglion through upper lumbar ganglia, in many cases forming networks of beaded varicosities around principal ganglion cells. Fibers and cells were associated with both interganglionic connectives and rami. In adjacent sections, the majority of cells were reactive for DBH. In several cells it was possible to demonstrate the coexistence of VIP- and DBH-immunoreactivity in the same cell body.

We conclude that VIP-immunoreactive cells are distributed primarily in the thoracic sympathetic chain ganglia and that fibers containing VIP are found in ganglia at all levels. At least some VIP-immunoreactive postganglionic neurons are noradrenergic. The possibility that noradrenalin acts synergistically with VIP upon bone cells is presently under investigation.

289.16 THE CATECHOLAMINE INNERVATION OF THE LUMBOSACRAL SPINAL CORD OF MALE AND FEMALE RATS. K.E. Mckenna. Dept. of Physiology, Northwestern Univ. School of Medicine, Chicago, IL 60611

Pelvic structures are innervated by sympathetic, parasympathetic and somatic efferents located in the lumbosacral spinal cord. These include sympathetic preganglionic neurons (PGNs) with axons in the hypogastric nerve, primarily located in the dorsal gray commissure (DGC) of segments L1-L3; sympathetic PGNs with axons in the caudal sympathetic chain, primarily located in the intermediolateral column (IML) of segments T11-L2; parasympathetic PGNs with axons in the pelvic nerve, located in the IML of segments L6-S1; somatic motoneurons (MNs) with axons in the pudendal nerve, located in the dorsolateral (DL) and dorsomedial nuclei of L5-L6. It has been shown that in the rat and other species, there are significantly more hypogastric and pudendal neurons in the male compared to the female.

The present study was undertaken to examine the catecholaminergic innervation of these efferent nuclei by immunohistochemistry (IHC) using antibodies to dopamine ß hydroxylase (DBH) (Eugene Tech). Male and female rats 250-400g were deeply anesthetized and perfused with Krebs solution, followed by 4% paraformaldehyde, pH 6.5, then 4% paraformaldehyde, pH 8.5. The lumbosacral spinal cord was removed and 30μ frozen sections cut. Tissue was processed for DBH IHC using a free floating technique and the avidin-biotin system with visualization using nickel-enhanced DAB.

Strong, specific DBH positive staining was seen in fibers throughout the lumbosacral spinal cord. No labelled cells were observed. Fibers were submicron in diameter with varicosities every 3-10μ. All pelvic efferent nuclei received strong DBH innervation. The strongest labelling was observed in the IML of the T13-L2 (sympathetic PGNs). The labelling on and around the PGNs was extremely heavy and also followed dendrite bundles of the PGN, both medially and laterally. The innervation of the hypogastric PGNs was less dense but formed a strong net-like pattern. Innervation of the DGC was strong throughout the lumbosacral cord. The parasympathetic PGNs also received a strong DBH innervation.

Motoneurons in the DL and DM displayed a dense concentration of labelled fibers covering the cells and coursing along their dendrites. A major difference was noted between the male and the female in these nuclei. The innervation to these nuclei in the male was obviously much heavier than in the female. The organization of the DBH innervation was qualitatively different as well. Differences in staining technique can be ruled out as the source of this apparent dimorphism because a nearby nucleus, the ventral cell column which innervates the pelvic diaphragm, displayed a heavy DBH staining in both males and females.

The DL and DM nuclei are only involved in the control of the anal and urethral sphincters in the female, while in the male they innervate two penile muscles as well. The present findings suggest that spinal catecholamines may play a role in sexual function.

289.17 SYMPATHETIC NERVE ACTIVITY AFTER COMPLETE SPINAL CORD TRANSECTION IN THE ANESTHETIZED RAT. R.F. Taylor* and L.P. Schramm (SPON: E. Young). Department of Biomedical Engineering, The Johns Hopkins School of Medicine, Baltimore, MD 21205

In a previous report from this laboratory it was demonstrated that renal sympathetic nerve activity significantly increased after spinal cord transection in the chloralose-anesthetized rats. The present experiments compare the effect of cervical spinal cord transection on renal, splenic and lumbar sympathetic nerve activity.

Chloralose-anesthetized, male Sprague Dawley rats were paralyzed with Flaxedil and artificially ventilated. Multi-unit nerve recordings were obtained from the renal, splenic and lumbar sympathetic nerves using stainless steel bipolar hook electrodes. The rectified and integrated activity from pairs of nerves was compared before and after complete spinal cord transection at C1. Arterial blood pressure was measured via a cannula placed in the right carotid artery. Nerve activity and arterial pressure were displayed on a polygraph and stored on magnetic tape for later analysis. The integrated nerve signal was sampled every 5ms for 50 seconds and expressed in arbitrary units. The mean activity over this period was compared for each pair of nerve (renal and splenic or renal and lumbar) before and after transaction using a paired t-test.

In 8 rats, transection increased mean renal nerve activity significantly from 209 $\pm$ 51 to 452 $\pm$ 50 units. In the same rats, splenic nerve activity increased from 157 $\pm$ 35 to 372 $\pm$ 75 units. Mean arterial pressure fell significantly from 99.6 $\pm$ 4.8 to 75.1 $\pm$ 4.5 mmHg. In contrast, preliminary results show that lumbar sympathetic activity (sympathetic chain at L6) decreased by 40% after cord transection (n=4).

We conclude that spinal cord transection in rats under these experimental conditions may interrupt a descending inhibitory pathway which results in a specific excitation of sympathetic activity to the abdominal viscera. At the same time, transection may reduce sympathetic activity to muscle beds causing a fall in arterial pressure. Supported by NIH grant HL16315.

289.18 SYMPATHOINHIBITION MEDIATED BY DORSOLATERAL SPINAL CORD IN THE RAT. L.P. Schramm, S.R. Livingstone*, R.F. Taylor*, and C.E. Palmer*. Department of Biomedical Engineering, The Johns Hopkins School of Medicine.

After spinal transection, renal sympathetic nerve activity in the chloralose-anesthetized rat is well-maintained. This activity is reduced by electrical stimulation in the region of the cervical dorsal horn and dorsolateral funiculus. These experiments determined the stimulus-response characteristics and pathways mediating the inhibitions and the distribution of the inhibition among sympathetic nerves. Male Sprague-Dawley rats were anesthetized with alpha-chloralose. Tracheal, carotid arterial, and femoral venous cannulae were placed for artificial respiration, measurement of arterial pressure, and infusions of drugs, respectively. The spinal cord was exposed via a C1 to C6 laminectomy. Renal, splenic, or lumbar nerves were dissected through a left flank incision. Bipolar, AC recordings from nerves were quantified by rectification and integration. Concentric bipolar electrodes were used for electrical stimulation of the spinal cord. Inhibitions were elicited by stimulation of the surface of the spinal cord ranging from the most lateral dorsolateral funiculus to the most medial portion of the dorsal horn. The lowest thresholds for inhibitions were observed most laterally and were often less than 10 microamperes. Stimulation at 5 and 10Hz elicited inhibitions which were 60 and 80% of maximal, respectively. On the average, maximal responses consisted of 60% reductions in renal nerve activity, and they ranged from 10 to 95%. Renal and splenic nerve activity were equally inhibited and exhibited similar stimulus-response curves. In preliminary experiments, activity in the lumbar sympathetic chain also was inhibited. The effects of both small and large knife-cuts, caudal to the site of stimulation, were used to trace the descending pathway for this inhibitory system. Spinal cords were subsequently fixed by perfusion, paraffin-embedded, sectioned serially, and stained to permit reconstruction of lesions. Inhibitions required the integrity of the dorsal portion of the lateral funiculus and the ventral portion of the dorsolateral funiculus. Other rats were kept in good condition for periods up to one month after production of both small and large C1 lesions. Inhibitions could be elicited caudal to the larger lesions from the region of the dorsal horn but not from more lateral cord. Intravenous delivery of antagonists to GABA, glycine, serotonin, and catecholamines were ineffective in preventing the inhibitions. We conclude that inhibitions elicited by cervical stimulation are mediated by both descending and propriospinal (or antidromically-activated, ascending) lateral spinal systems. The inhibitions affect many tissues. The transmitter mediating the inhibitions remains unknown. Supported by NIH grant LH16315.

289.19 TONIC DESCENDING INHIBITION OF SPINAL GRAY CELLS RECEIVING RENAL AFFERENT INPUT. M.M. Knuepfer, E.W. Akeyson* and L.P. Schramm. Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD 21205 and Dept. of Pharmacology, St. Louis Univ., St. Louis, MO 63104.

Renal afferents have been implicated in control of cardiovascular function and in the mediation of pain of renal origin. Studies in our laboratories have examined the spinal projections of renal afferents to better describe the pathways involved. The present experiments determined whether tonic descending modulation of neurons receiving renal input exists. Studies were done in 11 chloralose-anesthetized, artificially respired, male rats. The dorsal surfaces of the cervical (C1-C4) and thoracic (T10-T13) spinal cord were exposed and covered with mineral oil. The left renal nerve was exposed, crushed distally, placed on bipolar stainless steel electrodes and covered with oil. Double barrel, glass microelectrodes (tip diameter 3-5 µm) were used to record single unit activity in the lower thoracic spinal gray during electrical stimulation of the renal nerve (trains of 3 pulses at 200 Hz every 2s). Sixteen neurons responded to renal nerve stimulation (mean onset latency = 33 ms, mean duration = 27 ms). Six of these neurons had a second (late) response (mean onset latency = 51 ms, mean duration = 36 ms). The spontaneous firing frequency of the neurons ranged from 0.1 - 41 Hz (mean = 8.4 Hz). During recording of 10 of these neurons, cotton pledgets soaked in 0.5% lidocaine were applied to the cervical spinal cord. The resulting local anesthesia of cervical cord decreased activity in 4 neurons (mean fall of 46%) and increased it in 3 (mean increase of 1261%). Early responses to renal nerve stimulation were greater in 50% of the neurons tested (mean increase of 158%). Of 3 neurons with late responses, only one was affected (50% decrease). In 6 additional neurons, the affect of cooling the surface of the cervical spinal cord to 4-7° caused an average decrease in mean arterial pressure from 110 to 62 mmHg. Spontaneous activity rose in 4 neurons (mean increase of 828%) and decreased in 1 neuron (40%). The change in spontaneous activity during spinal block was correlated to the spontaneous firing rate before blockade (2.9 $\pm$ 1.3 Hz for cells excited and 20.1 $\pm$ 6.4 Hz for cells inhibited by spinal block). Early responses to renal nerve stimulation were increased in 50% of the neurons (547%). All three late responses exhibited an increase in spikes during cold block (mean increase of 274%). The majority of the neurons tested were also shown to be sensitive to both light touch and noxious stimulation of adjacent cutaneous fields. These results indicate that a number of spinal gray neurons receiving renal afferent input and cutaneous input are tonically inhibited by fibers descending from supraspinal levels. Fewer neurons may receive tonic descending facilitatory input. (Supported by NIH Grants HL16315, HL34446 and HL37224).

289.20 INVOLVEMENT OF NUCLEUS RAPHE MAGNUS IN THE SUPRASPINAL CONTROL OF SPLANCHNIC INPUT TO THE THORACIC SPINAL CORD OF THE RAT. E.W. Akeyson*, M.M. Knuepfer and L.P. Schramm. (SPON: J. Eberhart). Departments of Neuroscience and Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Previous investigations have demonstrated that dorsal horn neurons receiving somatic afferent input are influenced strongly by supraspinal sites including the Nucleus Raphe Magnus (NRM). A large number of dorsal horn neurons in the thoracic spinal cord also receive visceral afferent input. We studied the effect of visceral afferent input on dorsal horn neurons in the thoracic spinal cord of the rat and determined the level of supraspinal control of visceral-afferent evoked responses. Extracellular recordings were made of dorsal horn neurons responsive to electrical stimulation of the greater splanchnic nerve (GSN) in thoracic spinal cord segments T7-T10 of chloralose-anesthetized rats. Supraspinal influences were investigated by means of both electrical stimulation of NRM and reversible spinalization by placing a cold-probe at the C2 spinal cord level. Neurons responded to GSN stimulation with either a single spike or with one or two bursts of 2-7 spikes each. Spontaneous activity was present in 52% of the neurons at a mean rate of 1.7 spikes/sec. Of the neurons responding with bursts, the majority (76%) responded with a single burst with mean latencies of 20.0ms onset and 40.7ms offset. The remaining 24% responded to nerve stimulation with dual bursts having mean latencies of 9.0ms onset and 18.3ms offset for the first burst and 29.7ms onset and 65.3ms offset for the second burst. The response to GSN stimulation in the majority of neurons tested (12/13) was attenuated or abolished by short trains of NRM stimulation (10ms/200Hz) preceding the nerve stimulation. The remaining neuron was responsive to NRM stimulation only at high stimulus intensities. Typical stimulus intensities for 50% inhibition of the GSN response were 30-40uA, with total inhibition occurring with 80-100uA. The time course of the inhibitions often lasted in excess of 100ms. Upon reversible spinalization with cold-block, the neurons displayed changes in both spontaneous activity and GSN evoked response. In some cases, neurons with no measurable spontaneous activity became spontaneously active after cold-block. Spontaneously active neurons increased their firing rates 3-10 times. GSN evoked responses were 3-5 times greater after cooling. These data demonstrate that dorsal horn viscero-receptive neurons are tonically and phasically inhibited by supraspinal sites. The sites producing these inhibitions are functionally important since they play a role in modulating visceral afferent input at an early stage in its processing in the spinal cord. Supported by NIH Grant HL16315.

289.21 **BRAINSTEM ORGANIZATION OF VAGAL NUCLEI AND FIBER PATHWAYS IN THE RAT.** E.A. Fox, E. Baronowsky*, and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue Univ., West Lafayette, IN 47907.

Although excellent qualitative HRP descriptions of vagal brainstem organization exist, little quantitative information is available. Definition of the medullary coordinates of vagal afferent and efferent fiber distributions, which would allow selective intracranial manipulation of the axons, have not been provided. Additionally, the morphology and distribution of vagal motorneurons within the dorsal motor nucleus of the vagus (DMN) and the reticular formation have not been fully characterized. To provide such parametric analyses, 38 male Sprague Dawley rats were subjected to cervical vagal soaks with HRP and then their brains and spinal cords were processed with TMB. Complete sets of serial sections (frontal for one group of animals, horizontal for another) were examined.

All of the vagal nuclei have rostral borders 1.8mm anterior to the rostral pole of the area postrema (rAP). The bulk of labeled preganglionics in the DMN and reticular formation extend 4.5mm caudally (a limited number of cells continue through the upper cervical cord), while the nucleus ambiguus and the retrofacial nucleus extend caudally only 3.2 and 1.5mm respectively. Afferent axons enter the dorsolateral brainstem only at rostral levels as a single root composed of several fascicles 1.2mm anterior to the rAP, course medially, and then turn caudally to form the solitary tract. Some of these fibers were traced at least 7.3mm caudal, well into the spinal cord. In contrast, efferent axons exit the brain ventrolaterally as a series of rootlets distributed from rostrocaudal levels coincident with much of the DMN extent (3mm). These fibers issue from the lateral DMN pole and preserve rostrocaudal DMN organization by maintaining parallel trajectories, while they course ventrolaterally and caudally (initially at an angle of 30° and eventually 65°) in small fascicles that are relatively evenly spaced (~90µm apart) along the rostrocaudal axis.

Primary dendritic segments of DMN cells tend to remain within the nuclear boundaries as defined by the distribution of somata. In the rostral and caudal DMN the cells are fusiform and oriented longitudinally, while at mid rostrocaudal levels, where cell number and cross-sectional area are the greatest, cells are multipolar with initial dendritic segments extended medially, laterally and longitudinally. From the rAP caudally, larger somata predominate in the lateral pole of the DMN. Vagal preganglionics in the reticular formation are sparsely distributed, fusiform and oriented parallel to (and within) the efferent fascicles. Finally, several features of vagal organization are consistent with previous ontogenetic and phylogenetic analyses. (USPHS grant AM27627.)

289.22 **SELECTIVE AS WELL AS TOTAL SUBDIAPHRAGMATIC VAGOTOMIES CAN BE ASSESSED WITH A SENSITIVE AND VERSATILE TRACER TECHNIQUE.** T.L. Powley, E.A. Fox and H.-R. Berthoud. Lab. of Regulatory Psychobiology, Purdue University, West Lafayette, IN 47907.

While vagotomies are widely used, the absence of adequate verification tests for selective vagotomies has complicated the use of these more refined surgeries. A new protocol that provides a sensitive, highly reliable and practical test for completeness of a number of selective (as well as total) subdiaphragmatic vagotomies in the rat is described. The protocol is based on our earlier reports that the dorsal motor nucleus of the vagus (DMN) is composed of separate topographic cell columns corresponding to each of the branches of the abdominal vagus (Fox and Powley, Br. Res., 341: 269-282, 1985) and that essentially all vagal preganglionics innervating the abdominal viscera can be labeled by intraperitoneal administration of the fluorescent label True Blue (TB; Sterner et al., J. Neurosci. Meth., 14: 273-280, 1985). The vagotomy assessment employs a microscopic inventory of fluorescently labeled vagal efferents to determine which branches are intact and which have been destroyed.

Two to three days before vagotomized animals are to be sacrificed, 3mg of TB in a saline vehicle are injected or instilled into the peritoneal cavity. At sacrifice, the animal is perfused with 10% buffered formalin. The medulla is removed and stored overnight in 15% sucrose-buffered formalin at +4°C. Frozen, serial coronal sections are then taken throughout the extent of the DMN. Sections are collected onto uncoated microscope slides, air-dried, and stored dessicated in the dark at 4°C. All sections are examined for TB-labeled motor neurons with a standard uv (360nm) excitation filter system. Standardized coronal levels (area postrema as landmark) are then used to determine which of the five cell columns (each corresponding to one of the five abdominal branches) in the DMN are spared and which are eliminated. [The vagal branches must be transected by a cautery--or some other strategy used--to prevent cut axons from accumulating the tracer.]

The DMN profile of an animal can discriminate not only total vagotomies but also numerous--but not all--selective branch or trunk cuts and combinations of branch cuts. To date, we have used the protocol described in an extensive series (218 animals) to test the completeness of eight different types of selective vagotomy or combinations of vagal branch transections. Physiological validation experiments indicate that the True Blue test described is much more accurate and sensitive than visual inspection in predicting the outcome of selective or partial vagotomies. Strengths and limitations of the True Blue test for vagotomy will be identified. NIH Grant AM27627.

289.23 VAGAL INNERVATION OF THE RAT PANCREAS. L.M. Rinaman* and R.R. Miselis. Departments of Neuroscience and Animal Biology, The University of Pennsylvania, Philadelphia, PA 19104.

In order to identify vagal preganglionic parasympathetic neurons projecting to the pancreas, the retrograde tracer cholera toxin-horseradish peroxidase (CT-HRP) was injected throughout the pancreas of anesthetized rats. Tracer diffusion from injection sites was limited by isolating the pancreas from nearby viscera using sterile saline-soaked gauzes and by diluting the injection site surfaces. Control injections were made into the omental fat and the abdominal cavity, with the pancreas isolated to prevent tracer absorption. 48 hours later the animals were sacrificed for histological analysis. The medulla, viscera and spinal cord were reacted using the TMB protocol and analyzed for evidence of labeling. Our data indicate that the rat pancreas is innervated by approximately 50% fewer neurons in the left dorsal motor nucleus of the vagus than in the right, suggesting predominant innervation of the pancreas via the dorsal trunk of the vagal nerve. Many labelled cells sent dendritic projections into regions of the nucleus of the solitary tract, either terminating there or at the borders of the area postrema and fourth ventricle. Data from the control cases indicate that there was minimal tracer leakage from the injection sites. Further analysis using flourescent tracers and double-labelling techniques are underway in order to characterize the dendritic fields of the labelled neurons as well as to examine the possibility that some vagal motor neurons send axon collaterals to innervate more than one visceral organ. Supported by GM-27739 and an Office of Naval Research Fellowship to L.M.R.

289.24 **USE OF HORSERADISH PEROXIDASE TO LOCALIZE MOTONEURONS INNERVATING THE FELINE UPPER ESOPHAGEAL SPHINCTER (CRICOPHARYNGEAL MUSCLE), CERVICAL AND THORACIC ESOPHAGUS, AND LOWER ESOPHAGEAL SPHINCTER.** W.P. Norman, S. Pineo*, M. Visner*, S. Benjamin* and R.A. Gillis*. Dept. of Pharmacology and Anatomy, Georgetown University, Washington, D.C. 20007

The localization of motoneurons innervating the esophagus of the cat was studied by injecting 10 to 40 ul of 33% horseradish peroxidase (HRP) into the muscularis externis of the upper, mid- and lower portions of the esophagus of the cat. After injection of HRP into the cricopharyngeal muscle, labeled cells were found bilaterally in the retrofacial nucleus and the rostral part (i.e., 0 to 2.0 mm rostral to obex) of nucleus ambiguus. Injection of HRP into the cervical portion of the esophagus resulted in a similar degree of labeled cells in the retrofacial nucleus, but no labeled cells in nucleus ambiguus. After injection of HRP into the thoracic portion of the esophagus, labeled cells were prominent in the dorsal motor nucleus of the vagus (DMV) (rostral part from obex to 3.5 mm rostral to obex, and some scattered cells containing HRP were found in nucleus retroambiguus (NRA). Again, intense labeling of cells was observed in the retrofacial nucleus. With injection of HRP into the lower esophageal sphincter , the nucleus showing the most labeled cells was the DMV, with labeling occurring from 1.5 to 4.0 mm rostral to obex, and from 0.5 to 1.5 mm caudal to obex. Interestingly, very few cells were labeled on either side of the obex (i.e., from 0.5 mm caudal to obex to 1.5 mm rostral to obex). Labeled cells were also found in NRA. These results indicate that there is a topographic organization to the innervation of the esophagus by the medulla. (Supported by Grant AM-NS-29975).

289.25 THE MORPHOLOGY OF PELVIC GANGLIA IN THE DOMESTIC FOWL (Gallus domesticus). R.M. Kriebel, D.C. Foss* and S.L. Freedman. Departments of Anatomy & Neurobiology and Animal Sciences, The University of Vermont, Burlington, VT 05405.

The morphology of the avian pelvic ganglia and nerve plexus was studied using a variety of anatomical methods. All studies were done on adult, egg producing hens. The first phase of the work was to extend previous information reported by Freedman and Sturkie (Anat. Rec. 147:431, '63) on pelvic nerve formation and ganglia located at the uterovaginal junction. A modification of the Baljet and Drukker (Stain Tech. 5:31, '75) acetylcholinesterase gross nerve stain provided detail on ganglia and plexus organization previously not observed. Notable was the complexity of the left pelvic nerve plexus as compared to the right, likely related to functional development of only the left oviduct. Distally, the pelvic nerve contained a series of ganglia giving rise to numerous fine nerve branches. Ganglionic enlargements were observed at the uterovaginal junction and a large ganglion was observed beneath the adventitia in the vaginal region. A second part of the study was an immunohistochemical analysis of selected pelvic ganglia. Each ganglion contains large peripheral nerve fascicles and islands of postganglionic neurons. Peroxidase anti-peroxidase techniques showed immunoreactive nerve fibers labeled for substance P, vasoactive intestinal polypeptide, and cholecystokinin. Labeled nerve terminals for each of these peptides were seen in close association with the postganglionic neurons. Pelvic ganglia were studied with the electron microscope as a third method. The ganglia contained myelinated and unmyelinated nerve fibers. Postganglionic neurons in aggregations of a few to many were dispersed among these fibers. The dendritic processes of these neurons extended into the surrounding bundles of fibers and coursed parallel with them. In this "neuropil" the dendrites of the postganglionic neurons received multiple synaptic contacts from boutons containing clear vesicles and large numbers of granular vesicles. The presence of granular vesicles suggests a peptidergic content. The population of synaptic vesicles seemed to be polarized with the small clear vesicles adjacent to the synaptic density and the granular vesicles restricted to more proximal sites in the bouton. The neural components described above appear to be involved in the autonomic innervation of the reproductive tract. Peptides contained in the peripheral autonomic ganglia, as well as in the sacral regions of the spinal cord of hens (Freedman et al., Neurosci. Abs. 11:350, '85), suggests that these substances may be involved in regulation of avian reproduction. Studies continue which are designed to define the source of these peptides and to examine their role in mechanisms of egg production and oviposition.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM I

290.1 STRESS-INDUCED INCREASES IN PITUITARY CYCLIC AMP OCCUR IN THE ANTERIOR BUT NOT THE POSTERIOR LOBE. E.H. Mougey*, J.L. Meyerhoff, L.L. Pennington*, W.L. Gamble*, G.C. Driver*, and G.J. Kant. (SPON: F.J. Manning). Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307.

We have previously reported that various stressors increase levels of pituitary cyclic AMP in vivo in rats. Recently, we have suggested that the hypothalamic peptide, CRF, may be the prime stimulus for stress-induced increases in pituitary cyclic AMP since iv injection of CRF increases pituitary cyclic AMP levels, and injection of CRF antisera prior to stress challenge attenuates the pituitary cyclic AMP response. In addition, stress-induced increases in pituitary cyclic AMP are highly correlated with the release of three hormones regulated by CRF, i.e. ACTH, beta-endorphin and beta-LPH. In our previous work, we measured cyclic AMP levels in whole pituitaries following stress. Since the stress-induced release of ACTH, beta-endorphin and beta-LPH occurs primarily from the anterior lobe, the present study was conducted to determine if the stress-induced increase in pituitary cyclic AMP observed in vivo also occurs in the anterior lobe.

Male Sprague-Dawley rats weighing approximately 350 g were used in these experiments. Control rats were killed by decapitation immediately upon removal from their home cages. A second group of rats was exposed to 15 min of intermittent footshock (1.6mA, 5 sec shock duration, variable interval schedule with an average intertrial interval of 30 sec). These stressed rats were killed immediately following the footshock session. Pituitaries were immediately removed and dissected into anterior and posterior/neurointermediate lobes, weighed and placed into sodium acetate buffer (pH 6.2, 0.05M) at 90°C to prevent post-mortem changes in levels of pituitary cyclic AMP. Following sonication and centrifugation, the supernatants were stored frozen until assayed for cyclic AMP by RIA. Trunk blood was collected in heparinized beakers. Trasylol was added to plasma prior to freezing to inhibit peptidases. Plasma ACTH, corticosterone and prolactin were measured by RIA.

In control animals levels of cyclic AMP were similar in anterior and posterior/neurointermediate lobes: anterior (1.05 ± 0.11) and posterior (1.48 ± 0.33), pmoles/mg wet weight ± SEM. Footshock markedly increased levels of pituitary cyclic AMP in the anterior but not the posterior lobe: anterior (6.12 ± 2.14) and posterior (1.87 ± 0.49) pmoles/mg wet weight ± SEM. Footshock increased levels of plasma ACTH from 70 ± 8 to 363 ± 95 pg/ml ± SEM and plasma corticosterone from 4.6 ± 2.0 to 21.2 ± 1.6 ug/dl ± SEM. Footshock also increased plasma prolactin levels from 24.1 ± 3.9 to 148 ± 26 ng/ml ± SEM.

These results demonstrate that the stress-induced increases in pituitary cyclic AMP occur primarily in the anterior rather than in the posterior/neurointermediate lobe and support our hypothesis that these increases in cyclic AMP are related to release of hormones stored in corticotrophs of the anterior lobe and regulated by CRF.

290.2 REGULATION OF RECEPTORS FOR ADRENAL STEROIDS IN RAT HIPPOCAMPUS. B.S. McEwen, R.E. Brinton and L. Snyder*, Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021

Putatative receptors for adrenal steroids in rat brain are subject to regulation by adrenal steroids and by certain peptide hormones. Most of these effects are detected in hippocampus (HC), which has the highest in vivo uptake of 3H corticosterone (CORT) of any brain region. Recently it has become apparent that this 3H CORT uptake is due primarily to one receptor subtype ("CORT receptor") which is concentrated in HC (Reul and DeKloet, Endocrinology, 1985). The other receptor subtype (Glucocorticoid or "GC receptor") is not concentrated in HC but is found in other brain regions as well (Fuxe et al. Endocrinology, 1985). The "GC receptor" has a lower affinity for CORT than the "CORT receptor" (Veldhuis et al., Endocrinology, 1982) but becomes extensively occupied in vivo by stress levels of circulating CORT (McEwen et al., J. Neurosci. Met., 1980). Because previous studies of adrenal steroid receptor regulation in HC used 3H dexamethasone (DEX) or 3H CORT in vitro under conditions where both receptor subtypes were labeled, we have begun to reevaluate regulatory effects on each subtype separately using 3H DEX in vitro in the presence or absence of 100-fold molar excess of RU26988, a specific "GC receptor" ligand (Reul and DeKloet, 1985). HC, kidney, hypothalamus and pituitary cytosols were incubated for 4h with 10nM 3H DEX in the presence of 20 mM Na molybdate. "GC receptors" were assayed by competition with 100 x excess RU26988 while "CORT receptors" were measured as the difference between 3H DEX binding in the presence of RU26988 and 3H DEX binding in the presence of 100 x excess CORT. Hypothalamus, pituitary and kidney all have "CORT receptor" levels which are 0.1 to 0.25 those of HC while "GC receptor" levels across tissues are comparable to HC. We have compared receptor levels in HC and kidney of rats 18h after adrenalectomy (ADX) and 3d after ADX, with receptor levels in rats treated for 5d with CORT (30 mg Kg/day) prior to ADX (18h before sacrifice). HC and kidney cytosol "GC" and "CORT" receptors were lowest in CORT treated rats and highest in 3d ADX rats compared to 18h ADX controls. These results indicate that both receptor subtypes are negatively regulated by CORT. A comparison of "CORT" and "GC" receptors in male and female rat HC 3d after ADX revealed a sex difference, with males having more "CORT" receptors than females. Possible sex differences in regulation of "GC" and "CORT" receptors by adrenal steroids are currently being investigated, together with the role of gonadal hormones in determining the sex difference. (Supported by MH41256 and NS07080 to BMc and by NRSA MH09224 to REB).

290.3 TRANS-SYNAPTIC INDUCTION OF TYROSINE HYDROXYLASE mRNA IN RAT ADRENAL GLAND DURING COLD STRESS. M.K.Stachowiak*, E.M.Stricker, M.J.Zigmond and B.B.Kaplan. Dept. of Psychiatry, Psychology, and Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15213

Chronic cold stress increases the synthesis and release of catecholamines (CA) in the adrenal medulla. Adrenal chromaffin cells adapt to altered functional requirements by increasing the synthesis of tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine synthesis. We have previously demonstrated that the induction of TH activity during chronic cold stress (5-7 days) was accompanied by a parallel increase (4- to 5-fold) in the relative abundance and in vitro translation activity of TH mRNA (Stachowiak et al., Brain Res. 359: 356, 1985; Stachowiak et al., J. Neurosci. Res., in press). Increases in TH mRNA levels (approx. 90%) also were observed in the brain stem of cold-stressed animals. The change in TH mRNA appears specific, as no significant differences were observed in the content of adrenomedullary total RNA or poly(A)RNA. Additionally, the relative abundance of PNMT mRNA changed by only 20-40% under the identical conditions.

In recent studies, we examined the extracellular mechanisms underlying stress-induced alterations in adrenomedullary TH mRNA levels. Adrenal glands of male Sprague-Dawley rats were unilaterally denervated, and 2 wk later, animals were exposed to cold (5°C) for 5 days. Estimates of TH mRNA levels were obtained by RNA dot-blot hybridization and northern analysis using a cloned TH cDNA as probe. No detectable differences were observed in basal TH mRNA levels in the innervated compared to the denervated adrenal gland. In the innervated glands, cold stress increased TH mRNA levels 3- to 4-fold. Denervation abolished this effect, indicating that stress-induced alterations of TH mRNA levels require an intact splanchnic nerve. Interestingly, short-term exposure to cold (12 hr) resulted in a 5- to 8-fold increase in TH RNA levels. This change could be reduced 80-90% by treating stressed animals with the ganglionic blocker, chlorisondamine 1 hr before and 5 hr after the onset of cold exposure (2 times for 6 hrs each; 10 mg/kg,ip). The administration of chlorisondamine had little effect on the basal levels of TH mRNA (-20%; drug vs vehicle-treated controls). In contrast to these findings, the administration of the cholinergic agonists carbachol (8.2 umoles/kg,ip) or nicotine (5.0 mg/kg, sc), injected every 12 hr for 3 days resulted in only a small increase (approx. 50%) in the relative abundance of adrenomedullary TH mRNA. This finding suggests that activation of cholinergic receptors are necessary, but not sufficient, to mediate the effects of cold stress on adrenomedullary TH mRNA. Taken together, these data indicate that cold stress-induced alterations in TH RNA levels (1) require an intact sympathetic input, (2) are mediated, in part, by nicotinic cholinergic receptors, and (3) precede by 24-36 hr comparable alterations in TH enzyme activity.

290.4 ENVIRONMENTALLY-INDUCED MODIFICATION OF THE BENZODIAZEPINE RECEPTOR COUPLED CHLORIDE IONOPHORE. R. Trullas[1], H. Havoundjian[1], S. Paul[2], and P. Skolnick[1].
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We have recently demonstrated (H. Havoundjian, et al., Brain Res., 375:403-408,1986) a rapid stress-induced modification of the benzodiazepine/GABA receptor coupled chloride ionophore, manifest as an increase in the potency (decreased EC_{50}) and efficacy (increased E_{max}) of Cl^- to enhance [^{3}H]flunitrazepam (FLU) binding, and an increase in the apparent affinity and number of [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding sites. Neither GABA enhanced FLU binding nor FLU binding in the absence of halide ions was affected by this swim stress.

In order to determine whether the chloride ionophore is also under tonic regulation by the environment we have maintained animals in an environmental chamber ("protected environment") which has been postulated to be less stressful than conventional housing. A ten minute ambient temperature swim produced qualitatively similar changes in the chloride ionophore of animals maintained in either housing condition. In well-washed cerebral cortical membranes, the E_{max} of Cl^--enhanced FLU binding was increased by 76% (from 33 ±2% to 58 ±3%, p<.01) and 95% (from 20 ±3 to 39 ±1, p<.01) in the conventional and protected housing groups, respectively. Similarly, the B_{max} of TBPS was increased 34% in the conventional housing group (from 2030 ±56 to 2722 ±63 fmol/mg prot., p<.01) and 46% (from 1454 ±94 to 2131 ±59 fmol/mg prot., p<.01) in the animals maintained in the protected environment, while the K_d was significantly decreased by 22% (from 37 ±3 to 29 ±2 nM, p<.01) and 29% (from 35 ±2 to 25 ±2 nM, p<.01) respectively. Despite the qualitatively similar response to stress, both the E_{max} of Cl^- to enhance FLU binding and B_{max} of TBPS were significantly lower in the animals maintained in the protective environment both before and after swim stress (all p<.05). These results indicate that the chloride ionophore, which may be considered the effector component of the benzodiazepine receptor complex, is subject to both acute and tonic environmental control. The lower B_{max} of TBPS and E_{max} of Cl^- to enhance FLU binding observed in animals maintained in a protected environment suggests that changes in the chloride ionophore after stress represent a compensatory adaptive response.

290.5 LITHIUM AND STRESS RESPONSE PATTERNS OF SERUM CORTICOSTERONE, MELATONIN AND INTACT PINEAL BETA-ADRENERGIC ([H^{3}CGP-12177) BINDING IN RATS. Michael Wilkinson, Jo Seggie and Gregory M. Brown, Departments of Neurosciences and Psychiatry, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada and Departments of Physiology and Biophysics and Obstetrics & Gynecology, Dalhousie University, Halifax, Nova Scotia, Canada.

Lithium salts have been reported to alter the circadian pattern of resting levels of adrenal and pineal hormones. Historically, the hypothalamic-pituitary-adrenal-axis and more recently, the pineal gland have been noted for their responsivity to environmental stress. Pineal melatonin synthesis is under beta-adrenergic control. Since lithium has been shown to alter resting 24 hour levels of serum corticosterone and melatonin as well as intact pineal beta-adrenergic binding, it was decided to study the effect of lithium on the response to stressful stimulation of these three variables. Male, Long Evans Hooded rats were maintained in a controlled environment with free access to food and water under a 12:12 light:dark lighting cycle. For six weeks after adaptation, they were fed either normal rat chow or chow supplemented with 50 mg/kg of lithium chloride, which characteristically results in plasma lithium levels around 0.6 mEq/l in this strain. In the middle of the light phase, animals were sacrificed by rapid decapitation at rest or 0, 5, 15, 30 or 60 min after standing in an icewater slurry for 3 min. Trunk blood and pineal glands were frozen for future assay. Serum corticosterone was measured by a protein binding assay. Serum melatonin was measured by radioimmunoassay. Pineal beta-adrenergic binding was assayed using ([^{3}H]CGP-12177) as the ligand. Resting levels of serum melatonin, but not pineal beta-adrenergic binding or corticosterone were significantly elevated in lithium treated rats compared to controls at this time of day. Following exposure to cold water stimulation serum corticosterone levels were increased five fold from 5 to 60 min, serum melatonin levels were increased four fold during the first 15 min and pineal binding of [H^3]CGP-12177 were increased four fold from 5 to 60 min of observation. Following treatment with lithium, the pattern of the stress response of all three variables was significantly altered. The corticosterone response was potentiated throughout the period of observation. Melatonin and pineal responses, although not differing in absolute level, were attenuated in terms of the magnitude and latency of the response relative to resting levels. Since the pattern of corticosterone stress responses depends on the time of day at which stimulation occurs, these findings may be secondary to the effects of lithium on the resting circadian rhythms of these variables. (Supported by The Canadian Medical Research Council and The Ontario Mental Health Foundation.)

290.6 EFFECT OF STRESS ON CIRCULATING WHITE BLOOD CELLS IN RATS. D. B. Bower* and W. H. Vogel, Ph.D. Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107

Stress is known to cause changes in white blood count. To elucidate the effects of stress on white blood cells in more detail, we exposed rats to various stressors and measured total circulating leukocytes and their subtypes.

Male Sprague-Dawley rats, 250-300 g, were implanted with a jugular catheter to minimize stress during blood sampling and to allow repeated samples to be taken from the same animal. Results from catheter-drawn samples (lymphocytes, 73.0%; neutrophils, 23.9%) are significantly different from samples obtained from trunk blood at decapitation (lymphocytes, 90.4%; neutrophils, 6.7%; $p < .05$).

Stress was induced by either restraint (R), inescapable random footshock without prior training (RS), or escapable footshock in pairs of rats which could terminate (controllable shock, CS) or could not terminate the shock (non-controllable shock, NCS) after two training sessions. Control rats were left in their home cages and not stressed.

Total leukocytes increase during stress in all groups except NCS (R = +48%; CS = +49%; RS = +35%; NCS = -2%). Lymphocytes increase in number in all groups except NCS (R = +86%; CS = +54%; RS = +38%; NCS = -5%). Monocytes decrease in percentage during and after restraint (-70%) but increase in number in RS (+175%). Neutrophil percentage decreases during CS (-23%) but increases after RS (+50%). Eosinophil percentage decreases during and after restraint and NCS (R = -63%; NCS = -66%).

These results indicate that catheter-drawn blood samples are preferable to samples obtained by decapitation for blood cell counts. Acute stress causes an increase in total leukocyte count in all groups except non-controlling rats. The changes in differential vary with the nature of the stressor.

290.7 HYPOTHALAMICALLY-INDUCED STRESS AND PLATELET PHYSIOLOGY.
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Since recent experiments in our laboratory have suggested that stress in humans can profoundly effect platelet aggregation, the present study was undertaken to examine the effects of acute and chronic stress on cat platelet physiology and cardiovascular responses.

Adult cats were implanted with chronic electrodes aimed at the ventromedial hypothalamus, which when stimulated, elicited affective aggressive behavior including pupillary dilatation, arching of the back, piloerection, retraction of the ears, vocalization, and marked increases in blood pressure, heart rate, and plasma catecholamines. Additionally, each animal was implanted with an indwelling carotid cannula for measurements of cardiovascular responses and for atraumatic phlebotomies. The experimental design involved a comparison of the effects obtained prior to, during, and following hypothalamically induced emotional behavior. Analyses included: platelet count, platelet aggregation to various agonists, alpha-adrenergic receptor binding and cardiovascular responses.

Acute episodes of hypothalamically-induced stress generated an inhibition of platelet aggregation to epinephrine, collagen, adenine diphosphate, and arachadonic acid. In addition, acute stress caused a significant ($p<0.05$) decrease in alpha-2-adrenergic receptor binding. Chronic stimulation continued to display inhibitory effects on platelet aggregation. Moreover, chronic stimulation caused a stepwise decrease in the maximum number of binding sites per platelet over a period of 3 weeks. After 2 additional weeks during which the animals were allowed to rest undisturbed, the maximum number of binding sites per platelet gradually increased to their pre-stress values.

These studies demonstrate that periods of emotional arousal generated by hypothalamic stimulation have a marked effect upon platelet aggregation and alpha-2-adrenergic receptor binding. We hypothesize that normally, individuals are protected from thrombotic accidents during periods of stress, by mechanisms which reduce the sensitivity of platelets to aggregation agonists such as epinephrine. Accordingly, it may be assumed that in certain individuals the aggregation response to stress may be less effective and thus the likelihood of thrombus formation will be increased.

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290.8 PITUITARY-ADRENOCORTICAL AND ADRENOMEDULLARY RESPONSES TO ACOUSTIC STARTLE IN CONSCIOUS DOGS.
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The presentation of unexpected sensory stimuli to conscious animals results in a generalized motor or "startle" response. In conscious trained dogs, brief exposure to a 75dB tone with a frequency bandwidth of 0.25-8 KHz results in an alerting response that includes attending to the sound, tachycardia and hypertension. To assess the adrenomedullary and pituitary-adrenocortical responses to startle evoked by acoustic stimulation(AS), dogs trained to stand quietly in a modified Pavlov sling were prepared with adrenal venous and femoral arterial cannula. Experiments done 48-96h post surgery consisted of presentation of AS for 3min (n=6) or no presentation of AS(n=4). Samples were collected simultaneously from the adrenal vein and the femoral artery prior to and for 30min following the onset of AS. Samples were collected continuously during the initial 10min of this period. Adrenal venous concentrations of catecholamines (epinephrine (E) and norepinephrine(NE)) and of cortisol were determined by HPLC-EC and HPLC-UV, respectively; secretion rates were calculated as the product of hormone concentration and plasma flow rate. Arterial ACTH concentration was measured by RIA; adrenal presentation rate of ACTH was calculated as the product of ACTH concentration and plasma flow rate. In response to AS, mean arterial pressure increased at 1min($+18\pm2$mmHg; $p<0.01$) and returned towards baseline at 4min. There was no change in arterial pressure in control dogs. Adrenal secretion of E and NE increased at 1min (E:$+260\pm21$ng/min; NE:74 ± 18 ng/min; $p<0.01$) and remained elevated until 4min. Adrenal secretion of E or NE did not change in control dogs. In response to AS, there was a small, yet significant increase ($p<0.05$) in arterial ACTH(peak:+10pg/ml at 6min). However, adrenal blood flow increased ($p<0.01$) from 2-5min post AS (peak:+60% at 3min) resulting in a large increase ($p<0.01$) in ACTH presentation rate to the adrenal from 3-7min post AS (peak +79pg/min at 3min). The rise in ACTH presentation rate preceded an increase ($p<0.01$) in the secretion of cortisol which occurred from 7-15min post AS (peak:+10.7 ug/min at 9min). There was no change in arterial ACTH, adrenal blood flow, ACTH presentation rate or cortisol secretion in control dogs. These data show that AS presented to conscious dogs evokes a sequence of responses with rapid onset that include hypertension, activation of adrenomedullary secretion and increased adrenal conductance, followed by a delayed increase in adrenal secretion of cortisol. By amplifying the meager change in arterial ACTH, the rapid increase in adrenal conductance may be the primary element in augmenting the cortisol secretory response. These findings suggest that brief exposure of conscious dogs to unexpected sensory stimuli represents a potent activator of adrenocortical and adrenomedullary responses. Supported in part by NIH Grants AM2681 and GM27946.

290.9 DISSOCIATION OF STRESS-INDUCED HYPERCHOLESTEROLEMIA AND CORTICOSTERONE RELEASE.
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Chronic or subchronic exposure to immobilization stress elevates plasma and aortic cholesterol levels. Based on the ability to prevent these effects with naltrexone (NTX), and mimic them with morphine, we have suggested possible endorphinergic mediation of stress-induced hypercholesterolemia (Bryant et al., 1986). It was the purpose of this study to examine other stressors for NTX reversible hypercholesterolemic effects and to compare these effects to activation of the HPA axis as evidenced by plasma corticosterone levels. Exposure of cholesterol (1%)-cholic acid (0.5%) fed rats to a 5 day unpredictable electric footshock (FS) regimen (three bouts of 2 or 4 hr FS; 2 sec duration, 0.5 mA, average of 30 shocks/hr) had no effect on plasma or tissue cholesterol levels. Exposure to a single, 2 hr FS session led to a slight increase in plasma cholesterol levels. In contrast a 5 day unpredictable high intensity noise stress regimen (three bouts of 2 or 4 hr 100 dB wide band noise) increased plasma LDL and VLDL transported cholesterol. However, this effect was not prevented by pre-treatment with NTX (1.0 mg/kg, sc). A single, 2 hr noise stress session produced similar results, with the magnitude of the hypercholesterolemic response being smaller. Interestingly, only the FS stimulus led to significant elevations in plasma corticosterone levels, both following the 2 hr and 5 day exposure periods. NTX administration did not prevent FS-induced elevation of plasma corticosterone. Noise stress had no effect on plasma corticosterone. Additionally, only the 2 hr exposure period to immobilization stress elevated plasma corticosterone, and NTX administration did not prevent this effect. Morphine pellet implants (75 mg, 5 day period), which elevated plasma cholesterol levels, had no effect on plasma corticosterone. These studies indicate that NTX reversible hypercholesterolemia is not a response common to all stressors and that this effect is not dependent on the release of corticosterone. (Supported in part by the American Heart Association, Indiana Affiliate, Inc., USPHS BRSG 55861, and an AFPE HAB Dunning Memorial Fellowship to HUB.)

290.10 CATECHOLAMINE AND CORTICOID VARIATIONS ASSOCIATED WITH PREPARED AND CONTRAPREPARED DEFENSIVE RESPONSES.
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A series of studies assessed the influence of stressor controllability on the turnover and concentrations of brain norepinephrine (NE) and dopamine (DA), as well as plasma corticoids. Exposure to footshock was found to increase the utilization of NE in several brain regions, although the effects of the stressor were more pronounced in some regions than in others. Under conditions where control over stressor termination was possible, NE levels were unaltered. However, following an equivalent amount of uncontrollable shock levels of NE were reduced. The stressor-provoked alterations of DA utilization, as determined from the accumulation of DOPAC, were more regionally specific than those of NE, being most apparent in the nucleus accumbens and in the mesolimbic frontal cortex. The DA variations were readily elicited, particularly in frontal cortex, and even a limited amount of footshock increased DOPAC accumulation. These changes, however, appeared regardless of whether mice had received controllable or uncontrollable footshock. Finally, increased plasma corticosterone concentrations associated with footshock were evident irrespective of stressor controllability. Moreover, the time course for the corticosterone variations were comparable in mice that had been exposed to escapable or inescapable footshock. Likewise, increased corticosterone levels were noted in mice required to emit either a highly prepared or contraprepared response. Interestingly, as animals gained mastery over the prepared response the corticoid levels approached those of control animals, whereas acquisition of the contraprepared response was not associated with a reduction of corticoid concentrations. Thus, while the variations of NE levels appear to be a consequence of lack of control over a stressor, the corticoid changes are readily elicited and tend to diminish as animals gain mastery over the task. Such an effect, however, also appears to be dependent upon whether animals are required to emit a response consistent with or contrary to its natural defensive style.

290.11 CARDIOVASCULAR AND RENAL RESPONSES TO INCREASED DIETARY SODIUM AND BEHAVIORAL STRESS IN THE BORDERLINE HYPERTENSIVE RAT (BHR). B.J. Sanders,* R.H. Cox, & J.E. Lawler. Physiology Program and Department of Psychology, University of Tennessee, Knoxville, TN, 37996.

The F1 cross between the spontaneously hypertensive rat and Wistar-Kyoto (WKY) rat develops spontaneous borderline hypertension (HT), and is called the BHR. This animal develops HT when exposed to conflict schedules as well as increased dietary sodium. The purpose of this study was to characterize the cardiovascular (CV) and renal responses to classical conditioning and a high sodium diet in BHR and WKY. Ten male rats from each strain were placed on diets containing either normal (.8%) or high (8%) amounts of sodium (Na^+) for 8 weeks and were then exposed to 5 days of classical conditioning (tone-shock pairings). CV and renal data were collected from arterial and bladder catheters, respectively, in conscious animals during control and stress sessions. The results indicate that the combination of acute stress (1 wk) and a chronic high Na^+ diet does not produce a greater degree of HT in BHR than exposure to a chronic high sodium diet alone (c. 180-190 mmHg). In addition, BHR on a high sodium diet exhibited no increase in blood pressure reactivity (change score from control period) in response to stress compared to BHR on a normal diet (-2 mmHg vs 19 mmHg). With respect to WKY, a high Na^+ diet diminished the adaptation and reactivity of blood pressure during stress compared to WKY on a normal diet. Since both norepinephrine (NE) and epinephrine (E) responses were blunted in WKY on high Na^+ during stress compared to normal sodium controls (NE: 263 vs 767 pg/ml; E: 216 vs 723 pg/ml), these CV responses may be a reflection of reduced sympathetic functioning. In contrast, the NE responses to stress were comparable in BHR on normal and high sodium diets (818 vs 736 pg/ml, respectively). Finally, the renal function data indicate that a high Na^+ diet and stress interact in the BHR to decrease Na^+ excretion and renal plasma flow during stress compared to control session (3.8 vs 4.8 uEq/min/100 g BW; 3.6 vs 4.0 ml/min/100 g BW, respectively). We conclude that genetic and environmental factors, such as behavioral stress and increased dietary sodium, are important variables for the development of hypertension and may interact at the level of renal functioning to contribute to the disease process. (Supported by HL-19680 and HL-01395).

290.12 SOUND STRESS-INDUCED (SIH) HYPERTENSION IN THE FISCHER-344 RAT (CDF):NEUROENDOCRINE AND NEUROCHEMICAL EFFECTS. J.A. Rosecrans, L. Graves*, S.E. Robinson, MC. Roadle-Biber, J.S. Hong, J.H. Johnson, D.J. Mokler and K.C. Corley. Depts. Pharmacol., Physiol. and Anatomy, Va. Commonwealth Univ., Richmond, VA 23298 and NIEHS, Res. Triangle Park, NC 27709

Male CDF rats (90 days of age) were exposed to a sound stress consisting of a 2 sec 110 db tone (Radio Shack Sound Alarm No. 49-52) using a VI-60 sec schedule of presentation for 2 hrs (118/hr). Rats were exposed to stress 3 times/week (Mon. Wed. Th.) in groups of 3 for 8 consecutive weeks. Individual blood pressures were measured on Fridays of each week using a Natume (KN-209) Tail Cuff apparatus. Baseline blood pressures were determined over 3 sessions prior to the initiation of the chronic stress paradigm; initial BP's averaged 108-110 mmHG (N=27). SIH was evident within 2 weeks of stress (130 mmHG, N=18) and increased to 140-150 mmHG following 7 weeks of stress. BP values increased to an average of 192 mmHG following 24 stress exposures (8 wks); control BP values (N=9) averaged 100 mmHG through out this experiment. Rats were exposed to a 25th stress (Mon. wk 9) and all rats sacrificed 24 hrs later. Plasma, pituitaries and selected brain areas were collected and frozen for later neuroendocrine/neurochemical analysis. The major finding of this experiment indicated that cortical tryptophan hydroxylase (+47%) activity and pituitary immunoreactive beta-endorphin levels (+30%) were significantly increased 24 hrs post to 25 sound-stress exposures. However, whether serotonin and/or opioid systems are involved in the development of SIH needs further clarification (Supported by NIH grants 5-T35-HL073-09 and NS-14090-06.

290.13 THE SEROTONIN (5HT) ANTAGONIST LY53857, THE 5HT AGONIST 8-OH-DPAT, AND INTRA-MIDBRAIN RAPHE 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) LESIONS DO NOT AFFECT CONDITIONED FEAR INDUCED INCREASES IN RENIN SECRETION. S.A. Lorens, S.M. Sainati and L.D. Van de Kar. Department of Pharmacology, Stritch School of Medicine, Loyola University of Chicago, Maywood, IL 60153.

We previously have shown (Brain Res. 311:333, 1984) that electrolytic lesions of the midbrain dorsal raphe nucleus (DR) block, whereas electrolytic median raphe nucleus (MR) lesions potentiate conditioned fear (CER) induced increases in plasma renin activity (PRA). Neither lesion altered the stress induced elevations in plasma prolactin levels. Subsequently, we found that the increases in PRA and plasma renin concentration (PRC) produced by the 5HT releaser, fenfluramine, and by the 5HT agonist, MK-212, were blocked in a dose-dependent (0.3 - 1.0 mg/kg, i.p.) manner by the 5HT-2 selective antagonist, LY53857. We also have found that low doses (5.0 - 50.0 ug/kg, i.p.) of the selective 5HT-1A receptor agonist, 8-hydroxy-2-(di-N,N-n-propylamino)tetralin (8-OH-DPAT), led to significant reductions in PRA and PRC. In addition, we have reported (Life Sci. 36:1149, 1985) that the anxiolytic 5HT-1A and dopamine autoreceptor agonist, buspirone (0.5 and 2.0 mg/kg, i.p.; 33 - 48 min. before sacrifice), blocked CER induced renin secretion. We thus examined the effects of the following treatments on CER (12 min.) evoked increases in PRA and PRC: 1) intra-mesencephalic raphe injections of 5,7-DHT (10 ug in 1.0 ul vehicle; 45 min. after pretreatment with nomifensine, 15 mg/kg, i.p.); 2) LY53857 (0.3 - 3.0 mg/kg, i.p.; 90 min. before sacrifice); and, 3) 8-OH-DPAT (50 - 250 ug/kg i.p.; 30 min. before sacrifice). Neither 5,7-DHT lesions of the MR, or of the MR plus DR, nor LY53857 and 8-OH-DPAT pretreatments, affected the stress induced increases in PRA and PRC. These observations suggest that stress induced renin secretion is not mediated by DR and MR 5HT neurons or by 5HT-2 receptors, and that activation of 5HT-1A receptors does not modify the renin response to stress.

290.14 INCREASES IN PLASMA NOREPINEPHRINE AND EPINEPHRINE DURING NATURALLY-ELICITED DEFENSE IN THE CAT. S.L. Stoddard, P.E. Wilson*, V.K. Bergdall* and B.E. Levin. Dept. of Anat., Indiana Univ. Sch. of Med., Ft. Wayne, IN 46805 and Dept. of Neurosci., N.J. Med. Sch., Newark, NJ 07103.

The purpose of this study was to evaluate sympatho-adrenal (SA) activation, determined by plasma levels of norepinephrine (NE) and epinephrine (E), during naturally-occurring defensive behavior. Defensive behavior was elicited by both interspecific (barking dog) and intraspecific stimuli (hypothalamically-stimulated cat). Plasma samples were withdrawn from a cannula placed through the external jugular vein to the atrial appendage; plasma levels of NE and E were determined by radioenzymatic assay. A second cannula, placed through the common carotid artery to the aortic arch, was used for the continuous monitoring of heart rate (HR) and mean arterial pressure (MAP). The cannulae were secured to the calvarium and were passed out of an observation box through a spring during behavioral tests. Baseline blood samples were taken prior to the 15-sec behavioral stimulus, and at 0.25, 0.5, 0.75, 1.0 and 3.0 min following stimulus onset. Three trials were run with each behavioral stimulus. Responses were measured by determining the absolute change from the baseline mean for each parameter. Interspecific defense behavior was accompanied by adrenal medullary release of both NE and E, which decreased with successive trials. Intraspecific defense behavior was initially associated with release of E from the adrenal medulla; successive trials evoked the release of NE from the sympathetic nerves. Comparison of the responses evoked by a dog or stimulus cat on each trial revealed that on the first trial the increases in all parameters were significantly greater when the defensive behavior was evoked by a barking dog ($\bar{x} \pm$ SEM: $\uparrow$ NE = 583 ± 129 pg/ml; $\uparrow$ E = 926 ± 260 pg/ml; $\uparrow$ HR = 47 ± 8 bpm; $\uparrow$ MAP = 32 ± 4 mmHg) than when the behavior was elicited by a conspecific ($\bar{x} \pm$ SEM: $\uparrow$ NE = 244 ± 49 pg/ml; $\uparrow$ E = 155 ± 55 pg/ml; $\uparrow$ HR = 28 ± 6 bpm; $\uparrow$ MAP = 17 ± 4 mmHg). The data suggest that although the empirical concomitants of defensive behavior were similar, the cat interpreted the two stimuli as potentially requiring distinct behavioral responses ("fight or flight"), and responded by differential activation of the components of the sympatho-adrenal system.

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290.15 THE EFFECT OF STRESS ON RENIN AND CORTICOSTERONE SECRETION IS BLOCKED BY ELECTROLYTIC LESIONS OF THE MESENCEPHALIC DORSAL RAPHE AND HYPOTHALAMIC PARAVENTRICULAR NUCLEI.
K.D. Richardson-Morton*, L.D. Van de Kar, S.A. Lorens, J.M. Paris, J.H. Urban* and K. Kunimoto*. Loyola University Medical Center, Maywood, IL 60153.

We have previously reported that electrolytic lesions of the dorsal raphe nucleus prevent the stress-induced increase in plasma renin activity (PRA), plasma renin concentration (PRC) and corticosterone following a ten-minute conditioned emotional (fear) response paradigm designed to produce psychological stress (Richardson-Morton, Van de Kar and Lorens, Fed. Proc. 45:674, 1986). We recently found that bilateral electrolytic lesions of the hypothalamic paraventricular nucleus (PVN) also prevent the stress-induced rise in PRA, PRC and corticosterone levels. The parvocellular cells were destroyed in all the rats. In a few rats there were still intact magnocellular cells in the lateral part of the PVN which escaped destruction. This effect is restricted to the PVN as electrolytic lesions of the dorsomedial nucleus, just caudal to the PVN, do not prevent the stress-induced increase in either PRA, PRC, or corticosterone levels. PRA is a measure of the production of Angiotensin I (AI) from endogenous substrate, while PRC is a measure of AI production under conditions of saturating levels of exogenous substrate. Since PVN lesions lead to a reduction of corticosterone levels, it could be argued that this would diminish the secretion of angiotensinogen (the substrate for renin) from the liver. Therefore PRA would be lower even if renin levels were not affected. It is important to note that both the increase in stress-induced PRA as well as PRC was prevented by DR and PVN lesions. These results suggest that the effects of stress on renin secretion are mediated by a pathway from the PVN through the dorsal raphe nucleus possibly to cardioregulatory centers of the medulla. Alternately, the pathway for stress-induced corticosterone secretion may originate at a medullary site, travel through the dorsal raphe and terminate in the PVN.
(Supported by Bane Charitable Trust).

290.16 EXOGENOUS CORTICOTROPIN-RELEASING FACTOR INHIBITS STRESS-INDUCED GASTRIC ULCERATION.
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Stress-induced ulcers are a cause of gastrointestinal bleeding in man. Exposure to restraint plus cold causes stress ulcers in rats. Corticotropin-releasing factor (CRF) has been implicated in the mediation of other responses to stressors. Therefore, we studied the role of exogenous and endogenous CRF in the causation of stress ulcers.

In our first experiment, chronic indwelling cannulae were inserted into the lateral ventricles of male Sprague-Dawley rats 5 days prior to experimental procedures. 24 hour food-deprived rats were then treated with 5 µl of CRF (1 µg/µl) or saline icv prior to exposure to one of three stress conditions: (1) home cage x 2 hours; (2) restraint plus cold (4°C) x 1 hour; or (3) restraint plus cold x 2 hours. After stress exposure, rats were immediately sacrificed and stomachs removed for examination. Ulcers were counted. We found that exogenous CRF significantly decreased the number of stress-induced gastric ulcers in rats as shown in Table I.

	n	Saline	CRF
		(# of ulcers; mean ± SEM)	
Home Cage	6	0.3 ± 0.3	0 ± 0
1 hr restraint + cold	6	12.2 ± 2.6	3.9 ± 0.9*
2 hr restraint + cold	7	16.1 ± 3.3	8.3 ± 2.1*

In a second experiment, rats with indwelling, lateral ventricular cannulae were exposed to one hour of restraint plus cold after pretreatment with two 5 µl injections of the following combinations: (1) saline + saline; (2) saline + CRF (1 µg/µl); (3) saline + alpha-helical CRF (9-41) (10 µg/µl); and (4) CRF (1 µg/µl) + alpha-helical CRF (9-41) (10 µg/µl). Number of ulcers are shown below.

Treatment + Stress	Mean Number Ulcers ± SEM	
Saline + Saline	8.4 ± 2.2	n = 7
Saline + CRF	1.8 ± 0.7*	n = 7
Saline + alpha-helical CRF (9-41)	7.0 ± 2.6	n = 5
CRF + alpha-helical CRF (9-41)	10.9 ± 2.3	n = 7

Again, exogenous CRF resulted in decreased stress-induced ulcer formation in stressed rats. This effect of exogenous CRF was reversed by the CRF antagonist, alpha-helical CRF (9-41). However, the CRF antagonist alone did not increase the number of ulcers seen after stress. This suggests that endogenous CRF is not involved in stress-induced ulcer formation or that the stressor used was too great to be reversed by the antagonist. The ability of the antagonist to reverse the effects of lower levels of stress will be tested.

290.17 CIMETIDINE TREATMENT AND THE REDUCTION OF GLANDULAR ULCERATION DURING EXCESSIVE WHEEL RUNNING IN THE RAT.
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When a laboratory rat is maintained on a 1 hour per day restricted feeding schedule and housed in an activity wheel, the rat runs progressively more each day until wheel running becomes excessive, glandular ulcers form, and eventually leads to the demise of the animal. Previous research indicated that administration of cimetidine during restraint stress reduced the severity of acute glandular ulceration (Hemmer et al., Res. Exp. Med., 1980, 176, 207-217.); however injections of cimetidine, administered 14 hours following the feeding period, failed to reduce effectively the severity of ulceration in a wheel running situation (Pare et al., Pharmacol. Biochem. Behav., 1978, 8, 711-715). In the present study, the anti-ulcerogenic effectiveness of cimetidine during excessive wheel running was re-evaluated when injections were administered during the 6 hour period prior to the one hour feeding period.

To obtain baseline measurements of food and water intake, body weight, and running activity (recorded in three daily time periods), 14 naive female rats were housed in an activity cage for 5 days. Animals were then matched by mean baseline activity and divided into two experimental groups. One group received daily injections of cimetidine in doses of 100 mg/kg; the other group received ip injections of saline. Following baseline measures, animals re-entered the activity wheels and were fed for one hour between 1930 and 2030. Injections were administered twice daily at the following times: 1300 and 1630. All animals spent 18 days in the activity wheel or were sacrificed when the following signs were observed: a sharp peak in running activity accompanied by a reduction in daily food intake. Following sacrifice, the stomach was excised and later scored for gastric ulceration.

Results showed that both cimetidine and saline animals increased running above baseline rates during testing; the greatest increase occurred during the time period just prior to the one hour feeding period. Saline animals ran significantly ($p<.05$) more than cimetidine animals during the 1110-1830 running period. In addition, animals which received injections of saline had a significantly greater ($p<.05$) number of ulcers. The data from the present experiment demonstrate that cimetidine is beneficial in reducing acute gastric ulceration during times of excessive running and suggests that the anti-ulcerogenic properties of cimetidine buffer the stomach when it is most empty. Therefore, it is proposed that the gastric irritation may serve as an impetus for exaggerated wheel running.

291.1 RE-EXAMINATION OF THE INNERVATION OF THE THYMUS GLAND IN MICE AND RATS. D.M. Nance, D.A. Hopkins and D. Bieger. (SPON: P.C. Jackson). Dept. of Anatomy, Dalhousie Univ., Halifax, N.S. B3H 4H7 and Faculty of Medicine, Memorial Univ., St. John's, Newfoundland, A1B 3V6 Canada.

Central to the postulated relationship between the brain and the immune system has been evidence for the direct neural innervation of primary organs of the immune system. For example, a substantial innervation of the thymus gland by the 'retrofacial' nucleus and nucleus ambiguus of the brain stem with ancillary input from neurons in the dorsal motor nucleus of the vagus nerve and upper cervical spinal cord has been reported (Bulloch and Moore, Am. J. Anat., 162:157,1981). In view of the close anatomical proximity of the thymus to thoracic viscera and neck musculature known to receive motor fibers from these areas of the spinal cord and brain stem nuclei in question, we examined the possibility that labeled cells identified in the brain stem following injections of anatomical tracers into the thymus were secondary to the uptake of the tracers by the esophagus and surrounding tissue. Less than 1.0 ul of a 5% wheat germ agglutin - horseradish peroxidase solution (WGA-HRP) or else a 30% HRP solution was injected into the thymus, the adjacent esophagus and the mid-cervical vagal trunk of mice and rats. In addition, the thymus was also examined for the presence of nerve fibers containing catecholamines or cholinesterase activity. Finally, the effects of unilateral cranial vagotomy on cholinesterase activity in the thymus were examined. Tracer injections limited to the thymus gland produced little or no labeling in the brain stem. This result contrasted strikingly with the results obtained after injections into the esophagus in which numerous intensely labeled cells were observed in the nucleus ambiguus irrespective of the level of the esophageal injection, as well as in other regions previously thought to be related to innervation of the thymus (Bullock and Moore, 1981). Thus, it can be concluded that injections of tracers into the thymus that produce labeling in the brain stem are due to diffusion into surrounding tissue including the esophagus and neck musculature. The histofluorescence results confirmed a major sympathetic innervation of the thymus and the cholinesterase histochemistry revealed an elaborate subcapsular nerve plexus. The cholinesterasic fibers in the thymus were unaltered by a unilateral cranial vagotomy whereas cholinesterase activity was clearly altered in the brain stem nuclei by these surgeries. Anterogradely transported HRP was not detected in the thymus after injections of HRP into the cervical vagal trunk. In conclusion, the thymus does receive a substantial innervation which could be relevant to the control of the immune system, but the brain stem, in particular the ventral vagal complex, does not appear to contribute directly to this innervation. Supported by MRC of Canada.

291.2 ABSENCE OF A SEXUAL DIMORPHISM IN THE BRAIN OF THE ATHYMIC, NUDE RAT. D.F. Bloom* and P.E. Micevych (SPON: D. Whitmoyer). Depts. of Psychology and Anatomy, and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

The sexual differentiation of the nervous system is thought to result from the actions of gonadal steroids on the brain and spinal cord during a perinatal critical period. This differentiation results in distinctive male or female patterns of sexual behavior and LH release. Although the thymus gland has not previously been implicated in the process of neuronal sexual differentiation, it is recognized that genetically athymic (nude) mice or mice neonatally thymectomized exhibit reproductive abnormalities that include reduced fertility and diminished levels of circulating LH, FSH, and gonadal steroids. Since hypothalamic areas are involved in the control of these endocrine events and since the levels of LHRH are diminished in the nude mouse, a possible developmental abnormality in the hypothalamus is suspected. Interestingly, nude mice are also reported to be missing the usual sexual dimorphism in body weight, as well as the recognized dimorphisms present in kidneys and submaxillary glands. The present study examined the effect of athymia on brain sexual differentiation. In the normal rat, the sexually dimorphic nucleus of the preoptic area (MPNc) is a steroid-dependent nucleus that is measurably much greater in volume in male brains than in female brains. We examined the dimensions of this nucleus in a genetically athymic strain of rat. The brains of 10 homozygous nude male rats, 10 homozygous nude female rats, and 10 heterozygous male and female rats of the NIH strain were frozen sectioned at 50 µm thick in the transverse plane and stained with thionin. Three observers outlined the limits of the nucleus throughout its rostral to caudal extent using a camera lucida projection system. Volumes were statistically compared according to published methods (Gorski et al., J. Comp. Neurol., 193:529-540, '80). The brain of the heterozygous NIH rat was observed to have a frank sexually dimorphic MPNc; analysis of the homozygous, athymic brain revealed a diminished sexual dimorphism that was due to a decrease in the volume of the nucleus in the male animal. The cause of this diminished MPNc is unclear. However, it is possible that depressed testosterone levels are present in the nude rat (as they are in the nude mouse) during the critical perinatal period for brain masculinization. Such depressed steroid levels might inhibit the full expression of a masculine MPNc. Thus in the nude rat, the lack of a thymus and the absence of that gland's known interactions with the hypothalamic-pituitary-gonadal system, seem to prevent the normal development of the male brain.

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291.3 DENERVATION OF SPLENIC SYMPATHETIC FIBERS IN THE YOUNG ADULT RAT. D.L. Felten, D.L. Bellinger, K.D. Ackerman*, and S.Y. Felten (SPON: B. Weiss). Dept. of Neurobiol. and Anat., Univ. of Rochester, Sch. Med., Rochester, NY, 14642.

The origin of noradrenergic (NE) and cholinesterase-positive profiles in the spleen was examined in young adult male Sprague-Dawley rats. Surgical removal of the superior mesenteric-celiac ganglia and/or bilateral transection of the subdiaphragmatic vagus nerves was followed by a 5 day survival period. Sham operated and unoperated rats were used as controls for the procedure. The innervation pattern of NE fibers was similar in sham operated and unoperated controls; fibers distributed around the capsule, trabeculae, and vasculature, and within the parenchyma of the white pulp. Complete ganglionectomy resulted in total denervation of NE fibers in all compartments of the spleen including the vasculature, the capsule, the trabeculae, and the parenchyma. The distribution pattern of NE fibers in spleens of the vagotomized rats was similar to that observed in unoperated and sham operated controls. High performance liquid chromatography with electrochemical detection for catecholamines and stereological quantitative evaluation of the density of NE varicosities confirmed these observations. Cholinesterase-positive fibers were much less abundant than NE fibers in the spleens of controls, and were localized beneath the capsule or in the parenchyma near the capsule. Complete removal of the superior mesenteric-celiac ganglia resulted in total loss of these fibers. No loss of cholinesterase-positive fibers was seen following bilateral subdiaphragmatic vagotomy. In support of these findings, cholinesterase-positive neurons were found in the superior mesenteric-celiac ganglia, suggesting that they may be the source of the cholinesterase-positive fibers in the spleen. Since the distribution of cholinesterase-positive and NE profiles overlap in the spleen, it is possible that they coexist. It also is possible that the spleen is innervated separately by both NE and cholinergic neurons. The presence of both NE and cholinesterase-positive cell bodies in the superior mesenteric-celiac ganglia, and the disappearance of both NE and cholinesterase-positive fibers in the spleen following ganglionectomy, but not vagotomy, points towards a common origin in collateral ganglia for both adrenergic and cholinergic innervation of the spleen. The vagus nerve does not contribute NE or cholinesterase-positive fibers directly to the spleen. Both vagotomized and ganglionectomized animals demonstrated a significant reduction in body weight, probably due to reduced food consumption. The ratio of splenic weight/body weight was increased significantly by 35.8% in ganglionectomized animals compared with both control groups. This finding suggests that changes in splenic architecture, such as an alteration in the number of specific types of immune cells, may follow autonomic denervation of the spleen. Supported by Grant N00014-84-K-0488 from the Office of Naval Research, and by a MacArthur Foundation PrizeFellowship (DLF).

291.4 PARALLEL DEVELOPMENT OF CATECHOLAMINE INNERVATION AND T CELL DOMAINS IN THE RAT SPLEEN. K.D. Ackerman*, D.L. Bellinger, S. Livnat*, S.Y. Felten, and D.L. Felten (SPON: D.L. Felten). Dept. of Neurobiology and Anatomy, and Dept of Psychiatry, Univ. of Rochester, Sch. Med. Rochester NY 14642.

Sympathetic noradrenergic innervation is present in the parenchyma of several lymphoid tissues and appears to distribute extensively in T lymphocyte zones. At present it is unclear how these cellular components of the nervous and immune systems interact during development and in the adult. To address this issue, spleens from Long-Evans Hooded rat pups at 1,3,6,13,20, and 60+ postnatal days of age were examined with fluorescence histochemistry for catecholamines and with immunocytochemistry for surface markers on T lymphocytes.

In the young adult spleen, T cells were located primarily in the inner region of the periarteriolar lymphatic sheath (PALS), surrounded by other lymphoid cells and an outer marginal zone. Other putative T cells were scattered within the red pulp, mostly in subcapsular areas, but they represented a small percentage of the total spleen T cell population. The noradrenergic innervation of the spleen was associated with the capsule, the trabeculae, the vasculature (primarily the central artery and its branches), and the parenchyma of the white pulp. In 1 day old animals a separate PALS was not evident, and the T lymphocytes were evenly distributed. A large portion of the spleen was hematopoietic. The sympathetic innervation was confined to a few scattered fibers that traveled with blood vessels. By 3-6 days of age periarteriolar sheaths of T cells averaging less than 50 µm in diameter were detected, while outside of these areas, the T cells were randomly distributed. Sympathetic innervation remained sparse at this age, but was slightly increased in density compared with spleens at 1 day of age. Punctate profiles of noradrenergic nerves were first observed exiting from the vascular plexus into the parenchyma at 6 days of age.

As development progressed there was a relative increase in the absolute amount of white pulp with decline of the hematopoietic areas; T cells were less randomly distributed, and T cell zones became more distinct, associating with central arteries and their branches. Alternate sections stained for T cell markers suggest a close, but not exclusive relationship between noradrenergic fibers and lymphocytes in these T cell zones. By 14 days of age, the noradrenergic innervation extended into the trabeculae and capsule, and showed a progressive increase in density in all areas from 14 days to adulthood.

A growing body of evidence suggests neural modulation of immune function. Since lymphocyte development and migration are sensitive to changes in the microenvironnment, the ingrowth of noradrenergic fibers at the time when T cells and the PALS are developing may provide an important modulatory influence over the establishment of immune structure and function.

This work was supported by Grant N00014-84-K-0488 from the ONR, and by Grant T32GM07356 from the National Institute of Health.

291.5 SYMPATHETIC INNERVATION OF SPLEEN IN YOUNG ADULT and AGING RATS: A QUANTITATIVE STUDY. D.L. Bellinger, S.Y. Felten, T.J. Collier, AND D.L. Felten. Dept. of Neurobiol. and Anat., Univ. of Rochester, Sch. Med., Rochester, NY, 14642.

Sympathetic noradrenergic (NE) innervation of the spleen in 8 month old and 27 month old Fischer 344 rats was examined using fluorescence histochemistry, and high-performance liquid chromatography with electrochemical detection for measurement of NE. The 27 month old rats were subdivided into two additional groups based upon their response to novel gustatory stimuli (gustatory neophobia), in which an avoidance response has been associated with acute locus coeruleus lesions in young rats, and with diminished NE levels in central neurons in aging rats. This permitted us to ascertain whether diminished central NE function also was associated with diminished peripheral NE function of the spleen. In 8 month old rats, the spleens displayed abundant innervation. NE plexuses followed the vasculature and trabeculae into splenic white pulp, mainly in association with the central arteries and their branches. Areas of white pulp were large and well defined, and contained linear and punctate NE profiles that branched from vascular and trabecular plexuses into the parenchyma, and ended among T lymphocytes and other cell types. In both groups of 27 month old rats, sympathetic innervation was diminished in density and in overall intensity of fluorescence. Morphometric analysis revealed a significant decrease in the NE profiles associated with the vasculature (79.3%), trabeculae (68.5%), capsule (56.4%), and parenchyma (64.9%) compared with their younger counterparts. Splenic NE levels also were reduced significantly to approximately 50% of the levels in the spleens of 8 month old rats. White pulp in the 27 month old rat spleens was diminished in size, and often was difficult to delineate from red pulp. While central NE levels were selectively diminished in old rats that displayed gustatory neophobia, diminished peripheral sympathetic NE content in spleen appeared to be a consistent feature in aged rats. With age, diminished immune function is well documented, especially in the cellular arm of immunity. The demonstration of NE fibers that end preferentially among fields of T lymphocytes in spleens of young control rats provides a basis for neural modulation, possibly through an interaction with T lymphocytes. The association, in normal aging, of both a decline in immunocompetence and a decline in splenic NE innervation opens the possibility of a causal relationship between these phenomena. However, NE denervation may be compensatory rather than causal to immune dysfunction. Supported by Grant N00014-84-K-0488 from the Office of Naval Research, Grant MH 08829 (TJC), and by a MacArthur Foundation Prize Fellowship (DLF).

291.6 SYMPATHETIC NORADRENERGIC INNERVATION OF THE MURINE SPLEEN REMAINS STABLE FOLLOWING LOSS OF LYMPHOCYTES FROM TREATMENT WITH IMMUNOSUPPRESSIVE AGENTS. S.L. Carlson, S.Y. Felten, S. Livnat* and D.L. Felten. Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

The sympathetic noradrenergic (NE) innervation of the mouse spleen follows blood vessels and branches into the periarterial parenchyma of the white pulp, an area comprised primarily of T lymphocytes. These lymphocytes may be a target of the NE in the white pulp, with a paracrine-like secretion of NE from the varicosities into the parenchyma. Immunosuppressive drugs such as cyclophosphamide and the hormone corticosterone are known to deplete the spleen and other lymphoid tissues of lymphocytes, reducing the size of the white pulp. In this study, we have examined the effects of these agents on the innervation of the spleen.

Twenty-four young adult male C3H/HeJ mice were injected intraperitoneally with one of the following: a) saline, b) hydrocortisone acetate (2.5 mg/mouse), H; c) high dose of cyclophosphamide (250 mg/ kg), Cy-H; or d) low dose of cyclophosphamide (50 mg/kg), Cy-L. After two days, the animals were sacrificed, and the spleens were removed, weighed, and divided into three pieces for analysis of the anatomy, catecholamine innervation, and NE content.

The whole spleen wet weights were decreased 22% in the H group, 54% in the Cy-H, and 21% in the Cy-L group. Using HPLC for measurement of NE content, there was no change in pmol NE/whole spleen in any of the groups, suggesting that the total amount of sympathetic innervation did not change. However, as expected, because of the smaller spleen size, the pmol NE/mg protein increased 27% in the H group, 153% in the Cy-H group, and 6% in the Cy-L group. These data reveal that the sympathetic innervation is stable despite large changes in the splenic lymphocyte content induced by immunosuppressive agents.

SPG histofluorescence of the catecholamine innervation confirmed the presence of extensive innervation of the vasculature, and fibers that radiated into the white pulp following the various treatments, even with a marked overall reduction in the extent of the white pulp. In the spleens of drug or steroid treated groups, some fibers appeared more densely packed than in spleens of the controls. This presumably is due to the changing geometry of the white pulp induced by the immunosuppressive agents. In addition, some plexuses of NE fibers appeared particularly dense and tangled around the central artery and its branches in the drug or steroid treated groups. However, the overall pattern of compartmental distribution did not appear to be altered by drug treatment. If the activity of the splenic nerve fibers and subsequent release of NE remain constant following these immunosuppressive agents, then the effective concentration of NE in splenic white pulp may increase, perhaps playing a role in the recovery phase following loss of lymphocytes.

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291.7 USE OF *IN VIVO* DIALYSIS FOR EVALUATION OF SPLENIC NOREPINEPHRINE AND SEROTONIN. S.Y. Felten, J. Housel*and D.L. Felten. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The technique of *in vivo* brain dialysis developed by Clemens and Phebus (Life Sci. 35:671-677) has been used effectively for chronic monitoring of dopamine in the striatum of awake rats. We adapted this technique for use in chronic monitoring of rat splenic norepinephrine (NE) as part of a study of changes in splenic norepinephrine release during immune system challenges and manipulations. In the rat, splenic norepinephrine levels are normally substantial, averaging 76 pMol/gm wet weight (the average weight of an adult rat spleen is 0.7 gm). Histofluorescence studies show that the the white pulp is richly innervated with NE fibers indicating that at any given time, much of the NE is not available to its target without stimulated release. Therefore, measurement of total splenic NE gives little information about the concentration of NE in actual contact with target cells. Our *in vivo* dialysis system samples NE (and other small molecules) that have been released into the interstitium, providing a better approximation of the amount of NE bathing target cells.

Probes were constructed of cellulose dialysis fibers (~250 μm) containing a Diamel coated stainless steel wire (0.0013") cemented into Microline tubing (0.020" i.d.) with 1 cm of dialysis tubing exposed. A probe was threaded lengthwise through the spleen of a rat in such a way as to leave all the dialysis tubing inside the spleen. The tubing was brought through the body wall and threaded subcutaneoulsy to a stainless steel anchor located on the back between the scapulae. The tubing was then flushed with a small amount of distilled water and capped to prevent destruction by the animal. Rats were allowed to recover from surgery for 1 wk before measurements were made.

For our preliminary study to assess whether adequate NE could be collected, rats were anesthetized before each sample was collected. However, it is possible to attach the tubing to a swivel and collect samples from awake, behaving animals. Saline was perfused through the dialysis tubing at a rate of 5 μl/min for 20 min. The resulting sample was then injected directly onto our LCEC system. NE (and other monoamines and metabolites) was detected using a thin layer glassy carbon electrode and BAS amperometric controller. Sample recovery was estimated by perfusing dialysis tubing suspended in a solution of standards in saline, and averaged 25%.

NE levels corrected for recovery averaged 4.28±0.60 pMol or approximately 8% of total splenic NE. Serotonin was also measured and found to be 8.8±1.2 pMol. These pilot studies have shown that it is feasible to measure average concentrations of NE (and 5HT) available to target cells. Coupled with time based manipulations of the model, it can also provide an index of NE release. Combining splenic *in vivo* dialysis with manipulations of the immune system should greatly enhance our understanding of the role played by NE in neuroimmunomodulation.

This work was supported by grant N000 14-84-K-0488 from the Office of Naval Research.

291.8 NATURAL KILLER (NK) CELL ACTIVITY IN SPLEEN AND PERIPHERAL BLOOD AFTER HYPOTHALAMIC DAMAGE. C.P. Phelps, L. Chen* and J. Djeu*, Depts. Anat. and Med. Micro., Coll. Med., Univ. S. Fla., Tampa, FL 33612.

Destruction of the anterior hypothalamic area (AHA) alone, or in addition to the tuberoinfundibular region is reported to reduce spleen NK activity in rats (Cross, et al., Immunol. 51:399) and mice (Forni, et al., Nature 306:181) respectively. We have investigated whether there are correlations between the location of hypothalamic damage in mice and NK activity. Male mice (C56BL/6, 18-22g) received either 1) knife cuts (FC) stereotaxically located at the rostral border of AHA or 2) destructive lesions (FL) aimed at the AHA, both made by rotating knives (1.2 mm radii). Operations were performed aseptically under pentobarbital (35 mg/kg) and separate groups received sham surgeries consisting of midline knife descents to just above the AHA or remained intact. One month later all mice were rapidly asphyxiated in CO_2 and their spleens and a blood sample were removed for study of NK activity using YAC-1 target cells and natural cytotoxic (NC) activity using WEHI-164 cells. Brains were removed and fixed for histological study of lesion size and location. Statistical analysis of group mean body, spleen, thymus and adrenal weights revealed no significant overall treatment effects. Lesion of AHA resulted in an overall significant ($p<0.01$) effect on NK activity in peripheral blood (PB), including a significant increase ($p<0.03$) in PB mean % cytotoxicity after FL (25.2±5.8) when compared with sham FL (10.9±2.5). FL limited to AHA destruction based on histological examination was correlated with reduced spleen NK activity, whereas partial AHA destruction or more rostrally located lesions were associated with increased spleen and PB NK activity. Elevation of NK activity in the latter individual cases was consistently associated with a reduction in NC activity in spleen, but not in PB. However, if group mean NC activities after brain damage are considered, this relationship is not apparent. FC produced elevations in PB NK activity, but no change in group mean splenic NK activity. Viewed together, these results demonstrate a selective effect on PB NK activity 4 wk after relatively small lesions (300-600μ) located in the rostral AHA. When the latter was partially disconnected (FC) or partially destroyed there was a greater tendency for PB NK to become elevated. These findings suggest that rostral and dorsal afferents to the AHA or more posterior regions may include neural elements inhibitory to peripheral blood NK activity.

291.9 EFFECT OF CHEMICAL SYMPATHECTOMY ON ONTOGENY OF ANTIBODY RESPONSES IN THE RAT. W.L. Dempsey* and L.L. Ross. Dept. of Anatomy, Medical College of PA., Philadelphia, PA. 19129

Neurotransmitters of the peripheral autonomic nervous system are capable of modulating immune responses. The morphological link in this regulatory role has been established by the demonstration of the extensive innervation of lymphoid organs by the sympathetic nervous system (SNS). Autonomic regulation of the immune system may occur at many stages during the response to antigen as well as during ontogeny. Most studies of autonomic immune modulation have been concerned with adult end-stage immune responses. This present study is concerned with the neonate and examines the influence of the SNS on the ontogenetic development of immunocompetent cells.

Sympathetic denervation of the neonatal rat was obtained by the administration of 6-hydroxydopamine (6OHDA). Inbred Lewis rats were treated on days 1,3,5 and 7 of life with 6OHDA (100mg/Kg, s.c.). Antibody plaque-forming cell responses (PFC) to the T-dependent antigen, sheep red blood cells, and the T-independent antigen, pneumococcal polysaccharide type III (SSS-III) were measured at various times after birth. Antibody responses to SRBC began to appear by day 10 of life and reached adult levels by day 30 in both 6OHDA-treated and control animals. Although 6OHDA-treated and control animals exhibited similar SRBC-specific PFC responses on day 10, responses were depressed in 6OHDA-treated animals on day 20 of life. PFC responses in 6OHDA-treated animals, however, returned to control levels by day 30. In contrast, PFC responses to SSS-III did not appear until day 30 in sympathectomized or control rats. 6OHDA-treated rats exhibited lower SSS-III specific PFC responses than did controls on days 30 and 45 of life. No differences were observed in the total number of spleen cells between control and 6OHDA-treated rats suggesting that neonatal 6OHDA treatment does not exert any overall cytotoxic effects on spleen cells. These results are consistent with the hypothesis that the presence of an intact sympathetic innervation of the lymphoid organ is necessary for the normal ontogenetic development of antibody responses. The effects of sympathetic denervation vary with the inducing antigen system and may reflect a differential sensitivity of subpopulations of immunoreactive cells to products of the sympathetic nervous system.

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291.10 NEUROTRANSMITTER EFFECTS ON CELL SURFACE ANTIGENS V.P.CALABRESE, J.YOSHINO*, G.STONE*, AND D.MADDEN*, Depts of Neurology and Biochemistry, Med Col of Va, Richmond, Va. and Infec. Dis. Branch, NINCDS, NIH, Bethesda, Md.

Our laboratory has been studying the effects of neurotransmitters on human blood mononuclear cells and have shown that epinephrine and serotonin, as well as related drugs can markedly alter the DNA turnover of lectin stimulated cells. We have extended these studies to see what effects norepinephrine (NE) has on T subset antigens expressed on the cell surface.

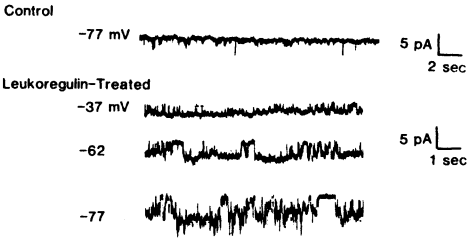
Human peripheral blood mononuclear cells were prepared by density gradient centrifugation and placed in culture flasks. NE (final conc $1x10^{-4}$ M) was added first followed within a few minutes by either pokeweed mitogen or concanavalin-A. At the end of the incubation period some of the cells were pulsed with ^{3}H thymidine and others treated with FITC monoclonal antibodies to surface antigens.

We found that seven days incubation with NE produced a 40-60% decrease in thymidine uptake. Analysis of surface markers showed only a modest decrease in the pan T marker OKT-3, OKT-11 and NEI-42 but a very significant decrease in both OKT-4 and OKT-8 markers. In one experiment there were less than 1% OKT-4 and 8 cells. When these cells were stained and observed microscopically they appeared to be mostly small lymphocytes whereas the lectin stimulated cells were large and plump. The IA marker remained unchanged but there was an increase in OKM1. When the study was repeated using NE with unstimulated cells we found no difference in surface markers between the cells which had NE and virgin cells. A time course study showed a marked decrease in OKT-4 by day three incubation.

These findings are consistent with the observation that NE inhibits the functional activity of mononuclear cells. Our finding may indicate that this depression may not be due to just decreased cell proliferation. In fact our cultures showed significant increase in cell numbers at seven days even though the thymidine uptake was depressed at that time. There may be a decrease in the functional capability of the cells. Further studies are being directed to answer this question. In addition, the effects of NE seem to occur only on cells which have been stimulated, i.e. they must be out of the G_0 phase.

291.11 LEUKOREGULIN, AN ANTITUMOR LYMPHOKINE, INDUCES SINGLE ION CHANNELS IN TARGET CELLS. P. A. Sheehy*, S. C. Barnett*, C. H. Evans* and J. L. Barker. LNP, NINCDS & LB, NCI, NIH, Bethesda, MD. (Spon: M. Henkart)

Leukoregulin (LR) is an immunologic hormone that inhibits the growth and increases the sensitivity of tumor cells to natural killer (NK) cell cytotoxicity. We have begun to investigate the mechanism of LR's action by characterizing its effects on the membrane permeability of target cells using the K562 cell-line model of NK-susceptible targets using the patch-clamp technique. Electrodes contained (in mM) 140 Kaspartate, 5 NaCl, 0.1 $CaCl_2$, 1.1 EGTA, 0.6 $MgCl_2$, 10 HEPES, pH 7.3. Discrete current steps induced by the addition of LR could be resolved while recording in the whole-cell mode. Soon after application (1-2 minutes by bath application, seconds by pressure pipette) large inward current steps were observed at a holding potential of -80 mV. Extrapolated reversal potentials (E_r) ranged between -20 & -30 mV. Using -25 mV as E_r, single channel conductance is approx 100 pS. Several qualitative features of the channel's kinetics are striking. i) superimposed openings have only been observed in 1 of 20 experiments. ii) resolvable openings diminish over 5-10 minutes, never to recur iii) the channel typically opens for periods on the order of hundreds of msec iv) cell is refractory to subsequent reapplication of LR. We conclude that LR has a direct action on K562 cells to induce the opening of transmembrane ion channels.



291.12 NEUROTRANSMITTER RECEPTORS IN AN AVIAN LACRIMAL GLAND K.L. Tencer*, J. Schmidt, M. Schneider* and B. Walcott
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The avian lacrimal gland is densely innervated by cholinergic and adrenergic fibers. These nerve fibers are branched and varicose among the large population of interstitial cells, most of which are lymphocytes. The majority of the lymphocytes have been defined as "plasmablasts" and are reported to produce the immunoglobulins in tears, the fluid secreted by the cortical epithelium of the gland. The anatomy, therefore, strongly suggests neuroimmunomodulation. To test this hypothesis and to examine the neural control of tear secretion, we have used radiolabeled ligands to establish the presence of cholinergic and adrenergic receptors on the two major cell types that make up the gland. Glands were disrupted by hand in a ground glass homogenizer, washed by ultracentrifugation and the resulting membrane pellet was resuspended. Aliquots of the membrane suspension were incubated to equilibrium with radiolabeled ligands with or without specific blockers. Portions of each sample were transferred to Whatman GF/C glass fiber filters where they were washed, dried and counted. [^{125}I]-bungarotoxin (BTX) showed no significant binding suggesting the absence of this class of nicotinic acetylcholine receptor (AChR) while [^{3}H]- quinuclidinyl benzilate (QNB) showed binding of 23.03 + 3.71 pMol/mg wet tissue. QNB binds with a K_D of 1.07 + 0.36 nM and is blocked by 1.5 uM atropine implying the presence of muscarinic AChR. The beta-adrenergic ligand, [^{3}H]-dihydroalprenolol (DHA), binds with characteristics suggesting the presence of both high and low affinity receptors. The high affinity receptors have 6.2 + 2.3 pMol sites/mg wet tissue and bind with a K_D of 2.26 + 0.33 nM. DHA binding is blocked by 10 uM propranolol. The spatial distribution of binding of the ligands (at concentrations corresponding to the K_D values) to cryosections of the gland was determined autoradiographically. BTX showed no significant binding to glandular tissue although discrete patches were seen on the adhering extraocular muscle. QNB and DHA bound specifically to both cell populations. These data confirm the absence of the BTX class of nicotinic AChR while confirming the presence of the muscarinic AChR and beta-adrenergic receptors which appear to be distributed over both the epithelial and lymphoid cell populations.

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291.13 THE INDUCTION OF INTERLEUKIN-2 RECEPTORS ON CULTURED HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS (PMC) BY NERVE GROWTH FACTOR. L.W. Thorpe*, K. Werrbach-Perez*, C. Beck*, and J.R. Perez-Polo. Dept. HBC & G, Univ. Tx. Med. Br., Galveston, TX. 77550

The concept of neuroimmunomodulation arose from studies on the immunoregulatory properties of certain hormones and neuropeptides, and other central nervous system effects on immune response. It has been suggested that nerve growth factor (NGF) might play a role in the regulation of immune function. NGF is a neurono- trophic peptide known to act on peripheral and central neural structures. We have demonstrated the presence of NGF receptors on lymphocytes and accessory cells and have also reported that NGF stimulated proliferation of cultured lymphocytes. Proliferation of lymphocytes is dependent on the interaction of the growth factor interleukin-2 (IL2) and receptors for IL2 (IL2R) on lymphocyte membranes. Prior stimulation of resting lymphocytes by antigen or mitogen is required for the induction of IL2R. Since IL2R induction is necessary for lymphoproliferation to occur, we examined the effects of NGF stimulation on IL2R induction on cultured human PBMC. Cells were cultured for 0-4 days with varing concentrations of either human or mouse beta-NGF (0.1-10 ug/ml). IL2R bearing cells were visualized and quantitated by fluorescent microscopy after staining by monoclonal anti-IL2R antisera and fluoresceinated second antibody. PHA stimulated cultures were used as positive controls. Over the 4 day period of culture there was an increase in the number of IL2R positive cells in NGF stimulated versus unstimulated cultures. The results of our findings are summarized in the following table and represent pooled data from 5 separate experiments.

PERCENT IL2-R POSITIVE CELLS

DAY	MEDIA	NGF	PHA
0	0	0	0
1	7±6	11±4	49±5
2	5±1	13±5*	92±10
3	12±5	25±7*	91±8
4	11±1	24±3*	92±12

*.statistically significant

Our results demonstrate that NGF is capable of inducing IL2R on cultured human lymphocytes in the absence of antigen or mitogen. Although the mechanism of this receptor upregulation is unknown, preliminary evidence would suggest that NGF is acting, at least in part, at the lymphocyte level rather than the accessory cell level. Continuing studies in our laboratory are currently focused on the mechanisms of NGF induced receptor expression, as well as the phenotypic specificity of IL2R bearing cells. Supported by NINCDS NS18708 and Robert Welch Grant H698.

291.14 CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN MOUSE SPLEEN: IDENTIFICATION AND CHARACTERIZATION. Elizabeth L. Webster*, Michael J. Kuhar, and Errol B. De Souza (SPON: Mathew Lo). Neuroscience Branch, Addiction Research Center, National Institute on Drug Abuse, Baltimore, Maryland 21224.

CRF is the primary physiological regulator of pro-opiomelanocortin (POMC)-derived peptide secretion from the pituitary gland. In addition, recent data suggest that endogenous CRF functions as a neurotransmitter in the CNS. There is a growing body of literature indicating that the CNS exerts an important regulatory control on the immune system. The neuroendocrine and immune systems share certain peptide hormones suggesting that the two systems may interact reciprocally. Blalock and coworkers have demonstrated that in vitro, CRF and immunostimulation elicit the secretion of POMC-derived peptides which in turn act to regulate immune responses (J. Immunol. 135: 858s, 1985). Furthermore, leukocyte-derived products (interleukin-I, hepatocyte-stimulatory factor) modulate POMC-derived peptide secretion from the pituitary (Science 230: 1035, 1985).

In order to examine whether the effects of CRF on immune function are mediated via specific receptors, we used mouse spleen to identify and characterize CRF binding sites. Binding was linear with membrane protein concentration, saturable, and of a high affinity with apparent K_d values of 460 + 42 and 305 + 58 pM and B_{max} values of 15 ± 2.5 and 8.6 + 0.3 fmoles/mg protein (mean + SEM; n = 3) for ^{125}I-Tyr^0-rat/human CRF and ^{125}I-Tyr^0-ovine CRF, respectively. The pharmacological specificity of the CRF binding site was examined in competition experiments. Rat CRF(1-41)NH_2, ovine CRF(1-41)NH_2, the putative weak receptor antagonist α- helical CRF(9-41)NH_2 and the biologically more potent analog α- helical CRF inhibited ^{125}I-Tyr^0-oCRF binding with IC_{50} values of 0.9 nM, 1.9 nM, 11.6 nM, and 0.7 nM, respectively. At 1 uM concentrations, oCRF(1-39)NH_2 inhibited 30% of the specific binding, whereas AcoCRF(4-41)NH_2 was equipotent with rCRF and oCRF. Other CRF-related (rCRF(1-20)OH, oCRF(1-22)OH) and -unrelated (VIP, Angiotensin II) peptides did not affect ^{125}I-Tyr^0-oCRF binding. These data suggest that CRF binding sites in spleen share the same structural characteristics as those in pituitary and brain. Currently we are examining CRF binding in slide-mounted sections of spleen by receptor autoradiography and in subpopulations of isolated mouse spleen cells in order to identify the specific cell type(s) that bind CRF.

In summary, we have identified and characterized specific high-affinity CRF receptors in spleen similar to those in brain and pituitary. These data provide evidence for and support the suggestion that CRF or a CRF-like peptide may play a physiological role in neuroimmunomodulation. Supported by USPHS grant MH 25951.

291.15 VISUALIZATION AND FUNCTIONAL DEMONSTRATION OF CCK RECEPTORS IN THE IMMUNE SYSTEM: COMPARISON WITH BRAIN RECEPTORS. P. Sacerdote, C.J. Wiedermann, M.R. Ruff and C.B. Pert* (SPON: M. Motta). Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA, Bethesda, Maryland 20892.

Evidence is rapidly accumulating to support the existence of a neuroimmune axis, suggesting a complex network involving different cell types and structures capable of emitting and receiving bi-directional signals. Neuropeptides and their receptors play a pivotal role in this network of communication. Thus, it seemed interesting to investigate the role in the immune system of cholecystokinin (CCK), a neuropeptide involved in a number of behavioral actions such as feeding behavior, analgesia, tolerance and dependence to morphine, and social behavior.

The ability of the peptide to induce monocyte chemotaxis was tested both in vitro and in vivo. In the in vitro assay the activity of different forms of CCK on human monocytes was studied over a broad concentration range, proving sulfated CCK8 to be the most potent attractant tested. CCK antagonists completely blocked the chemotactic activity. In the in vivo studies sulfated CCK8 was able to induce a peritoneal mononuclear infiltration in rat; the inactive form CCK4 was not. The order of potency of CCK analogs in inducing monocyte chemotaxis is consistent with the binding potency of these analogs to other tissues. This structure activity analysis and the ability of CCK antagonist to block chemotaxis suggest the presence of CCK receptors on monocytes.

In a second group of experiments, we have characterized CCK receptors in spleen, using autoradiography on frozen rat sections. Utilizing biologically active ^{125}I-labeled sulfated CCK8, a single class of high affinity specific binding sites for CCK have been detected. Scatchard and Hill plot analyses indicate a Kd of 0.1 nM and a maximum binding capacity of 0.25 fmol/mg protein. The relative potencies of various molecular forms of CCK in displacing specific binding were compared. The order of potency is: CCK8 sulfate > caerulein > CCK8 not sulfated > CCK4. Most interestingly this order of potency of CCK analogs in displacing CCK binding to spleen is comparable to their potency in chemotaxis induction. Combined with the finding of CCK innervation of spleen parenchimal tissue, the presence of CCK receptors in spleen and the CCK chemotactic activity suggest the possibility that the nervous system could control, through a release of CCK, immunological functions that may include the trafficking and homing of immune cells in different body compartments.

Studies are now in progress to compare CCK receptors in brain and spleen, using a crosslinking strategy in order to covalently affix reversible ligands to their recognition molecules for subsequent electrophoresis analysis.

291.16 THE EFFECT OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS ON THE CATECHOLAMINE CONCENTRATIONS IN THE SPLEEN. U. Wesselmann*, R.J. Konkol*, G.L. Leo*, D.L. Roerig*,+ and D.R. Harder* (SPON: Z. Bosnjak). Dept. of Neurology, Medical College of WI, Milwaukee, WI 53226; +Dept. of Anesthesiology, VA Medical Ctr., Milwaukee, WI 53295.

Experimental allergic encephalomyelitis (EAE) is a T-cell mediated autoimmune inflammatory disease which has been studied as a model for demyelinating disorders. EAE can be induced in animals by injecting myelin basic protein emulsified in complete Freunds adjuvant (CFA) into the hind footpads. Clinically, the disease begins about 10 days after inoculation with loss of tail tone and weakness of the hind legs. The peak clinical incidence occurs at day 13-14 with paralysis of the hind legs. Resolution of the disease then progresses rapidly, with full clinical recovery at day 21. Previous studies (J. Neurochem 40:1771-1773,1983; Experimental Allergic Encephalomyelitis, A.R. Liss, NY, 1984,pp:131-138) have shown a decrease in the catecholamine content of spinal cord and brain in animals with EAE. The purpose of the present study was to determine the effect of EAE on the catecholamine content of lymphoid organs. Male Lewis rats (3-4 months of age) were sacrificed 7,14 and 52 days after inducing EAE. Rats injected with only CFA served as controls. The concentrations of norepinephrine (NE), dopa (D), epinephrine (EPI) and dopamine (DA) in the spleen were measured by HPLC with electrochemical detection. No statistically significant differences (t-test) between the splenic catecholamine concentrations of the EAE group and the control group were found on day 7 and 52. However, on day 14 the D concentrations (128±13 vs 79±13)* were significantly ($P<0.05$) increased and the DA concentrations (30±2 vs 42±5)* decreased in the EAE rats compared to the controls. The NE (2529±101 vs 2291±239)* and EPI (32±11 vs 10±2)* concentrations tended to be elevated in the EAE group but did not achieve statistical significance. The results imply that there is a disruption in the splenic autonomic nervous system catecholamine metabolism during the peak of the clinical symptoms of EAE. These findings also highlight a possible role for the sympathetic nervous system at 14 days post inoculation, when α_1-adrenergic receptor blockade has been shown to suppress the clinical course of EAE (Proc. Natl. Acad. Sci. USA 82:5915-5919,1985).

*(ng/g tissue; mean ± SEM)

292.1 TEMPORAL INTEGRATION OF PHOTIC INPUTS TO A MAMMALIAN CIRCADIAN SYSTEM. D. Nelson* and J.S. Takahashi, Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, Illinois 60201.

In mammals retinal input to the circadian system is necessary for the entrainment of endogenous rhythms to light-dark cycles. To characterize the photoreceptive system which mediates photic information to a central pacemaker, we have used the phase-shifting response of the circadian pacemaker to discrete light stimulation as an assay. Using this approach the spectral sensitivity and stimulus-response relationship for phase advances have been determined for the hamster (M. auratus) locomotor rhythm (Takahashi et al., Nature 308:186, 1984). With 900-second stimuli this response was found to have a spectral sensitivity peak near 500nm and a relatively high response threshold. We have begun experiments designed to examine the properties of light adaptation and temporal integration in the photoreceptive system subserving the hamster circadian system. To test for possible sensitivity changes in the clock's photoreceptive system we examined the effects of an adapting background upon phase-shifting sensitivity. After 7 days in constant darkness a monochromatic (503 nm, HW = 20 nm) background of 1.6×10^{12} photons/cm^{2}sec was presented to the hamster for 600 seconds. Test stimuli of 300 seconds duration and various strengths were then superimposed upon the final 300 seconds of this background. Responses were plotted as the total response vs increment test irradiance and by the method of Normann and Pearlman (Vis. Res. 19:391, 1979). Results suggest that pacemaker responsivity to light stimuli is decreased by the adapting background. This decrease, however, seems primarily due to the compression or saturation of the response, with little or no contribution by "true" adaptation. In addition using stimuli of equivalent quanta/cm^2, we have found that the reciprocal relationship between stimulus irradiance and duration is maintained over an unusually long range. Varying the stimulus duration from 1 second to 2700 seconds, we find no significant changes in the magnitude of phase advances. Other experiments suggest that two one-second pulses of light given at circadian time 19 and 20 (separated by a one hour inter-pulse-interval) are interpreted as a single stimulus by the circadian system. Taken together these experiments suggest that the photoreceptive system subserving entrainment of the circadian pacemaker in the hamster is capable of integrating light inputs over quite long periods of time with changes in light responsivity due only to saturation of the phase-shifting response.

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292.2 THE EFFECTS OF FEEDBACK LIGHTING ON THE CIRCADIAN AND PHOTOPERIODIC RESPONSES IN THE IMMATURE DJUNGARIAN HAMSTER. J.S. Ferraro, K.A. Dailey*, H.N. Krum*, G.T. Wassmer*, and F.M. Sulzman*. Dept. of Biological Sciences, State Univ. of New York, Binghamton, NY 13901.

Feedback lighting (LD_{FB}) is a lighting condition where individual animals, otherwise in constant darkness (DD), are exposed to light in response to a monitored behavior. The specific purpose of this type of illumination is to expose the circadian cycle to light only during the subjective night. It has previously been shown, in three species of nocturnal rodents (Rattus norvegicus, Mesocricetus auratus and Mus musculis) and one species of diurnal primate (Saimiri sciureus), that the free-running period of the circadian cycle in LD_{FB} is similar to that in continuous light (LL) despite the relative absence of light during the subjective day. A group of immature male Djungarian hamsters (Phodopus sungorus) were placed in individual light-tight sound attenuated chambers where they were exposed to one of three lighting conditions (DD, LL or LD_{FB}) for at least 40 days shortly after weaning. The animals had free access to food, water and an activity wheel. Activity (wheel running) was monitored continuously on a microcomputer. The free-running periods for the animals exposed to DD, LL and LD_{FB} were 23.8 ± 0.2, 25.2 ± 0.2 and 24.6 ± 0.07 h respectively. Therefore it appears that LD_{FB} may not lengthen the period of the free-running activity rhythm as effectively as LL in this species. It is possible that this response stems partially from the immature nature of the subjects rather than a true species difference. However, since a phase-response curve has not been illucidated for the Djungarian hamster and due to the small sample size, variation in age at experimental onset, and preliminary nature of the data, it is difficult to draw firm conclusons. Reproductively, it appears that LD_{FB} may be photoinhibitory in the adolescent Djungarian hamster similarly to that seen in the adult Syrian hamster where LD_{FB} was unable to maintain reproductive funtion (Ferraro and McCormack, Biol. Reprod., 33:93, 1985). None of the hamsters exposed to DD or LD_{FB} had fluid in the atrophied seminal vesicles (suggesting a reduced testosterone level), whereas all hamsters exposed to LL had fluid in large seminal vesicles. Paired testis weights were 21.3 ± 3.1, 166.6 ± 45.9 and 212.1 ± 12.4 mg/10g total body weight for animals exposed to DD, LD_{FB}, and LL respectively. As a result of these preliminary experiments, it appears that LD_{FB} lengthens the free-running period of the locomotor activity rhythm and yet may not be capable of inducing reproductive function in immature Djungarin hamsters despite substantial light exposure during the subjective night.

292.3 EXPANDING THE RANGE OF ENTRAINMENT THROUGH THE USE OF LONGER DURATION LIGHT PULSES IN THE SQUIRREL MONKEY. K.C. Halperin*, F.M. Sulzman*, and J.S. Ferraro (SPON: N.E. Spear). Dept. of Biological Sciences, State Univ. of New York, Binghamton, NY 13901.

In order to examine the relative importance of the light pulse duration used to entrain the diurnal primate (Saimiri sciureus) to varying T-cycles, 11 squirrel monkeys were exposed to light-dark cycles consisting of 3 h light and X h dark (LD 3:X), where X varied from 18 h to 26 h. The results of this experiment were then compared to the range of entrainment derived from previous findings using a LD 1:X paradigm (Hoban, T.M. and F.M. Sulzman, Am. J. Physiol. 249:R274, 1985). The same group of monkeys was used for both experiments and were maintained in temporal and social isolation in light-tight sound attenuated chambers. The circadian rhythm of drinking activity was monitored through an electronic circuit and collected on an Apple computer. Sine waves were fitted to the data after eliminating the first 5 days as transients and the positively masked drinking activity. A regression line was fitted through the acrophases to determine the period of the rhythm. The range of entrainment for 3 h light pulses, defined as the last T-cycle to which >50% of the animals were entrained, was 22.5 (LD 3:19.5) to 27.5 h (LD 3:24.5). This range is twice as large as the range of entrainment found using a LD 1:X paradigm and still differs from the range predicted by the phase response curve generated for 5 of the animals. The predicted range of entrainment derived using 1 h light pulses is 21.9 to 25.7 h, whereas the experimental range was found to be 23.5 to 26.0 h. The results thus suggest that the range of entrainment in the squirrel monkey can be expanded by increasing the light pulse duration. However a phase-response curve using 3 h light pulses may be substantially different and therefore predict an even greater range of entrainment. Therefore the explanation of why the predicted range can not be obtained experimentally, at least for the short end, must await further investigation.

292.4 INTERACTIONS BETWEEN ONSET AND OFFSET COMPONENTS OF CIRCADIAN RUNNING WHEEL ACTIVITY IN HAMSTERS: FURTHER EVIDENCE FOR MULTIPLE OSCILLATORS. J.D. Hallonquist and N. Mrosovsky. Departments of Psychiatry and Zoology, University of Toronto, Toronto, Ontario M5S 1A1, Canada.

Fifteen adult male Syrian hamsters, Mesocricetus auratus, were housed in continuous dim light (LL, 3-6 lux) for 16 weeks with access to running wheels. In all 15 animals, the periodicity of activity onset was less than that of activity offset. As a result, the active portion (alpha) of the circadian cycle, as defined by the time interval between activity onset and offset, became progressively longer. When onset and offset components free-run at different periodicities, as they did in this experiment, eventually they must meet. We studied our animals sufficiently long to observe what happened when this point of collision was reached.

In 8 hamsters, collision of activity onset and offset was followed by disorganization of the circadian rhythms. In 2 other animals, decreases in the amount of activity made it impossible to provide a clear description beyond the collision point. In the 5 remaining animals there was a phase shift of one component. Most probably, the phase shifts resulted from interactions between two oscillators when in certain phase positions. Phase shifts cannot be readily explained by changes in thresholds for displaying activity, because the total amount of activity did not simply increase to occupy progressively greater portions of the record; rather it was alpha that increased.

In another experiment, chronic lithium administration increased the incidence of colliding of activity onset and offset in adult male Syrian hamsters housed in moderately bright LL (30-100 lux). Five of the 8 hamsters that received lithium carbonate (4.5g/kg food) for 6 weeks showed colliding during this period, compared to none of the 9 hamsters which received the control diet ($p < .01$, Fisher Exact Probability Test). It is suggested that the phenomenon of colliding of circadian activity's onset and offset may be valuable in studying the effects of drugs on the interactions and coupling between different oscillators.

292.5 CIRCADIAN TEMPERATURE RHYTHM IS PRESERVED IN THE BRATTLEBORO RAT. M.F. Mazurek*, M.T. Morton* & W.J. Schwartz. Dept of Neurology, Mass. Gen. Hosp. and Harvard Med. School, Boston, MA, 02114.

A circadian rhythm of vasopressin (AVP) concentration in cerebrospinal fluid (CSF) has been observed in several mammalian species. The physiological significance of this rhythm remains unclear. Among the putative actions ascribed to AVP in the central nervous system is a role in temperature regulation. Studies of the febrile response have indicated that the central release of AVP might serve an antipyretic function, acting to lower body temperature. We wondered whether the involvement of AVP in temperature regulation might extend to the control of daily temperature rhythmicity. To investigate this hypothesis, we examined whether Brattleboro rats, which are genetically incapable of synthesizing brain AVP, express a circadian temperature rhythm. Brattleboro and Long-Evans (LE) rats were surgically prepared with brain thermistors (Fenwal) lowered into the striatum and then fixed in place with stainless steel screws and dental cement. Each animal was entrained to a 12:12 h light-dark cycle in an isolation box that was maintained at 25 ± 1°C. After 3 wks, the rat was tethered to a spring assembly, with the thermistor leads run through the spring to an electrical swivel attached to the top of the cage. This was connected via a Wheatstone bridge to a chart recorder to permit continuous monitoring of brain temperature in the freely-behaving unstressed rat. LE rats showed daily fluctuations of brain temperature that were strictly entrained to the light-dark cycle, being approximately 1°C higher during the dark period. This rhythm persisted when the animals were housed in constant darkness. Brain temperature in the Brattleboro rats also displayed a clear-cut circadian pattern which persisted in constant darkness. The lack of AVP in these Brattleboro rats was confirmed by measuring drinking behavior (0.95 ± 0.10 cc/gm body weight/day in Brattleboro rats vs 0.10 ± 0.01 cc/gm body weight/day in LE rats) and by post-mortem radioimmunoassay of AVP in plasma and brain (e.g., undetectable levels in Brattleboro neurohypophysis vs 1-2 ng/neural lobe in LE rats).

These findings indicate that the circadian temperature rhythm, like other circadian rhythms which have been examined, is preserved in the Brattleboro rat. This would suggest that a CSF AVP rhythm is not necessary for the expression of circadian temperature rhythmicity.

292.6 CIRCADIAN VARIATIONS IN ACTIVITY OF NEUROANATOMICALLY DISTINCT RECOMBINANT INBRED MICE AS A FUNCTION OF GENETIC AND MEASUREMENT VARIABLES. D.F. Peeler, Department of Neurosurgery, University of Mississippi Medical Center, Jackson, MS 39216.

Recombinant inbred (RI) strains of genetically defined mice (C57BL/6 and BALB/c) have been reported to form two distinct groups with respect to the amount of activity they exhibit, giving support to the hypothesis that there is a single major gene determining such activity. These same strains have been found to dichotomize with respect to an aberrant development in area CA3 of the hippocampus (hippocampal lamination defect--Hld).

This study examined the relationship between genetic (and neuroanatomical) variables, activity, sex, and the time of day during which activity was measured. Male and female mice from C57BL/6, BALB/c and 7 RI strains derived from these progenitor (P) strains were tested in a runway for 4 minutes at one of 5 test periods. Traverses of the runway were counted by photocells; contacts with various objects spaced equidistantly along one side of the runway were counted independently. The groups consisted of 10 mice each, for a total of 900 mice.

There were significant second-order interactions for both activity measures. The reported finding of a dichotomy among the RI strains was not supported. As reported in numerous other studies, the C57BL/6 were significantly more active than BALB/c mice with respect to locomotion, but only at certain times during the day. The RI strains tended to form three or more groups, rather than two clusters about the P strains. Furthermore, the composition of these groups changed from one time of the day to another. Among RI strains, there are some which show little if any change in activity level as a function of time of day, whereas others show significant changes in one or both types of activity. There were similar findings with the object contact measure, except that the status of the P strains was reversed: C57BL/6 exhibited low object contact activity and BALB/c high activity. Sex was not a significant main effect for object contact activity, but it did interact significantly with strain and time of day. Test period was not a significant main effect for locomotor activity, but there was a significant interaction of strain, sex and test period. There was no apparent relationship between Hld and either measure of activity.

The present data offer no support for the hypotheses that activity is under the influence of a single major gene, or that the hippocampal aberration Hld affects in any measurable way the amount of either type of activity. Nevertheless, genetic composition of the individual is a powerful determinant of the amount of activity exhibited. The data do indicate very strong influence of circadian variables on both locomotor and object contact activities.

292.7 CIRCADIAN AND LIGHT-DARK CONTROL OF SLEEP AND BRAIN TEMPERATURE IN THE SQUIRREL MONKEY. Dale M. Edgar and Charles A. Fuller. Department of Animal Physiology and California Primate Research Center, University of California, Davis, CA 95616.

Light has differential influences on squirrel monkey arousal states and deep brain temperature (TBR). For example, slow wave sleep (SWS) is more sensitive to the inhibitory effects of light than is REM sleep, and light can influence the regulated level of TBR without invoking changes in arousal state. In this study we investigated the role of the circadian timekeeping system (CTS) in these responses.

Nine adult male squirrel monkeys (Saimiri sciureus) weighing 980-1150 g were surgically prepared with a miniature chronic skull implant permitting the continuous recording of TBR, cortical and muscle activity, and eye movement. Four of these animals also received histologically verified, complete lesions of the suprachiasmatic nuclei (SCNx) which eliminated endogenous circadian rhythms in all variables when monitored in constant light (LL). All animals were housed unrestrained within separate sound-attenuated, temperature controlled (27°C) chambers. Each animal was monitored in LL for several weeks prior to study. They were then subjected to 14 days of LD 12:12 followed by 48 hours of LD 2:2.

Light had potent modulating effects on sleep and TBR in SCNx monkeys. However, unlike intact animals, SCNx animals showed no circadian variation in light-dark (LD) sensitivity. Total sleep time (TST) was significantly ($p<0.01$) reduced in intact monkeys in LD 2:2 relative to that observed in LL. However, SCNx monkeys showed no difference in TST under LD 2:2 or LL conditions. SWS was inhibited by light in LD 12:12 and LD 2:2. Yet at no time did the peak levels of SWS in intact monkeys exceed that observed in SCNx monkeys. REM also showed LD modulations in LD 2:2, but remained arrhythmic in LD 12:12. During subjective night exposure to light, intact monkeys exhibited 6-7 times more REM sleep than that observed in SCNx monkeys at corresponding times. Brain temperature showed LD modulations of 0.6-0.8°C under both light regimens.

These results suggest that TBR, SWS, and REM are influenced by different aspects of photic stimuli which are normally mediated by the CTS. Thus, SWS and TBR are influenced by the tonic presence of light, whereas REM appears to be sensitive to phasic LD transitions. In addition, different mechanisms appear to underlie the circadian control of SWS and REM sleep. The subjective night levels of REM appear to be predominantly under the control of the CTS, while the regulation of SWS is more labile, and appears to be influenced less by the CTS.

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292.8 ENTRAINMENT OF MONKEY CIRCADIAN RHYTHMS: EFFECTIVENESS OF FOOD RESTRICTION AND TASK DEMANDS. W.N. Tapp*, D. Creighton*, T.A. Pritzel and B.H. Natelson (SPON:F.S. Knox). VA Medical Center and New Jersey Medical School, East Orange, NJ 07018.

Six adult, male rhesus monkeys were individually housed in chambers designed to enable us to control their environment. Three monkeys were required to perform a chained vigilance-discrimination task to obtain their food. Trials occurred on the average of every 2.4 min in the task. The other three monkeys merely had to press a key to obtain food. Activity, feeding and temperature rhythms were monitored in all monkeys.

In the first phase of the experiment (Constant Task/Free Feed), the task ran around the clock and food was available ad lib for non-task monkeys. During this phase, all monkeys exhibited normal free-runs with activity, temperature, and performance or feeding (in the non-task monkeys) showing the same period and stable phase relationships within a given monkey. In the second phase of the experiment, task and food availability were restricted to 8 hr/day. Both temperature and activity synchronized well to the time of task availability in monkeys that were working for their food. Synchronization was much poorer for the non-task monkeys, and one monkey even maintained a clear free-running component throughout the restricted feeding part of the experiment. Finally, monkeys were released to free-run in Constant Task/Free Feed. Monkeys were released on days when the onset of subjective day predicted from their initial free-run was about 12 hr out of phase with the onset of restricted task or feeding. Activity, temperature and performance all began to free-run smoothly from the phase of restricted task, indicating that the periodic task demands/restricted food combination truly entrained these rhythms. In contrast, non-task monkeys either consolidated their rhythms in the free-running component that was visible during restricted feeding or shifted toward the phase predicted by their initial free-run, indicating that restricted feeding alone did not successfully entrain activity, temperature or feeding.

We cannot tell whether task demands alone would entrain the rhythm or whether the task/restricted feeding combination is necessary, However, the results indicate that periodic cognitive or work demands can play a role in entrainment of primates.

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292.9 CHANGES IN THE CIRCADIAN RHYTHMS OF BODY TEMPERATURE AND ACTIVITY PREDICT THERMOREGULATORY DEFICITS IN ELDERLY RATS. E. Melanie W. Kittrell, Univ. Pittsburgh, WPIC-1516, 3811 O'Hara St., Pittsburgh, PA 15213

The most commonly reported age-related change in circadian rhythms is a diminished amplitude. Changes in phase and period of some rhythms have also been found. The functional significance of these changes is unclear although there is evidence that anomalies in biological rhythms are often associated with poor mental and physical health. Thermoregulatory deficits also accompany the aging process in humans and rats. The purpose of this study was to identify possible associations between changes in the circadian temperature rhythm (CTR) and thermoregulatory problems in aging rats.

An IBM computer measured body temperature (Tb) and general cage activity every five minutes in rats, via biotelemetry thermistors (Mini-Mitter, Sun River, OR) implanted into the peritoneal cavity. Six rats were studied continuously from 16 to 28 months of age and were compared with another 6 who ranged in age from 5 to 13 months over the course of the study. They were maintained on a 12-12 LD cycle. All were subjected to a 6 hour phase advance followed one month later by a 6 hour phase delay beginning when they were 27 and 11 months of age. All were tested for thermoregulation in the cold (5°C for 90 minutes) at 28 and 12 months of age. Our results can be summarized as follows:

1. CTR amplitude did not diminish gradually but fluctuated for several 4-6 wk periods between an exaggerated and a diminished amplitude. Just prior to death, there was a permanent decline in amplitude.
2. Activity closely followed temperature in both young and old rats, but in the old, the usual temporal pattern within a 24 hr period sometimes dissociated.
3. In the elderly rat, there were CTR instabilities characterized by sudden drops in T^b (3-5°C) that usually occurred at night and returned to normal T_b levels after 12-24 hours.
4. Many elderly rats CTRs "broke-up" when phase advancing. This was rarely seen in younger rats. Phase advances took longer to adjust to than phase delays regardless of age.
5. Rats whose CTRs appeared unstable and "broke-up" during phase advances were unable to defend their T_b against a cold challenge. Thus, CTR irregularities predicted thermoregulatory deficits.

The suprachiasmatic nuclei (SCN) brain regions of all rats were examined with cytochrome-oxidase staining and the histological results will be discussed.

292.10 REPRODUCTIVE SENESCENCE IN FEMALE RATS: NO EVIDENCE FOR DISRUPTION OF CIRCADIAN RHYTHMS. B.Tate-Ostroff* and R.S.Bridges. (SPON:Z. Boulos).Lab. of Human Reproduction and Reproductive Biology and Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115.

Aging in the female rat is associated with the loss of regular estrous cycles and the onset of a state of persistent vaginal estrus (PVE), which is similar to a state induced by treatments known to disrupt the circadian system of the rat. It has been proposed that age-related changes in the organization and functioning of the circadian system may play a role in the onset of ovarian acyclicity in the aged rat. The study described here simultaneously monitored spontaneous locomotor activity, body temperature rhythms and estrous cyclicity in young (3 month old) and middle-aged (9 month old) cycling female rats housed on lighting schedule LD14:10 for a period of several months. The characteristics of the circadian rhythms in activity and body temperature were analyzed when the middle-aged rats showed normal estrous cycles and again after these females entered a state of PVE. All animals were individually housed and implanted with telemeters. Activity rhythm and body temperature data were collected with the aid of the Dataquest III computer-assisted data collection system. Vaginal smears were taken daily. During the time of data collection, all middle-aged females entered PVE. Prior to this time, the proportion of days when animals displayed vaginal estrus during a ten day period was equal in both the young and old groups (26%).

Power spectra derived from Fourier analysis of activity and body temperature rhythms for young and old animals during this ten day period showed the major peak centered at 24 hours with no significant differences between spectra of old and young animals. As the animals aged, the older animals entered PVE and the percentage of days when middle-aged females displayed vaginal estrus rose to 78%. Power spectra of activity and body temperature rhythms for reproductively aged animals continued to show the major peak at 24 hours There was no significant change in rhythms of these females as they aged nor did they differ from those of young females. The data reported here indicate that age-related alterations in the circadian system may not precede the onset of PVE in the rat, and may instead occur months after the onset of PVE. The pattern of reproductive senescence seen in the aging rat may reflect a breakdown in the reproductive system specifically and not an initial deficit in the circadian regulation of reproduction. Supported by NSF Grant #DCB 85-08187 awarded to BTO and NIH Population Grant IP30H006645 awarded to the LHRRB.

292.11 DISRUPTION OF ACTIVITY RHYTHMS IN CARDIOMYOPATHIC HAMSTERS WITH SEVERE HEART FAILURE AND IN VERY OLD, NON-DISEASED HAMSTERS. J.E. Ottenweller*, W.N. Tapp*, C. Young*, T.A. Pritzel*, and B.H. Natelson. Primate Neurobehavioral Unit (127A), VA Medical Center, East Orange, NJ 07018.

Cardiomyopathic (CM; BIO 14.6) hamsters develop a slowly progressive heart failure and die with severe, uncompensated heart failure. There are similar signs of debilitation at the end of the lives of non-diseased, control (F1B) hamsters. We attempted to determine how the general debilitation of heart failure or old age affected the behavior and physiology of hamsters by examining activity rhythms and endocrine profiles in both CM and F1B hamsters throughout their respective lifespans of 13 and 27 months. Male hamsters were maintained on a 12 hr photoperiod (0600-1800 h) with mouse chow and tap water available ad libitum. Activity rhythms were monitored using tilt cages with activity being recorded in 10 minute bins throughout the day. Circadian activity rhythms broke down in 9 out of 10 F1B hamsters and 16 out of 19 CM hamsters before they died. In both groups, half the hamsters that broke down did so 1-2 weeks before death, and the other half had disrupted activity rhythms for 3-6 weeks before death. The total level of activity was similar before and after disruption of activity rhythms, but as rhythms broke down activity was distributed evenly throughout the day and night. These disruptions in activity rhythms predicted impending death well, in that hamsters died over a wide range of ages (from 8-18 months in CM and 19-42 months in F1B) and yet disruption of activity rhythms shortly preceded death in almost all cases. We have also measured plasma testosterone and thyroid hormone concentrations in the trunk blood of hamsters that were decapitated at various ages. As CM hamsters developed severe heart failure near the end of their lives, thyroxine (T4) levels fell from 3.3±0.2 ug/dl (mean±sem) in 5 month olds to 0.9±0.3 ug/dl in 13 month olds. In F1B hamsters, T4 declined from 3.4±0.2 ug/dl in 12 month hamsters to 1.0±0.2 ug/dl in 26 month hamsters near the end of their lives. Thus, in both old and sick hamsters, activity rhythms were disrupted and T4 levels declined. Whereas testosterone (T) declined in CM hamsters from 0.8±0.2 ng/ml in 5 month olds to 0.2±0.1 ng/ml in 13 month olds, T was the same in 12 month (1.7±0.3 ng/ml) and 26 month (2.3±0.8) F1B hamsters. Thus, testosterone may be implicated in the disruption of activity rhythms in sick CM hamsters. Inasmuch as thyroid function declined when activity rhythms were disrupted, the data suggested a relationship between low thyroid hormone levels and disruption of rhythmicity. Supported by VA Research Funds.

292.12 ANTITHYROID TREATMENT ALTERS CIRCADIAN PERIODICITY AND GONADAL ACTIVITY IN HAMSTERS. L. P. Morin. Dept. of Psychiatry and Behav. Sci., SUNY, Stony Brook, NY 11794.

The thyroid has been implicated in the regulation of hamster circadian period regulation (Beasely, L.J. and Nelson, R.J. Experientia, 28:870, 1982). However, our data (Morin et al., Am. J. Physiol., 250:R151, 1986) showed that a thyroid hormone blocker, propylthiouracil (PTU), would provide a dose-response lengthening in the hamster circadian period (tau), but not in the blockade of thyroid hormone release. We therefore, examined whether the effect of PTU on tau is dependent or independent of the thyroid gland. We also obtained data concerning the effect of antithyroid treatment on gonadal function.

Adult male hamsters were blinded, pinealectomized (to permit testicular maintenance in the absence of light), given powdered laboratory chow and access to a running wheel. When locomotor rhythms were stable, animals were divided into groups: Control--received the powdered diet; PTU--received a 0.6% PTU powdered diet; LoI--received a powdered diet low in iodine; I131--received the powdered diet plus a 500 uCi injection of sodium iodide-131. The latter is known to destroy rat thyroid glands. Rhythms were recorded from all animals for more than 10 half-lives of the I-131.

PTU lengthened tau by about 0.15 hr, but no other treatment was similarly effective. Thyroid weights were 105, 18, 6 and 5 mg for the PTU, LoI, I131 and Control groups, respectively. Thyroxine levels were nondetectable in the PTU group and 83% reduced in the LoI group. Triiodothyronine was normal in all but the PTU group for which it was nondetectable. On an individual basis, change in tau was not related to any index of thyroid function. Interestingly, both the LoI and PTU treatments resulted in greatly reduced testes size and activity in some animals. Further, testes size of the PTU animals was inversely related to the change in tau during PTU treatment (r=-.76). I-131 had no effect on any measure.

The data support the idea that PTU alters tau through a mechanism not necessarily involving thyroid function. The data also suggest that tau and gonadal activity may be influenced by a common mechanism not involving pineal activity. Supported by NIH grants NS22168 and AG05773.

292.13 *PROPYLTHIOURACIL DOES NOT AFFECT RUNNING RHYTHMS IN FEMALE HAMSTERS* H. Klemfuss, D. Swick* and D.F. Kripke*. Veterans Administration Medical Center, San Diego, CA 92161 and Dept. of Neurosciences, University of California, San Diego 92093.

Thyroid hormone levels may influence activity rhythms in rodents. Richter (Biological Clocks in Medicine and Psychiatry, Thomas, 1965) reported that thyroidectomy or antithyroid drug administration induced periodic decreases in locomotor activity in female Norway rats lasting 10-14 days at intervals of 20-45 days. We attempted to replicate these findings in the intact female hamster. Recent reports have indicated that experimental lowering of thyroid hormone levels lengthen tau and produce a phase delay in running wheel rhythms in male or ovariectomized hamsters.

We studied the wheel-running behavior of 20 female golden hamsters over a 3-month period. Sixteen hamsters were given drinking water containing 0.05% propylthiouracil (PTU), while the remainder received tap water. Half of the animals in each group were housed under a 16:8 light:dark cycle, with light corresponding to about 50 lux, dark less than 2 lux. Cage lighting for the other 10 hamsters followed an 8:16 light:dark schedule. Running-wheel activity was automatically counted for at least 10 weeks after PTU treatment was started. At the conclusion of the experiment, total serum thyroxine levels were determined from blood taken by cardiac puncture.

PTU treatment reduced serum thyroxine levels to 2.0 ± .23 (SEM) ug/dl, which was significantly less ($p<.001$) than control levels of 5.6 ± .92 (SEM) ug/dl. However, we found no evidence for any alteration in rhythmicity in PTU-treated hamsters. Acrophase, mesor, and amplitude of running rhythms of PTU-treated animals were all similar to rhythms of controls under the same light:dark schedule. Furthermore, examination of double plotted running-wheel records by an experienced observer blind to treatment revealed no characteristic abnormalities in activity levels or rhythmicity during 10 weeks of PTU treatment.

Although we did not replicate Richter's results with rats in the hamster, we cannot exclude the possibility that periodic variations in locomotor activity would develop with more than 3 months of observation or with greater thyroid suppression.

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292.14 CIRCADIAN PHASE RESPONSE CURVE TO DIAZEPAM FOR HAMSTERS IN CONSTANT LIGHT. T.A. Houpt*, Z. Boulos, M.C. Moore-Ede. Dept. of Physiology and Biophysics, Harvard Medical School, Boston MA 02115.

A pharmacological agent that phase shifts circadian rhythms can act either centrally on a circadian clock or peripherally on the pathways which mediate zeitgeber (e.g. light) input to the clock. With GABA receptors found in the SCN, LGN and retina, the GABA agonist benzodiazepines may act either directly on the clock or via light input pathways. Ralph & Menaker have reported that diazepam, a GABA agonist, blocks light-pulse-induced phase advances but not delays in hamsters kept in constant darkness, while bicuculline, a GABA antagonist, blocks light-induced delays but not advances (*Soc. Neurosci. Abs. 11* {1985}: 1140). They suggest that the phase advancing and phase delaying effects of light may be mediated by different neurological mechanisms. If diazepam's resetting action is confined to blocking light-induced phase advances, then a phase response curve (PRC) to diazepam in LL would be expected to comprise only phase delays in late subjective night. Male golden hamsters (90-100g) were housed individually in cages equipped with running wheels and enclosed in light-tight ventilated chambers under LL (140 lux). After at least 3 weeks in LL, 12.5 mg/kg diazepam was given i.p. Animals received a maximum of 2 injections at least 3 weeks apart at one of the following circadian times (CT): 0, 4, 8, 12, 16 and 20h. Diazepam caused phase advances during subjective day (CT 4 & CT 8) and phase delays during subjective night (CT 16 & CT 20). Both small advances and delays were seen at CT 0 & CT 12. Control saline injections had no effect. The PRC was similar to the PRC in hamsters to 2h dark pulses (Boulos & Rusak, *J. Comp. Physiol. 146*: {1982} 411-17). Since some animals ran intermittently for several hours after injection, this suggests that the phase shifts were not caused by a reduction in light input due to the animal being unconscious or with eyes closed. Thus diazepam either consistently blocks light input to the clock with each injection acting like a dark pulse, or it acts directly on the clock and its PRC cancels out the PRC to light pulses in DD. Since Ralph & Menaker's diazepam injections in DD at CT 13.5 & 18 did not produce phase shifts, this favors diazepam acting like a dark pulse. However, it is also possible that the PRC to diazepam in DD may have a different waveform than that in LL, with phase shifts occurring in DD at times other than CT 13.5 & CT 18.
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292.15 **THE SUBTERRANEAN MOLE RAT: AN ANALYSIS OF THE SUBDERMAL EYE.** **R. BreMiller*, J. Terkel*, E. Schabtach* and G.E. Pickard** (SPON:D. Kimble). Instit. Neuroscience, Univ. Oregon, Eugene, OR 97403 and Dept. Zool., Tel Aviv Univ., Tel Aviv, Israel.

Mammals which have radiated into the subterranean ecological zone have evolved structurally and functionally for burrowing and living underground. Mole rats of the family Spalacidae exemplify these specializations; body shape is tubular, the head, which is used for excavation, is flattened and blunt, the nose is broad and tough and the neck is short and massive. Although there is no external indication of an eye, minute eyes approximately 1mm in diameter are located subdermally adherent to a large Harderian gland. It would appear unlikely that the primary function of these tiny eyes is visual since they are located under a thick dermis (>1mm) in an animal which lives in underground burrows. However, it does appear that the mole rat uses photic signals to coordinate its behavior with the ambient light:dark cycle. Therefore, the mole rat may be a good experimental model for examining the structures and mechanisms underlying photic entrainment of circadian activity.

Feral mole rats of either sex were maintained in the laboratory for several months before the eyes were prepared for analysis. A complete series of consecutive sections (either 16μm cryostat or 2μm plastic) were prepared from several eyes. Examination of Nissl stained sections at the LM level revealed that the cornea, lens and anterior chamber are absent and that the neural retina forms a continuous sheet on the entire inner surface of the eye. The organization of the retina is similar to other rodent retinas with some modifications. A pigment epithelial layer covers the entire retina and is thickest at the anterior surface of the eye (the surface adjacent to the dermis). The photoreceptor layer contains short outer and inner segments and an outer nuclear layer (ONL) 4-6 cells thick. Immunocytochemical analysis performed with a cone specific monoclonal antibody (generously provided by V. Lemmon) indicated a small population of cone cells among the rod cell photoreceptors. Although an outer plexiform layer was clearly evident, many cells of the ONL were noted in the inner nuclear layer which gives the retina an immature appearance. Inward from the inner plexiform layer is a heterogenous layer of ganglion cells. The optic nerve was examined by electron microscopy; it is less than 100 μm in diameter and is composed of a number of fascicles of unmyelinated axons separated by oligodendrocyte processes. We estimate the optic nerve to contain <1000 optic fibers. Experiments designed to determine the site(s) of termination of the optic fibers are in progress.

It appears that the mole rat eye is ill-suited for visual perception although its morphology is that of a photosensitive organ. Supported by NIH grant NS-21165 (GEP).

293.1 SIZE-DEPENDENT BLOOD-BRAIN BARRIER OPENING DEMONSTRATED WITH 14C-SUCROSE AND A 200,000 DALTONS 3H-DEXTRAN, B.K. Armstrong*, P.J. Robinson* and S.I. Rapoport (SPON: M. Ottinger). Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, Md. 20892.

The blood-brain barrier (BBB) normally restricts the passage of water-soluble solutes and macromolecules into the brain. Osmotic opening has been used to augment the entry of these excluded blood-borne solutes into the brain in animals and humans. We previously examined the permeability of the BBB to 14C-inulin (5500 daltons) and 3H-dextran (79,000 daltons) (Ziylan et al., 1983,1984) and have now extended this study to elucidate the extent of BBB opening using a 3H-dextran (200,000 daltons).

The permeability of the BBB was determined simultaneously for two neutral water-soluble compounds: 14C-sucrose (340 daltons, r=5 A) and 3H-dextran (200,000 daltons, r=100 A) at different times after osmotic opening in Osborne-Mendel rats. The BBB was opened by a 30 s retrograde infusion of 1.8 molal L(+)-arabinose into the right external carotid artery and permeability-surface area (PA) products were calculated at 6, 10, 35 and 55 min thereafter or in uninfused rats (Rapoport et al., Am. J. Physiol., 238:R421-431, 1980) in 10 different brain regions. Control PA products for sucrose were approximately 10^{-5} s^{-1}, whereas the control PA products for dextran did not differ from zero ($p>0.05$). There were dramatic increases in PA for both sucrose and dextran at 6 and 10 min after BBB opening, with dextran permeability reaching about 10^{-4} s^{-1} at 6 min. PA for both substances fell significantly between 6 and 35 min after arabinose infusion ($p<0.05$). There were no significant differences between mean regional dextran PA values at 35 and 55 min, which had not returned to control values. Significantly lower PA products were found for dextran than for sucrose at all time points. Regional PA ratios, sucrose/dextran, were consistent with restricted diffusion through pores at 35 and 55 min after opening. At 6 and 10 min the regional PA ratio was less than the corresponding ratio of their free diffusion coefficients in water, indicating the presence of bulk fluid flow from blood to brain as high as 10^{-4} cm^3 s^{-1} g^{-1} brain in some regions.

These results further support a diffusion-bulk flow model for osmotic opening at the blood-brain barrier, with opening of pores within the cerebrovascular endothelium and a reduced pore size with time after arabinose infusion.

293.2 OSMOTIC OPENING OF THE BLOOD-BRAIN BARRIER IN BALB/C MICE. Wendy R. Fredericks* and Stanley I. Rapoport (SPON: P. Weinstein). Laboratory of Neurosciences, National Institute on Aging, Bethesda MD 20892.

Osmotic blood-brain barrier opening has been used in rats, rabbits, dogs, and monkeys to study brain entry of substances normally excluded from the central nervous system. However, a number of disease models have been developed in mice in which it would be useful to study central nervous system effects of drugs, enzymes, antibodies, etc. administered systemically. Therefore, a method was developed to unilaterally and reversibly open the blood-brain barrier in BALB/c mice, weighing 14-18g.

The technique is similar to that used in rats (Rapoport et al, Am. J. Physiol. 238: R421-R431, 1980), with changes in some conditions. A PE 50 catheter, with the end tapered to 0.4 to 0.5 mm in diameter by heating, was inserted retrograde into an external carotid artery and tied with surgical silk. A concentrated solution of L (+) arabinose in distilled water was infused for 30 sec into the internal carotid artery via the external carotid catheter at a rate of 0.64 ml/min. Evans blue dye, 2 ml/kg of a 2% solution in 0.9% saline, was injected into a saphenous vein 5 min before osmotic opening. The dye forms a complex with plasma albumin and is used as a marker of blood-brain barrier integrity. Most consistent dark staining (2+ to 3+) was achieved with infusion of 3.0 molal arabinose in water. The animals in this group had good survival, with over 90% surviving at least 2 h after blood-brain barrier opening. Animals allowed to recover for several days showed no signs of neurological dysfunction. Osmotic opening to Evans blue was reversible after 4 h. Dye injected iv 4 h after arabinose infusion did not produce staining of the brain.

Other concentrations of hyperosmotic arabinose in water were tested. 2.5 molal arabinose produced the gamut of staining from 0 to 3+. Infusions of 1.8 and 1.6 molal arabinose, the optimum in rats, caused consistent light (1+) staining. These results contrast with the sharp threshold for opening in the rat. The different concentration effects between mice and rats may be due to different pressure-flow relations of the viscous arabinose through the respective carotid circulations, as only a fraction of infusate concentration bathes the ipsilateral cerebral vasculature (Rapoport et al, Exper. Neurol. 91: 319-330, 1986).

293.3 CRYOFIXATION OF FROG BRAIN MICROVESSELS AFTER HYPEROSMOTIC AND COLD INJURY. Z. Nagy* and M.W. Brightman* (SPON: J.Fex). Lab. of Neurobiology, NINCDS, NIH, Bethesda, MD 20892.

The two purported routes by which large solutes move across a penetrated blood-brain barrier is via permeabilized tight junctions or vesicular transport. The role of vesicular transport has been questioned, in part, because many cytoplasmic vesicles appear to be created during aldehyde fixation of endothelium from different organs. Cryofixation reduces this artefact and was used to see the structural changes in cerebral endothelium made permeable to plasma solutes after perivascular exposure to hyperosmotic (2.5 -3 M) urea, or injury with a cold probe (-50°C). The frog was chosen because, unlike mammals, the pial or surface vessels are capillaries so that the endothelium is accessible for optimal freezing. The brains in untreated controls were immerse-fixed in aldehydes. Other controls and all other brains were rapidly frozen (RF), then substituted at low temperature with acetone-fixative. Hypothermic frogs, kept at 2 - 5°C for 5 days in order to minimize endocytosis, were also cryofixed. Large invaginations or pits, (about 0.08 - 0.32 μm wide) of the endothelial cell membrane characterize both RF and aldehyde-fixed vessels. The pits often appear as isolated vesicles in the cytoplasm, but serial sections reveal that many communicate with either the capillary lumen or subendothelial space. The number of pits in the aldehyde fixed and cold-injured specimens is about 3 times greater ($p<.01$) than in RF controls. Hyperosmotic exposure does not increase the number of pits whereas hypothermia reduces the number compared to normothermic controls. In the hypothermic frogs, a decreased fluidity of the cell membrane may account for the usually fewer plasmalemmal folds and pits, but it is not yet certain whether the junctions are less deformable in the more viscous lipid membrane. Significantly, the junctional cell membranes of overlapping endothelial cells in normothermic frogs given hyperosmotic urea are separated partially or completely along their entire length by a cleft 15 - 60 nm wide. A few endothelial processes are separated by very large pores about 0.14 - 0.2 μm wide. The open clefts and the pores may have been caused by cell shrinkage because, as determined morphometrically, the area (A) of these cells diminishes while their perimeter (P) remains about the same as in controls. The P/A ratio in the hyperosmotic group is thus greater ($p<.05$) than in the controls. There is a trend for an augmented P/A ratio ($0.1<p>.05$) in cold-damaged specimens because of an increase in P brought about by myriad pits or invaginations of the plasma membrane. Thus, the osmotic opening of the endothelial tight junctions has been directly shown. Reactive endothelium of cold-damaged brain can be highly plicated and invaginated. However, there is, as yet, no evidence of a parajunctional, transcytotic pathway in the stimulated capillary endothelium of the brain.

293.4 TRANSPORT RELATED MICRODOMAINS IN ENDOTHELIAL CELLS IN THE DEVELOPING, AGING AND INJURED MOUSE BLOOD-BRAIN BARRIER. A.S. Lossinsky, A.W. Vorbrodt* and H.M. Wisniewski*, Department of Pathological Neurobiology, New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314.

In the course of our previous studies, we have observed tubulo-canalicular structures, presumably representing a trans-endothelial transort mechanism which, in addition to vesicular transport,manifests in brain endothelial cells (ECs) of rodents following damage of the blood-brain barrier (BBB). The purpose of the present study was to: a) evaluate whether the tubulo-canalicular structures represent differentiated microdomains of the EC plasmalemma; and b) determine if these structures appear prior to BBB maturation in the mouse and/or whether they appear in the aged brain microvasculature. The following experimental groups of mice were used: (1) postnatal infants: day 1-day 14; (2) normal 60 day young adults; (3) young adult mice subjected to cold lesion injury and (4) aged mice (2 years or older). All animals received either intravascular injections of horseradish peroxidase (HRP) or in situ incubation with cationic ferritin (CF) in order to evaluate BBB permeability or EC anionic nature respectively. Ultrastructural analysis indicated that: (1) HRP leaks across developing MBVs focally up to ca. two weeks via vesiculo-canalicular structures and not through open EC junctions; (2) this leakage was similar to injured BBB transport, not observed in normal or aged mice, (3) the distribution of CF labeling in endothelia of developing brains was also remarkably similar to the CF profiles observed in ECs from injured microblood vessels. CF granules were observed in all experimental animals uniformly distributed along the luminal EC plasmamembranes with interruptions noticed at specific locations corresponding to vesicles or tubular profiles but were not present within the interior limits of either of these transport structures. More of these types of membrane interruptions were observed within ECs from developmental and injured brains and to a lesser extent in aged and normal brain ECs. Our results suggest that some mechanisms of increased BBB permeability are expressed before full brain development, become quiescent during normal adulthood and manifest in similar fashion following brain injury. Supported in part by a grant from the NINCDS, No. NS-18079-03

293.5 THE MORPHOLOGY AND PERMEABILITY OF THE BLOOD-NERVE BARRIER OF THE FROG SCIATIC NERVE DURING CHRONIC WALLERIAN DEGENERATION C.H.Latker*, A. Weerasuriya* and S.I.Rapoport (SPON: M. Carpenter) Lab of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

In the peripheral nerve, endoneurial blood vessels and the perineurium form a selective barrier, the blood-nerve barrier (BNB), which limits the entry of water-soluble substances in blood from reaching the endoneurial compartment. During Wallerian degeneration there is an increase in permeability of the BNB to tracers at the site of the transection, but little is known about changes distal to the lesion. We therefore investigated the morphological and permeability changes in the BNB distal to a transection in nerves in which there was no regeneration. Sciatic nerves of adult Rana pipiens were cut in the abdominal cavity, tied to prevent regeneration, and examined at several points distally in the thigh. At intervals from 3 days to 6 weeks permeabilities to horseradish peroxidase (HRP) and [^{14}C]sucrose were measured. Proliferation of cellular components was evaluated by the uptake of [^{3}H]thymidine. In endoneurial capillaries, an increase in the permeabilities to [^{14}C]sucrose and to HRP started by day 7, rose 4 to 6 fold at 3 weeks, and returned toward normal values by 6 weeks. The tracers leaked initially at the proximal portion of the nerve stump and later at the distal end. Uptake of [^{3}H] thymidine, which was evident within the first week, was found in endothelial cells, Schwann cells and the perineurial cells. The ultrastructure of endoneurial vessels appeared normal through the period examined. However by 3 weeks there was an accumlation of lipid and membrane debris within perineurial cells. This study suggests that: 1. Wallerian degneration is associated with altered endoneurial permeability distal to the lesion in the frog nerve, 2. there is a temporal-spatial pattern of permeability changes during the initial stages of degeneration, 3. endothelial cells are morphological unchanged by the degenerative process, but after 3 weeks perineurial cells appear to respond, 4. proliferation of non-neuronal components occurs without regeneration of neuronal elements, and 5. restoration of barrier function depends on stabilization of the endoneurial elements and is not dependent on intact neural components.

293.6 EFFECTS OF MECHANICAL BRAIN INJURY ON CEREBRAL VASCULAR PERMEABILITY. F.F. Samaha,* K. Meininger,* M.T. Shipley and P.A. Tornheim. (SPON: T. Mandybur) Dept. of Anat. Cell Biol., U. Cincinnati Coll. Med., Cincinnati, OH 45267.

The present experiments were designed to study the location and duration of altered vascular permeability in the cerebral hemispheres following mechanical injury to the brain. Anesthetized cats were either head injured (using impact, acceleration, and rotation of the skull) or sham injured. At 15 minutes and 7 days after trauma, horseradish peroxidase (HRP) was administered intravenously. Five minutes later, the animals were perfused briefly with a saline clearing rinse, followed by a buffered gluteraldehyde-paraformaldehyde solution. Adjacent serial sections at approximately 30 levels of the cerebral hemispheres were incubated with either 3'3'-diaminobenzidine (DAB) or tetramethylbenzidine (TMB) as a chromogen. Sections were examined by bright and darkfield microscopy for areas of HRP reaction product. Extravasation of HRP from permeable vessels was assumed to be represented by perivascular reaction product.

In animals with tissue hemorrhage, DAB reaction product was confined to the regions immediately surrounding the sites of contusions. By contrast, with use of TMB there was perivascular reaction product at a number of locations in hemispheres with contusion, including: 1) the cortex and white matter adjacent to contusion; 2) the area of interface between the lateral ventricle, white matter, and deep gray matter compartments; and 3) the areas of interface between the cerebral cortex and subcortical white matter. In addition, the vessel walls of both control and head-injured animals were lightly stained, allowing examination of vessel morphology. These findings were observed at both 20 minutes and 7 days after injury.

Our results suggest that TMB is qualitatively and quantitatively superior to DAB for localizing sites of cerebral vascular permeability to protein. With the TMB method, we observed breakdown of the blood-brain barrier to protein not only in tissue surrounding contusion but also at junctions between tissue compartments of the brain. The finding of protein leakage at the gray matter/white matter interface at 7 days following trauma was unexpected. This result suggests that severe brain injury can lead to entrance of plasma constituents into the extracellular space of the brain for prolonged periods at multifocal sites. We hypothesize that this type of vascular change results from mechanical damage to vessel walls secondary to shearing forces delivered at the time of injury.

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293.7 *NECROSIS ASSOCIATED WITH BLOOD-BRAIN BARRIER CHANGES FOLLOWING A SIMULATED SUBARACHNOID HEMORRHAGE.* *Priti S. Lacy, Department of Anatomy, University of Michigan Medical School, Ann Arbor, MI 48109.*

Abnormal post-contrast enhancement in cranial computerized tomography observed up to 20 days after subarachnoid hemorrhage has been reported in regions bordering the basal subarachnoid spaces (SS) suggesting it is not "subarachnoid" but is parenchymal and is possibly due to a breakdown of the blood-brain barrier (BBB) [Doczi et al. J. Neurosurg. 60:335, 1984]. Disruption of BBB was subsequently reported by Sasaki et al. (J. Neurosurg. 63:433, 1985) in major cerebral arteries. The present study sought to determine if presence of blood in the SS resulted in parenchymal damage associated with BBB changes.

Adult male Sprague-Dawley rats were anesthetized with Ketamine (90 mg/Kg, i.m.). Approximately 1.6 cm of a piece of silastic tubing was led to the ventral SS through a burr hole in the left frontal bone, maneuvering it manually towards the midline at the base of the skull. 0.3 ml of autologous blood was introduced through the tubing into the ventral subarachnoid space. Controls were either sham operated or were given 0.3 ml saline instead of blood. Animals were allowed to survive for 5 days. Brains were subsequently fixed via perfusion with FAM (formaldehyde, glacial acetic acid, methanol, 1:1:8). Following dehydration and embedding in paraffin, 7 µm sections were cut and stained with hematoxylin and eosin and evaluated for parenchymal damage.

Examination of skulls indicated either presence of small quantities of blood (n=5) or its absence (n=1). Gross examination of brains revealed dark (black, reddish brown, grey) areas on left side only (n=6). The dark areas corresponded to the location of blood present in the skull indicating disruption of BBB (n=5). In the remainder (n=1) dark grey area was present without evidence of blood in the skull. Brain sections revealed necrosis only in animals with simulated subarachnoid hemorrhage which was limited to the left side to cortex, striatum, amygdaloid nucleus, entorhinal cortex and subiculum. Hippocampus appeared normal on both sides. The results indicate that necrosis is associated with disruption of BBB and is due to presence of blood in SS. The absence of necrosis on the right side of the brains and infiltration of blood into brain surface on the left side only also indicated that parenchymal damage was associated with disruption of the BBB.

293.8 EFFECT OF NMR IMAGING ON BRAIN CORTEX MICROVESSELS OF THE RAT: INCREASED PERMEABILITY OF THE BLOOD-BRAIN BARRIER. R.R. Shivers, M. Kavaliers, F.S. Prato, and R.-M. Pelletier. Departments of Zoology, Nuclear Medicine and Anatomy. Univ. of Western Ontario, London, Ontario, Canada N6A 5B7.

The highly regulated, selectively permeable interface between the vascular and central nervous systems of vertebrates,the blood-brain barrier, has been shown to be functionally altered by a broad spectrum of physical and chemical insults. Increased permeability of this barrier to tracers such as horseradish peroxidase is the most frequently observed consequence of pathological agents, including purely physical ones (ie. stress, microwave irradiation, high blood pressure, heat shock).The present study on the integrity of the rat blood-brain barrier during magnetic resonance imaging (MRI), under conditions identical to those used to scan human patients, clearly shows that under the intense magnetic field of the MRI situation, the barrier becomes permeable to horseradish peroxidase, and is therefore presumed to be rendered "leaky". Electron microscopic examination of frontal cortex microvessels from which tracer had escaped into the adjacent intercellular compartment of the brain parenchyma, failed to demonstrate interendothelial tight junctions (zonulae occludentes) which had been completely permeated with protein tracer.These junctions are thus considered structurally intact - a conclusion supported by freeze-fracture, in which all tight junctions examined were structurally identical to those of unaffected control brain microvessels. On the other hand, the cytoplasm of brain capillary endothelia of MRI-treated rats contained numerous tracer-filled vesicles and tubulo-vesicular profiles. The vesicles were often attached to the luminal or abluminal plasma membrane as well as free in the cytoplasm. Occasionally, the tracer-laden tubulo-vesicular structures appeared to extend from the luminal to abluminal plasmalemma, to which they were fused, thereby creating trans-endothelial conduits for extravasation of tracer into the subjacent extracellular environment of the brain. Examination of platinum replicas of brain capillary endothelia from treated rats confirmed observations made on thin plastic sections and revealed vesicles and trans-endothelial channels identical to those found in other studies of blood-brain barrier dysfunction (eg.Shivers, Brain Res.170:509,1979;Farrell and Shivers, Acta Neuropathol.63:179,1984; Shivers and Harris, Neuropathol.Appl.Neurobiol.10:343,1984;Shivers et al, Acta Neuropathol.64:192,1984). The route of tracer extravasation observed in this study is therefore,likely via vesicular and tubulo-vesicular traffic;not by passage of tracer through disrupted interendothelial tight junctions. Results of this study strongly suggest that under the conditions of routine clinical NMR imaging, the blood-brain barrier of rats is rendered temporarily "leaky"- a condition that could jeopardize conserved and regulated functioning of the central nervous system. Supported by NSERC (RRS,MK).

293.9 ACUTE MICROWAVE IRRADIATION ALTERS IN VIVO MICRO-PINOCYTOTIC UPTAKE OF RHODAMINE-FERRITIN COMPLEX BY CNS CAPILLARY ENDOTHELIAL CELLS (CEC). J. Nierenberg*, R.A. Schilling* and D.G. Lange. Department of Anesthesiology/Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Acute exposure to microwave radiation (2.45 GHz, cw, 80 mW/cm^2, 30 min) in Sprague-Dawley rats (200-250 gm), has been shown to alter the amount of horseradish peroxidase staining seen by electron-microscopy. However, the existence of fixation artifact, and the poor flexibility of the assay system to perturbation by other agents, as well as the long turn-around time, prompted the evaluation of an alternate in vivo/in vitro micro-pinocytosis assay. Rhodamine-Ferritin complex has been demonstrated to estimate the uptake of fluorescent material, by pinocytotic mechanisms, into capillary endothelial cells isolated from epididymal fat pads (Wagner et al., Microvasc. Res., 14:6780, 1977). We have adapted this technique in both in vivo and in vitro assay systems employing CEC's isolated from microwave exposed animals.

In the in vitro assay, rats are exposed to microwave radiation, sacrificed, and the CEC isolated using a modified technique of Betz et al. (Am. J. Physiol., 236(1):96, 1979). After isolation, the CEC are incubated in the presence of Rhodamine-Ferritin complex (5-10 mg/ml). The microwave exposed CEC's showed a non-significant time-dependent increase in micro-pinocytotic activity by 45 minutes into the incubation. Since micro-pinocytosis in CEC's is predominantly a luminal membrane phenomenon (Broadwell et al., Proc. Natl. Acad. Sci. 80:7352-7356, 1983), the CEC's were exposed in vivo to the Rhodamine-Ferritin complex, in pentobarbital anesthetized rats, by a bolus intravenous injection (~ 30 mg/kg). Following either a 30 min sham or microwave radiation exposure, the CEC's were harvested from PBS perfused brains (40 ml at pH = 5.8), and assayed for Rhodamine-Ferritin complex content. Microwave exposure approximately doubled the amount of Rhodamine-Ferritin complex found in the isolated CEC's (0.005 μg/μg DNA vs 0.009 μg/μg DNA). This increase in uptake by the luminal surface of CEC's was associated with a significant increase in the rate of clearance of Rhodamine-Ferritin complex from the plasma (t 1/2 = 57 min in control vs t 1/2 = 30 min in microwaved animals). This data supports the hypothesis that microwave energy increases the micro-pinocytotic activity of the luminal surface of CEC's. (Supported by ES-03386).

293.10 **KINETIC ANALYSIS OF ORGANIC ACID HERBICIDE, 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T), UPTAKE BY THE RABBIT CHOROID PLEXUS.** **R.F. Keizer* and C.S. Kim*. (SPON: C.G. Lineberry). Dept. of Neurology and Biol. Sci. Res. Ctr., Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.**

The organic acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T, components of Agent Orange, were used extensively for weed control during Vietnam war. Increasing data has provided evidence for neurotoxic effects among people who have been exposed (IARC monogr. 15:111-138; 273-300, 1977). A distinctive transport system for 2,4-D has been described in the choroid plexus (Kim et al., J. Pharmacol. Exp. Ther. 225:699-704, 1983) and our present study provides further studies on its congener, 2,4,5-T. All kinetic studies were conducted in albino rabbits of either sex. Animals were sacrificed by decapitation and the choroid plexus (CP) was dissected free from the lateral and fourth ventricle (LV, FV), respectively. Tissues were incubated for 10 min in artificial CSF containing ^{14}C-2,4,5-T 0.02 μCi/ml (spec. act. 23 mCi/mmol). Tissue uptake was expressed as T/M ratio and kinetic values were determined by least squares regression line fitted to a Lineweaver-Burk plot using 2,4,5-T at seven different concentrations ranging from 0.01 to 1.0 mM. Analysis of the uptake revealed a greater V_{max} in LVCP (0.107 μmol/ml/min) than that of FVCP (0.086 μmol/ml/min) but no change in K_m (LVCP, 0.058 mM; FVCP, 0.057 mM). The larger T/M ratio (greater than 1) for 2,4,5-T indicates the intracellular accumulation in excess of the medium concentration and the uptake was concentration-dependent and inhibited by ouabain and hypothermia. Inhibition by organic acids was specific for those compounds which share the organic acid transport system. 2,4,5-T and 2,4-D were mutually inhibitory in a concentration-dependent manner and tyrosine failed to inhibit the uptake of ^{14}C-2,4,5-T by either FVCP or LVCP. The present results, therefore, strongly suggest that active transport of 2,4,5-T operates across the CSF-blood barrier by organic acid transport system, which may critically affect the neurotoxicity of the compound by determining the efficiency of its removal from brain. **Supported in part by NIH grants: HD-03110 and ES-03458.**

293.11 A PREVIOUSLY UNREPORTED PATHOLOGIC CONSEQUENCE OF ARGYRIA. Steve Landas*, Robert L. Schelper*, Richard Broadwell*[1], Lee D. Mitchell*, Lawrence D. Wilkin*, A. Kim Johnson*. (SPON: Alan Randich). Departments of Pathology, University of Iowa, Iowa City, Iowa 52242, and University of Maryland[1], Baltimore, Maryland 21201.

Argyria is systemic intoxication or overload with silver compounds. Silver absorption usually occurs following industrial or medicinal exposure. In the pre-antibiotic era large quantities of silver-containing agents were occasionally used in the treatment of some conditions. Consequences of argyria have long been thought to be limited to cosmetic alterations: intense gray discoloration of skin, sclera, and mucous membranes. We have studied the autopsy brain specimen of an elderly woman with argyria and have previously reported findings which demonstrate similarities in circumventricular organ vascular permeability between experimental animal and human brains. In addition, we have found evidence of silver deposition in another site of neural-hemal contact, the specialized olfactory epithelium. In consideration of animal models of anosmia induced by application of silver salts, the pattern of silver deposition in our patient, and the markedly shrunken anterior commissure in her brain, we believe we have found a specific, non-cosmetic pathologic consequence of argyria.. We postulate silver salts, in high concentrations, may cause cell death in the zones of neural-hemal contact in argyric patients with subsequent transynaptic degeneration. The distribution of silver in cranial nerve nuclei, spinal cord, and dorsal root ganglia is also examined and compared with animal models.

293.PO ISOLATION AND COMPOSITION OF A 4200 DALTON PEPTIDE FROM VASCULAR AMYLOID OF ALZHEIMER'S DISEASE. W.M. Pardridge, H.V. Vinters*, J. Yang*, J. Eisenberg*, T. Choi*, and W.W. Tourtellotte. Depts. of Medicine, Pathology, and Neurology, UCLA School of Medicine, Los Angeles, CA 90024 and Neurology Service, Wadsworth Veterans Administration, Los Angeles, CA 90073.

The cardinal lesions of Alzheimer's disease are neurofibrillary tangles, neuritic plaques, and vascular amyloid. The vascular amyloid generally involves small arterioles. Since recent studies show that intact microvessels can be readily isolated in high yield from autopsy human brain up to 40 hours postmortem (Pardridge et. al., 1985), the isolation of microvessels from Alzheimer's disease ia a new approach for isolating the protein comprising the vascular amyloid in Alzheimer's disease. Capillaries and small arterioles were isolated from three Alzheimer's disease brains and three control brains by mechanical homogenization, dextran flotation, 210μ nylon mesh seiving, and Percoll density centrifugation. One of the Alzheimer's disease brains showed extensive amyloid angiopathy in formalin-fixed, paraffin-embedded 6μ sections. The small arterioles isolated from this brain also showed extensive amyloid angiopathy after staining with 0.05% Congo red. These capillaries were solubilized in 90% formic acid and the supernatant was subjected to urea sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (PAGE). The Alzheimer subject with extensive congophilic angiopathy showed a unique 4200 Dalton peptide band after staining of the gel with Coomasie blue; the three controls and two Alzheimer's disease subjects without amyloid angiopathy showed no 4200 Dalton peptide on urea SDS-PAGE. The 4200 Dalton band was extracted from the gel and purified by C-4 reverse phase high performance liquid chromatography (HPLC). Only two prominent peaks eluted at approximately 42-45% acetonitrile, and these two peaks were individually evaporated to dryness, hydrolyzed by acid, and analyzed for amino acid composition using the Pico-Tag system (Waters). Both peaks eluted from the HPLC column were of essentially identical amino acid composition and contained 40 amino acid peptides that were particularly enriched in small aliphatic amino acids, such as glycine, alanine, and valine residues, i.e., amino acids expected for a β-pleated sheet peptide. The amino acid composition of the 4200 Dalton peptide isolated from vascular amyloid is very similar to the amino acid composition of the 4200 Dalton peptide recently isolated from neuritic plaques or neurofibrillary tangles or amyloid laden meningeal vessels of Alzheimer's diease. Therefore, all three cardinal lesions of Alzheimer's disease appear to be comprised of the same 4200 Dalton core peptide. Future studies on the origin of the 4200 Dalton peptide (i.e., brain versus blood origin) may lead to advances in the diagnosis and treatment of Alzheimer's disease.

294.1 MOLECULAR BASIS OF TARGET SITE RESISTANCE TO NEUROTOXIC INSECTICIDES. D. P. Rossignol and P. K. Pipenberg, (SPON: J.Carnahan). Biological Chemistry Group, Agricultural Products Dept., E. I. Du Pont De Nemours & Co., Experimental Station, Wilmington, DE 19898.

Persistent activation of voltage-dependent sodium channels is responsible for the neurotoxic effect of pyrethroids, DDT and several steroidal alkaloids. Resistance to pyrethroids and DDT insecticides in knockdown resistant (Kdr) insects is through decreased nerve sensitivity to these toxins. Analysis of neurotoxin binding to voltage dependent sodium channels and acetylcholine receptors in nerve membrane preparations from wild-type and Kdr houseflies indicates that the Kdr mutation results in a decreased number of voltage-dependent sodium channels.

A membrane fraction from housefly heads was purified by differential centrifugation. Neuronal membranes, as identified by ^{3}H saxitoxin binding, were enriched 5-6 fold in the high speed pellet, and had a buoyant density of 1.14 g/ml on sucrose density gradients.

Membranes from wild-type and Kdr flies contained a single, high affinity binding site for saxitoxin that was identical in the two strains (Kd=2.3nM at 22°C). However, maximum binding (Bmax) in Kdr flies was 125 + 51 fmoles/mg, a reduction of 40-60% when compared to wild type (Bmax=319 ± 33 fmoles/mg). If mutational alteration of the sodium channel polypeptide occurred, it might result in an effect on saxitoxin binding affinity. To test this, binding was assayed under mildly denaturing conditions such as various temperatures and pH. In addition, the dissociation rate of ^{3}H saxitoxin was measured. No significant differences between Kdr and wild type sodium channels were detected, suggesting that the Kdr mutation does not result in alteration of the sodium channel polypeptide.

In order to determine if this decrease in sodium channel density was due to a general decrease in the content of neuronal membrane proteins, we measured the binding of 125I-α-bungarotoxin to acetylcholine receptors. Membranes from wild-type and Kdr flies contained a single class of high affinity binding sites having identical Kd and Bmax values.

Thus, the nerve membranes from toxin resistant flies contain identical but fewer sodium channels than wild-type flies. In this case, when a neurotoxic agent causes persistent activation of sodium channels, reduction in the number of sodium channels is one mechanism of toxin resistance.

294.2 NEUROTOXIC INSECTICIDES ANTAGONIZE CHLORIDE UPTAKE AT THE MAMMALIAN $GABA_A$ RECEPTOR-CHLORIDE CHANNEL COMPLEX. J. R. Bloomquist, P. M. Adams*, and D. M. Soderlund*. Dept. of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456.

The $GABA_A$ receptor-chloride channel complex is an important mediator of inhibitory neurotransmission in the mammalian central nervous system. Competitive displacement of [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding by cyclodiene insecticides has suggested that the toxicity of these compounds is due to blockage of the chloride channel that is coupled to the $GABA_A$ receptor. Using a functional ^{36}Cl ion flux assay (Harris and Allan, Science 228:1108, 1985) we found that cyclodienes were effective inhibitors of GABA-dependent chloride uptake at concentrations comparable to those of the established chloride channel antagonists picrotoxinin (I_{50}=11 μM) and TBPS (I_{50}=1.3 μM) For a series of 7 cyclodienes, I_{50} values spanned a 140 fold concentration range in the following order of decreasing potency: 12-ketoendrin; isobenzan; endrin; dieldrin; heptachlor epoxide; aldrin; and heptachlor. I_{50} values in the chloride flux assay were closely correlated with toxicity (r=0.871) and inhibition of TBPS binding (r=0.966). Abamectin, an anthelminthic and insecticide with GABA-mimetic properties in several nerve and nerve-muscle preparations, did not stimulate ^{36}Cl uptake when applied alone at micromolar concentrations but inhibited GABA-dependent uptake with an I_{50} of 93 nM. This finding is consistent with our observation that intracerebral or intraperitoneal injection of just-lethal doses of abamectin results in transient hyperexcitation and convulsive behavior prior to the onset of its profound depressant effects. These results demonstrate the utility of this assay for describing and quantifying the disruption of normal $GABA_A$ receptor function by neurotoxic insecticides. Moreover, they provide evidence that this is an important target for the toxic action of these compounds in vivo.

294.3 ACTIONS OF GLYCINE ON DISSOCIATED MOUSE SPINAL NEURONES. M. R. Duchen*, D. L. Patterson* and T. J. Biscoe* (SPON: J. A. Stephens). Dept. of Physiol., Univ. Coll. London, Gower St., London WC1E 6BT. UK.

A combination of enzymatic and mechanical procedures have been used to obtain a preparation of dissociated neurones from the spinal cords of young (14-21 day old) mice (Biscoe, Duchen & Patterson, J. Physiol., in press). Cells retain their morphological features and range in size from 10-35 μm. The patch clamp technique has been used to study voltage- and transmitter-gated currents in these cells. A random selection of 50 cells had input resistances ranging from 300 MΩ to 2.5 GΩ (mean, 750 MΩ) and capacitances of 17.0 ± 4.13 pF (mean ± SD).

Stable whole cell recordings were most readily obtained when fluoride was present in the patch pipettes. Glycine, applied by ionophoresis to cells in which fluoride was the major anion present, evoked a response which reversed at -45 mV (filling solution (mM): KF, 130; $CaCl_2$, 1; EGTA, 11; Hepes, 7.5; pH 7.3). This indicates a relative permeability of F^-:Cl^- of 0.2:1. When combinations of F^- and Cl^- were used, the reversal potential of the response changed as predicted by the Nernst equation for Cl^-, given these relative permeabilities.

Currents evoked by application of glycine were typically associated with an increase in current noise. Analysis of current variance was undertaken on records consisting of small sustained responses that did not desensitise. A logarithmic plot of frequency against variance was invariably fitted best by the sum of 2 Lorentzian curves, with mean corner frequences of 2.16 ± 0.9 and 39.9 ± 23.6 Hz (mean ± SD, n = 121). Similar distributions were seen when single channel records obtained from outside-out patches were examined.

Total variance, calculated from the parameters of the Lorentzian best fit, was used to calculate mean single channel conductance, having defined the linearity of the variance/mean relation. Mean single channel conductance at a holding potential of -70 mV and with 50 mMCl, 80 mMF in the pipette, was 24 ± 13 pS. Attempts to define maximal evoked currents led to desensitising responses. The current variance seen during the desensitised response was less than expected from the variance/mean relation, suggesting that desensitisation leads to a low conductance state of the Cl^- channel.

At holding potentials close to the chloride equilibrium potential, application of glycine typically caused a fall in baseline noise. This was not seen when Cs^+ was used in the pipettes, suggesting that glycine may act to close K^+ channels as well as opening chloride channels.

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294.4 INTERACTION OF 4-AMINOPYRIDINE, TETRABUTYLAMMONIUM AND DEXOXADROL WITH THE RAT BRAIN PHENCYCLIDINE (PCP) RECEPTOR. R.G. Sorensen and M.P. Blaustein. University of Maryland, School of Medicine, Baltimore, Maryland 21201

PCP is a dissociative anesthetic and drug of abuse that blocks certain voltage-gated K channels (Bartschat & Blaustein, PNAS 83: 189, 1986). Consistent with an interaction of PCP with K channels, we have shown that K channel blockers compete with [^{3}H]PCP for binding to synaptic membranes (Biophys. J. 47: 384a, 1985). The dioxolane, dexoxadrol, which also binds to the brain PCP receptor, also blocks the same PCP-sensitive K channel (Soc. Neurosci. 11: 316, 1985). We now further characterize the interaction of these agents with the rat brain PCP receptor. Several aminopyridines (AP) and tetraalkylamines (TAA), compounds known to block K channels, displaced [^{3}H]PCP binding to rat brain synaptic membranes. The orders of potency (apparent IC_{50}, mM) were; for the AP's: 4-AP (2.6) ≃ 3,4-DiAP (3.1) > 2-AP (8.8) >> 3-AP (53.9); and for the TAA's: TBA (1.6) > TEA (6.7) >> TMA (33.6) (the tetrabutyl, tetraethyl and tetramethyl analogs, respectively). This agrees with their orders of potency for blocking K channels or increasing neurotransmitter release. 4-AP, TBA and dexoxadrol were further tested to determine the type of inhibition at the PCP receptor each produced. For these experiments, [^{3}H]TCP, a higher affinity and more behaviorally potent analog of PCP, was used. The effect of the test compounds on the apparent binding constants for [^{3}H]TCP was determined by measuring the displacement of [^{3}H]TCP from rat brain synaptic membranes by unlabelled TCP in the presence of selected concentrations of 4-AP, TBA or dexoxadrol. PCP was also included as a test compound for comparative purposes and as a control for "pure" competitive inhibition. All four test compounds specifically increased the apparent K_D for high affinity [^{3}H]TCP binding to the PCP receptor, with little change in the apparent B_{max}. This suggests that the inhibition by the four test compounds is competitive. ([^{3}H]TCP, as [^{3}H]PCP, also binds to low affinity sites. The binding constants of these sites were unaffected by the test compounds.) Schild plots of the data showed PCP, as expected, to be a pure competitive inhibitor (slope = -0.96), TBA to be a mixed (competitive and non-competitive) inhibitor (slope = -0.75), and 4-AP and dexoxadrol to be allosteric inhibitors (slopes ≃ -1.5). We propose that TBA binds to the same site as PCP: The non-competitive inhibition predicted for TBA probably results from its weak affinity for the PCP receptor. The Schild plot analysis predicts that 2 moles of 4-AP or dexoxadrol are required to displace 1 mole of PCP from the receptor. Thus, 4-AP and dexoxadrol should have binding sites distinct from that to which PCP binds. Supported by NIH grant NS-16106 and NRSA NS-07611.

294.5 EFFECT OF MASTOPARAN ON CA2+ ACTIVATED K+ CHANNELS IN BOVINE CHROMAFFIN CELLS. A. Joshi *2, J.M. Trifaró 3 and M.I. Glavinović 1,2, Depts. of Anaesthesia Research 1,Physiology2 and Pharmacology and Experimental Therapeutics3, McGill University, Montreal, Canada H3G 1Y6.

It is known that bee venom peptides (i.e. apamine) can affect Ca2+ activated K+ channels profoundly. It is also known that bee venom peptides affect calmodulin activity (Barnette et al., Biochem. Pharmacol. 32:2929-2933, 1984). It has been suggested that calmodulin is involved in activation of Ca2+ dependent K+ channels (Pape & Kristensen, Biochem. Biophys. Acta. 770: 1-6,1984). In this study we try to directly test whether mastoparan (bee venom peptide and potent inhibitor of calmodulin activity) affects Ca2+ activated K+ channels.

Experiments were done on excised inside out patches of bovine chromaffin cells. The pipettes were filled with Locke solution which consisted of (mM): 154 NaCl, 2.2 Cacl2, 1.2 MgCl2, 2.5 KCl, 2.15 K2HPO4, 0.85 KH2PO4 and 10 dextrose. The internal solution contained (mM): 160 KCl/KOH, 10 Hepes, 1 MgCl2, 0.5 EGTA and .279 CaCl2 at pH = 7.2, and nominally had 0.1 μM free Ca2+.

In control solutions, amplitude histograms usually show several 'peaks' indicating that the patch has several Ca2+ activated K+ channels. Addition of mastoparan (0.5 - 1 μM) to the internal solution alters amplitude histograms by diminishing the open-state probability. This leads to lower current flow through the membrane. Moreover, mastoparan also widens the 'peaks' and hence appears to act as a flickery blocker. Further studies will provide more information about the mechanism of action of mastoparan and about possible involvement of calmodulin in regulation of activity of Ca2+-activated K+ channels.

This study was supported by MRC and MDA (Canada).

294.6 BACLOFEN INHIBITS RAT LOCUS COERULEUS NEURONS IN BRAIN SLICES BY INCREASING K^+ CONDUCTANCE. S. S. Osmanovic* and S. A. Shefner. Department of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

Baclofen (β-p-chlorophenyl GABA) has been shown to bind specifically to $GABA_B$ receptors in the central nervous system. Baclofen effects on rat locus coeruleus (LC) neurons were studied with intracellular recording in a totally submerged brain slice preparation. Known concentrations of (±)-baclofen (1-100 μM), (-)-baclofen (100 nM-100 μM) and (+)-baclofen (10 μM-1 mM) were applied in the bath. LC neurons showed the usual regular pattern of spontaneous firing with firing rates of 0.1 to 2 Hz. (-) or (±)-baclofen (1-50 μM) reversibly decreased the rate of spontaneous firing in all neurons tested (n = 33), completely blocking spontaneous firing in 70% of these cells. In 91% of these cells inhibition of firing was accompanied by membrane hyperpolarization. Small reductions in apparent input resistance (3-16%) were observed at the peak of baclofen-induced hyperpolarizations (n = 12). The membrane potential was manually clamped back to its predrug value during these resistance measurements to control for changes in resistance secondary to membrane hyperpolarization. Hyperpolarizations in response to 10 μM (-)-baclofen ranged in amplitude from 1 to 23 mV. Hyperpolarization amplitude was found to be directly related to input resistance (correlation coefficient = 0.87, n = 12, sig. $p < .01$); i.e. cells with higher input resistances showed larger hyperpolarizations.

The mean reversal potential for the baclofen-induced hyperpolarization calculated from the intersection of voltage-current curves under control conditions and in the presence of baclofen was -111 mV (n = 14) and did not differ when measured with K chloride versus K acetate-filled electrodes. The baclofen reversal potential was similar to the mean reversal potential of the post-stimulus hyperpolarization (-114 mV, n = 4) and the K^+ equilibrium potential calculated from the Nernst equation given the K^+ concentration of the media (2.5 mM). Baclofen effects on LC neurons were blocked by extracellular barium (1 mM).

In cells tested with two or more baclofen concentrations (n = 11), effects on membrane potential, spontaneous firing and input resistance were dose-dependent. Responses showed stereospecificity; (-)-baclofen was much more potent than (+)-baclofen. Baclofen effects were not antagonized by bicuculline (40-100 μM) and persisted when transmitter release was blocked with media containing 0.25 mM Ca^{++} and 20 mM Mg^{++}. We conclude that baclofen acts on $GABA_B$ receptors to directly inhibit rat LC neurons by increasing the membrane K^+ conductance.

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294.7 STRAIN-SELECTIVE EFFECTS OF NICOTINE ON EVOKED RESPONSES IN MOUSE HIPPOCAMPUS. R. K. Freund and J. M. Wehner. Institute for Behavioral Genetics and School of Pharmacy, Univ. of Colorado, Boulder, CO 80309.

A number of differences regarding presumed nicotinic cholinergic transmission have been identified in DBA and C3H mouse strains, including sensitivity to Nic-induced seizures (C3H > DBA; Miner et.al., JPET, 231: 545, 1984) and number of alpha-bungarotoxin binding sites in the hippocampus (C3H > DBA; Marks et.al., JPET, 226: 291, 1983). We have examined electrophysiological effects of bath-applied Nic on synaptic transmission and cell firing in the CA1 region of hippocampal slices from DBA and C3H mice using extracellular recording techniques. Nic had little effect on evoked synaptic field potentials recorded from either strain at concentrations up to 6.4 mM, while the antagonist mecamylamine (Mec) completely inhibited field potentials with an IC50 of 2 mM. The effects of Nic on CA1 cell firing were examined by measuring evoked population spike amplitudes (PSAs). Complete input-output (stimulus intensity vs. PSA) curves were obtained several minutes before and after bath application of Nic. Nic (10-800 uM) increased PSAs elicited by relatively low stimulus intensities (i.e., stimuli which evoked PSAs < 50% of the maximum PSA) for both DBA and C3H mice. This effect of Nic was slow, reaching a maximum at 15-20 min. The Nic-induced increase was greatest for small PSAs (< 25% of the maximum PSA), and was apparently strain-selective: Nic (10-800 uM) induced an increase in PSAs from 0.2 to 6X for DBA mice, while this increase was 1-1.5X for C3H mice. Larger control PSAs (> 50% of maximum PSA) increased less after Nic treatment. Twenty minute pretreatment with a 10-fold excess of Mec partially inhibited (approximately 50% for both strains) the increase in PSA produced by 40 uM Nic for small PSAs. For larger PSAs, the Mec pretreatment was differentially effective. Nic in the presence of Mec resulted in a smaller average PSA than Nic alone for DBA mice, but Mec did not affect the Nic response for the C3H strain. Nic (≥ 100 uM) induced epileptiform activity (multiple evoked population spikes); the degree of this effect appears to increase with concentration, and is most easily quantified at higher concentrations. At a concentration of 800 uM, Nic induced more profound epileptiform activity in DBA than in C3H mice. These results suggest that Nic may differentially affect cell firing in hippocampal CA1 pyramidal cells from DBA and C3H mice. Nic induces a greater increase in cell firing for DBA mice than for the C3H strain. The data indicate that there are genetic factors regulating the electrophysiological responses to nicotine in the hippocampus, but the relationship between these responses and the behavioral sensitivity or binding data remain to be determined. (Supported by DA 07043 and a grant from R. J. Reynolds, Inc.)

294.8 INTERACTION OF PIPERIDINE DERIVATIVES WITH THE NICOTINIC CHOLINERGIC RECEPTOR FROM TORPEDO ELECTRIC ORGAN. T.K. Narayanan*[1], R.S. Aronstam[1], E.X. Albuquerque[2] and J.W. Daly[3] (SPON: A. Hitri). [1]Dept. Pharmacol. & Toxicol., Med. Coll. Georgia, Augusta, GA 30912, [2]Dept. Pharmacol. & Exp. Ther., Univ. of Maryland, Sch. of Med., Baltimore, MD 20201, and [3]Lab. Bioorganic Chem., NIADDKD, NIH, Bethesda, MD 20205.

The interactions of several piperidines with nicotinic receptor complexes from Torpedo californica electric organ were studied using radiolabelled probes for receptor and ion channel binding sites. Cis- and trans-2-methyl-6-n-undecanyl piperidines (C11), major constituents of fire ant venom, did not inhibit [^{125}I]alpha-bungarotoxin binding to the ACh binding site, but did inhibit [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) binding to sites associated with the receptor-gated ion channel (IC50's=0.08-0.24 uM). C11 affinity for the channel was doubled in the presence of 1 uM ACh. 2,6- (but not 3,5-) dimethylpiperidine was a moderately active inhibitor of ion channel binding (K_I=8.8 uM). 2-Methyl piperidine was considerably less active (K_I=600 uM), although it was more potent than 3- or 4-methylpiperidine. The ion channel affinities of 2,6-dimethylpiperidine and 2-methylpiperidine were decreased in the presence of 1 uM ACh. Carbamylcholine binding affinity was increased by up to 7 fold in the presence of 10 and 32 uM C11, but was decreased in the presence of 2,6-dimethylpiperidine and 2-methylpiperidine. The cis- and trans-isomers of C11 were equipotent in producing each of these effects. In these actions, C11 resembles a variety of other compounds derived from 2,6-disubstituted piperidines, including the histrionicotoxins, gephyrotoxins and pumiliotoxins. These studies establish the importance of alkyl substitutions in the ortho position of the piperidine ring in conferring ion channel specificity, and the importance of substantial alkyl side chains in conferring the ability of channel blockers to stabilize the nicotinic receptor complex in a high affinity, "desensitized" conformation.

Table 1. Piperidine Inhibition of [^{3}H]H_{12}-HTX Binding to Nicotinic Receptor Complexes.

Compound	K_I, uM	
	- ACh	+ ACh (1 uM)
Cis-C11	0.16 ± 0.03	0.13 ± 0.02
Trans-C11	0.24 ± 0.08	0.094± 0.008
2,6-dimethylpiperidine	8.8 ± 1.3	122 ± 26
3,5-dimethylpiperidine	394 ± 42	367 ± 25

Mean ± S.D., N=3.

294.9 ALPHA-BUNGAROTOXIN MODIFIES DIHYDROPYRIDINE BINDING BUT NOT THE K^+-INDUCED Ca^{2+} UPTAKE IN ANEURALLY CULTURED HUMAN MUSCLE. C. Desnuelle, V. Askanas, W.K. Engel. USC Neuromuscular Center, Los Angeles, CA 90017.

Dihydropyridine (DHP) derivatives belong to the class of Ca^{2+} antagonists, known to bind specifically and with high affinity to the voltage-dependent (VD) Ca^{2+} channel. We have recently shown that aneurally cultured human muscle has VD Ca^{2+} channels and that insulin treatment increases the maximum binding capacity for the DHP derivative (+)PN200-110 without change in its affinity (Desnuelle et al Neurology, 36, 171-172, 1986). Previously we shown that insulin also increases the total number of nicotinic acetylcholine receptors (AChRs) in aneurally cultured human muscle (Askanas, et al, Neurosci Lett., 61, 213-219, 1985). Since the magnitude of the AChR increase measured by ^{125}I-labeled alpha-bungarotoxin (^{125}I-alpha BT) binding was virtually the same as the increase of ^{3}H(+)PN200-110 binding sites (300% of control) and since other polypeptide growth factors, viz. fibroblast growth factor (FGF) and epidermal growth factor (EGF), that influence other muscle parameters (i.e. creatine kinase) did not influence either binding of alpha-BT or (+)PN200-110 to their respective receptors, we were interested to see if there is a relationship between AChRs and DHP receptors.

Human muscle cultures were established according to our described technique (Askanas and Engel, Neurology, 25, 58-67, 1975). $1x10^5$ cells were plated in 35mm Petri-dish in medium containing 10% fetal bovine serum (FBS), and supplemented with 10 ug/ml insulin, 25 ng/ml FGF and 10 ng/ml of EFG. After 48-72 hours, growth factors were removed and cultures were maintained in medium containing only 10% FBS. Twenty experiments from 10 different patient biopsies (total 170 cultures) were established. After 18-21 days of growth, experimental cultures were treated for 1 hour with various concentrations (10^{-10} to 10^{-5}M) of unlabeled alpha-BT and quantitative measurement of the total number of DHP receptors and nicotinic AChRs were made from 1/2 to 96 hours later. As expected, the total number of binding sites for ^{125}I-alpha-BT dramatically decreased (70-80%) after 1/2 h of $2x10^{-6}$M alpha-BT pretreatment; suprisingly, there was also 50-55% decrease of total binding sites for ^{3}H(+)PN200-110 binding. The magnitude of decrease of both ^{125}I alpha-BT and ^{3}H(+)PN200-110 binding sites was similar, related to the concentration of unlabeled alpha-BT used to block AChRs (IC_{50} value of 2.10^{-7}M and 6.10^{-7}M respectively). Total number of receptors for both alpha-BT and (+)PN200-110 increased exponentially and paralleled to each other during the 96 hours recovery period. K^+-induced Ca^{2+} uptake, measured in 40 mM K^+ and 5 mM K^+, was not affected by alpha-BT pretreatment, but was blocked when measured in the presence of unlabeled nitrendipine. Our studies 1) demonstrate the previously unknown correlation between binding sites of alpha-BT and DHP-derivatives, and 2) open a new avenue for investigating the realationship of the AChR to the VD Ca^{2+} channel function.

294.10 SINGLE CHANNEL ANALYSIS OF THE INTERACTION BETWEEN ANTIESTERASES AND CHOLINERGIC AGONISTS ON ACH RECEPTOR ACTIVATION IN RAT MYOTUBES. L.A. Merriam and J.F. Fiekers. Dept. Anatomy and Neurobiology, Univ. Vermont College of Medicine, Burlington, VT. 05405.

Anticholinesterase agents resemble cholinergic agonists in chemical structure and have recently been shown to activate the nicotinic cholinergic receptor in cultured rat myotubes. Previous studies from this laboratory have demonstrated that the simultaneous presence of low concentrations of both a cholinergic agonist and an anticholinesterase agent in the patch pipette produced channel activation which exceeded the activation achieved by either agonist alone. In the present experiments we have studied the interaction of acetylcholine, carbachol and suberyldicholine with antiesterase agents using the cell-attached and inside-out recording configuration of the patch clamp technique. Single channel currents were recorded at 20 degrees C from rat myotubes cultured in DMEM and 10% horse serum using standard cell culture conditions. Neostigmine (0.1uM) increased the frequency of channel opening when present simultaneously in the patch pipette with either ACh or Sub. In the presence of both agents small amplitude channel activation was also frequently observed which increased the baseline noise level. Pretreatment of the myotubes with methane sulfonyl fluoride (10mM) for one hour followed by a one hour wash inhibited the acetylcholinesterase present but did not alter the synergistic agonist interaction. The frequency of channel activation was concentration- dependent increasing with increasing concentrations of either agent. High concentrations of antiesterase agents produced numerous brief closures within the open channel with both ACh and Sub. Mean open time histograms with low concentrations of either the agonist or the antiesterase agent alone were well fitted by single exponential functions. However, the simultaneous presence of both agents frequently yielded open time histograms which required two components for an adequate fit. In low concentrations (<1uM) neostigmine prolonged the mean open time of channels activated by ACh and Sub. These results indicate that neostigmine may bind to a site on the nicotinic receptor-channel complex to modify channel activation produced by cholinergic agonists. (supported by the Muscular Dystrophy Association).

294.11 MECHANISM OF INTERACTION OF THE OXIMES 2-PAM AND HI-6 WITH ORGANOPHOSPHATE-POISONED FROG MUSCLE. M. Alkondon*, K.S. Rao* and E.X. Albuquerque (SPON: J.E. Warnick). Dept. Pharmacol. & Exper. Therap., Univ. Maryland Sch. Med., Baltimore, MD 21201.

The acute toxic and lethal effects of organophosphorus (OP) compounds have been ascribed to an irreversible inhibition of cholinesterase (ChE). Accordingly, oximes thought to reactivate the phosphorylated enzyme were developed as antidotal drugs. However, our recent studies have suggested that mechanisms other than the reactivation of ChE could be responsible for their therapeutic effectiveness. Thus, we have attempted to test the above hypothesis by studying the actions of oximes 2-PAM and HI-6 on endplate currents (EPCs) of frog muscles poisoned by the irreversible OP compounds VX, soman, sarin or tabun. EPCs were recorded from the frog sciatic nerve-sartorius muscle preparations. OP poisoning was achieved by perfusing the preparation for 1 h with Ringer's solution containing 20 μM of OP and then with normal Ringer's for 1 h to remove any excess of OP. This procedure resulted in a maximum inhibition of ChE as evidenced by a marked prolongation of EPC decay (>3 times that of control) with minimal direct effects of these compounds on the endplate. The prolongation of EPC decay by OP poisoning was seen even up to 4 h after OP-wash in control experiments and this allowed us to test the effect of oximes on such preparations. Both 2-PAM and HI-6 (50 μM to 1 mM), at membrane potentials between -100 and + 50 mV, caused a voltage-independent reduction in the time constant of EPC decay (τ_{EPC}) in OP-poisoned muscle, which nearly reached control (pre-OP) levels. In addition, especially with HI-6 at hyperpolarized potentials, a steep voltage-dependent decrease of τ_{EPC} was observed. On removal of the oximes, τ_{EPC} again increased to a level nearer to pre-oxime values. Qualitatively similar results were obtained when 2-PAM was administered after the muscle was exposed to OPs for a brief period of 10 min. Both these oximes also accelerated the EPC decay in preparations pretreated with the reversible ChE inhibitor (-)-physostigmine (10 μM). To assess whether the acceleration of EPC decay is due to a decrease in channel opening frequency or to a shortening of single channel open times, these oximes were tested at low temperature. When the oximes were applied alone either at 20 or 10°C, the main effect was the voltage-sensitive shortening of τ_{EPC} at hyperpolarized potentials. Only at very high concentrations, HI-6 produced an additional voltage-independent decrease in τ_{EPC} accompanied by a curvature in the current voltage relationship. These findings suggest that, in the presence of OPs, in addition to a process similar to desensitization, the oximes produce an open-channel blockade strongly dependent on voltage. (Support: U.S. Army Med. Res. & Devel. Comm. Contract DAMD17-84-C-4219).

294.12 EFFECT OF AN ORGANOPHOSPHORUS INHIBITOR OF ACETYLCHOLINESTERASE ON CHLORIDE CONDUCTANCES IN APLYSIA CALIFORNICA GANGLIA. Margaret G. Filbert*, James P. Apland, and Michael Adler. US Army Medical Research Institute of Chemical Defense, Neurotoxicology Branch, Physiology Division, APG, MD 21010-5425.

Organophosphorus (OP) inhibitors of acetylcholinesterase (AChE) are known to produce epileptiform activity. While the mechanisms underlying OP-induced seizures are not well understood, the abnormal electrical discharges could be the direct consequence of acetylcholine (ACh) accumulation. In the absence of transmitter hydrolysis, persistent ACh might produce epileptiform activity by reducing steady potassium current in a manner similar to the potassium channel blockers 4-aminopyridine and tetraethylammonium (Krnjevic et al., J. Physiol. 215:247,1971; Lebeda, & Rutecki, Neurobiology of Acetylcholine, Ed. N.J. Dun, Plenum, 1986). Other convulsant drugs are believed to exert their effects by interfering with chloride-dependent inhibitory synaptic activity mediated by the neurotransmitter γ-aminobutyric acid (GABA). Barnes (personal communication) has shown that paraoxon (O,O,-diethyl O-4-nitrophenyl phosphate) displaces specific binding of the cage convulsant t-butyl-bicyclo-phosphorothionate (TBPS), a GABA channel blocker, from binding sites on rat and chicken cerebral cortical membranes. Diazepam, a modulator of GABA-mediated inhibitory transmission, has been shown to afford significant protection against OP toxicity. To test the possibility that OP inhibitors interact with chloride conductance channels, we have examined the effects of paraoxon on chloride responses of cell R-2 in the abdominal ganglion of Aplysia californica. R-2 was chosen because the ACh and GABA receptors of this cell are coupled to chloride, but not to sodium or potassium channels (Yarowsky and Carpenter, Life Sci 20:1441, 1977). ACh and GABA were applied alternately from separate barrels of a triple-barrel iontophoretic pipette under control conditions, in the presence of paraoxon and after washout. The resulting chloride currents were recorded with a single-electrode voltage-clamp amplifier (Dagan 8100-1). Paraoxon (10^{-6} - 10^{-4} M) depressed both ACh- and GABA-mediated chloride currents with approximately equal efficacy. IC_{50} values were 9.6×10^{-6} M and 8.9×10^{-6} M for ACh and GABA responses, respectively. Paraoxon reduced the slopes and maxima of ACh and GABA dose response curves. This implies that the paraoxon blockade is noncompetitive. Over the concentration range examined, paraoxon was without effect on resting membrane potential, membrane input resistance, or chloride reversal potential. These studies indicate that paraoxon interferes with GABAergic as well as cholinergic neurotransmission, either or both of which might contribute to OP-induced seizures.

294.13 AZAPROPHEN (6-METHYL-6-AZABICYCLO[3·2·1]OCTANE-3α-OL 2,2-DIPHENYLPROPIONATE) AS A NOVEL AND HIGHLY POTENT ANTIMUSCARINIC AGENT. P. K. Chiang, R. K. Gordon*, H. W.-K. Yeung*, T. Alonso* J. M. Witkin*, P. Abraham* and F. I. Carroll*. Walter Reed Army Institute of Research, Washington, DC. 20307-5100, and Research Triangle Institute, Research Triangle Park, NC 27709.

Azaprophen (6-methyl-6-azabicyclo[3·2·1]octane-3α-ol 2,2-diphenylpropionate) is a novel and highly potent muscarinic antagonist. The potency of azaprophen to act as an antimuscarinic agent, in comparison to other standard antimuscarinic agents, was measured by its ability to inhibit acetylcholine-induced contraction of guinea pig ileum, to block α-amylase release from pancreatic acini cells induced by carbachol, and to inhibit [N-methyl-^{3}H]scopolamine ([^{3}H]NMS) binding to the muscarinic receptors of N4TG1 neuroblastoma cells, which lack the M_1 muscarinic subtype receptors. Among the compounds tested, K_i values for the inhibition of [^{3}H]NMS binding in N4TG1 neuroblastoma cells correlated positively with I_{50} values for the inhibition of carbachol-induced α-amylase release from pancreatic acini cells and with K_B values for the inhibition of acetylcholine-induced contractions of guinea pig ileum. Azaprophen was 50 times more potent than atropine as an antimuscarinic agent as measured by the inhibition of acetylcholine-induced contraction of guinea pig ileum, and 10 times more potent than atropine in its ability to block α-amylase release from pancreatic acini cells induced by carbachol. The rank order of potency for the inhibition of [^{3}H]NMS binding to the muscarinic receptors of N4TG1 neuroblastoma cells was: quinuclidinyl benzilate (QNB) > quinuclidinyl xanthine-9-carboxylate (QNX) > atropine > benactyzine > azaprophen > adiphenine > aprophen > pirenzepine. From these compounds, atropine, aprophen, azaprophen, and pirenzepine were selected for behavioral studies. Lever-press responses of male rats were maintained by food delivery under a schedule which required 10 responses for each food presentation. Response rates under this schedule were decreased in a dose-dependent manner by all 4 compounds. The order of potency for this behavioral effect (ED_{50}) was: atropine > azaprophen > aprophen > pirenzepine. Behavioral depressant actions of the antimuscarinic agents correlated with their efficacies in inhibiting [^{3}H]NMS binding. The comparable potency of azaprophen and atropine in altering operant behavior indicate that azaprophen may show a wider separation between muscarinic receptor blockage and undesirable side-effects than existing centrally-acting antimuscarinic drugs.

294.14 MUSCARINIC RECEPTOR COUPLING TO G PROTEINS IN RAT HEART IS ALTERED BY VOLATILE ANESTHETICS. R.L. Dennison, Jr.*, B.L. Anthony* and R.S. Aronstam (SPON: S.H. Hobbs). Depts. of Anesthesiol. and Pharmacol. & Toxicol., Med. Coll. of Georgia, Augusta, GA 30912, and Anesthesia Service, Vet. Adm. Med. Ctr., Augusta, GA 30910.

Volatile anesthetics produce deleterious effects on myocardial function and alter the actions of many cardioactive agents. To determine whether volatile anesthetics affect muscarinic systems in the heart, the influences of halothane and enflurane on muscarinic receptor binding and coupling to G proteins were determined.

Muscarinic receptor binding was measured in membranes prepared from the atria of adult male Wistar rats using [^{3}H]methylscopolamine ([^{3}H]MS). Anesthetic-gas mixtures were blown over membrane suspensions for 20 min before, and then during, the binding assays.

Halothane and enflurane at concentrations up to 8% did not affect the binding of [^{3}H]MS (dissociation constant, number of sites, or rate constants of association and dissociation). Carbamylcholine affinity for cardiac receptors was similarly unaffected (Table 1). The ability of 5'-guanylylimidodiphosphate, a stable analogue of GTP, to lower carbamylcholine affinity was greatly reduced in the presence of either anesthetic (Table 1). The elimination of the guanine nucleotide effect may reflect a stabilization of the receptor-G protein complex due to either a direct action on the G protein or to an alteration of the physical state of the membrane. Alternately, the ability of the G protein to bind guanine nucleotides may have been compromised.

Table 1. Influence of Halothane and Enflurane on Carbamylcholine Binding to Cardiac Muscarinic Receptors

	Gpp(NH)p	B_H	K_H	K_L
Control	0	0.64±0.05	0.13±0.04	15± 6
	10 uM	0.43±0.03*	0.72±0.08*	32±10
Halothane 2%	0	0.65±0.05	0.12±0.03	12± 4
	10 uM	0.52±0.04*	0.62±0.04*	26±11
Enflurane 2%	0	0.66±0.02	0.12±0.04	18± 5
	10 uM	0.50±0.03*	0.50±0.15*	41±18

All values are means ± S.D., N=3.
*Decreased in the presence of Gpp(NH)p ($p<.05$, t test).
B_H, fraction of receptors displaying high affinity carbamylcholine binding, K_H and K_L, dissociation constants (uM) for receptors displaying high and low affinity carbamylcholine binding, respectively. K_H was reduced in the presence of both anesthetics compared to control, K_L was unchanged.

294.15 THE EFFECT OF HALOTHANE ON CNS CAPILLARY ENDOTHELIAL CELL MEMBRANE FLUIDITY AND TEMPERATURE DEPENDENT PHASE-TRANSITION CHARACTERISTICS. A.M. Phelan*, J. Nierenberg*, Cecelia Frederick* and D.G. Lange (SPON: T-P. Su). Depart. of Anesth./Crit. Care Med., The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

The effect of Halothane on CNS capillary endothelial cell (CEC) membrane characteristics was conducted using electron spin resonance (ESR) techniques. The spin-label agent, 12-doxyl-stearic acid (12-DS), was selected because it reports on membrane fluidity characteristics near the middle of the extended fatty acyl chain of phospholipids. This location on the acyl chain appears to be an extremely sensitive reporter of changes in fluidity induced by exogenous agents (propranolol, cholesterol, temperature, ETOH, etc.), and reports multiple phase-transitions in biological and model membrane systems.

Following isolation from Sprague-Dawley rats (200-250 gm, n=7/group), the CEC's (~ 200 mg) were incubated in 2 ml of PBS buffer (pH = 7.40) containing 40 μM 12-DS. After a 30 min incubation in O_2 saturated PBS, the capillaries were washed 3x with PBS at 500 xg and resuspended in 2 ml of PBS which had been previously equilibrated with various concentrations of Halothane. After equilibration, 20-30 min, the capillaries were pelleted (500 xg, 10 min), placed in a 20 μl pipette, and the membrane fluidity characteristics evaluated over a temperature range of 274°K to 316°K. At all temperatures examined, Halothane produced a more rigid environment, although this decrease in fluidity was attenuated at higher temperatures.

Interestingly, there was a dose-dependent change in one of the phase-transition temperatures observed by 12-DS (control - 299°K; 1% Halothane - 300°K; 3% Halothane 302°K). This was predominantly produced by a change in membrane fluidity responsiveness of the CEC over the temperature range of 289-301°K. Linear regressions over this temperature range demonstrated a significant increase in temperature-dependent fluidization of the acyl chains expressed as a slope (b) function (control: b = 1.65; 1% Halothane: b = 2.47; 3% Halothane: b = 2.71). No changes in temperature-dependent fluidization were observed over other temperature ranges. The interaction of Halothane with membrane cholesterol and protein was evaluated in model liposomes, while the functional significance of the Halothane induced changes in membrane fluidity was evaluated by measuring CEC mitochondrial respiration. This data suggests that the membrane effects of the inhalational anesthetic, Halothane, are more complex than through simple membrane fluidization (HL-32134).

294.16 KINETICS OF RECOVERY OF ELECTRICAL EXCITABILITY IN AXON MEMBRANE FOLLOWING NERVE IMPULSES IN THE PRESENCE OF BUPIVACAINE, LIDOCAINE, AND CHLOROPROCAINE. S.A. Raymond and M.T. DiFilippo*, Anesthesia Research Labs., Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Local anesthetics (LA's) cause agent-specific transient shifts in blocking action following action potentials that are thought to reflect increased binding to particular conformations of channel molecules that exist when channels are in open or in inactivated states (Kendig, Anesthesiology 62:382-284, 1985). For sub-blocking doses in frog nerve axons, this "use-dependence" appears as a transient subexcitability relative to the resting threshold for electrical stimulation. We compared the kinetics of this subexcitability for 3 LA's.

Electrical stimulation was given to a region of excised frog sciatic nerve with sheath intact located more than 5 cm from the suction electrode used for recording a single myelinated axon. The entire conduction path was exposed to Boyle-Conway solution containing the LA. Tonic effects of LA were assessed by monitoring resting threshold and conduction latency for current pulses administered every 20 seconds. To assess phasic effects, threshold and latency were measured as a function of delay from the last pulse of a train of 8 conditioning pulses.

The LA's differed in potency for phasic action. Although lidocaine (MW 234) and bupivacaine (MW 288) are similar amide-linked, tertiary amine LA's, the transient subexcitability with bupivacaine was greater in magnitude and longer lasting. Subexcitability for all LA's peaked within 300-400 msec. Magnitude and time course depended strongly on dose, though even for near blocking doses of lidocaine and chloroprocaine, subexcitability recovered completely within 1 second. With 0.01 mM bupivacaine (2.88 μg/ml) tonic threshold rose less than 10%. However, phasic threshold in some units rose so high that for 1-2 seconds after the train no impulse could be generated regardless of the intensity of stimulation. Recovery was complete only after 10-15 seconds in all 15 units tested with bupivacaine. A 10-fold variation was observed among fibers in phasic susceptibility. The results support the possibility that difficult resuscitation following cardiac arrest with bupivacaine is related to an extraordinary phasic action of this agent (Clarkson and Hondeghem, Anesthesiology 62:396-405, 1985). Autorhythmic electrical propagation at the normal rhythm may be impossible to induce (e.g. by defibrillation) or to sustain in the presence of bupivacaine because of pronounced subexcitability that will occur after depolarization of Na channels in cardiac Purkinje fibers. Supported by USPHS GM35647.

295.1 TARGETING LIPOSOME AND NANOPARTICLE DRUG CARRIERS TO SPECIFIC RECEPTORS. Jeffrey S. Nye and Solomon H. Snyder, Departments of Pharmacology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Drug carriers are the subject of recent investigation for their potential uses as targetable carriers of therapeutic or diagnostic materials. Liposomes, or synthetic lipid vesicles, and nanoparticles of biologically compatible polymers make effective microcarriers for a wide variety of substances including drugs, enzymes, and radioopaque dyes. These microcarriers can be administered systemically and will deliver their contents selectively to predetermined organs or tissues. Targeting strategies have employed antibodies coupled to the liposomal or particle surface, as well as magnetic or heat-sensitive carriers.

Carriers can be directed to cells and tissues which bear drug-receptors by attaching high affinity receptor ligands to the carrier's surface. In our strategy reactive analogs of drugs are coupled to lipid molecules which are then incorporated into liposomes. Using ligand analogs for the beta-adrenergic receptor, the adenosine (A1) receptor and the opiate receptor, we have developed drug-lipid conjugates that retain high affinity for their respective receptors. Liposomes incorporating these conjugates show avidity for cell surface receptors. Examples of drug and fluorescent dye delivery in vitro demonstrate that modified liposomes transfer their contents specifically to cultured cells bearing the appropriate surface receptors.

In a similar strategy, we have designed nanoparticulate drug carriers which possess avidity for drug receptors. Reactive drug-ligands may be covalently attached to the surface of polymeric particles. The resulting particles show affinity for drug receptors, and should allow delivery of trapped contents to cells expressing receptors.

For therapeutic or diagnostic purposes receptor-targeted drug carriers should offer a number of advantages over other targeting schemes. In contrast to targeting schemes with antibodies, receptor-targeted carriers require no proteins and thus avoid the problem of immunological reactions. Furthermore, a wide range of normal and diseased tissues may be chosen because drug and hormone receptors are ubiquitous but display characteristic tissue distributions. For example, certain tumors such as brain glioma and bronchogenic carcinoma frequently express specific beta-adrenergic and peptide receptors, respectively.

295.2 BINDING PROPERTIES OF 3H-CYANOIMIPRAMINE ON SLIDE-MOUNTED SECTIONS OF RAT BRAIN CORTEX. G.B. Kovachich and D.J. Brunswick*, Veterans Administration Medical Center and Dept. of Psychiatry, Univ. of Pa. School of Med., Philadelphia, PA 19104.

Experiments were conducted with 3H-cyanoimipramine (CN-IMI) to determine its suitability for autoradiographic mapping of the serotonin nerve terminals, a proposed site of action of several tricyclic antidepressants.

Sprague-Dawley rats (250-350g) were used. Coronal sections were prepared from the frontal cortex, after the olfactory bulb had been removed. From each animal 65 sections of 20 micron thickness were used immediately rostral from the forceps minor of the corpus callosum (a block of tissue lying approximately between 12.0 and 13.5 mm from the interaural line). The sections were randomized and incubated in quadruplicate at 0-4° C, in a medium of 50 mM TRIS-HCl, 150 mM NaCl, pH 7.4, in the presence of 0.02 - 5.0 nM CN-IMI. Incubation of 24 hrs was necessary for equilibrium to be attained. The sections were wiped from the slides with filter disks and counted in a liquid scintillation counter. Nonspecific binding was determined by 100 uM desimipramine, and was found to be less than 10% of total binding at all concentrations of CN-IMI. Saturating experiments (0.02 - 5.0 nM CN-IMI) were analyzed by the method of Scatchard. The results showed a single high affinity binding site, with a Kd of 0.14 nM. Competition studies (0.2 nM CN-IMI) with serotonin and serotonin uptake inhibitors, analyzed by the PROPHET method, yielded simple displacement curves with Hill coefficients of approximately 1.0 and the following IC_{50} values: norzimelidine 7 nM; sertraline 8 nM; fluoxetine 9.5 nM; imipramine 50 nM; desimipramine 900 nM; serotonin 1300 nM. Propranolol and spiperone had no effect at 10^{-4} M. The stereospecificity of binding was verified with the Z and E forms of zimelidine (IC_{50} = 85nM and 1600 nM, respectively). The binding of CN-IMI was markedly reduced 7 days after intraventricular administration of 200 ug 5,7-dihydroxytryptamine (5,7-DHT). Desimipramine was administered to these rats (25 mg/kg, ip., 15 min prior to 5,7-DHT) The results are in agreement with the assumption that the binding site for CN-IMI is associated with the nerve terminals of serotoninergic neurons.

Our findings indicate that CN-IMI is a suitable ligand for autoradiographic mapping of presynaptic sites associated with serotonin re-uptake: it satisfies pharmacological criteria for selectivity, it has low Kd and low non-specific binding. Furthermore, both Scatchard and displacement analyses indicate that under our conditions CN-IMI binds to a single high affinity site, in contrast to the reported binding properties of its parent compund, imipramine. **Supported by research funds from the Veterans Administration and from NIMH (MH Grant - 36761).**

295.3 DOES 8-OH-DPAT INHIBIT 5-HT SYNTHESIS BY STIMULATING CELL BODY (5-HT_{1A}) AUTORECEPTORS? S. Hjorth*, T. Magnusson* and A. Carlsson* (SPON: J.A. Engel), Dept. Pharmacol., Univ. Göteborg, Göteborg, SWEDEN.

We have previously shown that the putative 5-HT receptor agonist 8-OH-DPAT, potently inhibits various aspects of in vivo 5-HT neuronal activity in the rat CNS (Hjorth et al., J. Neural Transm. 55: 169, 1982). For example, there is a marked, dose-dependent reduction of the 5-HT synthesis following 8-OH-DPAT. The question arises as to whether this effect is mediated via 5-HT autoreceptors on the cell bodies or terminals, and/or via postsynaptic 5-HT receptors.

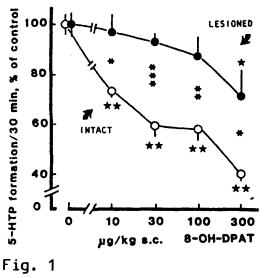

Fig. 1

8-OH-DPAT was administered SC (0.01--0.3 mg/kg) to acutely unilaterally axotomised rats, or infused locally (1 µg) into the dorsal raphe (DR) of intact rats. The in vivo 5-HT synthesis (5-HTP formation per 30 min, after decarboxylase inhibition by means of NSD 1015) in the striatum was subsequently determined in either of the two experiments. While markedly inhibiting striatal 5-HT synthesis on the intact side of the hemisectioned animals, 8-OH-DPAT failed to significantly alter this measure on the axotomised side until the highest dose was reached (Fig. 1) Following DR application of 8-OH-DPAT there was also a clearcut reduction (~40 %) of the striatal 5-HT synthesis.

Taken together, the 5-HT synthesis-inhibiting action of 8-OH--DPAT appears predominantly (though not exclusively) dependent upon intact impulse flow in the 5-HT neurons. Analogising with e.g. DA systems, the findings thus seem to indicate that there is no major **terminal** 5-HT autoreceptor involvement in this action of the compound. On the other hand, the data suggest that 8-OH-DPAT can inhibit **terminal** 5-HT synthesis by acting at 5-HT **cell body** autoreceptor sites. Whether or not there is also a possible contribution from postsynaptic 5-HT (5-HT_{1A} ?) receptors in the biochemical effects observed after systemic 8-OH-DPAT cannot be decided from these data.

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295.4 PERMISSIVE ROLE OF D1 RECEPTORS ON THE D2-DOPAMINE MEDIATED FUNCTION. BIOCHEMICAL AND BEHAVIORAL STUDIES. O. Gandolfi, R. Dall'Olio, A. Vaccheri, P. Roncada and N. Montanaro. Institute of Pharmacology - University of Bologna, Via Irnerio 48, I-40126 Bologna-Italy.

Until now the major problem in determining a functional role for D1 and D2 DA brain receptors has been the lack of selective drugs. This study concerned the behavioral and biochemical effects of acute and repeated treatments with DA specific agonists and antagonists. The behavioral responses employed were: hypermotility evoked by the D2 agonist LY 171555, stereotyped behavior induced by the mixed D1/D2 agonist apomorphine and the grooming behavior elicited by the selective D1 agonist SKF 38393. All these behaviors were blocked by the acute pretreatment with the D1 selective antagonist SCH 23390, suggesting a role of D1 receptors in these responses. However this antagonism appeared to be not specific for the D1 receptors; in fact even the D2 receptor blocker YM 09151 antagonized hypermotility, stereotypy and grooming; this suggests an interaction between D1 and D2 receptors. In order to clarify this view, rats were daily treated with SCH 23390 or YM 09151 for 3 weeks and the characteristics of striatal DA receptors were assayed 5 days later. Repeated administrations with SCH 23390 increased the number of D1 but not D2 receptors, while the chronic treatment with YM 09151 increased the Bmax only of D2 receptors in rat striatal synaptic membranes. Moreover, groups of D1 or/and D2 supersensitive rats were challenged with threshold doses of LY 171555, SKF 38393 or apomorphine. Only the apomorphine-induced stereotyped behavior was increased in D1 receptor supersensitive rats. On the contrary, D2 supersensitive rats exhibited increased responses to both apomorphine-elicited stereotypy and LY 171555-elicited hypermotility. The SKF 38393-induced grooming did not change following any of the 3 pretreatments. "In vitro" studies indicated that SCH 23390 is a weak competitive inhibitor of 3H-spiroperidol specific binding to D2 sites in striatal membranes, ruling out direct pharmacologic interactions as an explanation for the behavioral effects of the drugs used. Our findings suggest that D1 receptors are as important as D2 receptors for the expression of behavioral responses; in the nigrostriatal dopamine system the D1 receptors might serve to modulate the D2 receptor mediated function.

295.5 BROMOCRIPTINE REQUIRES CONCOMITANT D1 DOPAMINE RECEPTOR STIMULATION FOR THE FULL EXPRESSION OF ITS HYPERKINETIC ACTION IN MICE. D. M. Jackson and M. Hashizume*. Dept. Pharmacol., Univ. Sydney., NSW 2006., Australia.

A peculiarity of bromocriptine's (BRC) pharmacology is that it requires intact stores of dopamine (DA) for the expression of many of its effects, despite having high affinity for the D2 receptor and producing contralateral rotation in "Ungerstedt" rats. We recently showed that BRC potentiated apomorphine's stimulant effects in rats and mice, even under conditions where BRC itself was inactive, and potentiated the effects of DA when both were applied together to the nucleus accumbens of rats (Jackson, D & Jenkins, O., J. Neural Transm., 62:219, 1985; Jenkins, O & Jackson, D., Neuropharmacol., in press). We suggested that BRC either lacked intrinsic activity at the D2 receptor or that BRC required concomitant D1 stimulation for its behavioural effects to be elicited. The present study shows that BRC-induced stimulation in naive mice is potentiated by the D1 agonist SKF38393, and that the ability of DA depletion to block BRC's hyperkinetic effects is reversed by concomitant SKF38393 administration. Naive mice were challenged with BRC and SKF38393, and their cumulative locomotion determined 105 to 300 min. after injection (Table A). In other mice, BRC and SKF38393 were administered 4 h after reserpine (10 mg/kg) plus alpha-methyl-p-tyrosine (200 mg/kg) and cumulative activity measured for the following 3 h (Table B). All injections were IP, doses are in mg/kg and data are means ± SEMs of (n) replicates.

DOSE OF SKF38393	DOSE OF BRC		
A-In Naive Mice	0	5	10
0	501 ± 107 (10)		1580 ± 272 (13)
6	658 ± 156 (9)		3073 ± 684 (12)
B-In DA-depleted mice			
0	35 ± 9 (6)	17 ± 11 (5)	52 ± 16 (7)
6	31 ± 13 (6)	973 ± 324 (10)	2887 ± 455 (10)

SKF38393 plus BRC reversed DA depletion-induced akinesia, despite each drug being inactive by itself. Moreover, SKF38393, despite its inactivity in naive animals, potentiated the stimulant effect of BRC. These data suggest that BRC requires concomitant D1 stimulation for the expression of its behavioural effects and that DA depletion blocks BRC by removing DA which is necessary for D1 stimulation. A requirement for both D1 and D2 stimulation may underlie the oft-reported synergism between BRC and L-DOPA in the clinic and it is proposed that BRC will be more effective in Parkinson's disease if combined with a D1 agonist. Sandoz and Smith, Kline & French are thanked for drug donations.

295.6 ACUTE EFFECTS OF PIMOZIDE ON RADIOLIGAND BINDING PROPERTIES OF DOPAMINE D_2 RECEPTORS. L.H. Tecott and S.J. Peroutka. Departments of Psychiatry, Neurology and Pharmacology, Stanford University School of Medicine, Stanford, CA 94305

We have recently reported that daily administration of the diphenylbutylpiperidine neuroleptic pimozide for a 7 day period produces a decrease in dopamine D_2 receptor binding as measured with ^{3}H-spiperone in rat striatum. This effect is opposite to the receptor increase produced by chronic administration of equally potent neuroleptics such as haloperidol. In the present study, we examined the time course of this effect.

Pimozide was administered daily (10 mg/kg i.p. injections) for 1, 3, 5, and 7 days. Radioligand binding assays using ^{3}H-spiperone in rat striatal homogenates were performed 24 hours after the final injection. A 50% decrease in ^{3}H-spiperone binding was observed in animals treated for 7 days, while reductions of 51%, 63%, and 63% occurred in animals that had received 5, 3, and 1 days of drug administration, respectively.

Scatchard analysis was then performed 24 hours following a single dose of pimozide, haloperidol, or vehicle. Haloperidol treatment produced no significant change in K_D or B_{max}, whereas pimozide treatment produced a 50% reduction in B_{max} without a significant change in K_D relative to control values. We further studied the time course of this effect, with receptor assays performed 1½%, 4, 7, 19, and 48 hours following a single 10 mg/kg i.p. dose. An initial 85% reduction in binding was seen 1½ hours after injection. Binding thereafter increased with longer postinjection intervals so that by 48 hour postinjection, binding was only reduced to 12% below control values. Saturation analysis again revealed marked decreases in B_{max} without significant changes in K_D at the above postinjection intervals.

The results suggest that a single dose of pimozide causes a marked and rapid decrease in dopamine D_2 receptor binding density. The mechanism of this effect is currently under investigation.

295.7 RECEPTOR STUDIES AFTER CHRONIC LOXAPINE, ALPRAZOLAM OR BUSPIRONE TREATMENT IN THE RAT. Tyrone Lee, John Bruni*, Bruce Campbell* and Cathy Knowles*. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada.

Loxapine, alprazolam and buspirone have been implicated to have antipsychotic action. Loxapine, a tricyclic dibenzooxazepine which has been used as an effective neuroleptic, has an unusual potent down-regulating effect on central serotonin receptors (Lee & Tang, Psychiat. Res. 12:277, 1984). Alprazolam, a triazolobenzodiazepine, has been shown to reduce negative symptoms in chronic schizophrenics when used alone (Csernansky et al J. Clin. Psychopharmacol. 4:349, 1984) or in combination with other neuroleptics (Wolkowitz et al., Am. J. Psychiat. 143:85, 1986). Buspirone, a non-benzodiazepine anxiolytic, was shown to have dopamine agonist and antagonist action and is a potential atypical neuroleptic (Wood et al., Life Sci. 33:269, 1983). The present paper is intended to determine if there is a common mode of action on brain receptors after chronic treatment of these agents in the rat.

Four groups of adult male Wistar rats were injected daily (i.p.) with the following drugs at the corresponding doses for 21 days: loxapine (5 mg/kg), alprazolam (10 mg/kg), buspirone (10 mg/kg) and vehicle control (0.1 M tartaric acid, 1 ml/injection). Brain receptors were assayed after the animals were sacrificed 48 hours after the last injection using ^{3}H-spiperone (for dopamine D_2), ^{3}H-8-OH-DPAT (for serotonin S_{1A}), ^{3}H-ketanserin (for serotonin S_2), ^{3}H-DHA (for adrenergic β_1), ^{3}H-clonidine (for adrenergic α_2) and ^{3}H-flunitrazepam (for benzodiazepine B_z). Preliminary results are:

	% Change in Receptor Density					
	α_2	β_1	D_2	S_{1A}	S_2	B_z
Loxapine	-7	-13	+34#	-4	-38@	+3
Alprazolam	-19*	-10	-12	-9	+3	-8#
Buspirone	+10	-13	-7	+6	-3	-4

@P<0.01; *P<0.02; #P<0.05, Student's t-test.

Under the conditions of the present study, the drugs did not seem to share any common action on neurotransmitter receptors being studied, although all three drugs appeared to lower β_1-adrenergic receptor density but this effect was not statistically significant. (Supported by the Clarke Institute of Psychiatry Research Fund.)

295.8 NONCOMPETITIVE INHIBITION OF [^{3}H]NIMODIPINE BINDING IN MOUSE BRAIN BY CHLORPROMAZINE: MODULATION BY VERAPAMIL, DILTIAZEM AND CALCIUM IONS. V. Ramkumar* and E.E. El-Fakahany. Dept. of Pharmacology and Toxicology, Univ. of Maryland Sch. of Pharmacy, Baltimore, MD 21201.

The interaction of chlorpromazine with mouse brain [^{3}H] nimodipine binding sites was studied. Chlorpromazine decreased the maximum binding at lower concentrations and increased the equilibrium dissociation constant at higher concentrations. Schild plot of saturation data showed a slope of 0.8. Increasing the [^{3}H]nimodipine concentration resulted in a shift to the right in the chlorpromazine displacement curves but inhibition constants obtained from these curves showed a non-linear relationship to the radioligand concentration. Chlorpromazine accelerated the rate of dissociation of [^{3}H]nimodipine in a dose-dependent fashion, showing a two-fold increase in the rate in presence of 30 uM chlorpromazine. This effect was attenuated in the presence of verapamil, but not diltiazem. The potency of chlorpromazine for interacting at [^{3}H]nimodipine binding sites was reduced in the presence of Ca^{2+} and diltiazem in a dose-dependent fashion. This effect of Ca^{2+} was observed in both normal and EDTA-washed membranes. In addition, the ability of Ca^{2+} to restore [^{3}H]nimodipine binding in EDTA-washed membranes to control levels was reduced by chlorpromazine. Chlorpromazine, in a dose-dependent manner, shifted verapamil displacement curves to the right. Furthermore, it returned towards control level [^{3}H]nimodipine binding inhibited by verapamil. These results indicate a complex interaction between chlorpromazine at various subunits of the Ca^{2+} channel and suggest potential drug-drug interactions with Ca^{2+} channel blockers when these drugs are administered concurrently with neuroleptics.

(Supported by a grant from Miles Laboratory)

295.9 QUANTITATIVE AUTORADIOGRAPHY OF REGIONAL FRACTIONAL OCCUPANCY OF OPIATE RECEPTORS IN VIVO DURING ^{3}H-NALOXONE PRECIPITATED MORPHINE WITHDRAWAL. W.A. Geary, II and G.F. Wooten. Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908.

Estimates of naloxone fractional occupancy (FO) of opiate receptors in 20 brain regions were made using quantitative autoradiography of in vivo and in vitro ^{3}H-naloxone binding. For in vivo studies, morphine dependent rats received intravenous pharmacologic doses of ^{3}H-naloxone corresponding to 5, 25, and 105µg/kg (200 µCi of 50-57 Ci/mmole ^{3}H-naloxone combined with unlabeled naloxone); doses were chosen based on known dose-response relations for naloxone precipitated withdrawal. Rats were killed by pentobarbital overdose 16 min after ^{3}H-naloxone injection (the time of maximal specific binding in vivo as measured in tracer kinetic studies) and perfused transcardially with heparinized saline (0-4°C; 1 min). Brains were frozen in Freon (-50°C). Total elapsed time for perfusion to freezing was 4-6 min. In vivo binding (fmol/mg) was estimated by quantitative autoradiographic methods; all data were corrected for differential autoabsorption of tritium. Specific binding was calculated by subtracting cerebellar binding from regional total binding. Coronal sections at the level of the rostral striatum typically bound 2500-4500dpm after in vivo ^{3}H-naloxone injection. For in vitro Bmax studies, adjacent sections from the same rats were rinsed in phosphate buffered saline (1 liter X 3) until residual tritium was undetectable. Total binding was defined using 10nM ^{3}H-naloxone alone (57Ci/mmole, Amersham); nonspecific binding was estimated using 10µM sufentanil. Fractional occupancy was calculated as [specific binding in vivo/Bmax in vitro]X100. Pharmacologic doses of ^{3}H-naloxone produced FO values in 20 brain regions that corresponded with naloxone dose response curves for behavioral measures of precipitated morphine withdrawal. The mean±SD[range] of FO values for 20 regions pooled was 28±10[20-54], 46±11[26-67], and 93±15[52-114] for 5, 25, and 105 µg/kg naloxone doses, respectively. The in vitro Bmax values were equal to in vivo specific binding values for the 105 µg/kg dose of naloxone; this verified the correspondence of in vitro Bmax and plateau FO values in vivo. Studies in naive rats yielded FO values equal to those in dependent rats (55±12 and 90±19 for 25 and 105 µg/kg naloxone, respectively). The data suggested: 1) an absence of spare receptors for naloxone during precipitated withdrawal and 2) that precipitated withdrawal mechanisms were attributable to post-binding events in vivo. Quantitative autoradiographic studies of in vivo and in vitro binding provided new data concerning specific regional receptor occupancy states in vivo and measured drug effects. This work was supported by grant NIH DA03787.

295.10 IN VITRO DEMONSTRATION OF A PHYSIOLOGICALLY ACTIVE COMPARTMENT FOR PHENYTOIN ACCUMULATION
Lothman, E.W., Geary, W.A., II, Wooten, G.F., and Perlin, J.B.*, Dept. Neurology, Univ. Virginia, Charlottesville, VA 22908.

The mechanisms by which phenytoin (PHT) is distributed in brain are incompletely understood. We sought to test for specific brain binding sites for PHT and to map the pattern of PHT distribution when variables imposed by systemic pharmacokinetics were removed. In vitro binding studies were conducted with radiolabeled PHT in the absence or presence of excess non-labeled PHT as a competitive displacer to determine total (T) and nonspecific (NS) binding respectively in either sections ("dead" tissue) or slices ("living" tissue) obtained from rat brain. Sections (20 µm) were prepared from fresh-frozen brains, cut at -15°C, and stored over silica gel dessicant at -70°C until the time of experimentation. Brain slices (hippocampus or ventral or dorsal forebrain at the level of the striatum) were prepared by conventional techniques, cut at 400 µm with a McIlwain tissue chopper and maintained at 37°C in a CO_2-buffered artificial CSF. Binding experiments were done under high affinity (T=4-9 nM radiolabeled PHT; NS with additional 4 µM nonlabeled PHT) or low affinity (T=1 µM radiolabeled PHT; NS with additional 100 µM nonlabeled PHT) conditions. Sections were incubated in Tris-buffered (pH 7.4) saline or artificial CSF solutions containing PHT. Accumulation of radiolabeled PHT was determined by liquid scintillation spectroscopy or quantitative film autoradiography with calibrated standards and corrections for regional autoabsorption. Kinetic studies revealed a steady state for PHT accumulation by 10 minutes in sections and by 30 minutes for slices. No specific binding was found in either sections or slices. For sections there was no appreciable difference in PHT accumulation at 22 vs 37°C or in Tris-buffered saline vs artificial CSF. Autoradiographs of sections showed a greater accumulation of PHT in gray than in white matter structures (white:gray ratio 0.7); in contrast, slices showed the converse (white:gray ratio 1.5). A white:gray ratio >2 was found previously in our experiments in which radiolabeled PHT was administered in vivo (Neurology 35:S155, 1984). We conclude that there are no specific binding sites for PHT in the brain. The distribution of PHT in white relative to gray matter differs, however, depending on the functional state of the tissue. For "living" slices exposed to PHT in vitro, the drug preferentially accumulates in white matter, the distribution seen in situ. This suggests the presence of an active process, perhaps involving glial cells, that serves to accumulate or bind PHT by a non-saturable process.

295.11 EFFECT OF MILACEMIDE, AN ANTIEPILEPTIC AGENT, RELEASED IONTOPHORETICALLY ON RAT AND CAT CNS CELLS IN VIVO.

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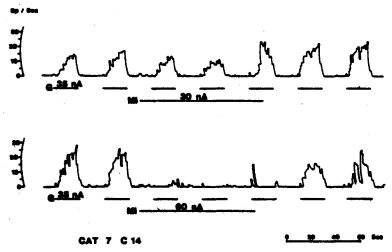
Milacemide (Mi) exerts anticonvulsant activity. Following its administration, elevated levels of the inhibitory amino-acids GABA and glycine have been noticed in some brain areas. This observation prompted the suggestion that the antiepileptic activity of Milacemide could be accounted for by these increased amounts of inhibitory amino-acids.

In this study, extracellular recordings were obtained from neurons in the cerebral cortex, thalamus and hippocampus of cats and rats anaesthetized with urethane. Milacemide was released iontophoretically either on cells firing spontaneously, or on cells excited by regular iontophoretic pulses or by continuous iontophoretic application of glutamate (G), or acetylcholine.

Milacemide was capable of depressing the firing of practically all cells examined; this depressant activity appeared reversible and dose-dependent. By comparison, Milacemide was less powerfull than GABA applied with similar iontophoretic currents. However, when the ejected amount of GABA induced a submaximal depressant effect on firing rate, the simultaneous iontophoretic release of GABA and Milacemide inhibited cell firing more profoundly than when GABA alone was applied.

It is concluded that Milacemide itself is capable of depressing cell activity and that its depressant effect may be additive to the inhibitory action of GABA.

With support from FNRS and Banque Nationale de Belgique.



295.12 EFFECT OF AGE ON THE RESPONSE OF THE GAMMA-AMINOBUTYRIC ACID RECOGNITION SITE TO ETHANOL IN THE PRESENCE AND ABSENCE OF DIAZEPAM.
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Rat brain tissue above the cerebellum was examined for age-related changes in low affinity ^{3}H-gamma-aminobutyric acid (^{3}H-GABA) binding after a single injection of the vehicle, ethanol and/or diazepam (180 ug/kg). Young (3-4 mo. old), mature (12-15 mo. old) and senescent (28-31 mo. old) male Fischer 344 rats were injected (0.05 ml/100 grams of body weight) intravenously with one of the above treatments five minutes prior to decapitation. To expose the brain tissue of all age groups to similar ethanol concentrations, young and mature rats were injected with 60% ethanol, while senescent rats were injected with 45% ethanol.

The brain homogenate was washed thoroughly prior to being used in the binding assays. Incubations were conducted at 0-4°C in a 1 ml final volume containing aliquots of homogenate, ^{3}H-GABA, and various concentrations of unlabeled GABA. Incubations were terminated by rapid centrifugation.

An acute injection of ethanol, with and without diazepam, produced an age-dependent alteration in low affinity ^{3}H-GABA binding. For example, ethanol pretreatment did not alter the affinity of ^{3}H-GABA binding sites in young rats. In contrast, the affinity of the ^{3}H-GABA binding sites in ethanol-pretreated mature rats was significantly lower than that of ethanol-pretreated young rats and vehicle or diazepam-pretreated mature rats. In senescent rats, the affinity of ^{3}H-GABA binding sites in ethanol-pretreated rats was significantly lower than diazepam-pretreated rats. Interestingly, the maximum number of binding sites was not significantly altered by an acute ethanol injection in any age group. However, in young rats a single injection of ethanol with diazepam altered both the affinity and maximum number of ^{3}H-GABA binding sites compared to diazepam-pretreated rats. In mature and senescent rats pretreated in this manner, neither the affinity nor the maximum number of ^{3}H-GABA binding sites were altered.

The present results indicate that ethanol in the presence and absence of diazepam elicits within minutes an age-dependent alteration in low affinity GABA binding. The age-related effects of acute ethanol administration on GABA binding may even play a role in the discrepancies reported in the literature regarding the effects of ethanol on GABA receptor binding.

Supported by USPHS grant AG-04710 and MSP funds College of Medicine at Rockford.

295.13 EFFECTS OF ETHANOL ON PINEAL BETA-ADRENERGIC RECEPTOR FUNCTION.
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Ethanol, in vitro, was shown to enhance isoproterenol (ISO)-stimulated adenylate cyclase (AC) activity in mouse cerebral cortical membranes but after chronic, in vivo, ethanol treatment, the response to ISO was diminished. The rat pineal gland is a model system in which activation of β-adrenergic receptors by agonists, in vitro, stimulates melatonin synthesis and cAMP formation. The sensitivity of pineal β-adrenergic receptors to activation by agonists, however, was shown to vary with a circadian rhythm. In the first set of experiments, rats were given ethanol chronically to induce physical dependence, and ISO-stimulated AC activity in pineal gland membranes was measured during ethanol withdrawal, at 1400 and 0200 hours. In pineal membranes from control rats, the stimulation of AC activity by 10^{-4}M ISO was higher at 1400 hours (184.3 ± 20.3 pmol cAMP/mg protein/min) than at 0200 hours (79.6 ± 29.3 pmol cAMP/mg protein/min). In ethanol-withdrawn rats, however, this diurnal variation was not apparent (stimulation of pineal AC activity by 10^{-4}M ISO at 1400 and at 0200 hours = 113.9 ± 18.6 and 93.4 ± 22.2 pmol cAMP/mg protein/min, respectively). Thus, chronic ethanol treatment and withdrawal appears to result in a subsensitivity to ISO of pineal β-adrenergic receptor-coupled AC activity at the 1400 hour time point.

To assess whether ethanol directly affects pineal production of melatonin, pineal glands were cultured in media for 48 hours and then transferred to fresh culture media with and without various concentrations of ethanol, submaximal concentrations of ISO (10^{-9}M) or both ethanol and ISO. Media was changed every 30 minutes for six hours. Melatonin content in each 30-minute collection sample was determined by RIA. ISO (10^{-9}M) increased basal melatonin production by 138% and peak stimulation was achieved by three hours (basal melatonin production at 3 hours = 1.11 ± 0.79 ng/gland/30 min; n=4). Ethanol (5-50mM) alone did not appear to affect basal melatonin synthesis. However, ethanol (50mM) significantly potentiated the stimulation of melatonin synthesis by 10^{-9}M ISO (stimulation over basal at 3 hours = 854%). These data suggest that ethanol alters pineal β-adrenergic receptor-mediated production of melatonin, and that the cultured pineal gland may provide a simple model system to elucidate the effects of ethanol on the cascade of cellular events from receptor stimulation to physiological output signal.

MUSCLE II

296.1 FINE STRUCTURE AND FUNCTION OF SEA HORSE (HIPPOCAMPUS) SKELETAL MUSCLE CELLS.
M. Morales*, D. S. Smith* and J. del Castillo*. (SPON: G. Escalona de Motta). Lab. of Neurobiology, U.P.R., Blvd. del Valle 201, Old San Juan, P.R. 00901 and Dept. of Zoology, U. of Oxford, South Parks Road, Oxford OX1 3PS, U.K.

In addition to the slow contracting myotome muscles, which make up most of their skeletal muscle mass, the sea horse (Hippocampus) possess highly specialized fast muscles that move the dorsal fins at a rate of 32 Hz. We are conducting a morphometric and functional comparison between these two types of muscle fibers.

In studying the fibers of the fin muscles, Rollett [Arch. mikr. Anat. 32: 233-266, 1888] provided the first clear histological distinction between sarcoplasm and myofibrils in a vertebrate cell. These fibers are highly unusual both in the extent of the sarcoplasm and in the disposition of the contractile system; myofibrils appear, in transverse sections, as sinuous ribbons. The ratio of myofibrils (including T-system and SR), to extrafibrillar sarcoplasm and to mitochondria is ca. 6:3:1. Lanthanum tracer studies show that the T-tubules, aligned in Z-level triads, originate via caveolae and follow an irregular course through the sarcoplasm, which is filled with granular cisternae involved in glycogen synthesis. Mitochondria are conspicuously aligned at the Z-band level of the myofibrils.

Average membrane potential of fin muscle fibers determined, as in other teleosts (cf. Hagiwara & Takahashi J. Physiol. 190: 499-518, 1967), by the K^+ conductance, is -92.2 mV ± 5.1 (S.D.). Action potentials are only abortive in some preparations while in others they exhibit a well developed overshoot (+20 mV). Passive electrical constants are similar to those of frog skeletal muscle fibers. These muscles are profusely innervated, each junction involving two or more terminal axons.

Myotome muscle fibers differ markedly in their fine structure. The contractile system is incompletely divided into myofibrils and fills the cell, restricting the sarcoplasm to a minimal volume. Mitochondria are sparse. Sarcomere length is similar to that of fin muscle, but the triads are situated at the A-I level. Myoneural junctions are seldom seen in myotome muscle.

(This work was supported by NIH grants NS-07464 and NS-14938).

296.2 VARIABILITY IN SDH ACTIVITY AMONG DIAPHRAGM MUSCLE UNIT FIBERS.
M. Fournier and G.C. Sieck. Dept. of Respiratory Diseases, City of Hope Medical Center, Duarte, CA 91010 and Depts. of Anatomy and Kinesiology, UCLA School of Medicine, Los Angeles, CA 90024.

Recent studies in rat (Nemeth, P.M., J. Physiol. 311: 489, 1981) and cat (Nemeth, P.M., J. Neurosci. in press) hindlimb muscle indicate that enzymatic activities among muscle unit fibers are virtually identical. However, in these studies only a small number of microdissected unit fibers were assayed microbiochemically. In the present study, succinate dehydrogenase (SDH) activity of unit fibers in the cat diaphragm (DIA) was determined microphotometrically, using a quantitative histochemical method which facilitates the analysis of a much larger sample of fibers. The purpose of this study was to compare the variability of SDH activity among muscle unit and non-unit fibers.

DIA units were isolated by dissecting cervical ventral root filaments. Muscle units were characterized physiologically as type S, FR, Fint or FF. Unit fibers were identified histochemically, using the glycogen depletion method. Muscle fibers were also classified as type I (light) or type II (dark) based on myosin ATPase staining.

The number of fibers analyzed per unit ranged from 42 to 89, and their SDH activities were compared to at least an equal number of non-unit fibers of the same histochemical type. The extent of variation of SDH activity within a single fiber was also determined from multiple serial sections. The intra-fiber coefficient of variation (CV) for SDH ranged from 3 to 5%. The mean CV for SDH among type S unit fibers was 16% compared to a mean CV of 23% for non-unit type I fibers. The mean CV for SDH among type F units fibers was 26% compared to a mean CV of 58% for non-unit type II fibers. The overall range of SDH activity was greater for type II fibers compared to type I. Within this overall range, the SDH activity of type FF and Fint unit fibers was restricted to a greater extent than that of type S or FR unit fibers.

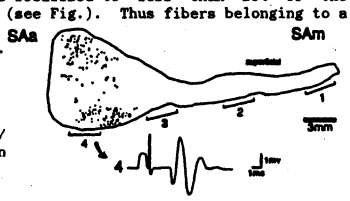
These results indicate that among muscle unit fibers of the cat DIA there is a relative homogeneity of SDH activity. However, compared to intra-fiber variability, the variation in SDH activity among muscle unit fibers is still significant.
(Supported by NIH Grant H129999-03)

296.3 SINGLE MUSCLE UNIT TERRITORIES IN THE CAT SARTORIUS. C.A. Pratt and C.M. Chanaud. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20892.

The cat sartorius (SA) muscle is an anatomically complex muscle that has been shown to be functionally heterogenous. The medial portion of SA (SAm) flexes the hip and knee. The anterior portion of SA (SAa) is bifunctional; it also flexes the hip but extends the knee. During treadmill walking both SAa and SAm generate a burst of EMG during the swing phase, but SAa also is active during the stance phase. Direct evidence showed that separate groups of motoneurons (MNs) generated the two bursts of EMG in SAa, and indirect evidence suggested that the swing phase EMG in SAm was produced by a third independent group of SA MNs (Hoffer et al., J. Neurophysiol. In press). The proposal that the SA MN pool is partitioned into 3 functionally independent subsets of MNs (task groups) during walking presumes that the temporally overlapping swing phase bursts in SAa and SAm are produced by anatomically separate muscle units. This presumption was tested in the present experiments.

In acute experiments under pentobarbitol anesthesia, single SA motor axons were isolated in cut ventral root (VR) filaments. An EMG patch electrode with 4 bipolar electrodes (3x3 mm, 4-5 mm interelectrode spacing, 1-4 in Fig.) was placed across the width of SA, and a bipolar cuff electrode was placed on the intact SA nerve. The quadriceps and sciatic nerves were cut. Single units were identified by the presence of unitary orthodromic ENG and EMG potentials in the SA nerve cuff and muscle patch electrodes, respectively, and unitary antidromic potentials in the VR filament. The cross-sectional distribution of a single muscle unit's territory was mapped electrically by recording from all patch electrodes and confirmed, for some units, histologically by the glycogen depletion method (stimulation paradigm: 40 Hz for 330 ms, 1 train/sec for 60-120 min). Multiple single units were mapped electrically only in undepleted muscles.

SA unitary EMG potentials (N=12) were restricted to 1 or 2 of the patch electrodes (ie.,the SAa unit's EMG in the Fig. was confined to electrode #4). SA muscle unit territories, as revealed by PAS staining (N=4), were localized to less than 20% of the cross-sectional area of SA (see Fig.). Thus fibers belonging to a motor unit likely share similar skeletal lever arms. This is consistent with the suggestion that the partitioning of MN pools into task groups reflects the need to preserve functional/kinematic homogeneity within a motor unit (Loeb, J. exp. Biol. 115:1985).



296.4 ASSOCIATIONS OF BIOCHEMICAL AND PHYSIOLOGICAL PROPERTIES IN SINGLE MOTOR UNITS. T.M. Hamm, P.M. Nemeth, L. Solanki*, D.A. Gordon, R.M. Reinking* and D.G Stuart. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013; Depts. of Neurology and of Anatomy and Neurobiology, Washington Univ. Med. Sch., St. Louis, MO 63110; and Dept. of Physiology, Univ. of Arizona Health Sci. Center, Tucson, AZ 85724.

Motor units from the cat tibialis posterior muscle were examined for associations between physiological and biochemical properties. Functionally isolated motor units were categorized on the basis of their physiological properties. This was followed by quantitative microchemical analysis of single muscle fibers from each unit, identified in cross-sections using the glycogen-depletion method. The fatigue resistance of the motor units was well correlated with the activities of malate dehydrogenase and β-hydroxyacyl-CoA dehydrogenase. These correlations were evident between fatigable (type FF) and fatigue-resistant units (types FR and S) and, moreover, were evident within the fatigue resistant group. The activities of both lactate dehydrogenase and adenylokinase were higher in fast- than in slow-contracting units. The association between physiological and biochemical properties substantiates the idea that biochemically distinct groups of motor units correspond to physiologically identified groups.

Table 1. Mean Values (±S.D.) of Physiological And Biochemical Properties of Physiologically Characterized Motor Units.

	Physiological Properties			
Unit Type	Progressive Fatigue Index ($F_{2'}/F_{0'}$)	Twitch Contraction Time (ms)	Tetanic Force (g-wt)	Axonal Conduction Velocity (m/s)
FF(n=6)	0.04±0.02	24.7±4.6	80.3±35.5	72.7±6.6
FR(n=2)	0.89±0.08	22.0±0.6	11.7±1.3	89.0
S (n=2)	0.96±0.0	42.4±1.4	2.9±0.8	80.0±0.0

	Enzyme Activities (mol/kg dry weight/hr)			
Unit Type	Adenylo-kinase	Lactate Dehydrogenase	Malate Dehydrogenase	β-hydroxyacyl-CoA Dehydrogenase
FF	220.8±19.3	251.0±53.2	4.4±1.2	1.7±0.5
FR	181.5±7.8	285.0±48.1	13.7±0.6	8.6±1.7
S	33.0±0.0	36.5±7.8	16.4±1.3	9.8±0.3

Supported by USPHS grants NS18387, NS29544, and HL07249, and by a grant from the Muscular Dystrophy Association.

296.5 **TRANSIENT ATROPHY OF SKELETAL MUSCLE FIBERS AFTER SPINAL CORD INJURY.** L.C. Maxwell* and E. Eidelberg. (SPON: M. A. Biedenbach). Department of Physiology and Division of Neurosurgery, The University of Texas Health Science Center, San Antonio, TX 78284.

Transection of the spinal cord in adult rats results in paralysis caudal to the transection site. Initially, segmental reflexes are severely diminished or abolished ("spinal shock") for a period which may last from a few days to weeks. There is evidence showing death of some motor neurons caudal to the lesion soon after transection. Ultimately, reflexes may recover and exceed normal characteristics. This suggests that the initial decline in reflex function may be related to denervation, as motor neurons are damaged or killed. The subsequent recovery may include reinnervation of orphaned motor units. We asked whether these changes in the motor neuron population would be reflected in time-dependent alterations of the characteristics of muscle fibers in the paralyzed muscles. Soleus (SOL), extensor digitorum longus (EDL), and plantaris (PLN) muscles from rats with complete transections of the spinal cord at T_6-T_8 were studied by histochemical techniques after up to 60 days post-transection. Fibers were classified based on enzymatic profile, and fiber area was determined by digital morphometric analysis. In each of the muscles, mean fiber area declined during the first three weeks to approximately 40% of control values, but made substantial recovery over the next five weeks. The percentage of fibers of various types was relatively unaffected in EDL over this time course. In PLN, declining percentages of Type I and IIa fibers and increasing percentages of Type IIb and IIc fibers were evident during the later part of the recovery period. In SOL, the initial predominance of Type I fibers was ultimately replaced by a predominance of Type IIa fibers. These changes in histochemical profile began during the third week post-transection and were most pronounced during the eighth week post-transection. As these changes were occuring, Type IIc fibers were observed; they may represent fibers in which the myosin isozymes are in transition. At least part of the fiber transformation may result from reinnervation of orphaned motor units by motor neurons of different characteristics. Whatever the mechanism, SOL was by far the most susceptible to alteration of fiber type following spinal transection, while EDL was the least affected. All three muscles showed partial recovery from the initial fiber atrophy. Supported by grants from the Veterans Administration and the NINCDS (14546).

296.6 MORPHOLOGICAL INTEGRITY OF SKELETAL MUSCLE AFTER CESSATION OF CHRONIC ELECTRICAL STIMULATION. B.C. Marts*, K.M. Klueber and J.W. Brown*, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405.

A cardiac assist device (CAD) powered by an autologous skeletal muscle would enable many patients with end-stage heart disease to survive. Chronic electrical stimulation has been used for the purpose of converting a fatiguable skeletal muscle of heterogeneous myofiber populations to a fatigue-resistant muscle of homogeneous myofiber population, after which, it has been used to power a CAD. The effect of chronic electrical stimulation on skeletal muscle has been examined histochemically, morphometrically and ultrastructurally. Recent interest has focused on the myofibrillar integrity of skeletal muscle that has been subjected to such an electrical stimulation regimen and then discontinued. The purpose of this study was to examine the myofibrillar integrity of the skeletal muscle after cessation of chronic electrical stimulation. The 2nd and 3rd superior most innervating segmental nerves of the right rectus abdominis (RA) from 3 mongrel/female dogs (mean weight - 21.7kg) were electrically stimulated with an implantable cardiac pacemaker for a mean period of 97 days (4.9V, 2Hz, .2msec duration). After the stimulation period, the pacemaker was removed and the muscle was left intact for a mean period of 155 days. Biopsies from 4 sites along the length of the RA were obtained prior to electrical stimulation, at the end of the stimulation period, and once again at the completion of the experiment. A portion of the biopsy was embedded in Epon for ultrastructural analysis, while the remainder of the specimen was reacted for acid and alkaline pre-incubated myosin ATPase for histochemical and morphometrical analysis. At the biopsy site located between the 2 stimulated nerves, 49% of the myofibers were Type I (mean area: 2066.3u^2) before stimulation. After stimulation, greater than 99% of the myofibers present were Type I (mean area: 1843.6u^2) and after the last biopsy was taken, 98% (mean area: 2076.0u^2) of the myofibers were Type I. A significant degree of atrophy in the Type I fibers occurred during electrical stimulation. After electrical stimulation had been discontinued, the Type I myofibers had increased in size, and were not signficantly different from the normal Type I myofibers. The Type IIa myofibers present after the stimulation had been discontinued were significantly ($P<.05$) smaller than those before electrical stimulation (2704.8u^2 and 4159.8u^2 respectively). Thus, the canine skeletal muscle has not reversed its fiber type profile up to 5 months following cessation of electrical stimulation, but myofiber size returns to near normal for Type I fibers during this period. (This research was funded by the Department of Surgery.)

296.7 EFFECT OF INTRAFUSAL FIBER DAMAGE ON MUSCLE SPINDLE DYNAMICS
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The dynamic response of the muscle spindle referred to here corresponds to the velocity sensitive portion of the spindle's response. The mechanism which enables the spindle to show this velocity sensitivity is not known; it could be due to an active property of the intrafusal muscle fibers (such as stretch activation), and/or it could be due to the spindle's transduction properties.

The contribution of the intrafusal muscle fibers to the dynamics was tested by damaging the intrafusal fibers and monitoring the spindle's response to see whether the dynamics remained. The fibers were damaged by applying a pinch to the fibers just inside the capsular region. This is a modification of a previously reported technique of cutting the intrafusal fibers which resulted in severe degeneration of the fibers (Quick,D.C. (in press) J.Neurosci.). The pinch was used instead of cutting the fibers because we needed to maintain some continuity between the capsular and peripheral regions of the spindle in order to apply sinusoidal stretches. The pinch modification was not as successful as the cutting technique, in that it always resulted in some damage to the muscle fibers, (as assayed by light microscopy), but the extent of the damage was not consistent.

The dynamic response was determined by applying sinusoidal stretches in the range of 0.3-4.0 Hz. The resulting Bode plot yields a break point at about 1 Hz that has been attributed to the spindle's velocity-sensitive dynamic properties. In preliminary experiments, we have found that damage to the intrafusal muscle fiber had no effect on the Bode plot in this frequency range. If the fibers had been responsible for the dynamic response, then we would have expected a change in the Bode plot following muscle damage. This suggests that the mechanism giving rise to this aspect of the spindle's dynamics is not the active contraction properties of the intrafusal muscle fiber, and is consistent with the hypothesis that the sensory transduction mechanism is responsible for these dynamics.

296.8 MUSCLE DISUSE INDUCED ALTERATIONS OF HIGH-ENERGY PHOSPHATES IN RAT SLOW AND FAST TWITCH MUSCLE.
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Many properties of skeletal muscle are known to be altered by modifications of neural influences. Analysis of the changes in content of high-energy phosphates during exercise and in pathological conditions has been an important tool in establishing muscle characteristics. Experiments were carried out to investigate changes in high-energy phosphate compounds such as phosphocreatine (PC) and the adenine nucleotides; adenosine triphosphate (ATP); adenosine diphosphate (ADP); and adenosine monophosphate (AMP) following muscle disuse induced by either denervation or hindlimb-suspension. During a two week period of muscle disuse, the soleus developed a significant atrophy (50% of control weight) while extensor digitorum longus (EDL) did not show any change in weight. Both muscles, however, developed similar atrophy following denervation. Quantitative changes in PC and ATP in rat soleus and EDL muscle were determined using reversed phase-HPLC. A significant decline of PC to 50% of control occurred within three days of denervation in both muscles without significant changes in creatine concentration. This reduced level of 50% was maintained in soleus over a fourteen day period while in EDL a further reduction to 25% was seen. Detailed analysis of adenine nucleotides indicated that following 3 days of denervation a marked decrease in the ratio of ATP/AMP (88%) compared to ATP/ADP (44%) occurred. This was a result of a decrease in ATP with a concomitant threefold increase in AMP. Suspension-induced muscle disuse (intact innervation) caused a smaller and slower decrease in PC content following the seventh day of disuse (30%), with a further decrease only in soleus PC to 56% of control at the end of the fourteen day period. The ratio of ATP/AMP was reduced to 50% without a significant change in ATP/ADP. No changes were seen in the adenine nucleotides of EDL during the fourteen day suspension period. It is concluded from the present experiments that neural innervation as well as loadbearing activity have regulatory effects upon high-energy nucleotides in skeletal muscle. Supported by NASA Grant #NAG 2-301.

296.9 BIOSYNTHESIS OF GLYCOSPHINGOLIPIDS, GLYCOPEPTIDES AND GLYCOSAMINOGLYCANS DURING IN VITRO MYOGENESIS OF RAT L6 MUSCLE CELLS.
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Glycoconjugate biosynthesis was examined as rat L6 myoblasts fused to form multinucleated myotubes. L6 muscle cells were cultured in DMEM + 10% fetal calf serum and triplicate dishes were harvested at days 2, 3, 4 and 6. 3H-galactose was added to the growth medium 24 hr prior to collection of cells. At day 2, cultures consisted of replicating single-celled myoblasts, which reached confluency and membrane contact (cell-cell recognition) at day 3. By day 4 opposed membranes began fusing and 70 to 80% of the total nuclei were contained within multinucleated myotubes by day 6. Lipids were extracted, saponified, isolated by reverse-phase chromatography and separated by chromatography on DEAE-Sephadex. The lipid-free protein residue was subjected to exhaustive digestion with papain and glycosaminoglycans separated from glycopeptides in the supernatant by precipitation with cetylpyridinium chloride. Recoveries of all classes of glycoconjugates were greater than 93%, determined by addition of either 3H-lactosylceramide, 3H-GM1 ganglioside or 3H-asialosyl-fetuin to unlabeled samples.

Labeling of glycopeptides increased slightly as myoblasts achieved contact and then was lowered so that labeling by myotubes was approximately equal to that of myoblasts. Glycosaminoglycan biosynthesis increased steadily during differentiation, with a plateau during the times of membrane contact and the onset of fusion. Final levels of GAG labeling by myotubes were five times higher than values from myoblasts, but absolute labeling was always one order of magnitude lower than that of glycopeptides.

Incorporation of label into neutral glycolipids was equivalent to glycopeptides by myoblasts, but decreased steadily as myotubes were formed. Biosynthesis of neutral glycolipids by myotubes was one-half as great as that observed with myoblasts during cell contact. As membrane fusion began, labeling decreased significantly. Conversely, ganglioside biosynthesis doubled when myoblasts first achieved cell contact and membrane opposition. Ganglioside labeling then decreased as myotubes formed so that the final levels were slightly lower than those seen with myoblasts. Qualitatively, L6 cells biosynthesized large amounts of ganglioside GM3 during all myogenic phases. Major neutral GSL products were lactosylceramide and paragloboside (nLcOse4Cer). Few changes in TLC autoradiographic pattern were noted during differentiation.

These results demonstrate an increase in sialic acid during myoblast fusion and suggest a role for membrane gangliosides during L6 muscle cell recognition during differentiation.

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296.10 IMMUNOLOCALIZATION OF BASAL LAMINA IN SKELETAL MUSCLE DEGENERATION AND REGENERATION.
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Skeletal muscle fibers are surrounded by extracellular matrix components that form a discrete coat called the basal lamina. Recent evidence strongly suggests that the basal lamina plays an important role during development and maintainance of various tissues. In the skeletal muscle during degeneration and regeneration as well as in in vitro myogenesis, basal lamina components are dynamically involved in the process of myofiber formation. Fibronectin and laminin are two non-collagenous glycoproteins that act as adhesive proteins during muscle regeneration. In this study we have localized fibronectin and laminin by immunofluorescence in rat skeletal muscle undergoing degeneration and regeneration under three different conditions. 1. Experimental tenotomy in animals induces certain degenerative and regenerative changes in the *muscle fiber that mimic those seen in some human* denervative disorders. Yet in the animals, tenotomy neither damages muscle fibers directly nor interferes with their nerve supply. 2. The myotoxin marcaine, when injected into the muscle, induces rapid degenerative and regenerative changes in myofibers. 3. Adult denervation with marcaine injection is another condition in which both muscle and nerve are traumatized. It is interesting to compare the immunolocalization of laminin and fibronectin under these different types of regenerative efforts. The pattern of laminin and fibronectin distribution resembles that seen in the normal adult skeletal muscle and differs from that seen in marcaine induced regeneration.

296.11 SODIUM WITHDRAWAL CONTRACTURES IN TONIC MUSCLE FIBERS OF THE FROG. M. Huerta, J. Muñiz & E. Stefani*. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Apdo. Postal 199, 28000 Colima, Col., México and *CINVESTAV-IPN, Apdo. Postal 14-740, México, D.F.

Contractile response due to alterations in $[Na]_o$ was investigated in tonic muscle fibers. Tension was isometrically recorded from bundles of cruralis muscle of Rana pipiens. Experiments were done at room temperature (20-22°C). Control solution was (mM): NaCl 117.5, KCl 2.5, $CaCl_2$ 1.8, pH was adjusted to 7.4 with Imidazole-Chloride. Low Na or Na-free solutions were prepared by replacing sodium chloride with an osmotically equivalent amount of Tris-Cl, TEA-Cl, TMA-Cl, or glucamine-Cl. All experimental solutions contained d-tubocurarine (50 uM) to prevent a possible acetylcholine-like effect. Sodium withdrawal contractures were evoked in solutions where the $[Na]_o$ was reduced to 90, 60, 30 or 0 mM. The initial tension slowly developed and reached a maximum, failing to reach a plateau. On return to control solution there was a second rise in tension which relaxed spontaneously. The second response was larger than the initial response. The amplitude of the initial tension ranged from 6 to 8% of the tension elicited by 40 mM of K^+, and the second response from 10 to 14%. The relationship between the amplitude of sodium withdrawal contractures and $[Na]_o$ shows that the tension rose along an S-shaped curve when $[Na]_o$ was reduced and it reached the maximum at about 0 mM of Na^+. Contracture threshold was about 90 mM of Na^+.

These results suggest that a reduction of the $[Na]_o$ has an effect on the link between excitation and contraction in tonic muscle fibers.

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296.12 CONTRACTILE PROPERTIES OF HINDLIMB MUSCLES IN THE DEMYELINATING NEUROPATHY OF THE TWITCHER MOUSE. R.F. Mayer[1], E. Potes[1], B. Dyas[1], A.M. Yeager[2], H.W. Moser[2], and E. Toyoshima[1]. [1]Dept. of Neurology, VA Med. Cntr. & Univ. of MD Sch. of Med., Baltimore, MD 21201, [2]Dept. Oncol., Pediatr., & Neurol., and J.F. Kennedy Inst., Johns Hopkins Sch. Med., Baltimore, MD 21205

The Twitcher (twi) mouse, an authentic animal model of globoid cell leukodystrophy (Krabbe's disease), is an autosomal recessive deficiency of galactosylceramidase resulting in progressive demyelination of both the central and peripheral nervous (PNS) systems. In the PNS, axons are not involved (Jacobs et al, 1982). Motor function and conduction velocity (MCV) appear normal at birth but MCV becomes very reduced and the motor responses reduced in amplitude and temporally dispersed as the demyelinating neuropathy progresses (Toyoshima et al, 1985). Hematopoietic cell transplantation (HCT) prolongs life from 50 to over 120 days and, although remyelination occurs in the PNS (Yeager et al, 1984), MCV remains reduced. Hindlimb muscles become atrophic but there is little change in composition or arrangement of fibers within the soleus even after 16 weeks in HCT-treated animals. Type IIA muscle fibers (mean areas) become more atrophic (34% contols) than type I fibers (64% controls) and approximately 4% of type IIA fibers appears to change to IIC fibers that may be transitional (Toyoshima et al, 1986). The greater effect on type IIA fibers than type I may reflect the inability of demyelinated nerve fibers to conduct trains of impulses rapidly rather than de- or reinnervation. In order to study the fuctional capacity of muscle in this progressive demyelinating neuropathy, the contractile properties were examined. C57BL/6J - twi of the both sexes were studied at the early (20-25 days of age) and late (over 35 days of age) stages of the disease without treatment. Under 4% chloral hydrate (10µl/g BW, i.p.) anesthesia, isometric contractile properties of whole soleus or gactrosoleus muscles were measured in vivo using established techniques. Following the recordings, muscle weights were obtained. The data from the soleus muscles showed that there was little change in the early stage. In the late stage, the muscles were atrophic, 1.4±0.2 mg. vs. controls of 3.3±1.3 mg (mean ± SD, 3 animals), mean twitch tension was reduced, 0.2±0.1 g wt vs. 1.5±1.3 g wt, with little change in twitch: tetanus ratios 0.16 vs. 0.19 or contraction times, 20.0±4.7 ms vs. 19.9±3.0 ms, suggesting that PNS demyelination does not alter muscle contractile properties except those that can be secondary to muscle atrophy.

Supported by the Veterans Administration Research Service.

296.13 Progressive Equine Myotonia Resembling Myotonia Dystrophica of People. G.A. Hegreberg, S.M. Reed,* and W.M. Bayly.* College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

A neuromuscular disorder accompanied by clinical, electrophysiological, and pathological features of myotonia dystrophica was observed in three foals. This progressive disorder was apparent as early as 1 month of age and was characterized by proximal muscle hypertrophy and hypertonicity of the rear limbs and muscle stiffness and weakness of all limbs. The muscle stiffness decreased in functional severity with exercise. The proximal muscle hypertrophy and hypertonicity was observed in the semimembranosus, semitendinosus, and gluteal muscles of the hind limb. Prolonged dimpling of these large rear-limb muscles was elicited by percussion. Pain in the affected muscles was not a prominent feature of the disease. Bilateral contracture of the deep and superficial digital flexor tendons developed. One foal was undersized for his age and had testicular hypoplasia. Other clinical changes included early cataract formation and borderline glucose intolerance.

Myotonic discharges were identified by electromyography in the semimembranosus, semitendinosus, and gluteal muscles of the hind limb, the triceps, supraspinatus, and infraspinatus muscles of the forelimb and the axial musculature of the back and abdominal wall. No significant change was noted in the peripheral nerve conduction velocities. Percussion dimpling and the typical myotonic discharges persisted after neuromuscular blockade with d-tubocurarine.

Distinctive histologic muscle changes included ringed fibers and sarcoplasmic mass formation. Other skeletal muscle changes were variation in fiber diameter size, internal positioning of sarcolemmal nuclei, and an increase in endomysial connective tissue.

These cases of myotonia in the horse resembling myotonia dystrophica of people represents the introduction of a previously undescribed form of myotonia in animals. A number of important components of this disease remain undefined, including the influence of genetic factors and the definition of the primary cellular abnormality. Further investigation of this progressive form of myotonia will provide insight into the human counterpart and will provide a greater understanding of the underlying disease processes that occur in myotonias.

This study was supported in part by NIH grant RR00515, the Equine Orthopedic Research Fund, and Washington State University.

296.14 RECORDING SOUND FROM MUSCLE. C.F. Bolton,* T. Parkes,* R.T. Thompson,* M.R. Clark,* C.J. Sterne.* SPON: (J.D. Brown). Departments of Clinical Neurological Sciences and Nuclear Medicine, The University of Western Ontario, London, Ontario, Canada. N6A 4G5

Recent studies suggest the recording of sound from muscle may be a new way of studying muscle function (Oster, G., Scien Amer., 250:108-115, 1984; Barry, D.T., Geiringer, S.R., Ball, R.D., Muscle & Nerve, 8:189-194, 1985). We studied muscle sound waves by several different techniques. Signal distortion, attentuation, movement artifact and environmental noise resulted from poor coupling of the microphone to the recording surface, notably from microphones which required direct contact with the skin or air intervening between the skin and the microphone. The most satisfactory recordings were made using a piezoelectric microphone encased in a plexiglass bell. It was filled with ultrasound gel and attached firmly to the skin. The microphone's output was amplified and lowpass filtered at 1,000 Hz, the response being flat from 10 to 400 Hz.

Six healthy subjects were then studied. The single twitch method was used first since, as opposed to recordings during continuous muscle contraction, it allows observations on the temporal relationships of electricity, sound and force. It is reproducible, so that technical variations can be examined. The median nerve was stimulated at the wrist and recordings were made of the compound muscle action potential over the thenar motor point (reference on the tendon), of sound from various sites on the hand, and of force from a strain gauge attached to the thumb. The onset of electricity, sound and force were at 3.8±0.2, 9.3±0.1 and 13.4±1.8 msec., respectively. The sound wave was biphasic, compression and rarefaction, was a differential (varied with the rate of change) of the force. With the thumb restrained, the sound amplitude was maximal over the center of the muscle and declined rapidly to near zero outside the boundaries of the muscle. Fourier analysis revealed peak frequencies at about 4 and 14 Hz, 4 Hz dominating. Recording sound only while the biceps muscle was continuously contracting revealed multiple frequencies, varying with methods of filtering and frequency analysis, but the majority falling below the auditory range.

Thus, "sound" from muscle is closely related to the rate of change of the force for a single muscle twitch, but it is a more complex phenomenon during continuous contraction. Its true value in studying muscle function requires further investigations.

297.1 OCULOMOTOR IMPAIRMENT FOLLOWING COMBINED LESIONS OF PARIETO-OCCIPITAL CORTEX AND FRONTAL EYE FIELDS IN RHESUS MONKEYS.
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The frontal eye fields (FEF) and the association cortex of the inferior parietal lobule and dorsal prestriate region (IPL-PS) of primates have been implicated in the control of purposive eye movements through several lines of evidence. However, if the FEF or the IPL-PS are surgically destroyed, only relatively mild deficits in learned oculomotor tasks result. In order to study the contribution of each of these regions to the overall neural control of eye movements, a series of experiments was performed to measure the oculomotor impairment following FEF lesions, IPL-PS lesions, and combined lesions of both the FEF and the IPL-PS cortex. The results of experiments in which lesions were limited to the FEF or the IPL-PS cortex were reported earlier. In the present series of combined lesions, 4 rhesus monkeys were trained to fixate and follow a 0.1° light target projected from the rear of a 52° square tangent screen. Eye position was measured with chronic electro-oculogram electrodes and was digitized and stored on computer disk for later calculation of performance parameters. Oculomotor performance was measured during horizontal and vertical saccades ranging from 8° to 24° in amplitude; during horizontal and vertical visual pursuit at velocities of 10°/sec to 40°/sec; and during the steady fixation of stationary targets at various locations on the screen. Three monkeys received bilateral FEF lesions, were tested during recovery, then received bilateral IPL-PS lesions, and were tested further. One monkey received a bilateral IPL-PS lesion first, followed by a unilateral FEF lesion. All of these animals had more severe oculomotor deficits than did any monkey after bilateral lesions of prefrontal or parieto-occipital cortex alone. Saccade latencies doubled or tripled in some monkeys, saccade velocity was reduced, and accuracy was much worse than normal. Both horizontal and vertical saccades were affected. Recovery to pre-lesion levels of performance took longer than after either FEF or IPL-PS lesions, and in some cases did not occur. Both horizontal and vertical pursuit gain was reduced and pursuit was accomplished by successive small saccades. The oculomotor deficits recovered to different degrees in different animals. The results suggest that there is normally some overlap in the oculomotor roles of the FEF and IPL-PS cortex and that each may contribute to the absence of a major oculomotor deficit when the other is destroyed. The recovery that follows lesions of both areas together may be mediated by the dorsomedial prefrontal cortex, superior temporal cortex, primary visual cortex, or by subcortical structures alone.
[Supported by grants from NIH (EY-04159) and the Vaughan Stroke Research Trust.]

297.2 THE PRIMATE FRONTAL EYE FIELDS ARE NECESSARY FOR PREDICTIVE SACCADIC TRACING.
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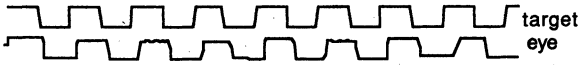
When a target moves in a regular manner the human oculomotor system can anticipate such movements. In the case of square wave motion the human initially tracks with saccades of normal latency (~200 ms), but soon begins to predictively track the target's motion with little or no time lag. Eye movements of macaque monkeys are very similar to those of humans; however, monkeys had appeared to lack predictive tracking (Fuchs, J. Physiol., 193:161, 1967). In response to conventional square wave motion monkeys fail to reduce their phase lag and continue to make saccades with normal (~150-200 ms) latencies.

We found that the monkey is capable of predictive tracking during a modified square-wave task such that the target is turned off for a brief gap before each step. Turning off the current fixation point appears to give the monkey permission to anticipate the next target locus, whereas leaving it on obligates him to wait. This task is similar to the "express saccade" paradigm in which turning off the fixation light can reduce visually-guided saccade latencies to ~100 ms; however, in our experiment saccades are made before the visual target appears. The traces below show a monkey predictively tracking a such a 'gap' square wave, with a frequency of 1 Hz, a gap of 250 ms, and an amplitude of 10°.



We then tested the ability of monkeys with bilateral frontal eye field lesions to track both types of square waves. For conventional square wave tracking, the lesioned monkey is indistinguishable from the normal monkey: Both track well, even at very high frequencies (up to 3 Hz), but neither engages in predictive tracking. However, the lesioned monkey fails to reduce phase lag in the modified square wave task. Although some short latency "expresss saccades" are made, latencies are never reduced to near zero as in the normal monkey . Thus, a monkey without its frontal eye fields seems incapable of predictive tracking. The traces below show the monkey with a bilateral frontal eye field lesion tracking the same waveform as above, but failing to show predictive tracking.



We conclude that the monkey is more stimulus-bound than the human, and that lesions of the frontal eye fields compound this difference. Our finding also supports the idea that one general role of the frontal lobes is to direct behavior using predictable aspects of the environment.
Supported by EY04740.

297.3 THE ACCESSORY OPTIC SYSTEM, VISUAL CORTEX, AND VERTICAL OPTOKINETIC NYSTAGMUS: VISUAL INPUTS CONTRIBUTING TO THE UP/DOWN ASYMMETRY.
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In our earlier investigations of the feline accessory optic system we reported that, in normal cats, approximately equal numbers of cells within the lateral terminal nucleus (LTN) of the accessory optic system are direction selective for either upward or downward vertical whole field motion. In addition, velocity selectivity of LTN cells is distributed across the population in a systematic fashion: upward direction selective LTN units prefer higher stimulus velocities, while downward direction selective cells prefer slower stimulus speeds. Both high velocity preferences and upward direction selectivity are severely diminished by decortication, dark rearing and monocular deprivation, suggesting that these properties are provided by the substantial visual cortical input to the LTN. For these reasons, we have posutlated that the visual sensitivities of LTN cells might underly the up/down asymmetry observed for the slow phase gain of vertical optokinetic nystagmus (VOKN) in which the response to upward stimulus motion is more vigorous and sensitive to a broader range of velocities that VOKN response to downward motion.

In light of these considerations, we tested directly the effects of cortical lesions on VOKN by measuring vertical eye movements in cats using the magnetic search coil technique before and after visual cortex ablation. Large field stimuli rear projected onto a tangent moved either upward or downward over a range of velocities between 5 and 30 deg/sec. In normal cats, the gain (eye velocity/stimulus velocity) of slow phase eye movement for upward motion is between 0.8 and 0.9 for stimulus velocities from 5 to 15 deg/sec. At higher velocities of upward movement (from 15 to 30 deg/sec) gain falls to between 0.5 and 0.4. For downward motion, slow phase gain usually drops below 0.5 for stimulus velocities of 10 deg/sec or less, reaching lower limits of near 0.2 for velocities of 15 to 30 deg/sec. Following decortication, the gain of upward VOKN is attenuated by as much as 35%, especially at velocities of 20 deg/sec or more. In contrast, the gain of downward VOKN is much less affected by cortical lesions. This high velocity deficit in upward VOKN is consistent with the notion that the visual cortex (via the LTN) makes a direction specific contribution to VOKN sensitivity. However, the residual upward VOKN response following decortication argues for the existence of an additional source of upward direction selective information to the vertical optokinetic system of the cat.

297.4 INTRAVITREAL PICROTOXIN AFFECTS OPTOKINETIC AND VESTIBULAR EYE MOVEMENTS VIA A SUBCORTICAL PATHWAY IN CAT.
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Synaptic drugs which block GABA inhibition, such as picrotoxin, have a dramatic effect on directionally-sensitive (DS) retinal cells. When GABA is blocked, DS retinal ganglion cells in rabbit or turtle lose their directional tuning and respond equally to visual targets moving in any direction. It has been suggested that DS neurons at the retinal level serve as a direct input to the accessory optic system for rapid stabilization of eye position. We expected therefore that direct application of a GABA antagonist to the retina would disrupt sensory control mechanisms of eye stabilization.

Electrodes were surgically implanted around the eyes of each cat to measure electrooculograms (EOGs). A head bolt was fixed with dental acrylic to stabilize the cat's head to the turntable without discomfort. EOGs were recorded either during monocular optokinetic stimulation while the turntable remained fixed or during vestibular stimulation in total darkness. The optokinetic stimulus was a checkerboard pattern drifting horizontally (20 deg/sec). The vestibular stimuli consisted of horizontal sinusoidal rotation (0.1 Hz, peak velocity 5-50 deg/sec). Before the drug, EOGs were recorded during visual stimulation of each retina and during vestibular stimulation. A monocular intravitreal injection was made of 100-200 u L of 3.3 mM picrotoxin under local anesthetic. Within a short time, EOGs were collected using conditions identical to those presented before the injection. Experiments were repeated in cats in which cortical areas 17, 18, 19, and large areas of the lateral suprasylvian gyrus were ablated.

During the drug's effect, there was a spontaneous horizontal nystagmus with its slow phase toward the side contralateral to the injected eye. This nystagmus was more vigorous after cortically ablation. Spontaneous vertical eye movements occurred if the cat was rolled onto its side. All the drug effects lasted for at least several hours before recovery.

Optokinetic nystagmus driven by the uninjected eye was less affected by this spontaneous drift than the optokinetic nystagmus driven by the injected eye. This drug-induced drift was superimposed on vestibularly driven eye movements, even in total darkness. In some cases, the drug-induced nystagmus would even block stimulus-induced slow phase eye movements towards the injected eye. These findings suggest that directional information is processed in the cat retina to strongly influence both visual and vestibular control of eye position via subcortical pathways.

297.5 MODIFICATION OF THE VESTIBULO-OCULAR REFLEX DURING BEHAVIORAL AND AMPHETAMINE AROUSAL IN CATS BEFORE AND AFTER 6-OHDA INDUCED NOREPINEPHRINE DEPLETION. J.G. McElligott and W. Freedman. Dept. of Pharmacology, Temple U. School of Medicine, Phila, PA. 19140 and Bioengineering Program, National Science Foundation, Washington, D.C. 20550

The vestibulo-ocular reflex (VOR) is a reflex that operates to stabilize the eyes in space during movements of the head. The system has been described to have a gain of -1 since stimulation of the semi-circular canals brought about by head movements will have the effect of causing the eyes to rotate an equal amount in the opposite direction. The reflex has also been shown to be plastic since the gain of the reflex can be respectively increased or decreased by wearing either magnifying lenses or reversing prisms. Change in the gain of the VOR reflex has been put forth as a model to study plasticity in the central nervous system. Since numerous studies have implicated norepinephrine (NE) in neuroplasticity and modifiability of neural circuits, we attempted to determine the effect of NE depletion (via a 6-OHDA intra-cisternal injection) on the modifiability of the VOR.

Ten cats were implanted with preformed ocular search coils in order to measure the eye movements. Modification of the VOR over a 4 hour period was accomplished by oscillating (pseudo-random; linear combination of frequencies, 0.07 to 0.4 Hz @ ± 12.5 deg.) head restrained animals in the horizontal plane while an illuminated optokinetic drum was rotated 180 degrees out of phase with the animals' head. Estimates of VOR gain were made by oscillating (0.25 Hz @ ± 10 deg.) the animals in the dark for 1 minute at half hour intervals. A computer program automatically removed saccades and selected the 'best' 40% (i.e., highest VOR gain) in each data set. The actual value of the VOR gain was estimated by determining the power (via a Fast Fourier Transform) at the oscillating frequency (0.25 Hz) for both the eye position signal (with the saccades removed) and the table signal (Gain = FFTeye/FFT table @ 0.25 Hz). Arousal levels of each animal were estimated by counting the number of saccades made during the period of modification. High levels of behavioral arousal were maintained by auditory stimulation. Amphetamine arousal was accomplished by a subcutaneous injection (5 mg) 30 minutes prior to the initiation of VOR modification. NE depletion in the animals was acchieved by injecting 6-OHDA intra-cisternally (dose range 1.2 - 2.4mg). This resulted in a significant reduction of cerebellar norepinephrine (20 % of normal levels). Modification of the VOR in the ten animals was carried out under 4 separate conditions: 1) behavior arousal/pre 6-OHDA injection, 2) amphetamine arousal/pre 6-OHDA injection, 3) behavior arousal/post 6-OHDA injection, 4) amphetamine arousal/post 6-OHDA injection. The post 6-OHDA modification and testing of the VOR was carried out 2 weeks after the injection.

The average gain change for the ten animals was 1.3 during behavioral arousal/pre 6-OHDA injection. There was a statistically significant (2 way ANOVA $p < 0.05$) decrease in the ability of the animals to modify their gain after NE depletion over the 4 hour period. No changes in arousal level (as estimated by counts of saccadic eye movements) were detected during the 4 hour period either before and after NE depletion. Average gain changes under amphetamine in cats before and after NE depletion were not significantly different (2-way ANOVA $p > 0.05$) during the 4 hour period. In fact, the gain change under amphetamine even though the animals were at a higher level of alertness was less than that obtained in the behaviorally aroused non-depleted animals. These data indicate that decrease in NE levels correlates with a decrease in the ability of cats to modify (increase) their VOR gain. This effect cannot be attributed to a decrease in arousal levels or other changes in the VOR. The area with the highest NE depletion was the cerebellum. However, these present experiments cannot role out the contribution of other areas in the brain since there were also reductions in NE in these other areas. (Supported by Grants BNS # 8410231 and BRSG # SO7 RR05417)

297.6 DEFICIENT EYE VELOCITY STORAGE AFTER KAINIC ACID LESION OF THE INTERSTITIAL NUCLEUS OF CAJAL IN THE CAT. M.Le Taillanter* and J.H.Anderson (SPON:E.Lambert). Depts.of Otolaryng.& Physiol., Univ. of Minn., Mpls., MN 55455

The interstitial nucleus of Cajal (INC) in the mesencephalon seems necessary for the normal control of the vertical component of eye movement, based on single unit recording of INC neurons (cf., King et al, J.Neurophysiol. 46:549, 1981) and impaired eye movements after lesions. Kainic acid lesions in the region of INC impaired the ability to maintain vertical gaze during fixation and the dynamics of the vestibulo-ocular reflex which suggested that INC was necessary for normal functioning of a neural integrator (Anderson et al, Neurosci.Let., 14:259, 1979; King and Leigh, Func.Basis Ocular Motil.Dis., p.267, 1982). The present work indicates a role of INC specifically for mediating the vertical component of eye velocity storage.

Eight adult cats were used. In four cats lesions were made by injecting 1.0 ul (0.5 ugm/ul) of kainic acid into the region of INC on both sides. Eye movements were recorded from 2 days to 5 months thereafter. For recording the animals were comfortably restrained and positioned on their sides. The optokinetic stimulus (OKS) was an irregular pattern which was rotated around the animal. The slow phase eye velocity was calculated and fitted by a second order function during optokinetic afternystagmus (OKAN). The average eye velocity was calculated during the 100 msec just before (VB) and after (VA) each quick phase.

In normal cats the vertical component of individual slow phases during OKN was approximately linear for stimulus velocities of 2-60 deg/s. During OKAN the time constant (Tc) of the function fitted to the decay in eye velocity was 10-20 sec for up and 2-12 sec for down OKAN. After lesioning INC, the individual upward slow phases were significantly curved, with VA being greater than VB, for stimulus velocities of 20-60 deg/s. This was also the case during OKAN and suggests that the integrator for upward velocity storage was impaired. During OKAN the up Tc could not be estimated by the function fitting because of the curvature of each slow phase; the down Tc was 1-6 sec. For the up OKAN the decay over time of VA and VB was different: VA decreased over 5-12 sec and VB over 0-2 sec.

These results show that kainic acid lesions in the region of INC result in a marked deficit in the velocity storage mechanism for vertical eye movements. Both upward and downward eye movements are affected; the up more so. Also, the quick phases seem to strongly interact with some component of the integrator network.

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297.7 LOSS OF HEAD MOVEMENTS ACCOMPANIES LOSS OF EYE MOVEMENTS RESULTING FROM KAINIC ACID LESIONS IN THE PONTINE RETICULAR FORMATION IN THE RAT D.W. Sirkin, B.J.M. Hess*, J. Lannou*[1], R.H.I. Blanks[2], and W. Precht*. Institut für Hirnforschung der Universität Zürich, 8029 Zurich, Switzerland, Laboratoire de Neurophysiologie Sensorielle, Université de Rouen, 76130 Mont-Saint-Aignan, France (1), and Department of Anatomy and Surgery, University of California, Irvine, CA 92717 (2).

Eight years ago at this meeting it was reported that electrolytic lesions in the medial pontine reticular formation (PRF) in the rat abolished not only saccadic eye movements, as was well documented in other species, but also the analogous head movements, i.e. voluntary lateral head movements (and body turns) and quick phases of head nystagmus [for a recent follow-up, see Sirkin et al., Exp. Brain Res. 58: 503 (1985)]. This effect of PRF lesions on head movements has not been noted in other species in clinical or experimental reports. Recently, monkeys whose saccadic eye movements were abolished by kainic acid (KA) lesions in the PRF were observed to have no apparent deficits in head movements (V. Henn, personal communication).

We inspected 27 rats, which had been injected with KA in the PRF for an eye movement study (Hess, Blanks, Lannou, and Precht, in preparation), for loss of head nystagmus quick phases. Also, from three of these rats, head movement recordings during optokinetic stimulation were made with a search coil. As did the earlier electrolytic lesions, KA lesions in the dorsomedial PRF rostral to the abducens nucleus impaired or abolished saccadic (quick phase) movements of both eye and head.

Conclusions:

1. Head and eye movements are mediated by the same, or closely neighboring, cells in the rat PRF.
2. The importance of the PRF for head movements is much greater in the rat than in primates.

297.8 CATECHOLAMINE DEPLETION PRODUCES IRREPRESSIBLE SACCADIC EYE MOVEMENTS IN NORMAL HUMANS. L. Tychsen. Ophthalmology Branch, United States Air Force School of Aerospace Medicine, Brooks AFB, San Antonio, TX 78235.

Extrapyramidal pathways play a major role in the control of eye movements in primates (Hikosaka and Wurtz, J Neurophys, 49: 1230, 1983) and extrapyramidal neurotransmission is known to be mediated in part by catecholamines. I recorded the eye movements of normal humans who were pharmacologically depleted of catecholamines and found that they made irrepressible saccades. These irrepressible saccades are similar to those seen in monkeys who have chemical lesions of the substantia nigra (Hikosaka and Wurtz, J Neurophys, 53:292, 1985).

Three normal adults were given a placebo for seven days and then alpha-methyl-p-tyrosine, orally, 3 grams/day for three days. Methyltyrosine inhibits tyrosine hydroxylase, and thus depletes the CNS and the periphery of dopamine and norepinephrine. The drug resulted in a 45-60% reduction in the urinary metabolite of catecholamine (MHPG). Steady fixation of a visual target, saccades to predictable target steps, smooth pursuit of predictable target motion, and vestibulo-ocular reflex eye movements were recorded by infrared oculography on a placebo day, and on the first and last methyltyrosine days.

By the last methyltyrosine day, all subjects made irrepressible saccades. The amplitude of these saccades ranged from 3-12X, and the frequency from 4-7X that of the tiny saccades seen during steady fixation on placebo days. Intervals between saccades were of normal duration (approximately 200 ms). Saccadic latency and velocity were within control range. Irrepressible saccades intruded on smooth eye movements, but the gain of pursuit and the vestibulo-ocular reflex were within control range.

These results suggest that catecholamines mediate some aspects of supranuclear control of saccadic eye movements in primates.

297.9 THE MOTOR PROGRAMS OF MONKEY'S SACCADES: AN ATTENTIONAL HYPOTHESIS. L. Bon* and C. Lucchetti*. (SPON: M. Goldberg). Istituto di Fisiologia, Università di Trieste, Via Fleming 22, 34127 Trieste, Italy.

Many reports suggest that vigilance level modifies the spatial and temporal characteristics of saccades. In this study we asked if visual input itself could modify the motor programs of saccades or whether that input must have an attentional meaning. One monkey (Macaca nemestrina) was trained to perform visual fixation and make saccades to visual targets. Eye position was recorded with a magnetic search coil. Signals were digitally filtered and analyzed off-line by a digital computer using a low pass non-recursive FIR filter which did not introduce phase shift or phase distortion. We studied saccades executed spontaneously in the dark (SPONT-DARK), spontaneously at the normal ambient light level of the laboratory (SPONT-LIGHT), and while the monkey performed the visually guided saccade task (TASK). By plotting instantaneous velocity against time we described three different velocity patterns: (1) symmetrical, when the time to reach peak velocity equals the time to return to zero velocity; (2) asymmetrical, when the rising and falling velocity phases are unequal; (3) irregular, when the peak velocity reaches a plateau or the velocity profile has multiple maxima or inflection points. The normalized frequency distributions of the three different velocity patterns were different among the three experimental conditions, as follows:

	SPONT-DARK			SPONT-LIGHT			TASK		
	1	2	3	1	2	3	1	2	3
HOR	3.5	29.5	67.0	4.5	42.5	53.0	64.5	35.5	0.0
VER	9.0	57.0	34.0	10.5	56.0	33.5	58.5	41.5	0.0

There was no substantial difference in the frequency distribution of saccade types between the two rest conditions, SPONT-DARK and SPONT-LIGHT, with irregular saccades occurring half the time in each condition, and very few symmetrical saccades. In the TASK condition there were no irregular saccades and a majority of symmetrical saccades. This suggests that light alone cannot modify the motor programs of saccades, unless the light comes from an attentionally meaningful stimulus. Alternatively the modification could be related to the artifacts of reward in the task, and not to the process of attention. To distinguish between these two alternatives we switched on a spot of light during a fixation task. The new stimulus evoked unrewarded saccades, whose the velocity profiles were exclusively types 1 and 2. This result agrees with Dichgans' (1973) suggestion that visual input requires an attentional meaning to modify motor programs.

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297.10 HEAD-EYE COORDINATION IN ALERT CATS. Carol K. Peck and Franklin S. Wartman III.* School of Optometry, University of Missouri-St. Louis, St. Louis, MO 63121.

In monkeys, saccades are programmed to take the eye to a target regardless of whether the head moves or not. Cats have an oculomotor range (OMR) of only about ± 25° although their visual field extends to about ±90°. Thus, in orienting to peripheral targets, cats face a different set of problems than do primates. We have examined two questions concerning eye-head coordination in cats: 1. the frequency with which cats make single gaze shifts larger than their oculomotor range and 2. the frequency with which both large and small gaze shifts consist of solitary saccades unaccompanied by head movements. We have made similar analyses for multiple-step gaze shifts.

Head and eye movements were studied in three cats that had been trained to fixate targets (either visual or auditory) in order to obtain food reward. The target locations were arranged horizontally in front of, and equally distant from, the cat. Within the OMR, the task could be accomplished with either single or multiple-shift eye saccades or with coordinated eye-head movements (gaze saccades); beyond the OMR, the task could be accomplished with either single gaze saccades or multiple-shift gaze saccades. Eye movements were recorded with a scleral search coil. Head movements were recorded, in the horizontal plane only, with a low-torque potentiometer.

When the size of the gaze shifts was small (less than 5°), cats made single eye saccades about as often as they made single gaze saccades. This differs from Guitton et al. (J. Neurophysiol., 52: 1030, 1984) who reported that cats never made single eye saccades unaccompanied by head movement even when well within the OMR. As the size of the gaze shift increased within the OMR, the frequency of eye saccades alone decreased until they comprised less than 10% of single gaze shifts between 21-25°.

Multiple-step gaze shifts were infrequent when the total gaze excursion was well with the OMR. For all amplitudes of total gaze excursion, multiple-shift gaze saccades were far more common than multiple-shift eye saccades, and the ratio of gaze saccades to eye saccades increased as the amplitude of the total excursion increased. Outside of the OMR, single gaze shifts and multiple-step gaze shifts were made about equally often.

These results are consistent with the view that cats may employ different motor strategies at different times during the performance of the "same" oculomotor task. Whether these different motor strategies reflect differences in the summation of head and eye movements remains to be determined.

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297.11 THE EFFECT OF DIFFERENT WAYS OF ATTENDING TO A TARGET ON THE SUPPRESSION OF OPTOKINESIS J. Pola, H.J. Wyatt and M.L. Golomb*, Schnurmacher Institute for Vision Research, SUNY State College of Optometry, 100 East 24th St., New York, NY 10010.

By fixating a stationary target a person can easily suppress optokinetic eye movements. One account for this suppression is that the smooth pursuit system holds the eye on the fixation target against the optokinetic movements, where the stimulus for fixation is target offset or motion relative to the fovea. In previous work (Vis Res '84) we found that when subjects viewed a target, foveally stabilized against a moving background field, optokinesis was largely suppressed. This suggests that target offset or motion is not the sole determinant for suppression, and that the act of viewing the target may also play a substantial role in suppression.

We have begun further investigation of this type of suppression of optokinesis. In these experiments we asked subjects to view a foveally stabilized target presented against horizontal sinusoidal motion of a background field (e.g., 0.5 Hz). An important feature of these experiments is that we varied the mode of attending to the target: the subjects were instructed either to "gaze", to "look", or to "hold". In the "gaze" condition the subjects were asked to regard the target passively, gazing ahead without attending to the target. In the "look" condition, subjects observed the target attentively. In the "hold" condition, subjects attempted to maintain the target at a location directly in front of them.

We found that by varying the viewing task we notably changed the characteristics of the suppression of optokinesis. In the "gaze" condition, optokinesis was substantially suppressed, but typically there were small amplitude slow movements roughly in phase with the field motion. In the "look" condition, a form of hyper-suppression occurred, where the eye moved in counterphase to the field, sometimes quite substantially. The "hold" condition showed the best suppression, in the sense that the eye was relatively still, neither moving with nor against the field motion.

In addition to the above, we asked subjects to Imagine a target in the plane of the moving field, and to "hold" the imaginary target at a fixed position in front of them. Surprisingly, all subjects showed little or no suppression of optokinesis. This indicates that besides "paying attention", the actual presence of the target is important. This is different from suppression of VOR, where an imagined target can have a marked influence.

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297.12 PREDICTIVE BEHAVIOR OF PASSIVE OPTOKINETIC EYE MOVEMENTS H.J. Wyatt and J. Pola, Schnurmacher Institute for Vision Research, SUNY State College of Optometry, 100 East 24th St., New York, NY 10010.

It is well established that the smooth pursuit eye movement system is capable of predictive behavior if target motion is "predictable" (e.g. periodic). Recently, Yasui and Young ('84) suggested that optokinetic slow phases also show predictive control. They moved an optokinetic stimulus pattern (90°w x 50°h) either sinusoidally or quasi-randomly using a sum of sines. Responses to the quasi-random stimulus showed increased phase lags relative to sinusoidal stimuli.

There is some difficulty, however, in interpreting data from experiments with sum-of-sines stimuli: they bring out distinctly nonlinear smooth eye movement behavior. Pursuit and optokinetic responses to sinusoidal motion show low-pass behavior: as frequency increases, gains fall off and phase lags increase monotonically. On the other hand, responses to sums of sines show marked departures from such simple behavior. We have observed such departures in passive optokinetic responses.

Lisberger et al ('81) devised a different quasi-random pursuit stimulus: targets moved in a random sequence of halves of sine waves (one frequency and amplitude per trial). Responses to complete sinusoidal cycles which happened to occur in the sequence were analyzed and compared to data obtained with sinusoidal stimuli. This technique yielded simple low-pass gain and phase curves for both quasi-random and sinusoidal stimuli; however, the responses to the quasi-random stimuli were degraded relative to the responses to sinusoids.

We have applied a similar "random walk of half sine waves" technique to optokinesis. Subjects gazed ahead passively while a full-field optokinetic stimulus moved either sinusoidally or in a series of half sines. At frequencies of 0.5 Hz and above, the "random walk" stimulus gave lower gains and larger phase lags than sinusoidal stimulation. This argues for a form of predictive control during optokinesis.

In a second experiment, we asked if predictive control is localized to central retina: sinusoidal and "random walk" stimuli were again used, but the central 40° of the stimulus field was masked off. The effect of the mask was to reduce all gains; however, making the stimulus non-predictable produced changes in gain and phase that were approximately the same as those that occurred when the full field stimulus was made non-predictable.

Optokinesis, even when driven entirely through peripheral retina, appears to employ a form of predictive control.

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297.13 CORDINATES INTRINSIC TO THE SEMICIRCULAR CANALS AND THE EXTRAOCULAR MUSCLES IN THE RAT. W.J.Daunicht and A.J.Pellionisz. Dept. Biophysics, Div. Biocybernetics, Univ. Düsseldorf, D-4000 Düsseldorf and Dept. Physiol. Biophys. N.Y.U. Med. Ctr.,New York 10016,U.S.A.

To treat the geometrical aspects of the rat's vestibulo-ocular reflex quantitatively the right semicircular canals (SCC) of 3 pigmented rats were measured in stereotaxic coordinates (intraaural line - incisor bar horizontally) and the vectors of maximal sensitivity were determined using the principal component method. Then the coordinate system was pitched upward by 30.3° to abolish the roll component for the horizontal SCC and the sensitivity vectors were recalculated (Fig. 1). The misalignment of 'coplanar' SCC can be as large as 15°. In the same coordinate system muscle insertions, muscle fibre directions, and bulbus surface of the right eye were measured. Based on these data the directions of the axes of rotation were calculated (Fig.2). Misalignment of 'antagonistic' muscles can be as large as 40°.

The orthogonal projection rule applies to the measurement of the vestibular components as well as of the extraocular proprioceptive components, while the parallelogram rule applies to the execution of (infinitesimal) eye movements. Based on the geometrical data the metrics of respective intrinsic coordinate systems have been calculated and, as proposed by Pellionisz and Llinas (1985, Neuroscience 16: 245) a tensorial treatment of the neuronal networks mediating the vestibulo-ocular sensorimotor transformation in the rat has been performed. This opens the way towards a quantitative interpretation of the role of proprioception in sensorimotor mechanisms.

Fig.1
VESTIBULUM

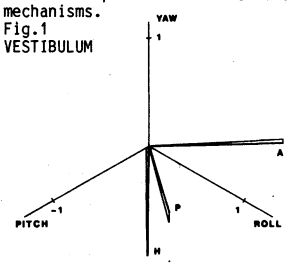
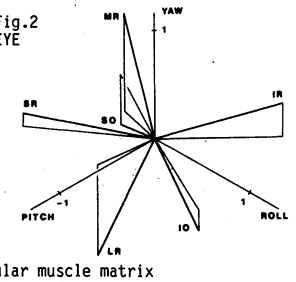


Fig.2
EYE



Vestibular matrix

	H	A	P
PITCH	-.022	.768	-.589
YAW	-1	-.031	.093
ROLL	0	.640	.802

Extraocular muscle matrix

	LR	MR	SR	IR	SO	IO
PITCH	-.540	.098	-.577	.682	.421	-.423
YAW	-.839	.905	.121	.313	-.463	-.196
ROLL	-.066	-.415	-.807	.661	-.779	.885

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297.14 FUNCTIONAL ORGANIZATION OF PRIMARY AND SECONDARY OPTOKINETIC AFTER-NYSTAGMUS. C. Maioli* (Spon: C. Terzuolo). Istituto di Fisiologia dei Centri Nervosi-CNR, via Mario Bianco 9, 20131 Milano, Italy and Institut für Hirnforschung der Universität Zürich, August-Forelstr. 1, CH-8029 Zürich, Switzerland.

The absence of covariation between primary and secondary optokinetic after-nystagmus (OKAN I and II) in a number of experimental paradigms led various authors to propose that OKAN is generated by two opposing storage elements, independently charged during optokinetic stimulation. The finding that following bilateral labyrinthine lesions OKAN II can be retained in spite of a complete loss of OKAN I gave further support to this view. However, analysis of OKAN time course strongly suggests that the two storage elements are mutually interconnected so that one exerts a negative feedback on the other, resulting in a second order underdamped system (Maioli and Precht, Exp. Brain Res., 55:494-506, 1984). A computer simulation was undertaken to verify compatibility between this model and available literature data. Furthermore, optokinetic responses were recorded in acute and chronic labyrinthectomized cats and interpreted on the basis of model predictions.

As a first step, the comparison between model predictions and OKN recorded in normal cats revealed that the dynamic properties of the optokinetic system during visual stimulation and during its discharge in the dark are different. Responses can be modelled by assuming that the storage element responsible for OKAN II is charged during stimulation but has a little or no effect on OKN time course, due to a variable coupling between OKAN II and I storage elements. A second order underdamped model, provided with such a feature, can predict, with a proper parametric adjustement, OKAN I and II behavior in situations which have been used to demonstrate an independent charge of the two storage elements; i.e. prolonged stimulus duration, repetitive stimulation and brief visual fixation periods during OKAN I.

Bilateral labyrinthectomy acutely induces loss of OKAN I and II. With time, in the cat a mild degree of compensation occurs. In the chronic stage, turning off the light during optokinetic stimulation can result in an almost immediate appearance of OKAN II. However, a slow build-up in OKN slow phase velocity is concomitantely seen. In addition, a partial coming back of the slow OKAN component can be inferred also from sinusoidal optokinetic responses. Taking into account the above-mentioned context dependent changes of OKN dynamic properties and the low gain of responses in labyrinthectomized cats, also these data can be easily modelled without assuming the independency of OKAN I and II storage elements.

297.15 VESTIBULO-OCULAR REFLEX GAIN ASSESSMENT DURING ACTIVE HORIZONTAL HEAD MOVEMENTS IN RHESUS MONKEYS. R.D.Tomlinson* and P.S.Bahra* (SPON:R.Harrison). Playfair Neuroscience Unit and Dept. of Otolaryngology, University of Toronto, Toronto, Ont. M4T 2S8

Recent experiments have demonstrated the presence of a velocity saturation in the human vestibulo-ocular reflex when head velocity exceeds 350 deg/s (Pulaski, Zee and Robinson, Brain Res.222:159,1981). No similar saturation has been demonstrated in the monkey; however, the recent interest in eye head coordination makes such information of considerable importance since high head velocities are often achieved during active head movements, particularly when the animals are highly motivated or excited. In order to investigate this possibility we recorded eye and head movements in two juvenile rhesus monkeys during horizontal combined eye-head gaze shifts. The animals' heads were coupled to a vertically oriented shaft via a damped universal joint. This arrangement allowed the animals complete freedom of movement in the horizontal plane but greatly restricted vertical mobility. Eye and head position were both measured using a Collewijn-type coil system (CNC Engineering) which was linear over the entire range of movements.

Rhesus monkeys were found to make head movements with velocities which occasionally exceeded 1000 deg/s. However, such high velocities were only seen to occur during large combined eye-head gaze saccades where the VOR is known not to be functioning (Tomlinson and Bahra, J. Neurophysiol. submitted for publication). When the measurements were made during the compensatory roll-back of the eyes following the saccade, head velocities were found to exceed 300 deg/sec only very rarely and were never seen to exceed 500 deg/s. During these movements, no examples of saturation of the VOR were seen to occur. It seems highly unlikely that monkeys are able to generate vestibular eye movements with peak velocities of more than 1200 deg/s (the peak head velocity observed) however, such head movements only occur during saccades, outside of such times the head velocity is always sufficiently low that saturation does not occur.

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297.16 THE LINEARIZING EFFECT OF NYSTAGMUS ON SLOW PHASE VOR RESPONSES - A POTENTIAL STRATEGY FOR TRIGGERING QUICK PHASES. H.L. Galiana. Biomedical Engineering Unit, McGill University, Montreal, Que. Canada H3A 2B4.

Many second-order vestibular cells, lying in the vestibular nuclei (VN), and combining both eye movement and vestibular information, have been shown to serve as the central link in the three-neuron vestibulo-ocular reflex arc (VOR) [Tomlinson & Robinson, JNP 51:1121-1136,'84; Nakao et al, EBR 45:371-385,'82]. The resting rates of such cells in mammals are less than half that of the primaries arising from associated semicircular canals, and yet the VOR has a linear range of operation at least as large as that of the primary. This is due in part to the fact that during vestibular nystagmus, eye-movement related VN cells show a readjustment in their firing rate with each fast phase, which raises the activity level on the side contralateral to the rotation direction, and hence delays the silencing of these cells during the next slow-phase response segment. This is one of the functional advantages of combining eye position and vestibular information at an early pre-motor level. Hence the question addressed here is whether one aspect of the strategy controlling the timing of quick phases (or saccades) may be the preservation of linearity in central VOR responses.

A bilateral model of the VOR [Galiana & Outerbridge, JNP 51: 210-241,'84] was simulated on a DEC 11/73 computer: it provides for the representation of central cells modulated by both the vestibular signal and an efference copy of eye position, and exhibits the observed resetting effect of fast phases on the firing rates of such cells. A simple strategy for the triggering of fast phases was implemented: enable the quick phase whenever one side approaches cut-off. As a result 1) the linearity of the VOR was extended beyond that expected from the lov central resting rates, 2) quick phase amplitude and frequency increased with head velocity, 3) for fixed peak head velocity and sinusoidal inputs, the number of quick phases per cycle decreased with increasing rotation frequency, 4) the quick phase pattern could vary from cycle to cycle without requiring changes in the triggering threshold.

These simulation results are all compatible with published observations and suggest that the strategy for the triggering of fast phases should not be deduced from eye position records. Rather, experiments should attempt to look for a threshold strategy on the firing levels of premotor vestibular neurons modulated by eye movements.

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297.17 QUICK-PHASE EYE MOVEMENTS OF GOAL-DIRECTED VESTIBULO-OCULAR REFLEX REDUCE GAZE ERROR. B.N.Segal♦ & A.Katsarkas*, Dept. Otolaryngol.,Sir Mortimer B.Davis-Jewish Gen.Hosp. & McGill Univ., Montreal, Canada, H3T 1E2.

To clearly see an object during rapid head movements, the eyes must be approximately stabilized with respect to that object. If a subject is briefly turned in total darkness while trying to "look" at a just-viewed world-fixed target, both slow- and quick-phase eye movements tend to stabilize the eyes on the actual target (Segal, Physiol.Can. 16:211, 1985). We now report that such quick-phase movements acted to reduce gaze error.

Subjects, seated with head fixed to a rotating chair, were told to look at a world-fixed point. All lights were extinguished, the chair was rapidly turned to a new position and stopped, while the subject continued to "look" at the remembered point. After 1.6s, the lights were turned on again and the point was re-fixated, if necessary. To characterize the functional capabilities of such stabilization, the chair was turned by hand, 20-60 times, with profiles that approximated those of active head movements (peak velocity: ±20-225°/s; duration: ≈0.5 s). For each turn, the net change in eye position (B), as well as the cumulative slow-phase change in eye position (C, where C=B-(effects of quick-phases)), were measured just before re-fixation. The eye-position change required to exactly maintain gaze on the target was obtained from the change (A) between initial and final eye positions in the light. Thus, "net stabilization" (B/A), "cumulative slow-phase stabilization" (C/A) and "cumulative quick-phase stabilization" ((B-C)/A) were calculated for each turn. Note that unity stabilization indicated perfect gaze stabilization.

In each of 5 subjects, average net stabilization was close to, but was usually significantly different from, unity (i.e., perfect stabilization). However, error in net stabilization was always less than the corresponding slow-phase error, indicating that quick-phases had reduced gaze error. To clarify how this occurred, we examined the relationship between slow- and quick-phase eye movements elicited in a given subject during individual head turns. Slow- and quick-phase stabilization were negatively correlated, with correlations being significantly ($p<0.001$) non-zero (-0.56to-0.94) in all but one subject. Also, a straight line appropriately fitted to a plot of slow-phase stabilization vs quick-phase stabilization had a slope that was proportional to slow-phase error, in all subjects. Moreover for the subjects with significantly negatively correlated slow- and quick-phases, quick-phase stabilization tended to equal slow-phase error.

Thus quick-phases of the goal-directed vestibulo-ocular reflex elicited in darkness, tended to be similar to memory-contingent visual saccades, which leads to speculation that both may employ common mechanisms.

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297.18 CHRONIC GAZE STABILIZATION DEFICITS FOLLOWING UNILATERAL LOSS OF PERIPHERAL VESTIBULAR FUNCTION. A. Katsarkas* & B. N. Segal♦, (SPON: D. Watt), Dept. Otolaryngol., Sir Mortimer B. Davis-Jewish Gen. Hospital & McGill Univ., Montreal, Canada, H3T 1E2.

When a normal subject is turned in total darkness while trying to "look" at a just-viewed world-fixed target, inadequate slow-phase eye movements are supplemented by quick-phase eye movements which correct ocular deviation from the actual target[a]. Based on animal studies, patients who have compensated for complete unilateral-loss of peripheral vestibular function should have reduced vestibulo-ocular function during rapid head movements towards the side of the lesion, but this has not been consistently observed. Therefore, we examined slow-phase stabilization in such patients to determine whether it was reduced, and if so, whether quick-phase eye movements corrected for this deficit.

Three subjects were tested. All had their vestibular nerve sectioned during removal of small acoustic neuromas, 3-24 months prior to testing, and all had normal oculomotor function pre-operatively and at the time of testing. All were judged to have "clinically" compensated to their dysfunction, on the basis of history, clinical examination, and spontaneous nystagmus. Testing and analysis methods were identical to those used on normal subjects[a]. Briefly, we characterized the functional capability of the patient's vestibular system to stabilize gaze during passive head movements (± 20-160 °/s peak velocity and ≈0.5 s duration) that approximated active head movements.

In contrast to normal subjects[b], slow-phase stabilization in all patients was highly dependent on head velocity, typically declining by about 50 % during rapid head movements directed towards the lesion. Such reduced slow-phase stabilization was supplemented by quick-phases which made net stabilization less dependent on head velocity in 2 subjects, and made it independent of head velocity in the third who had the longest post-operative period. As in normals, quick-phase movements always reduced gaze error because quick-phase stabilization tended to be proportional to slow-phase error. However in contrast to normals, quick-phases tended to correct only about 40 % of the slow-phase error, despite the presence of significant negative correlations between slow- and quick-phases elicited during individual head turns.

Thus complete unilateral loss of peripheral vestibular function appeared to cause two functional deficits of vestibular gaze stabilization: (1) deficient slow-phase stabilization of gaze during rapid head turns towards the lesion, and (2) deficient quick-phase capability to correct errors in gaze stabilization. However during natural behaviour, vision may compensate for these deficits.

[a]Segal & Katsarkas, this meeting; [b]Segal, Physiol.Can.16:211,1985

♦Supported by McGill Fac. Med. and Dept. OTL of JGH.

297.19 DOES THE QUICK PHASE SUPPLEMENT OF GOAL-DIRECTED VESTIBULO-OCULAR REFLEX (VOR) REFLECT THE ADAPTIVE STATE OF A MODIFIABLE INTERNAL SPATIAL MAP? J. Bloomberg*, G. Melvill Jones & B. Segal. AMRU, Dept. of Physiol., McGill Univ. Montréal, Canada H3G1Y6.

When a subject in total darkness attempts "visual" fixation on a remembered earth-fixed target during passive whole body rotation, the slow phase of compensatory VOR is normally supplemented by intermittent quick phase eye movements which tend to correct for instantaneous gaze error (Segal & Katsarkas, this meeting). The present experiments investigated this goal-seeking quick phase response after adaptive attenuation of the VOR. The adaptive stimulus comprised prolonged (2 hr) visual suppression of VOR, with the subject viewing a full-field scene moving with the head during horizontal sinusoidal rotation at 1/6 Hz and 40°/s peak vel. To determine the effect of this adaptive stimulus on net ocular stabilization (cumulative slow phase plus quick phase eye movements), subjects were exposed in total darkness to brief step changes of rotational position (20°; 40°/s peak vel.) whilst they tried to maintain their gaze (direction of regard) on a previously seen, but now remembered, earth-fixed target. Each of 7 human subjects underwent 40 such control tests, and 60 post-adaptation tests. The direction of turns was randomly chosen.

Mean control values of cumulative slow phase and quick phase compensatory eye movements, expressed as percentage of the ideal total value, were 81% and 15% respectively, representing a net stabilization of 96%. After adaptation the corresponding slow phase value was reduced to 56% with a quick phase contribution of 19%. Thus the net post-adaptive stabilization of 75% (i.e. 56+19%) was considerably less than the control value of 96% ($P<<0.001$). Consequently after adaptation there was sizeable undershooting of the target's location in external space. Particularly noteworthy is the fact that in both the control and adapted states there was a highly significant ($P<0.001$) negative correlation between quick and slow phase contributions, indicating that a smaller slow phase output tended to be supplemented by a larger quick phase contribution, and vice versa.

In conclusion, during control tests quick phase supplements generally ensured good net stabilization of gaze on the real target, despite an inadequate slow phase response. Presumably, this reflects a properly calibrated internal representation of the external spatial map. However after adaptation, the quick phase supplements consistently undershot the position of the real target. Perhaps this modified positional "goal" of the supplementary quick phases reflects adaptive contraction of the horizontal component of the internal spatial map.

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J. Bloomberg is a Canadian NSERC Scholar.

297.20 VISUALLY INDUCED REVERSAL OF VESTIBULAR NYSTAGMUS - PRIMATE DATA AND MODEL REALIZATION: U.Schwarz* and W.Waespe* (SPON:M.C. Hepp-Reymond. Neurology Dept. Univ., 8091 Zuerich, Switzerland.

Visual-vestibular interaction (VVI) has been investigated as conflict stimulation or foveal fixation. During rotation of the subject together with a subject-stationary surround or a single target, slow phase eye velocity (SPV) of vestibular nystagmus (VN) is suppressed. To determine the effect of eye velocity on the velocity storage mechanism we used a paradigm consisting in visually inducing a reversal of SPV for short periods during VN.

Normal subjects were rotated in darkness to evoke VN while the enclosing optokinetic drum was rotated into the same direction but with higher speed. After switching the lights on, the SPV reversed within 0.2 to 0.5 s and attained a gain of 0.5 to 1.0 during the visually induced response. When lights were turned off, SPV reversed its direction again within 1.0 to 5.0 s and decayed exponentially as before the visual stimulus.

We examined the paradigm for different relative optokinetic stimulus velocities (from +50 deg/s, i.e. in the direction of the vestibular SPV, to -150 deg/s, i.e. opposite to the direction of the vestibular SPV), and different light exposure times (1.0 to 15.0 s). Contrary to present concepts of VVI, an increasing discharge effect on the velocity storage mechanism was not found for stimulus velocities between zero (i.e. conflict) and -150 deg/s despite the increasing eye velocity directed oppositely to the vestibular SPV. The response of our paradigm can be simulated on existing models by using a very short time constant for the velocity storage mechanism or a very low gain for the 'indirect visual pathways' both implying a functional disconnection of visual and vestibular signals mediated by the common velocity storage element.

In the Rhesus monkey, we found a behavior qualitatively the same and quantitatively similar to that of human subjects.

To determine the underlying neural mechanism we are currently doing single cell recordings in the vestibular nuclei.

297.21 REBOUND NYSTAGMUS. S.E. Gordon*, T.C. Hain*, D.S. Zee and M. Fetter*. (SPON: S. Herdman). Dept. Ophthalmol. and Neurol., Johns Hopkins Hospital, Baltimore, MD 21205

Rebound nystagmus (RN) is a transient jerk nystagmus that appears in primary position after prolonged eccentric gaze. It commonly occurs in patients with cerebellar lesions. It can also be demonstrated in normals if they are deprived of a fixation target when they return their eyes to the primary position. Accordingly, RN may reflect a normal physiological process that is seen in patients because of defective pursuit/fixation mechanisms.

To better understand RN we studied 5 normal subjects. RN was elicited after varying degrees and durations of eccentric fixation of a continuously-illuminated light emitting diode (fixation LED). After eccentric gaze subjects returned their eyes to a flashing LED (10 msec duration, 1 Hz). This stimulus enabled the subjects to keep their eyes near a specific orbital position but provided little retinal slip information for fixation/pursuit mechanisms to suppress RN.

Thresholds and amplitudes of RN were determined with the flashing LED located at 0 deg. Eccentric gaze for a constant duration (16 sec) elicited RN with an initial slow-phase velocity of 0.3 deg/sec after fixation at 30 deg. Initial eye velocity increased to 6.8 deg/sec after fixation at 60 deg. With a constant eccentricity (55 deg), RN increased from 0.3 deg/sec after 4 sec of gaze-holding to 5.8 deg/sec after 16 sec and then remained about 6 deg/sec for longer durations. RN also depended on orbital position implying that a velocity bias alone could not account for RN. When the flashing LED alternated between $\pm$ 20 deg subjects showed a centripetal drift superimposed on the bias. The centripetal drift presumably reflected a decrease in the time constant (Tn) of the central gaze-holding network (the neural integrator). By measuring the velocity of RN at $\pm$ 20 deg we determined both Tn and the velocity bias and then calculated the shift in the null (0 deg/sec drift) position. The development of the bias (up to 8 deg/sec), the decrease in Tn (to 4-5 sec) and the calculated shift in the null (as far as 75 deg) varied with the degree and duration of prior eccentric gaze. As RN decayed, the velocity bias disappeared well before Tn returned to normal.

Conclusions: RN reflects a shift of the null in the direction of prior eccentric gaze; it may reflect the actions of a physiological mechanism to optimize ocular stability in eccentric positions of gaze. The shift in the null reflects two processes: development of a velocity bias -- which causes the null to move-and a decrease in the time constant of the neural integrator-- which, together with the bias determines the exact location of the null. Since, after eccentric gaze, the time course of the decay of the bias and of the recovery of Tn were different, the two components of RN may reflect separate neural mechanisms.

297.22 APPARENT MOTION REVEALS SEVERAL PROCESSES UNDERLYING PURSUIT EYE MOVEMENTS IN MONKEYS. K. Logan* and S. G. Lisberger. Dept. of Physiology, Div. of Neurobiology, University of California, San Francisco, CA, 94143.

We have used apparent motion, the sequential presentation of stationary targets, in order to elucidate mechanisms involved in visual motion processing during smooth pursuit eye movements. Two Rhesus monkeys tracked a vertical bar, 1°-.3° wide and 1° high, that was flashed along the horizontal meridian to produce an apparent motion of 30°/sec. In each individual trial, the time interval between flashes (delta-t) was set to a value between 5 and 90 msec. Delta-t varied randomly from trial to trial.

We found that the response has at least three different components that can be distinguished by varying delta-t and the number of flashes: 1) motion detection; 2) response latency; and 3) response magnitude. We studied motion detection by analyzing the parameters necessary to elicit a response. Four flashes were required to initiate pursuit when delta-t was 10 msec, but only 2 when it was 25 msec, indicating that detection was not a function of the number of flashes. However, the duration of apparent motion (number of interflash intervals times delta-t) was similar in these two cases (30 and 25 msec) and was also similar to that required for continuously illuminated targets to initiate pursuit. This suggests that detection is a function of stimulus duration.

Latencies for pursuit initiation were longer for apparent than for real motion and increased as a function of delta-t. The shortest latencies (approximately 110 msec) occurred when delta-t was 10 msec. For each 5 msec increase in delta-t there was a 15 msec increase in latency. Latency was not affected by increasing the number of flashes at a given delta-t.

Response magnitude was measured by computing the smooth eye acceleration during the first 100 msec of the response (the open loop interval). We observed an early and late component. Eye acceleration during the first 20 msec was always about 100°/sec^2 and was not affected by varying the parameters of apparent motion. In contrast, in the 20 to 100 msec interval after pursuit initia-(the late component), eye acceleration was greatest when delta-t was 10 msec, and decreased sharply as delta-t was increased. For the two monkeys peak eye accelerations were 337 and 180 deg/sec^2. Eye acceleration in the late component was also a function of the number of flashes and the motion duration.

Thus, varying the parameters of apparent motion distinguishes among motion detection, response latency and response magnitude in initiation of pursuit. These three processes may be subserved by different physiological systems. (Supported by NIH grant EY03878).

297.23 ENHANCEMENT OF ANTICIPATORY SMOOTH EYE MOVEMENTS BY TARGET EXTINCTION. D.K. Boman* and J.R. Hotson. Institute for Medical Research-Santa Clara Valley Medical Center, San Jose, and Stanford University School of Medicine, Stanford, CA 94305.

Anticipation of predictable target motion has been recognized since early recordings (Dodge, 1930). Previous studies have shown that prior to ramp or step stimulus motions, anticipatory smooth eye movements (ASEM) are produced that actually move the fovea away from the target in the direction of expected target motion (Kowler and Steinman, 1979a,b; 1981). These ASEM are very low in velocity (<1 deg/sec) requiring a high resolution eye movement monitor to detect them. It was also found that ASEM were abolished when there was no target present. We have also observed ASEM before the initiation of ramp stimulus motion. With a target continuously present, however, we found that the smooth eye movements were regularly interrupted by refixation saccades that returned the fovea to the target. The occurrence of this 'anticipatory' nystagmus led us to reexamine the dependency of ASEM on a visual target.

ASEM were elicited by predictable 2-10 deg/sec ramps that had the target visible or extinguished at the onset of stimulus motion. Eye movements were recorded with a double Purkinje image eyetracker. When the target was not extinguished, averaged ASEM velocities were always less than 1 deg/sec, consistent with previous studies. In contrast, ASEM velocity was enhanced, not attenuated, by extinguishing the target prior to the initiation of the ramp, reaching velocities as high as 4 deg/sec. Peak ASEM velocity increased as the duration of the target's disappearance increased from 0 to 600 msec and as the expected ramp speed increased from 2 to 10 deg/sec. Smooth eye movement velocity also decreased before the ramp stimulus was expected to stop. Averaged smooth eye movement velocity dropped from 10 deg/sec to as low as 4 deg/sec prior to the termination of a 10 deg/sec ramp.

These findings show that ASEM are not dependent on the presence of a visual target during ramp stimulus presentations and extend the dynamic range of velocities that ASEM can attain, thus making them easier to detect and study.

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297.24 SMOOTH PURSUIT IN PSYCHIATRIC DISEASE: ARE THERE SPECIFIC DEFECTS IN PATIENTS AND THEIR RELATIVES? L.A. Abel, R.W. Hertle* and H.Y. Meltzer, Ocular Motor Neurophysiology Lab, Cleveland VA Medical Center, Department of Biomedical Engineering, University of Akron and Departments of Neurology and Psychiatry, Case Western Reserve University and University Hospitals of Cleveland, Cleveland OH 44106

In the past decade, numerous papers have appeared examining the eye movements of psychiatric patients. Schizophrenia has been the disorder most often studied, although a number of reports have dealt with the affective disorders. The overwhelming majority of these studies evaluated their data qualitatively, using a variety of global performance measures. Only rarely have measures such as pursuit gain or the presence of inappropriate saccades been assessed. This has particularly been the case in those studies examining the eye movements of patients and their relatives. The present investigation involved the assessment of smooth pursuit in 33 patients with Research Diagnostic Criteria diagnoses as follows: 15 schizophrenic or schizoaffective, primarily schizophrenic, 9 bipolar or schizoaffective, primarily bipolar and 9 unipolar affective disorder. Constant-velocity targets of 5, 10 and 20°/sec were presented and eye movements recorded using infrared oculography. Smooth pursuit gains were below normal in all patient groups, although miminally so in the unipolar depressives. In the bipolar patients, gain was lower in those taking lithium carbonate. No ocular motor effects of neuroleptic or antidepressant medication were observed in patients tested both on and off medication. Square wave jerks were abnormally frequent in the schizophrenic, schizoaffective (mainly schizophrenic) and bipolar affective patients. The anticipatory saccades that we had previously noted in a study of the relatives of schizophrenics were only prominent in a few patients, all of whom had bipolar affective disorder. Why an abnormality should be present in patients' parents but not in the patients themselves is unclear.

Identical abnormalities (low gain and square wave jerks) were seen in patients and their relatives; this was true for both schizophrenic and affective disorder patients. The possibility of a genetic link in the abnormal eye movements of affective disorder patients is of particular interest, since it has been proposed that defects of tracking in this population are state, rather than trait, related. Impairments seen in bipolar patients have been ascribed to lithium treatment; this would not account for our observed familial occurrence. It may be that the more global assessement techniques used in previous studies were insufficiently sensitive to detect the deficits we observed.

297.25 NORMALIZATION OF SMOOTH PURSUIT TRACKING BY DARK ADAPTATION: EVIDENCE OF CEREBELLAR DYSFUNCTION IN PSYCHOSIS. R.T. Pivik, F.W. Bylsma* and P. Cooper*. Lab. of Psychophysiology, University of Ottawa, Ottawa, Ontario K1H 8L6.

Psychotic patients have been shown to evidence both reduced suppression of vestibular nystagmus by visual fixation and a higher incidence of disordered smooth pursuit tracking. Cerebellar structures play a significant role in fixation suppression and affect smooth pursuit performance. Furthermore, the cerebellar influence is largely inactive in darkness. The purpose of the present investigation was to determine if disordered pursuit tracking evident in psychotic patients under light-adapted conditions would normalize during dark adaptation when the cerebellar influence on eye movement control is reduced.

Twenty psychiatric patients--predominantly schizophrenics (n=11)--and twenty normal control subjects were studied. Diagnoses were based on two independent psychiatric evaluations using DSM-III criteria, hospital diagnosis, and information from patient history charts and psychological testing. Patients were medicated and none had overt signs of organicity or motor abnormalities. Control subjects did not have personal or family histories of psychosis and were free from medication known to affect eye movements. Participants signed forms of informed consent.

Horizontal and vertical eye movements were recorded while subjects visually tracked a target light oscillating at 0.45 Hz (2.2 sec periods). Tracking trials (15-20 oscillations/trial) were recorded under both light- and dark-adapted conditions ($\geq$10 min duration) with order of conditions randomized across subjects. Pursuit accuracy was determined using two measures of deviation, i.e., the incidence of velocity arrests (VAs) and the general correspondence of tracking and target patterns (root-mean-square error deviations: RMS).

Patients' tracking performance was significantly impaired relative to that of controls under light-adapted conditions (VA: $p<.0002$; RMS: $p< .02$). Within-group comparisons of tracking performance across conditions were of borderline significance for controls (VA and RMS light-dark comparisons, $p<.10$), but were highly significant for patients (VA: $p<.0001$; RMS: $p<.002$). Patients' dark-adapted performance was not significantly different from that of controls under dark- or light-adapted conditions.

The observation that a functional manipulation may normalize the smooth pursuit tracking dysfunction in actively psychotic patients is unprecedented. The results are consonant with the hypothesis that cerebellar dysfunction contributes significantly to disordered smooth pursuit but do not exclude the contribution to disordered tracking from other influences.

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TROPHIC AGENTS: NERVE GROWTH FACTOR RECEPTORS II

298.1 MOLECULAR CLONING AND CHARACTERIZATION OF THE RAT NERVE GROWTH FACTOR RECEPTOR. M. J. Radeke*, T. P. Misko* and E. M. Shooter. Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

In order to better understand the mechanism of action of nerve growth factor (NGF) it is essential to know the structure and function of the NGF receptor (NGFR), since it is the binding of NGF to its receptor which initiates cellular differentiation. To achieve this, we cloned the rat NGFR. To carry out the cloning of the NGFR a two step approach was used. First, genomic DNA mediated gene transfer was used to produce five mouse L cell lines which expressed NGFRs, one which had greater than 2 x 10^6 NGFR/cell (PCNA). The second step was to rescue the NGFR gene in a cDNA form based on the difference in gene expression between L cells and the $NGFR^+$ L cell transfectants.

To recover the NGFR gene a cDNA library was constructed in pUC9 using PCNA $polyA^+$ RNA. The library was then screened with a receptor enriched probe. The probe was made by annealing ^{32}P-cDNA prepared from PCNA $polyA^+$ RNA to L cell $polyA^+$ RNA. Constitutive L cell cDNAs which hybridized to the L cell RNA were then removed by hydroxylapatite chromatography. The remaining single stranded ^{32}P-cDNA was used to screen 30,000 clones. From this screening a few hundred positive clones were identified. Cross hybridization analysis of these initial clones revealed a family of related clones, each of which was strongly positive when rescreened with the enriched probe. When a clone from this family was used to probe total RNA from various cells which express the NGFR a common 3.7 Kb message was detected in each. No mRNA was found in RNA from cells that do not express NGFRs. To determine whether these clones really were NGFR clones, a clone containing a near full length 3.5 Kb insert was isolated from the original family. The insert was subcloned in either orientation into pCDL1, a SV40 based eukaryotic expression vector. These two constructs were cotransfected into L cells and stable transfectants were selected by growth in HAT-media. Analysis of the transfectants using anti-NGFR binding, ^{125}I-NGF binding and SDS-PAGE of immunoprecipitated radioiodinated cell surface proteins revealed that the insert did, indeed, code for the rat low affinity NGFR. This near full length NGFR clone pNGFR.1 has been sequenced by the Sanger Method. Preliminary analysis suggests that there is one open reading frame which covers approximately half of the mRNA with the remaining 3' region being untranslated. Further, it appears that the protein has only one membrane spanning region and that the amino terminus is fairly cysteine rich. The significance of the NGFR sequence regarding structure-function relationships will be discussed.

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298.2 TRANSFECTION OF PC12 DNA INTO MOUSE L CELLS: SELECTION AND CHARACTERIZATION OF LINES EXPRESSING THE NERVE GROWTH FACTOR RECEPTOR. T. P. Misko*, M. J. Radeke* and E. M. Shooter. (SPON: B. Wallace) Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The first event in nerve growth factor (NGF) induced differentiation of responsive cells is the binding of NGF to its receptor Thus, knowledge of the structure and function of the nerve growth factor receptor (NGFR) will give important clues regarding the mechanism of action of NGF.

This abstract reports the transfection and the expression of the rat NGFR gene in mouse L cells. We have transfected mouse LTK^+ cells with purified PC12 DNA and DNA coding for thymidine kinase. Cells surviving HAT (hypoxanthine,aminopterin,thymidine) selection were stained with an anti-receptor monoclonal antibody, MC192 (Chandler et. al., J. Biol. Chem., 259: 6882, 1984) followed by goat anti-mouse IgG-FITC. Cells which expressed the NGFR were isolated using a fluorescence activated cell sorter. Five independent transfectants which specifically bound NGF were obtained. Four of the $NGFR^+$ lines had low levels of NGFR expression (1-5 x 10^4 NGFR/cell). NGFR expression in the other line proved to be amplifiable. After 10 rounds of cell sorting a stable cell line, PCNA, that expressed 2.3 x 10^6 NGFR/cell was obtained. Binding analysis showed that these lines express predominately low affinity (Ka = 2 pM), fast ($t_{1/2}$ = 30 s) NGFR. Photoaffinity crosslinking of ^{125}I-NGF to the PCNA line with HSAB (hydroxy-succinimidyl-4-azidobenzoate) also produces a NGFR-NGF complex of 100 KD, which is identical to the low affinity form of the NGFR in PC12s. A purification of the NGFR from octylglucoside solubilized PCNA cells using affinity chromatography on an anti-NGFR monoclonal column has been accomplished. Analysis of the purified NGFR using SDS polyacrylamide gel electrophoresis under reducing conditions revealed two molecular weight species of 82 and 85 KD which appear to be present in approximately equal mass. These same two species were observed when cell surface radioiodinated PC12 and PCNA cells were solubilized with 0.5% NP40 and subjected to immunoprecipitation using the anti-NGFR monoclonal. An approximately 220 KD species was also observed after immunoprecipitation; however, the amount relative to the lower molecular weight species varied.

In conclusion, we have succeeded in getting expression of the rat NGFR by L cells into which PC12 DNA had been transferred. One of these lines, PCNA, possesses over 2 x 10^6 NGFR/cell and has been used as a source for NGFR purification. Finally, because of the large number of NGFR in this cell line, we will be able to use it to clone the NGFR.

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298.3 NGF RECEPTOR DENSITY IN DEVELOPING RATS. Qiao Yan* and Eugene M. Johnson, Jr. (SPON: D. Harris). Washington Univ. Med. Sch., St. Louis, MO 63110

To study the developmental expression of nerve growth factor (NGF) receptor, a quantitive immunoprecipitation assay was conducted using a monoclonal antibody (192-IgG) against rat NGF receptor. Either homogenates or membrane preparations from isolated tissues or whole embryos were bound with ^{125}I-NGF and cross-linked by EDAC. The receptor was then solubilized by 2% octylglucoside, bound with 192-IgG and immunoprecipitated by a secondary antibody against mouse IgG prebound with Pansorbin. The ^{125}I-NGF receptor-Ab-Staph A complex was separated from free ligand by rapid centrifugation through a 0.15M sucrose cushion. The tube was frozen and the tip containing the radioactivity was cut and counted. In some cases, the pellet was resuspended in SDS-PAGE sample buffer and subjected to a SDS-PAGE autoradiographic analysis. The NGF receptor was found in whole embryo homogenates as early as embryonic day 11 (E11) (earliest age examined). Its content in whole embryos increased 3.5-fold from E11 to E18 (35 fmol/mg protein). SDS-PAGE autoradiography showed that the molecular weights of ^{125}I-NGF bound receptor from different ages were the same as that of adult (a major band 92KD and a minor band 220KD). The NGF receptor content in sciatic nerve homogenate decreased 23-fold from new born rats (276 fmol/mg protein) to adult rats. The change of NGF receptor level in hindleg muscle had a similar profile as that in sciatic nerve. The NGF receptor content in DRG and SCG homogenates were expressed in two ways: 1) On per mg protein basis: In DRG, the receptor density was relatively constant from E15 through P0 (average of 100 fmol/mg protein) and then dropped 6.7-fold in adults. In SCG, it decreased slightly from E20 (80 fmol/mg protein) to adulthood (50 fmol/mg protein). 2) On per ganglion basis: In DRG, it increased 9-fold from E15 to P0 (1.7 fmol/DRG) and then stayed constant through adulthood. In SCG, it increased 4.4-fold from E20 to adult (7.9 fmol/SCG). The NGF receptor was also found in CNS. In brain membrane, it dropped 11-fold from E15 (68.9 fmol/mg protein) to adulthood. In spinal cord membrane, it dropped 7-fold from E18 (174.5 fmol/mg protein) to adulthood.

The present study shows that: 1) The NGF receptor appears very early in the development and the receptor molecular weights do not change. 2) Prenatally, the changes of NGF receptor content vary with different tissues: It increases slightly in whole embryo, stays relative constant in DRG, SCG and spinal cord, and decreases in brain and muscle. 3) NGF receptor density dramatically decreases postnatally in all the tissues we examined except SCG. These data suggest that alterations in NGF receptor density may play a role in changes in tissue responsiveness to NGF during development.

298.4 NERVE GROWTH FACTOR (NGF) PROMOTES CELL SURVIVAL AND NEURITE OUTGROWTH OF RAT CNS NEURONS IN CULTURE: CORRELATION WITH ONTOGENESIS OF NGF RECEPTOR IMMUNOREACTIVITY. S. Koh*, M.D. Notter and R. Loy (Spons. R.J. Joynt) Depart. of Neurobiology and Anatomy, Univ. of Rochester, Rochester NY14642

NGF sensitive cells in the Peripheral Nervous System (PNS) respond to NGF by increased survival, fiber outgrowth and selective induction of tyrosine hydroxylase and dopamine ß-hydroxylase. In the CNS, a selective increase in choline acetyltransferase (ChAT) is observed in septum, hippocampus and cortex of the neonatal rats following intraventricular administration of NGF. Also, dispersed fetal septal neurons in culture respond to exogenous NGF by an increase in ChAT activity. In analogy to the PNS, it would be expected that the NGF sensitive CNS neurons in culture respond to NGF by an increase in survival and fiber outgrowth. The present study addresses whether such NGF effects and NGF receptor expression are developmentally regulated. NGF receptor was visualized immunocytochemically using the monoclonal antibody 192 IgG (characterized and provided by E. Johnson) at E12, E15, E18, E20, PD1, PD4, PD6, PD8 and PD 21. At E12, fetal forebrain is devoid of NGF receptor immunoreactivity. By E15, a few NGF receptor positive neurons are first visible in the medial septal area and nucleus basalis (NB) region. By E18-20, these neurons are quite numerous and appear as well defined cell groups in the medial septum, diagonal band of Broca and NB. In addition, NGF receptor immunoreactivity is widespread throughout the developing brain. Most notably, fiber projections from the basal forebrain neurons to hippocampus and to neocortex are intensely immunoreactive. At PD 1-4, the pattern of staining is similar to fetal tissue. However, by the end of the first postnatal week, the staining pattern starts to resemble that of the adult; intense staining is limited to the cell bodies and dendrites of basal forebrain magnocellular neurons. In order to correlate NGF responsiveness with NGF receptor immunocytochemistry, prelliminary *in vitro* studies were undertaken. Dissociated telencephalic neuronal cultures were prepared from embryonic day 12 fetuses, seeded at $1x10^6$ onto poly D-lysine coated 35 mm cultrure dishes and grown in serum-free, growth medium (Optimem®) in the presence or absence of 50 ng/ml NGF. At 3 days and 6 days in culture, cells were stained for NGF receptor , and the number of immunoreactive cells was counted. An NGF effect on cell survival is apparent at 6 days in culture, but not at 3 days. NGF treated cultures show a 1.5 fold increase in the number of NGF receptor positive cells. To study the NGF effect on neurite outgrowth, septum from E 15 fetuses were dissected and allowed to attach to a collagen-coated, multi-welled culture dish supplemented with Optimem® ± NGF, and observed at 2 days and at 4 days. Short neurite growth from one of the NGF treated septal explants was detected at 2 days in culture. By 4 days, all three of the NGF treated explants showed cellular migration and neurite elongation, while none of the controls grown in the absence of NGF did. Also, septal explants from PD10 pups failed to respond to NGF.

The results indicate that the NGF receptor immunoreactivity undergoes time dependent changes, reaching a peak at around E18-PD4. Correspondingly, the receptor-bearing cells appear to respond to NGF during that period by increased survival and fiber outgrowth. NGF sensitive neurons in the CNS may be behaving similarly to peripheral sensory ganglia in that they respond to NGF only in a certain window of time during development. Supported by NSF grant BNS-8511564 (RL) and NS-19711 (MFDN)

298.5 THE TIMING AND SITE OF NGF SYNTHESIS IN DEVELOPING CUTANEOUS TARGET FIELDS IN RELATION TO INNERVATION AND NGF RECEPTOR EXPRESSION. Alun M. Davies, Christin Bandtlow, Rolf Heumann, Sigrun Korsching, Hermann Rohrer and Hans Thoenen Department of Anatomy, St. Georges Hospital Medical School, Tooting, London SW17 ORE and Department of Neurochemistry, Max Planck Institute for Psychiatry, Martinsried, 8033 Munich, West Germany.

The trigeminal ganglion and part of its peripheral target field (the maxillary process, from which the whisker pad develops) were studied in closely staged mouse embryos. In normal development the earliest nerve fibers emerge from the ganglion at 9½ days gestation (E9.5, Theiler stage 15) and reach the epithelium of the maxillary process by E11. The numbers of trigeminal fibers and neurons increase to reach a maximum at E13 after which over half are lost during a phase of cell death.

Using a sensitive two-site enzyme immunoassay, NGF was first detected in the maxillary process at E11 and increased 20 fold per process by E13 after which the target field content of NGF fell. NGF was first detected in the ganglion at E12 and increased in amount thereafter. Hybridization of Northern blots of target field mRNA with a ^{32}P-labelled cRNA probe revealed that NGF mRNA first appears in the target field just 12 hours before NGF protein and increases with the latter from E11 to E12, suggesting that post-translational processing is not the major rate-limiting step in NGF synthesis in development. From E12 to E12.5 there was a substantial increase in the target field content of NGF mRNA which coincides with the differentiation of whisker follicles, the most densely innervated sensory organs in the integument. The epithelial and mesenchymal components of the target field, separated by enzymatic digestion of the basement membrane, both contained NGF mRNA but with a several fold higher concentration in the epithelium. This was consistent with dense labelling of surface and follicular epithelium in autoradiographs of sections of whisker pads hybridized with an ^{35}S-labelled cRNA NGF probe.

Autoradiographs of trigeminal neurons incubated with ^{125}I-labelled NGF after 24 hours in culture revealed no labelled neurons in E9 cultures, less than 30% in E10 cultures, and a progressive increase in cultures of older neurons to reach 100% by E14. After 48 hours in culture, however, almost all early neurons became labeled, suggesting that receptor expression is independent of target field innervation.

The absence of NGF receptors on early nerve fibers at the stage when they are growing to their targets together with the absence of NGF in the target field prior to innervation strongly suggest that NGF does not guide sensory nerve fibers to their targets by chemotaxis during development.

298.6 IMMUNOCYTOCHEMICAL LOCALIZATION OF FIBERS STAINING FOR NERVE GROWTH FACTOR RECEPTOR IN THE HIPPOCAMPAL FORMATION OF THE RAT. J. E. Springer and R. Loy Department of Neurobiology and Anatomy, University of Rochester Medical Center. Rochester, New York. 14642.

The hippocampal formation contains one of the highest levels of nerve growth factor (NGF) and messenger RNA encoding for NGF in the central nervous system. Many studies have suggested that NGF is synthesized in the hippocampus, taken up selectively by septal/diagonal band terminals, and transported retrogradely to the cells of origin, where NGF may have trophic effects in maintaining cell survival. Several lines of evidence suggest that the trophic effect of NGF may be on central cholinergic neurons in the medial septum. Although it is not known which cell population(s) may be producing NGF, we have used a monoclonal antibody to the NGF receptor (192-IgG, characterized and kindly provided by E. Johnson) to localize fibers in the hippocampal formation which may transport NGF. Female Sprague-Dawley rats were perfused with 0.1M PBS (pH 7.4) containing 0.5% paraformaldehyde and 1.0% glutaraldehyde and 50 µm Vibratome sections were prepared for NGF receptor (NGF-R) immunoreactivity using the ABC method. Control sections were run without primary antibody, or by substitution with a monoclonal antibody to epidermal growth factor receptor.

Fibers which are immunoreactive for NGF receptor (NGF-R) are found throughout the hippocampal formation, with the densest staining being associated with the granule cell and pyramidal cell layers. Specifically, fibers are observed immediately above and below the granule cell layer resembling the innervation pattern of septal/diagonal band projections. Minimal staining is seen in the granule cell layer itself. Fibers are also found, although to a lesser degree, in stratum moleculare and stratum polymorphe. NGF-R positive fibers are found in all levels of Ammon's horn (CA1-CA4), and are observed to penetrate the pyramidal cell layer. The densest pattern of fiber staining is found in CA3a subregion.

We have previously reported that following transections of the fimbria/fornix, there is a loss of cells in the septum/diagonal band which stain for NGF-R. We suggested that this disappearance of cells was due to the separation of the septal/diagonal band from the source of NGF in the target regions of the hippocampus. From the results reported here we suggest that the target region(s) of these projections are located throughout the hippocampal formation, and may represent those fibers which transport NGF in a retrograde fashion to cell bodies in the septum/diagonal band.

Supported by Grant No. DA-05274 (JS) and BNS-8511564 (RL).

298.7 RAT SCHWANN CELL-SCHWANNOMA HYBRID CELLS BIND NGF AND EXPRESS THE NGF GENE. I.Taff*, K.H. Sonnenfeld*, J. Fisher*, AND J.E. Pintar*. (SPON: M.D. Yahr). Dept. of Neurology, Mt. Sinai School of Medicine, N.Y., NY 10029, and Dept. of Anatomy and Cell Biology, Columbia University, N.Y.,NY 10032.

Rat Schwann cell-Schwannoma hybrid cells possess properties of Schwann cells which make them useful models for studying processes associated with Schwann cell function. For example, the hybrid line RNS-5 has the ability to bind axolemma (Barbara Kreider and David Pleasure, personnal communication). These cell lines were tested for their ability to specifically bind NGF and express the NGF gene. Four hybrid cell lines were tested for their ability to bind ^{125}I-NGF. Cultured cells were harvested and incubated in suspension in binding medium (RPMI 1640 with BSA, 5mg/ml, and HEPES, 25 mM) containing ^{125}I-NGF. Non-specific binding was assessed in parallel incubations containing unlabeled NGF (10 ug/ml). Specific binding of ^{125}I-NGF was present on all cell lines tested. When RNS-5 cells were incubated in the presence of ^{125}I-NGF (5 ng/ml) at 37°C, approximately 80% of specifically bound ^{125}I-NGF dissociated rapidly when the cells were transferred to 4°C and unlabeled NGF (10 ug/ml) was added. This dissociation is consistent with binding of ^{125}I-NGF to fast NGF receptor sites. Competition experiments demonstrated that ^{125}I-NGF binding to RNS-5 cells has inhibited by unlabelled NGF with an IC50 of 3nM.

Northern blot analysis demonstrated the presence of NGF mRNA in RNS-5 cells. Total RNA was isolated from these cells using the guanidine isothiocynate-LiCl procedure and poly A$^+$ RNA isolated by oligo dT chromatography. Poly A$^+$ RNA's were separated by agarose gel electrophoresis, transferred to Gene-Screen (NEN), hybridized with ^{32}P labeled NGF cRNA (synthesized following subcloning into SP64 of a Pst fragment from an NGF cDNA clone originally obtained from Axel Ullrich), and washed to a final stringency of 0.05X SSC at 68°. A single band of approximately 1300 base pairs, similar in size to NGF mRNA demonstrated in tissues and cell types, was revealed following autoradiography. These results indicate that Schwann cell-Schwannoma hybrid cells can both bind NGF and express the NGF gene. The possible role of NGF in the regulation of NGF gene expression by these cells is presently being investigated. Taken together, these results suggest that these cells may serve as a potential model for studying the modulation of NGF receptors and NGF synthesis in Schwann cells.

Supported by an NIH training grant NS07245 (I.T), the Neurofibromatosis and Dysautonomia Foundations (K.H.S.) and NIH grant NS-21970 (J.P.).

298.8 PRESENCE AND/OR ABSENCE OF NERVE GROWTH FACTOR (NGF) RECEPTORS ON NEURON-LIKE CELLS IN LONG-TERM NEURAL CREST CULTURES. P. Bernd. Department of Anatomy, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. 10029.

This laboratory has previously demonstrated that a subpopulation of cultured neural crest cells undergoing differentiation have receptors for Nerve Growth Factor (NGF), and exhibit specific binding of iodinated NGF (^{125}I-NGF; dissociation constant of 3.2 nM). These cells are likely to be targets of NGF during the early stages of embryonic development. This study attempted to identify the phenotypic characteristics of the cultured neural crest cells that bear NGF receptors.

Neural crest cultures were prepared from the trunk region of 48 hr quail embryos, and maintained for 1 week in complete medium containing 5% chick embryo extract (CEE) and 15% fetal bovine serum. Under these conditions, neuron-like process-bearing cells were identified which possessed phenotypic characters such as tyrosine hydroxylase-like, serotonin-like, or vasoactive intestinal polypeptide-like immunoreactivity (TH-LI, 5-HT-LI, VIP-LI). Antibodies were used at a dilution of 1:1000, 1:15,000, and 1:2000, respectively. In order to determine if cells possessing these phenotypic characters had receptors for NGF, cultured neural crest cells were rinsed with CEE-free tissue culture medium (2 hr; 37°C), incubated with ^{125}I-NGF (5 ng/ml, 130 cpm/pg; 1 hr; 37°C), rapidly rinsed six times with ice-cold phosphate buffered saline, fixed (4% paraformaldehyde in 0.1M sodium phosphate buffer; 1 hr; room temp.), and processed for immunocytochemistry using an avidin-biotin-peroxidase technique. The cultures were subsequently embedded in epon and sectioned (1 µm). Examination of light microscopic radioautographs revealed that neither TH-LI, 5-HT-LI, nor VIP-LI cells had NGF receptors as evidenced by a lack of silver grains, although some cells in the same section did bind ^{125}I-NGF. The identity of the NGF receptor positive cells remains to be determined.

Neural crest cultures were alternatively grown for 1 week with continuous exposure to nonradioactive NGF (50 ng/ml), in addition to the complete medium described above. Co-localization studies of such cultures revealed, in contrast to the above results, that some of the TH-LI cells possessed NGF receptors and are likely to be responsive to NGF. These results indicate the critical nature of the microenvironment in determining the differentiation pathway of neural crest cells in early development. Ongoing studies are examining whether the 5-HT-LI or VIP-LI cells have NGF receptors when grown in the presence of NGF, and whether high affinity NGF binding (0.03 nM) can be detected under these conditions. Supported by grants from the NIH (#HD 17262) and the Dysautonomia Foundation.

298.9 MOST CATECHOLAMINE-CONTAINING NEURAL CREST CELLS LACK NGF RECEPTORS IN VITRO. C. A. M. Greiner* and G. Guroff. Committee on Neurobiology, University of Chicago; Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892.

We have previously demonstrated that a subpopulation of neural crest cells express specific receptors for nerve growth factor (NGF) after 3-1/2 to 4 days in vitro (Soc. Neurosci. Abst. 1985: 937); many of these cells are still mitotically active after 9 days in culture. As a prelude to determining the possible role of NGF in the development of these cells, we sought to identify the phenotype of the NGF receptor-positive cells. Since NGF-responsive neural crest derivatives in vivo include catecholamine-containing cells, i.e., sympathetic neurons, small intensely-fluorescent (SIF) cells, and adrenal chromaffin cells, initial studies examined the possible co-expression of catecholaminergic traits and NGF receptors by neural crest cells. Primary cultures were established from the trunk region of 2-day quail embryos and grown for 4-5 days in α-MEM, 15% horse serum, and 10% chick embryo extract. Cells were then dissociated and replated at low density onto collagen-coated chamber slides or 35mm dishes. Following an additional 1-5 days growth, cells were incubated with ^{125}I-NGF for 1 hr at 37°C, washed rapidly, and fixed with 4% formaldehyde, 0.5% glutaraldehyde in 0.1 M PO_4 buffer, pH 7.4 for 2 hr at room temperature (FAGLU method). Following exposure and development for autoradiography, cultures were examined using phase, fluorescent, and brightfield optics.

Fluorescent cells varied in morphology and included small, highly fluorescent cells with and without processes. Occassionally, larger cells with long processes and weaker fluorescence were observed. Regardless of morphology and culture age, catecholamine-containing cells generally lacked NGF receptors, although a significant number of non-fluorescent cells in the same cultures were specifically labeled with ^{125}I-NGF. A cell positive for both NGF receptors and catecholamines was observed only once in an examination of cells from over 80 primary cultures.

The general lack of NGF receptors on catecholaminergic neural crest cells indicates that NGF is not required for the initial expression of this trait in vitro. Furthermore, NGF is not necessary for the maintenance of this expression during the culture period examined. Since catecholaminergic neural crest derivatives in vivo do exhibit specific NGF receptors, the data additionally suggest that the catecholamine cells in these neural crest cultures are (a) developmentally immature, (b) represent a subpopulation of catecholamine cells that are NGF-independent, or (c) lacking an environmental/developmental stimulus normally encountered by neural crest cells in vivo and required for extensive NGF receptor expression.

298.10 FLUORESCENT NERVE GROWTH FACTOR BINDING TO CELLS. M. Kasaian* and K.E. Neet. Dept. of Biochemistry, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

The interaction of fluorescein-labelled nerve growth factor (NGF) with rat pheochromocytoma cells (PC12) has been studied. The NGF was modified at carboxyl groups with iodoacetamidofluorescein, to a level of about 1 fluorescein per NGF dimer. The modified NGF showed full binding and biological activities as compared to unlabelled NGF. Binding to unfixed, live cells was assayed using the fluorescence-activated cell sorter (FACS). This method offers the advantage that unbound ligand need not be separated from that which is cell-associated, thus avoiding perturbation of the binding equilibrium. Furthermore, analysis of each cell of the population by the instrument allows accurate and extensive statistical analysis. The main limitation of the method appears to be its relative lack of sensitivity as compared to the binding of radiolabelled NGF. The limit of fluorescence detection for PC12 cells corresponds to the binding produced by about 5 nM NGF. Therefore, study of the low affinity receptor is possible, but not the high affinity receptor. Binding of the fluorescein-NGF was largely specific and was saturable. Scatchard analysis indicated a Kd for binding of 5-7 nM. Time-based data acquisition allowed a continuous time course for binding to be generated. Binding reached a steady state level within 5 minutes of exposure of the cells to the ligand. Both the Kd and time course determined using fluorescein-NGF agree well with data for binding to the low affinity receptor produced by ^{125}I-NGF binding stuides.

Additional experiments were done to investigate the effects of chloroquine and colchicine on the binding of both fluorescein-labelled NGF and epidermal growth factor (EGF) to PC12 cells and to A875 human melanoma cells. The A875 cells contain the fast NGF receptor, but do not appear to internalize the ligand. These cells are also able to bind fluorescein-EGF. Chloroquine had no effect on the binding of either ligand to PC12 or A875 cells at either 4° or 37°C. Colchicine, however, caused decreased binding of both ligands to PC12 and A875 cells at 4°C. This effect may be due to decreased receptor recycling during the 3 hour pre-treatment with the drug at 37°C. (Supported by NSF Grant 83-14309).

298.11 RECEPTORS FOR NERVE GROWTH FACTOR ON PRIMARY SENSORY NEURONS: QUANTITATIVE RADIOAUTOGRAPHIC OBSERVATIONS. V.M.K. Verge*, P.M. Richardson and R.J. Riopelle. Div. of Neurosurg., McGill Univ., Montreal H3G 1A4 and Div. of Neurol., Queen's Univ., Kingston K2L 2V8.

NGF-binding sites on the perikarya of rat primary sensory neurons have been studied by quantitative radioautographic techniques. Frozen sections of fifth lumbar dorsal root ganglia (L5 DRG) have been incubated with ^{125}I-NGF, fixed, defatted, dipped in radiosensitive emulsion and developed after 2-7 days exposure in the dark. In 40-45% of neurons, grain counts clearly exceed background levels for this labelling in vitro as for previous studies with retrograde axonal transport. The perikaryon of a large sensory neuron contains approximately 100,000 binding sites and the dissociation equilibrium constant for the receptor is in the order of 60pM by displacement methods. In radioautographic studies and in classical binding studies, the number of binding sites in L5 DRG is reduced by sciatic nerve transection. Also, by analysis of retrograde axonal transport after intraneural injection, the uptake of ^{125}I-NGF by axonal receptors is diminished. The reduction in NGF receptors on axotomized sensory neurons could reflect either selective death of NGF-receptive neurons or decreased synthesis of NGF receptors on individual neurons. The observations are relevant to the regulation of NGF receptors and the possible functions of NGF after neural injury.

TROPHIC AGENTS: NERVE GROWTH FACTOR

299.1 ISOLATION AND SEQUENCING OF A CLONE OF CHICKEN GENOMIC DNA ENCODING A PROTEIN HIGHLY HOMOLOGOUS TO MOUSE BETA NERVE GROWTH FACTOR. D.L. Shelton, L.F. Reichardt and E.M. Shooter. Dept. of Neurobiology, Stanford University, Stanford, CA 94305 and Dept. of Physiology, UCSF, San Francisco, CA 94143.

Beta nerve growth factor (NGF) is a protein which is necessary for the normal development and maintenance of sympathetic and sensory neurons in vivo and in vitro. Recent evidence suggests that there are other populations of neurons which may also respond to NGF, but at an early stage of development. Avian embryos are much easier to manipulate than mammalian embryos at these early stages, but very little is known about avian NGF. In order to learn more about the structure of avian NGF, a chicken genomic clone encoding the protein has been isolated and sequenced.

Southern analysis of chicken genomic DNA revealed bands which hybridized strongly to mouse NGF cDNA under stringent conditions. On the basis of this analysis chicken genomic DNA was cut with Bam HI and fractionated by size using sucrose gradient sedimentation. Fractions enriched in the hybridizing DNA were used to construct a partial genomic library in lambda EMBL4. Screening of this library revealed two clones which strongly hybridized to the mouse NGF cDNA. These were plaque purified and analyzed in greater detail.

Restriction site mapping and Southern analysis showed that all of the sequence which hybridized to the NGF cDNA was contained in a 2.2 kb Pvu II fragment. This fragment was subcloned into M13 and sequenced using the Sanger dideoxy technique. The nucleotide sequence showed an open reading frame (ca. 0.8 kb), which corresponds to the 3' end of the mouse NGF cDNA. This region contains the entire sequence corresponding to mature mouse NGF, and is highly conserved in amino acid sequence. The 5' end of the open reading frame ends at a consensus RNA splice site, and so presumably marks an intron-exon junction. This site matches the known intron-exon junction in the human NGF gene.

The isolation and characterization of the NGF gene from chicken should enable further studies on the possible role of NGF in early development.

299.2 NERVE GROWTH FACTOR mRNA IN PERIPHERAL AND CENTRAL RAT TISSUES AND IN THE HUMAN CENTRAL NERVOUS SYSTEM: LESION EFFECTS IN THE RAT BRAIN AND LEVELS IN ALZHEIMER'S DISEASE. M. Goedert*, A. Fine+, S.P. Hunt* and A. Ullrich+, *Medical Research Council Laboratory of Molecular Biology and *Medical Research Council Molecular Neurobiology Unit, Hills Road, Cambridge CB2 2QH, UK; and +Department of Molecular Biology, Genentech Inc., 460 Point San Bruno Boulevard, San Francisco, California 94080, USA.

Using RNA blot hybridisation nerve growth factor (NGF) mRNA was found to be widely distributed throughout peripheral and central rat tissues and throughout the human central nervous system. In the rat, high levels of NGF mRNA were found in cerebral cortex, hippocampus and thalamus/hypothalamus, medium levels in striatum and brainstem and low levels in cerebellum and spinal cord. The hippocampal levels did not change 1-4 weeks following the surgical transection of the septohippocampal pathway; similarly, the ibotenic acid-induced lesion of the nucleus basalis magnocellularis did not affect the amounts of NGF mRNA in the cerebral cortex. NGF mRNA was also present in high amounts in human cortex and hippocampus, with only low levels in septum/nucleus basalis magnocellularis, suggesting that NGF may also function as a retrograde trophic messenger in the human central nervous system. No evidence was obtained for an insufficient production of NGF mRNA in senile dementia of the Alzheimer type (post mortem delay 4-6 h). In peripheral rat tissues, the highest concentrations of NGF mRNA were found in vas deferens, heart, sciatic nerve, submandibular gland and skin, with low levels in tissues such as trigeminal ganglion and pituitary gland.

299.3 LEVELS OF NERVE GROWTH FACTOR AND ITS mRNA DURING DEVELOPMENT AND REGENERATION OF THE PERIPHERAL NERVOUS SYSTEM. S.I. Korsching, R. Heumann, A. Davies*, and H. Thoenen. (SPON: W.V. Bleisch) *Dept. of Anatomy, St. Georges' Hospital Med. Sch. London SW17 ORE, U.K. and Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033, Martinsried, F.R.G.

Nerve growth factor (NGF) plays an essential role in the development and maintenance of structure and function of the sympathetic and sensory nervous systems. We have analyzed levels of NGF and its mRNA during development and in the injured nerve using a two-site enzyme immunoassay (PNAS 80: 3513, 1983) and quantitative Northern blots with an RNA-probe (EMBO J. 3: 3183, 1984), respectively.

In the mouse whisker pad NGF is first detected at embryonic day (E) 11, along with the first target encounter of the innervating trigeminal sensory neurons, and in the submandibular gland, which is sympathetically innervated, at E13. At E12 and E14 NGF is detected in the corresponding ganglia. The absence of NGF in prospective target organs prior to their innervation by sensory and sympathetic neurons makes it unlikely that NGF is used in the organism for initial guidance of outgrowing nerve fibers toward their targets. It is interesting that during the period of sympathetic neuronal death, NGF levels in heart ventricle and submandibular gland are in the same range as those of normal adult sympathetic target organs (i.e. in the ng NGF/g wet weight range). The male mouse submandibular gland, an exceedingly rich source of NGF in the adult animal, shows first signs of an abnormal behavior after postnatal day 18, when NGF levels increase 100-fold within the following three days.

To study a possible role of NGF in regenerating nerves we analyzed NGF and NGF mRNA levels in the adult rat sciatic nerve 6 h to 2 weeks after a cut. In agreement with results obtained earlier (Neurosci. Lett. 39: 1, 1983), up to 1 day after lesion the NGF increase is confined to the distal nerve segment adjacent to the cut, due to accumulation by retrograde axonal transport initially persisting in the distal axon stumps. Three days after the cut, when axonal transport had ceased in the distal nerve stump, NGF accumulation builds up also in the segments of the distal stump farther away from the lesion site, indicating onset of local NGF synthesis in the injured nerve. This interpretation is confirmed by analysis of the NGF mRNA levels. Three days after lesion, NGF mRNA is detected in all segments of the distal stump analyzed and, surprisingly, also in the proximal stump directly adjacent to the cut, where NGF levels are below control values. We conclude that NGF synthesis is induced locally in the injured nerve (presumably in glial cells) and that NGF synthesized in the proximal stump is removed by axonal transport to the neuronal cell bodies, thereby possibly attenuating the effects of target organ deprivation.

299.4 NERVE GROWTH FACTOR IMMUNOHISTOCHEMISTRY: ON THE SPECIFICITY AND DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM AND IN GLANDS OF DIFFERENT POLYCLONAL AFFINITY-PURIFIED ANTIBODIES AGAINST MOUSE NGF. L. Olson*, C. Ayer-LeLievre*, T. Ebendal*, Å. Seiger*, I. Strömberg*. (SPON: L. Olson, Member ENA). Dept. of Histology, Karolinska Inst., 104 01 Stockholm, Sweden, Dept. of Zoology, Uppsala Univ., 751 22 Uppsala, Sweden.

Polyclonal antibodies were raised in rabbits and sheep against mouse submandibular gland NGF. Several polyclonal affinity-purified antibodies have been characterized that show a strong NGF-dependent binding to the secretory granules of tubular cells in the male mouse submandibular gland with excellent signal-to-noise ratios. NGF immunoreactivity can also be detected in several closely related glands in mice and rats and with one of the antibodies in the mouse testis. Before tested in brain tissue, antibodies had to pass rigid specificity controls including "affinity-purified" antibodies derived from serum prior to immunization, rejects from various affinity purifications, antibodies adsorbed to hydrophilic or hydrophobic chick proteins, normal rabbit sera, and hyperimmune sera from rats immunized with nonrelated chick protein. Further controls included preadsorbing antibodies with NGF and pretreatment of sections with NGF. In adult rat brain two different patterns of immunoreactivity were found: most antibodies reveal a widespread distribution of immunoreactivity (type A) in nerve fiber bundles and fiber tracts as well as finer fiber-like structures, e.g. in cerebral cortex parallel and perpendicular to the cortical surface. This pattern disappears upon preabsorption of the antibodies with NGF. Affinity-purified antibodies from some of the immunized animals show a distinctly different pattern (type B) characterized e.g. by positive pyramidal cell bodies and ascending bundles of apical dendrites in cortex. This type B-immunoreactivity remains after preabsorption of the antibodies with NGF. When affinity-purified antibodies that show type A immunoreactivity are preabsorbed with NGF and type A is no longer seen, type B appears. Moreover, serum from which type A antibodies can be affinity-purified sometimes also contain antibodies that do not bind to the affinity columns and which can give rise to type B staining. When NGF is injected into brain tissue, all antibodies can detect the injected NGF and preabsorption always causes disappearance of such fluorescence. We conclude that immunization with salivary gland-derived NGF generates antibodies that give rise to at least two different staining patterns in the central nervous system. Studies now in progress including in situ hybridization and the use of antibodies directed against specific peptide sequences in the NGF molecule should clarify the relationship between these staining patterns, salivary gland NGF, and the regional presence of NGF in the brain. (Supported the Swedish MRC and NFR.)

299.5 SEX DIFFERENCES IN NERVE GROWTH FACTOR LEVELS IN SUPERIOR CERVICAL GANGLIA AND PINEALS. L.L. Wright, C. Beck*, and J.R. Perez-Polo. Boston University School of Medicine, Boston, MA 02115; and Univ. Texas Med. Br., Galveston, TX 77550.

There is a normal sex difference in the numbers of neurons in the rat superior cervical sympathetic ganglion (SCG), with the males having 20-30% more SCG neurons than females at 60 days of age. No sex difference is present on the day of birth (Wright & Smolen, 83,85). NGF administered during the early neonatal period is known to increase the numbers of neurons in the rat SCG (e.g., Hendry & Campbell, 76). The current studies were undertaken to determine whether endogenous levels of NGF differed for males and females at critical times during the development of SCG neurons. Such differences might help explain the development of the normal sex difference in these sympathetic neurons. The levels of nerve growth factor (NGF) in the SCG and pineal gland of the rat, which is innervated by the SCG, were studied using a one site radioimmunoassay. The ages studied were 5 days postnatal, which is at the peak of normal neuron death in the SCG, 15 days, which is after the majority of normal neuron death, and 30 days.

The pineal gland and the SCG showed similar patterns of development of NGF levels. In females, the NGF levels rose between 5 days and 15 days, then declined by 30 days. In males, the NGF levels were highest at 5 days.

The presence of higher NGF levels in pineals and SCGs of males than in those of females at 5 days, when neuron death is occurring, is consistent with the presence of more surviving neurons in males following normal neuron death. The significance of the rise in NGF levels in female SCGs and pineals at 15 days, and subsequent fall is not understood. The presence of almost twice the amount of NGF in the SCGs of males at 30 days than in SCGs of females is consistent with the presence of more neurons in the SCGs of males at this age.

Mean NGF levels in ng/gland or ganglion (standard deviation)

	d5	d15	d30
PINEALS			
females	0.319 (.040)	0.414 (.030)	0.131 (.001)
males	0.393 (.020)	0.121 (.015)	0.181 (.001)
SCGS			
females	0.263 (.270)	0.456 (.050)	0.045 (.003)
males	0.439 (.012)	0.180 (.020)	0.083 (.004)

This work has been supported in part by NIH grants NS21577 to LLW and NS18708 to JRP.

299.6 NERVE GROWTH FACTOR STIMULATES THE HYDROLYSIS OF PHOSPHOINOSITIDES IN PC12 CELLS. M. L. Contreras, and G. Guroff. Section on Growth Factors, NICHD, NIH, Bethesda, MD 20892.

Nerve growth factor (NGF) induces the morphological and biochemical differentiation of PC12 cells into sympathetic neuron-like cells. The second messenger systems involved in the differentiation of PC12 cells has not been clearly elucidated. In an attempt to determine the second messengers involved in the action of NGF, the effect of NGF on the metabolism of phosphoinositides was examined.

NGF stimulated the breakdown of phosphoinositides in PC12 cells. Addition of NGF to PC12 cells, prelabeled with ^{3}H-inositol, resulted in a rapid formation of tritium-labeled inositol trisphosphate (^{3}H-IP$_3$), inositol bisphosphate (^{3}H-IP$_2$), and inositol monophosphate (^{3}H-IP). The formation of these inositol phosphates was detected as early as 15 sec after the addition of NGF. In the presence of LiCl, which inhibits the breakdown of IP to inositol, the accumulation of ^{3}H-IP was measured to monitor the hydrolysis of phosphoinositides. The time course of accumulation of ^{3}H-IP was linear for at least 15 min. The NGF-stimulated accumulation of ^{3}H-IP was dose-dependent with half-maximal stimulation occurring at 0.34 nM. Hydrolysis of phosphoinositides in response to NGF was dependent on the presence of extracellular calcium. In a calcium-free buffer containing EGTA, the basal level of ^{3}H-IP accumulation was lower than that observed in the presence of calcium. Additionally, an NGF-dependent accumulation of ^{3}H-IP was not seen in the absence of extracellular calcium. In the presence of lanthanum, an inhibitor of calcium influx, both the basal and NGF-stimulated accumulation of ^{3}H-IP was inhibited in a dose-dependent manner. Whereas blockade of calcium influx inhibited NGF-stimulated accumulation of ^{3}H-IP, an increase in calcium influx stimulated phosphoinositide breakdown. The calcium ionophore, A23187, in the absence of NGF, stimulated the accumulation of ^{3}H-IP in a dose-dependent manner with half-maximal stimulation occurring at 190 nM. These results suggest that NGF stimulates the breakdown of phosphoinositides in PC12 cells and that this NGF-dependent hydrolysis of phosphoinositides occurs by a calcium-dependent mechanism.

299.7 REGULATION OF CYTOSKELETAL PROTEIN PHOSPHORYLATION BY NGF IN PC12 CELLS. G.E. Landreth and L.K. Williams*. Dept. of Neurology, Medical University of South Carolina, Charleston, SC 29425.

Nerve growth factor (NGF) directs the biochemical and morphological differentiation of its target cells, however, the mechanisms subserving these actions are unknown. We have investigated the NGF-stimulated phosphorylation of a 250 kDa cytoskeletal protein by a protein kinase which is also associated with the Triton X-100 insoluble cytoskeleton (J.C.B. 100:677, 1985). This was detected initially by treating the cells with NGF, followed by detergent extraction of the cells and incubation of the resulting cytoskele tons with ^{32}P-ATP. We report here that the NGF-stimulated phosphorylation can be detected in vitro following solubilization of both the 250 kDa protein substrate and the kinase from the PC12 cytoskeleton. PC12 cells were treated with NGF for 5 min at 37°, extracted in 0.15% Triton and the cytoskeletal proteins solubilized by addition of 2M urea/1% NP40. When the solubilized proteins were incubated with ^{32}P-ATP, the labeling of the 250 kDa protein was dramatically enhanced in extracts obtained from NGF-treated cells compared to untreated controls. The 250 kDa protein kinase activity eluted in two distinct peaks following gel filtration; one coeluting with the 250 kDa substrate and a second with an apparent MW of 60kDa. The 250 kDa kinase activity was inhibited by Na+ and K+. The phosphorylation of the 250 kDa protein was greater in the presence of Mn++ rather than Mg++.

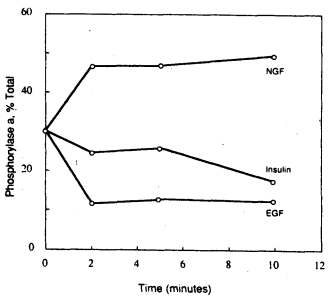
The 250 kDa substrate is present at very low levels in PC12 cells. We have prepared an antiserum to this protein. Western blot analysis demonstrated that the 250 kDa protein was exclusively associated with cytoskeletal elements, both in control and NGF-treated cells.

In summary, we have demonstrated that NGF rapidly elicits stable changes in cytoskeletally-associated protein kinase activity which persists in vitro. The retention of these effects in a soluble extract will permit characterization of both the NGF-regulated kinase and the 250 kDa substrate.

299.8 NERVE GROWTH FACTOR ACTIVATES GLYCOGEN PHOSPHORYLASE IN PC12 CELLS. L.H. Davis* and F.C. Kauffman. Dept. Pharmacology and Experimental Therapeutics. Univ. Md. School of Medicine, Baltimore, MD. 21201.

Glycogen phosphorylase in PC12 exists in two forms analogous to those found in brain and muscle. The active phosphorylated form of the enzyme, phosphorylase-a, represents about 20-30% of total glycogen phosphorylase in PC12 cells measured in the presence of 1 mM AMP. Conversion of phosphorylase-b to phosphorylase-a provides a convenient index of increases in cytosolic Ca^{2+} or cyclic AMP since both events lead to activation of phosphorylase kinase and phosphorylation of phosphorylase-b in intact cells.

Incubation of PC12 with 100 ng 7S NGF/ml resulted in a rapid increase of phosphorylase-a (Figure). The increase in this phosphoprotein appears specific for NGF since insulin (6 ng/ml) or EGF (6 ng/ml) produced decreases in phosphorylase-a. Activation of phosphorylase-a by NGF was not accompanied by increases in the cellular content of cyclic AMP over the time course of the experiment. Removal of extracellular Ca^{2+} or incubation of cells with calcium channel blockers, verapamil or diltiazem, inhibited the activation of glycogen phosphorylase in PC12 cells by NGF. Based on these results, it is concluded that increases in phosphorylase-a activity in PC12 cells represents an early response to NGF and this response is mediated by elevation of cytosolic free Ca^{2+}.



(Supported, in part, by USAMRICD Contract No. DAMD-17-85-C-5091 and USPHS Grant No. 16596).

299.9 EFFECTS OF LAMININ AND FIBRONECTIN ON NGF-DEPENDENT SURVIVAL AND NEURITE OUTGROWTH OF PURIFIED SENSORY NEURONS. A.I. Millaruelo*, M. Nieto-Sampedro and C.W. Cotman. (SPON: R.A. Bradshaw). Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

Nerve growth factor (NGF) is known to promote survival and neurite outgrowth from explanted or dissociated sensory and sympathetic ganglia in culture (Physiol. Rev. 48: 534-569, 1968). However, a relatively small percentage of sensory or sympathetic neurons survive in the presence of NGF on a polylysine substrate, in the absence of non-neuronal cells (PNAS USA 77: 1199-1203; EMBO J. 3: 1463-1469, 1984). The survival and outgrowth of neurites from sympathetic neurons in response to NGF is potentiated by the basement membrane protein laminin (EMBO J. op.cit.). The present work evaluates the role of laminin and fibronectin on both survival and neurite outgrowth from sensory neurons cultured in the presence of NGF.

Eighty to ninety percent pure dorsal root ganglion (DRG) neurons were obtained from E8-10 chick embryos. Survival and neurite outgrowth were determined after 48 h in culture in medium containing 10% fetal calf serum, on different substrates.

At E8, both laminin and fibronectin enhanced NGF-dependent survival from 20% (polylysine substrate) to 75% (laminin or fibronectin substrate). At E10; the response to laminin was increased to 80-90%, while the response to fibronectin was slightly decreased (to 60%). Both laminin and fibronectin potentiated NGF-mediated neurite outgrowth to virtually 100% (from 40-55% on polylysine), at E8 as well as E10. The effects of laminin and fibronectin were dose-dependent. Preliminary experiments suggest that their effects on NGF-mediated survival may be additive. Neurite outgrowth (estimated at 24 h) was evoked more quickly by laminin than by fibronectin, with this difference being more marked at later developmental stages; neurite outgrowth was practically nonexistent on polylysine at 24 h. We have obtained evidence that addition of antisera against laminin and fibronectin reduced NGF-mediated survival to less than 5% of seeded neurons, suggesting an essential role of these proteins for survival of sensory neurons.

Our results suggest a cooperative effect of NGF with laminin and fibronectin on developing sensory ganglia, and support the role of these extracellular matrix proteins as survival-promoting factors.

Supported by grant MH 19691 and FIC/NIH fellowship F05 TW03302.

299.10 STUDIES ON NERVE GROWTH FACTOR AND LAMININ SYNTHESIZED BY MOUSE L929 CELLS IN CULTURE. N.J. Pantazis and R. Jensen*. Dept. of Anatomy, Bowen Science Bldg., Univ. of Iowa Medical School, Iowa City, IA. 52242.

The mouse fibroblast cell line, L929, synthesizes nerve growth factor (NGF) and releases it into the feeding medium, producing conditioned medium (CM). The L929 NGF is identical to the beta-NGF found in the mouse submandibular gland. However, the alpha and gamma subunits which are associated with the beta-NGF in the submandibular gland to form 7S-NGF are not present in the L929 CM. In this study, characterization of the L929 CM on gel filtration columns, using an NGF radioimmunoassay (RIA) to locate NGF, showed that the L929 NGF was associated with binding components. These binding components changed with time in culture and type of media used. When DMEM medium was used, two NGF containing molecules were seen, one with a molecular weight (MW) >150,000, the other with a 50,000 MW. The cells were refed several times and several cycles of CM were obtained. In DMEM, the binding components disappeared by cycle 5, resulting in an NGF peak at 26,000 MW, the unassociated MW of NGF. With DMEM/F12 medium or MCDB 105 defined medium, the >150,000 MW complex was again evident; however, the 50,000 MW material was missing and instead uncomplexed NGF at 26,000 MW was seen. The >150,000 MW peak did not disappear with time in culture with either DMEM/F12 or MCDB 105.

The gel filtration peaks were pooled and examined in immunoblotting experiments to determine whether they contain NGF. Laminin, an extracellular protein which may be involved in neurite outgrowth, was also examined. Results indicated that NGF was present in all peaks. Laminin was detected in the >150,000 MW peak.

Was the neurite outgrowth stimulated by L929 CM due to NGF, laminin or both? Samples were tested on pheochromocytoma PC12 cells. The NGF containing peaks produced neurite outgrowth which was completely inhibited by anti-NGF antibody; less inhibition of outgrowth occurred with the anti-laminin antibody.

In conclusion, NGF synthesized by L929 cells was associated with binding proteins, which changed with time in culture and type of media used. NGF was present in multiple complexes in the CM which were biologically active. Laminin was detected by immunoblotting; however, NGF appears to be the principal component for stimulation of neurite outgrowth.

(Supported by an NIH grant, GM 28644, to NJP)

299.11 MODULATION OF TUBULIN mRNA LEVELS AND NEURITE GROWTH BY NERVE GROWTH FACTOR. P. Fernyhough and D.N. Ishii. Physiology and Biophysics Dept., Colorado State University, Fort Collins, CO 80523.

Nerve Growth Factor (NGF) has profound effects on the development of the vertebrate nervous system, including the capacity to enhance neurite elongation and growth towards higher concentrations of NGF. Microtubules, comprised of alpha- and beta-tubulin subunits, are important structural elements of axons. NGF can increase the formation of microtubules in neurites. We, therefore, investigated the effects of NGF on alpha- and beta-tubulin mRNA levels in cultured E-13 embryonic chick sympathetic, and rat pheochromocytoma PC12 cells. RNA was extracted from cultures, and equal quantities were analyzed on Northern blots with cDNA probes containing the coding regions for the tubulins. Sympathetic ganglion cells were cultured in media containing 2 µg/ml cytosine arabinocide to virtually eliminate the non-neurons. Tubulin transcript levels were higher in sympathetic neurons cultured in medium containing 0.4 nM than in 0.04 nM NGF. The low concentration was included because NGF is essential for sympathetic cell survival. The NGF concentrations which enhanced tubulin mRNA levels were similar to those which stimulated neurite outgrowth. This relationship was also found for the elevation of tubulin transcript levels by NGF in PC12 cells, in which NGF-supplementation is not needed for cell survival under normal culture conditions. The alpha-tubulin transcripts accumulated more rapidly than did the beta-tubulin transcripts in PC12 cells. Following treatment with actinomycin D, the rate of loss of alpha-tubulin transcripts was monophasic in the absence of NGF, but was biphasic in its presence. A class of transcripts with a very slow rate of loss was observed in the NGF-treated PC12 cells. This suggested that NGF may elevate tubulin transcript levels by a) stabilizing certain existing transcripts, or b) inducing a more stable class of transcripts. We have previously shown that neurite outgrowth enhanced by insulin and insulinlike growth factor-II is correlated with increased tubulin mRNA levels (J.F. Mill, M.V. Chao and D.N. Ishii, Proc. Natl. Acad. Sci. USA 82: 7126, 1985). Taken together, these data suggest that the elevation of tubulin mRNA levels is a common prelude to neurite elongation stimulated by NGF and the insulin-insulinlike growth factor family of neuritogenic polypeptides. Supported in part by NIADDKD grant RO1 AM32841, and NIH BRSG 2S07 RR05458-23.

299.12 EFFECTS OF AXOTOMY OF THE PERIPHERAL AND CENTRAL PROCESSES AND OF NERVE GROWTH FACTOR TREATMENT ON THE POSTNATAL DEVELOPMENT AND SURVIVAL OF DORSAL ROOT GANGLION NEURONS. H. K. Yip and E. M. Johnson, Jr., Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Previous studies have shown that axonal injury of either peripheral or central process of sensory neurons in the dorsal root ganglion (DRG) of newborn rats results in a significant (50%) reduction of neuronal number. The results indicate that immature DRG neurons require both peripheral and central target areas for trophic support. Treatment with nerve growth factor (NGF) for 5-7 days temporarily prevents the loss of neurons in both cases.

The present study examined the postnatal dependence of DRG neurons on either peripheral or central targets for survival and the utility of NGF as a pharmacological agent to prevent permanently the cell loss after axotomy. The response of rat DRG sensory neurons was studied two months after the axonal injury. Axotomy of either peripheral or central process was carried out at 0, 3, 7, 14, 21, or 28 days post partum and treatment with NGF was for 1 to 3 weeks in animals lesioned on day 0.

During normal postnatal development there is no decrease in the number of neurons in the DRG; axotomy of peripheral process in the sciatic nerve of the newborn rat (post partum day 0) resulted in a 50% loss of cells in the DRG. The loss of neurons decreased to 20% if the axotomy was done at 21 or 28 days post partum. There was no significant difference in the degree of cell loss following a peripheral nerve crush or cut. However, complete transection of the central processes in the dorsal root resulted in a greater loss (60%) of DRG neurons when compared to a crush lesion in newborn rats.

The cell loss after peripheral nerve crush in newborn rats is decreased by daily administration of NGF (30 ug/kg) for 3 weeks but not for 1 week. Treatment with NGF (1 or 3 weeks) in dorsal rhizotomized animals completely prevented the cell loss after the injury. The difference in the ability of exogenous NGF to prevent cell loss after peripheral, as compared to central, process axotomy is probably because of a progressively greater dependence of DRG neurons on peripheral target for survival as the animal develops.

Thus the decrease of cell loss observed after NGF administration appears to be because of the survival of cells that otherwise would have degenerated after axotomy. In conclusion, these results are consistent with a role for NGF as a trophic substance supporting DRG neurons during the postnatal period and demonstrate that NGF is capable of permanently preventing sensory neuronal loss caused by axonal injury in the neonatal rat.

299.13 NERVE GROWTH FACTORS ENHANCE NEURITE REGENERATION BUT ARE NOT REQUIRED AS SURVIVAL FACTORS FOR ADULT SENSORY NEURONS IN CULTURE. R. M. Lindsay,* (SPON: G. Raisman). The Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN.

There is broad concurrence from in vivo and in vitro studies to indicate that nerve growth factor (NGF) is essential for the survival of developing sensory neurons of vertebrate dorsal root ganglia (DRG). Recent in vitro studies have shown, however, that NGF is not a unique neurotrophic factor for peripheral neurons, as the survival of certain populations of primary sensory neurons can also be promoted by a brain-derived neurotrophic factor - BDNF (Barde et al. EMBO J. 1, 549-553, 1982; Lindsay et al. Dev. Biol. 112, 319-328, 1985; Davies et al. J. Neurosci. 1986, in press). Although there is considerable evidence to support a role for NGF in regulating certain functions of fully differentiated DRG neurons, there is presently little consensus as to whether or not the survival of adult sensory neurons is dependent on NGF or other neurotrophic factors. I report here that neither NGF nor BDNF are required for the long term (30d) survival of the vast majority of adult (3-6 months old) rat DRG neurons when maintained in vitro as essentially pure neuronal cultures (1-5 x 10^3 neurons/laminin coated 35mm dish). Both NGF and BDNF do, however, enhance the regeneration of neurites from cultured adult DRG neurons, especially on a laminin substrate. By minor modification of existing procedures (Scott, J. Neurobiol. 8, 417-427, 1977; McCarthy & Partlow, Brain Res. 114, 391-414, 1976), we have devised a procedure which leads to enrichment (85% + neurons) and high yield (80%) of adult rat DRG neurons after dissociation with collagenase and trypsin and separation from non-neuronal cells and myelin debris by differential adhesion to a polyornithine coated substrate. Although at 24h after plating both NGF and BDNF increase the number of process-bearing neurons when compared to control levels, this difference largely disappears by 2-3 days in culture, when a uniformly high level of survival (60-70% of the neurons plated) is seen in the presence or absence of NGF, BDNF or NGF antibodies. Neurite outgrowth was, however, visibly greater in NGF treated cultures (compared to controls) for at least 10 days. While elimination of non-neuronal cells was essential to observe any survival activity that NGF or BDNF might exert towards adult DRG neurons, we observed that the presence (more than 95% of the cells) or absence of non-neuronal cells (less than 10% of the cells at the outset, and further reduced to less than 1% by cytosine arabinoside treatment) did not noticeably affect the level of neuronal survival during the first few days in culture.

299.14 THE EFFECT OF NERVE GROWTH FACTOR ON THE DEVELOPMENT OF DENDRITIC ARBORS IN THE RAT SUPERIOR CERVICAL GANGLION. William D. Snider, Departments of Anatomy and Neurobiology and Neurology, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110

Factors that regulate the shape and extent of dendritic arbors of mammalian neurons are poorly understood. The traditional view is that afferent fibers exert the primary influence. However, studies in autonomic ganglia have not supported this idea and arbors of sympathetic ganglion cells develop almost normally in the absence of afferent innervation (Voyvodic, 1986). Another idea about regulation of dendritic arbors is that interactions with targets may be involved, presumably mediated via the uptake of trophic molecules. One such molecule, nerve growth factor (NGF), has been identified and has broad effects on neuronal survival, cell size, and ability to synthesize transmitter enzymes of sympathetic and dorsal root ganglion cells.

In order to assess the effects of nerve growth factor on dendritic development I have administered daily subcutaneous injections of NGF (kindly provided by E. M. Johnson and P. A. Osborne) at a dose of 5 mg per kg to new-born rats for a period of six days. On postnatal day seven the animals were sacrificed, the superior cervical ganglia removed and dendritic arbors were studied by intracellular staining with horseradish peroxidase. Forty-five neurons from treated animals were compared with forty neurons from controls at the same age.

In addition to the expected effects on neuronal size, nerve growth factor had a striking effect on dendritic arbors. Total dendritic length was increased by more than 60% over control neurons (948u compared to 589u). The greater dendritic complexity was due to a small increase in the number of primary processes (8.7 compared to 7.0) and a larger increase in the length and branching of these processes. Some neurons extended fine processes which appeared to be supernumerary axons; furthermore, the primary axons of several neurons gave off fine branches that appeared to arborize within the ganglion. Such structures were never observed in control ganglia.

In summary, exogenously-administered nerve growth factor promotes the growth of dendrites in sympathetic ganglion cells. These results are consistent with the idea that target interactions mediated by neurotrophic molecules may be among the factors that regulate normal dendritic development.

Supported by NIH Grants NS 11699 and 18629 to D. Purves.

299.15 EFFECT OF NERVE GROWTH FACTOR ON SEPTAL CHOLINERGIC NEURONS IN MIXED GLIAL-NEURONAL CULTURES. J. Hartikka* and F. Hefti. (SPON.: R. Keane). Dept. of Neurology, University of Miami School of Medicine, Miami FL 33101.

We have studied the effects of NGF on survival, fiber outgrowth and expression of transmitter specific enzymes of forebrain cholinergic neurons in primary cell cultures. We earlier reported that NGF stimulates synthesis of choline acetyltransferase (CAT) but fails to affect survival and fiber growth of cholinergic neurons in pure neuronal cultures (Hefti et al., Neurosci. 14:55,1985). We have now studied the effects of NGF on cholinergic neurons grown in cultures containing both, neurons and glial cells.

Cultures of dissociated cells were prepared from fetal (E 17) rat septum. Cells were plated at a density of 300,000/sqcm in poly-lysine coated dishes and grown for 5-15 days in a modified L-15 medium. Proliferation of glial cells resulted in mixed glia-neuron cultures. Using immunocytochemical methods, app. 50% of the cells were identified as astrocytes and app. 50% were identified as neurons after ten days in culture. Cultures were treated with NGF (100ng/ml) or with an anti-NGF serum and then taken for cytochemical or biochemical analysis. Cholinergic neurons were identified using CAT immunocytochemistry and acetylcholinesterase (AChE) cytochemistry. The entire fiber network of cholinergic neurons could be visualized with AChE cytochemistry.

NGF treatment markedly increased the density of fibers of cholinergic neurons. There was a slight but far less pronounced increase in the number of AChE-positive cell bodies. Increased fiber growth was reflected by a twofold increase in AChE activity as compared to control cultures. In addition, NGF treatment resulted in a fivefold increase CAT activity. Anti-NGF serum slightly decreased the number and fiber density of AChE-positive neurons in these cultures. However, anti-NGF treatment did not decrease the activities of CAT and AChE as compared to untreated cultures.

Results indicate that, in mixed glial-neuronal cultures, NGF increases the activity of CAT and fiber growth of cholinergic neurons. The effects of NGF on cholinergic neurons therefore seem to be more pronounced in this culture system than those previously found when cholinergic cells were grown in absence of glial cells.

299.16 TROPHIC EFFECTS OF GLIAL CELLS AND ITS RELATION TO NERVE GROWTH FACTOR. T. Amano and M. Takata*. Department of Neuroscience, Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan.

Monoclonal antibodies to mouse nerve growth factor (NGF) recognized the antigens in astrocytes in the regenerating rat brain (T. Amano, Soc.Neurosci.abstr., 11, 934, 1985). Choline acetyltransferase (CAT) activities of the brain homogenates derived from injured rat brain did not change significantly after 7, 14 days of the stab wound of the brain. On the other hand, CAT activities were induced about two-fold in culture of embryonic neurons of the rat septum (H. Gnahn et al., Dev.Brain Res.,9, 45-52, 1983). To analyze further the mechanism of CAT induction by NGF, serum-free culture of G. Sato's method was introduced for culture of neuronal cells.

Brain extracts were prepared by centrifugation of 100,000 x g. 60 min after homogenization. Conditioned medium of astrocytic cells were made by culturing astrocytic cell (GFAP positive) derived from 7-day-old rat brain in serum-free culture for 7 days. In low cell number of culture (less than 1,000 cells/cm^2) neuronal cells (anti-neurofilament positive) did not survive in SFDF in the presence of insulin 10 µg/ml, transferrin 10 µg/ml, progesterone 20 nM, Putrescine 100 µM and selenium 30 nM, for 5 days culture. In the presence of brain extracts (100 - 10 µg/ml) neuronal cells survived more than 7 days. Conditioned medium of rat astrocytes were also effective for the survival of neurons. NGF in the range of 100 - 10 ng/ml of culture medium did not extend the survival of neurons. In higher cell number of cultures (> 106/cm^2), CAT activities increased two-fold in the presence of NGF. Brain extracts and astrocyte-conditioned medium also induced the CAT activities about two-fold. This increase of the enzyme activities was blocked about 50 % in the presence of polyclonal anti NGF.

These results suggest that for the functioning of cholinergic neurons in the central nervous system other factors such as glial elements may be necessary besides NGF.

299.17 Partial reversal of lesion induced deficits in cortical cholinergic markers by nerve growth factor. V. Haroutunian*, P.D. Kanof*, J. Segarra* and K.L. Davis*. (SPON:C.Sorenson). Bronx VA Medical Center and the Mount Sinai School of Medicine. 130 W. Kingsbridge Road, Bronx, N.Y. 10468.

Recent studies have shown that nerve growth factor (NGF), in addition to its effects on peripheral sympathetic and sensory neurons, may also exert an influence on the central nervous system where its affinity appears to be most pronounced for cholinergic neurons (Mobley,W.C. et al,Science,229,284,1985). This action of NGF is especially significant in light of the involvement of cholinergic projections in Alzheimer's disease (Appel,S.H., Ann.Neurol.,10,499,1981). The present study tested the hypothesis that NGF administration may reverse some of the effects of experimentally induced lesions of basal cholinergic neurons (nbM) in the rat.

Twenty-eight male rats (250-275 g) received ibotenic acid (4ug in 1ul) lesions of the nbM (bregma-0.5, ML+/-2.8, DV-8.0). A cannula, directed at either the right or the left lateral ventricle, was implanted at the time of lesion. Fourteen rats received sham operations. One half of the nbM lesioned rats received lateral ventricular injections of 5ug of NGF in 10 ul of saline on post-operative days 2, 4, 8 and 12. The remaining nbM lesioned rats received lateral ventricular injections of saline alone. Seven sham operated rats, 7 nbM lesioned saline injected rats and 7 nbM lesioned NGF injected rats were sacrificed 14 days post-operatively. The remaining 7 rats in each of the three groups were sacrificed 6 weeks post-operatively. Cortices from each rat were assayed for choline acetyltransferase (CAT) and acetylcholinesterase (ACHE) activity.

Lesions of the nbM led to a 32% depletion of cortical CAT activity relative to sham operated controls irrespective of post-lesion survival times. Cortical ACHE was depleted by 33% two weeks following the lesion and by 29% six weeks after the lesion. Administration of NGF had no effect on cortical CAT or ACHE activity at the two week post-lesion interval. There was however a dramatic NGF-induced reduction in cortical CAT and ACHE depletion at the 6 week post-lesion interval (Fs[CAT & ACHE at 6wks]>16.0, ps<.001). Cortical CAT and ACHE depletions were reduced by approximately 50% in rats which had received intraventricular injections of NGF. CAT and ACHE in the NGF injected group were significantly (ps<.01) depleted relative to sham operated rats, but these depletions were not as great as those observed in the non-NGF treated lesioned group.

299.18 NERVE GROWTH FACTOR ATTENUATES LESION-INDUCED DEGENERATION OF SEPTAL CHOLINERGIC NEURONS. C.N. Montero* and F. Hefti (SPON: W.A. Sheramata). Dept. of Neurology, University of Miami School of Medicine, Miami FL 33101.

Data accumulated in recent years affirm that nerve growth factor (NGF) acts as neurotrophic factor for cholinergic neurons of the basal mammalian forebrain. We have earlier found that NGF is able to prevent the lesion-induced degeneration of these neurons after lesions of the septo-hippocampal pathway. In these earlier studies cholinergic neurons were visualized using acetylcholinesterase histochemistry after DFP pretreatment. In the same experimental situation, we now visualized cholinergic neurons using choline acetyltransferase (CAT) immunocytochemistry and studied the expression of NGF receptors (NGFR) by these cells.

A hemi-transsection of the septo-hippocampal pathway was performed in female adult rats by cutting the fimbria. A cannula was implanted ipsilateral to the lesion through which 2.5S NGF (10ug twice weekly), or an equal ammount of a control protein was injected intraventricularly during four weeks. The animals were then perfused intracardially, the brains frozen and sections were taken for immunohistochemical visualization CAT and NGFR (using monoclonal antibody 192; Chandler et al., J. Biol. Chem. 260:3360,1985).

In control animals fimbrial transections resulted in a pronounced reduction of CAT-positive and NGFR-positive cells in the medial septal nucleus and the vertical limb of the diagonal band of Broca. Staining of adjacent sections revealed that the same population of neurons was stained for both, CAT and NGFR in the basal forebrain. In the NGF treated animals there was only a slight difference in the number of CAT-positive and NGFR-positive cells between lesioned and unlesioned sides in these areas. These findings confirm those of previous studies in which cholinergic neurons were identified with acetylcholinesterase histochemistry. A study of the time-course of lesion-induced changes revealed that the number of NGFR-positive cells was reduced starting 2-5 days after lesioning, reaching minimal levels after 2 weeks.

The findings indicate that fimbrial transections result in degeneration of septal cholinergic neurons as indicated by a loss of CAT and NGFR immunoreactivity. They furthermore confirm that chronic intraventricular injections of NGF are able to attenuate this lesion-induced degeneration.

299.19 INTRACEREBRAL INJECTIONS OF INSULIN AND NERVE GROWTH FACTOR (NGF) IN RATS WITH NIGROSTRIATAL LESIONS.
A.T. Salvatierra, F. Hefti, and W.J. Weiner. Department of Neurology, University of Miami School of Medicine, Miami FL 33101.

Recent findings have shown NGF to be a neurotrophic factor for forebrain cholinergic neurons. There are similarities between the amimo acid sequence of NGF and that of insulin and insulin-like growth factors. These proteins have also been found to have neurotrophic properties in culture. In addition, insulin receptors are widely distributed in the rat brain. We have earlier reported that NGF promotes the survival of septal cholinergic neurons after fimbrial transection. In this study, partial lesion of the nigrostriatal system was used as an animal model of Parkinson's disease. This type of lesion results in retrograde degeneration of dopaminergic neurons in the substantia nigra. We examined the effects of insulin and NGF on this lesion-induced degeneration.

Female Wistar rats with unilateral transections of the nigrostriatal system were implanted with chronic injection cannulae at the site of the lesion. Animals were given either: 1) bovine insulin (25ug); 2) mouse salivary gland 2.5S NGF (5ug); or 3) cytochrome c (5ug) which was used as a control protein. Insulin injections were given daily, while NGF and cytochrome c were given twice a week. Animals were treated for two weeks and then perfused intracardially. Frozen section (30 um) were stained with cresyl violet to verify lesion site and cannulae placement. Tyrosine hydroxylase (TH) histochemistry was employed on consecutive 45 um sections of the substantia nigra. The number of TH-positive cells in the substantia nigra was visually counted on the lesioned and intact sides.

Hemitransection of the nigrostriatal pathway in control animals resulted in an 80-90% loss of TH positive cells in the substantia nigra on the side ipsilateral to the lesion. Insulin treated animals showed a slightly lower loss of cells on the lesioned side as compared to lesioned control animals. NGF treated rats showed no significant prevention of cell loss on the lesioned side. These results suggest that chronic intracerebral injection of insulin or NGF at the site of the nigrostriatal lesion does not promote survival of TH positive cells in the substantia nigra. Results also support previous literature suggesting NGF as a neurotrophic factor specific for cholinergic neurons.

TROPHIC AGENTS II

300.1 **A VIP-RELATED PEPTIDE IN EMBRYONIC SKELETAL MUSCLE: A PUTATIVE TROPHIC FACTOR.**
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We have found that the developing myotome of chicks and rats contains a peptide that is antigenically related to, but distinct from, vasoactive intestinal peptide (VIP). Rabbit antisera directed towards the amino-terminal end of VIP reacts with myogenic cells whereas antisera directed to the carboxy- terminal end of VIP do not. Using a competitive radioimmunoassay, we have shown that the cross-reacting material in the myotome is soluble in physiological salt solution and that it elutes from a gel-filtration column as three peaks with molecular weights of approximately 20, 10 and 6 kD; no immuno-reactive material is detectable in the region where synthetic VIP (3.5 kD) elutes. The peptide(s) in the myotome therefore seem to be novel, but with some homology to the amino end of VIP. We have called it intra-muscle peptide (IMP).

IMP is first seen in chick myogenic cells at St. 14 (50-52 hours of incubation) and later in striated myotubes. The intensity of immunolabelling decreases with embryonic age so that it is barely visable at E12. This suggests that IMP may be present in primary myotubes but not in secondary myotubes which start to form at E12. IMP is sometimes seen in the distal end of peripheral nerves raising the possibility that it is retrogradely transported. IMP is also present in cultured myotubes and is secreted in large amounts into the culture medium.

These findings raise the possibility that IMP may function as a trophic factor during nerve-muscle development.

300.2 STRIATAL DERIVED NEURONOTROPHIC FACTOR: BIOLOGICAL CHARACTERIZATION AND PRODUCTION OF MONOCLONAL ANTIBODIES.
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We have previously reported that adult bovine caudate nuclei contains neuronotrophic factors (NTFs) capable of increasing survival and maturation of mesencephalic and striatal neurons in serum-free culture conditions (Dal Toso, R. et al., Neuroscience Meeting, abs. 279.14, p. 949, 1985). We now report that further purification procedures, involving molecular seive fractionation and cation exchange HPLC chromatography, indicates that the NTF activity is associated with a protein (14,400 molecular weight; pI<10) which is not NGF. Moreover this striatal derived NTF (SDNF) was utilized to immunize mice for the production of monoclonal antibodies. Ascitic fluids obtained from three hybridoma clones, showing the highest immunoreactivity for the purified SDNF, were utilized for the production of immunoglobulin fractions. When conjugated to a CNBr-activated Sepharose resine, two of these monoclonals were able to reduce, with respect to appropriate controls, the biological activity present in the crude acid precipitated dialyzed supernatant, obtained from bovine caudate nuclei.

300.3 THE EFFECTS OF BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) ON THE SURVIVAL OF RAT RETINAL NEURONS IN CULTURE.
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Brain-derived neurotrophic factor (BDNF) is a protein (Mr-12kd, pI-10) purified from the mammalian brain which supports the survival of peripheral sensory neurons. In the present study, we have investigated the effect of BDNF on the survival of retinal neurons cultured during various stages of development. At E17, BDNF supported the survival of a small subpopulation (7%) of retinal cells which could only be detected with the help of cell specific markers. Using both immunofluorescent labeling of Thy-1 and prior retrograde lebeling, these BDNF dependent cells were identified as retinal ganglon cells. About 80% of the original number of Thy-1 positive cells (as determined 3 hours after plating) could be supported by BDNF for at least 2 weeks in culture. No effects of BDNF were detected on either the total number of cells or the total number of tetanus toxin positive neurons in these cultures. Neither NGF (50 ng/ml) nor acidic or basic FGF (25 ng/ml) could substitute for the survival activity of BDNF (1 ng/ml) on Thy-1 positive neurons. In addition, unlike acidic or basic FGF, BDNF treatment did not affect the number of retinal cells labeled with H^3-Thymidine in culture. Postnatal ganglion cells labeled both with Thy-1 immunofluorescence and by prior retrograde labeling with HRP could be enriched approximately 80 fold on a metrizamide density gradient (Sarthy et al., 1983, J. Neurosci. 3, 2832-2544). These purified postnatal ganglion cells were also responsive to BDNF, however unlike labeled embryonic ganglion cells, their survival was only transiently increased with BDNF. We conclude therefore that BDNF, a CNS-derived neurotrophic protein, acts directly on a specific subpopulation of CNS neurons - the retinal ganglion cells.

300.4 BASIC AND ACIDIC FIBROBLAST GROWTH FACTORS INCREASE SURVIVAL OF, AND NEURITE OUTGROWTH FROM, HIPPOCAMPAL NEURONS IN VITRO.
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Dissociated hippocampal pyramidal neurons from 18 day fetal rats were grown in a modification of Bottenstein and Sato's serum-free medium (PNAS 76(79):514-9) at a density of 16,000 cells/cm^2. Under control conditions, neuronal survival at 24 hours was 60-95% depending on the substrate used, but nearly all neurons died by 7 days in vitro.

In the presence of basic fibroblast growth factor (bFGF), neuronal survival after 7 days in vitro on a plastic substrate was increased 4-fold to almost 60% of cells present at 1 day. With a more adherent substrate, nearly 100% of the neurons could be maintained for 7 days. The change in neuronal survival was not secondary to growth of the nonneuronal cell population, which remained at about 10% of the total cell population as judged by a variety of standard cell markers. Acidic FGF (aFGF) also increased neuronal survival, though less effectively than bFGF. Comparison of the number of neurons surviving 7 days in acidic versus basic FGF yielded a ratio of 78.6% ± 5.8% (SEM) for 17 determinations on different substrates. Additivity could not be demonstrated during the 7 day assay, but cultures maintained 2-4 weeks contained more surviving neurons in a combination of aFGF and bFGF than in either alone. Half-maximal survival occurred at 15-30 pg/ml bFGF (1pM). Titration of aFGF produced a curve with two plateaus of cell support, presumably reflecting subpopulations with differing requirements for aFGF. Half-maximal survival for one occurred at 0.30 ng/ml (20 pM), for the other at 1.25-2.75 ng/ml (150 pM).

Basic FGF bound to plastic or polyornithine-heparin (Phep) substrates significantly increased neurite extension at 24 hours in vitro. On Phep, bFGF decreased the proportion of neurons without neurites from 29% to 8%. Of neurons with processes, 2/3 were shorter than 30 µm in the control. With bFGF 88% had processes longer than 30 µm, and 10% had processes longer than 100 µm. The concentration of bFGF required for stimulation of neurite outgrowth was 8-fold higher than that required for survival. In contrast, aFGF had much less effect on neurite outgrowth: mean process length on Phep increased from 23.9 ± 1.5 µm in controls to 29.1 ± 2.5 µm in the presence of aFGF.

Both bFGF and aFGF have been purified from adult brain. Although they contain about 55% sequence homology, they appear to be distinct gene products (Esch, F. et al., Biochem. Biophys. Res. Comm. 133(85):554-562). Our observations suggest that both are capable of supporting survival of hippocampal neurons and that bFGF also strikingly enhances neurite extension. The differences between the effects of aFGF and bFGF suggest that they are probably two distinct neurotrophic factors, possibly with distinct receptors.

300.5 EFFECTS OF EPIDERMAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR ON POSTNATAL MAMMALIAN NEURONS IN CULTURE.
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Epidermal growth factor (EGF) and the basic form of fibroblast growth factor (bFGF) are potent mitogenic polypeptides which have been identified in mammalian brain tissue. We have previously demonstrated that both bFGF and EGF exert trophic effects on postnatal mammalian central neurons in culture. bFGF has been shown to affect cultures derived from the cerebral cortex of 1 day old rats while EGF has been shown to influence neurons from the subneocortical telencephalon. In the present study we have examined the possibility that bFGF and EGF affect different neuronal subpopulations. Neocortices were removed from 1 day old rats and highly purified dissociated neuronal cultures were prepared. Cells were first plated in the presence of 10% fetal calf serum + .5µM cytosine arabinoside and then shifted to a chemically defined medium in the presence or absence of bFGF, EGF, or both. Neurons treated with either factor exhibited prominent process outgrowth, while very few control neurons survived beyond 5 days. However bFGF-treated neurons were multipolar with a greater number of primary processes than EGF-treated neurons, which generally possessed 2-3 primary processes per cell. Incubation with bFGF and EGF together at optimal concentrations for each factor increased the total survival of neurons by approximately 60%. When cultures were first treated with EGF and then shifted to medium containing bFGF, the neurons assumed a multipolar morphology similar to those originally treated with bFGF. These data are consistent with the hypothesis that discrete populations of neurons exist which express receptors for EGF and bFGF. In addition, another population is apparently responsive to both factors.

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300.6 NEURONOTROPHIC FACTORS RELEASED BY C6 GLIOMA CELLS
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Glial cells have previously been shown to synthesize and release factors that promote survival of central and peripheral neurons (neuronotrophic factors, NTFs). We have investigated the release of NTFs by C6 glioma cells under different modes of conditioning. Protein content of medium conditioned by C6 cells (C6CM) was estimated to be about 1 - 3 µg/ml, corresponding to a protein secretion rate of about 0.5 pg per cell and day. Using the respective antisera we found that C6CM contained the neurite promoting factor laminin (about 15 ng/ml) and, in early passages, also S-100. The cytoskeletal protein GFAP, and nuclear proteins (histone H1 and HMG 14) could not be detected. This suggests that C6CM proteins result from release rather than cell death. NTF activity of C6CM was tested on embryonic chick sympathetic (SG), sensory (DRG), and ciliary ganglion (CG) neurons using a 24h in vitro survival assay with NGF or ciliary neuronotrophic factor (CNTF) as positive controls. For "normal" conditioning C6 cells were grown to confluence in medium containing 10% fetal calf serum (FCS), washed twice with FCS-free medium, and conditioned for 1 - 4 days with serum-free medium. NTF activity of C6CM addressing SG (embryonic day 8 = SG_8) and DRG_{10} neurons increased from day 1 to day 2 but decreased during the following 2 days of conditioning. Only little activity was found for DRG_8 on day 1 and no activity on days 2 - 4. In contrast, a linear increase in NTF activity for CG_8 was found from day 1 to day 4. These results indicate that C6CM may contain several NTFs, which seem to be regulated independently from each other. On separation of C6CM proteins by FPLC gel filtration three fractions with different NTF activities were obtained, two of which did not conform to any of the established NTFs in terms of the spectrum of target neurons beeing addressed. The third fraction resembled NGF.

Electrophoretic analysis of C6CM prepared as described above showed that about 70 - 80 % of the proteins present in C6CM were residual FCS proteins. Therefore we prepared C6CM with only minor FCS-contaminations by excessive washing of cells prior to conditioning with serum-free medium. C6CMs produced under these conditions totally lack NTF activities for any of the neurons tested. Also C6CM from cells grown in the presence of N1-supplement instead of FCS showed drastically reduced NTF activities. This indicates that FCS may contain factors that stimulate NTF-expression and -release by C6 cells. In extracts of C6 cells no NTF activity for any of the neurons tested could be detected.

Further studies concerning the regulation and characterization of the different NTFs in C6CM are in progress.

300.7 ASTROGLIAL AND FIBROBLAST GROWTH FACTORS PROMOTE IN VITRO SURVIVAL OF NEURONS FROM THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM.

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Survival and differentiation of embryonic and postnatal neurons may be regulated by neuronotrophic factors (NTFs; prototype NGF). There is increasing evidence to suggest that a variety of trophic agents with survival promoting properties may exist.

Astroglial growth factors (AGFs) 1 and 2 isolated from bovine brain have initially been characterized by their capacity to stimulate proliferation and differentiation of astroglial cells in vitro by their resemblance to the acidic and basic fibroblast growth factors (aFGF, bFGF) (Pettmann et al., FEBS Letters 189: 102, 1985). We report now that these mitogenic growth factors also support the in vitro survival of distinct populations of peripheral and central neurons. AGF 2 and bFGF promoted the survival of purified ciliary ganglion neurons dissociated from E8 chick embryos without addressing embryonic chick sympathetic and sensory neurons. Half-maximal survival was achieved with 1-5 ng/ml of AGF 2. Neuronal survival was maintained over 7 days at plateau values identical to those seen with ciliary neuronotrophic factor. Peripheral neurons showed no or very little response to AGF 1 and aFGF. These data point at dissimilarities between aFGF and bFGF irrespective of their significant structural homology (Esch et al., Proc. Natl. Acad. Sci. USA 82: 6507, 1985; Gimenez-Gallego et al., Science 230: 1385, 1985). Both aFGF and bFGF as well as the corresponding AGFs supported the survival of spinal cord neurons from E6 chick embryos in long-term culture. The surviving neurons also included motoneurons that had been retrogradely labeled with rhodamine isothiocyanate prior to dissociating the spinal cord. Two other mitogenic growth factors, EGF and PDGF, failed to enhance the in vitro survival of any neuron tested.

Work in progress aims at evaluating whether (i) motoneurons are preferentially supported by FGFs, (ii) other CNS neurons also respond to these factors, and (iii) choline acetyltransferase activity is affected.

These results reveal that two groups of mitogenic growth factors may affect neuronal cell survival in vitro. The localization of FGFs in neurons (Pettmann et al., Neurosci. Letters 1986, in press) further supports the notion that neurons may store and release NTFs (cf. Unsicker and Lietzke, Soc. Neurosci. Abstr. 11: 1083, 1985).

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300.8 TYPE I ASTROCYTES PROMOTE THE SURVIVAL OF NEURONS AND UNIQUELY INCREASE THE CELL HETEROGENEITY IN FETAL RAT HINDBRAIN CULTURES.

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A neurotrophic agent comparable to nerve growth factor (NGF) in peripheral nervous system development has yet to be recognized for the central nervous system (CNS). Most neurons of the CNS do not have peripheral processes and so local cells become important candidates for extrinsic support. Indeed, isolated CNS neurons do not grow well in culture. In preliminary experiments with mixed cell-type cultures, we found that neurons grew particularly well when in proximity to astroglia. Therefore we chose to explore whether astrocytes can provide unique diffusible neurotrophic agents by using cell cultures enriched in CNS neurons or astrocytes.

Neuronal cultures were prepared using day 17 embryonic rat hindbrain. Dissociated cells were plated at low density on a collagen gel in a serum-free defined medium as previously reported (Dobrenis, K. & Utermohlen, V., Fed. Proc. 42 (4):990,1983). Dispersed cells only gave rise to morphologically recognizable small and intermediate-sized neurons which bound tetanus toxin (TT^+) and did not contain glial fibrillary acidic protein ($GFAP^-$). Virtually pure rat cerebral cortex astrocyte cultures were prepared by a slight modification of the method of McCarthy and deVellis (J. Cell Biol. 85:890,1980). These cultures were composed of flat protoplasmic-like astrocytes ($GFAP^+$, TT^-). Defined medium was conditioned for 24 hrs. by exposure to astrocyte (ACM), rat fibroblast, rat skeletal muscle or L1210 cultures at equivalent nuclei/ml of medium ratios. Neuronal cultures were plated and maintained with the various conditioned media or different control media.

Cultures with ACM demonstrated statistically significantly greater long-term (1 wk. or more) survival or process-bearing neurons than control medium cultures. This effect was not reproduced by other cell-conditioned media or other appropriate controls. The results support the concept that astrocytes are a source of diffusible neurotrophic agents in the CNS. Certain other cells also consistently appeared only in ACM cultures. These cells included ones resembling oligodendrocytes and small, phase-bright, bi- or tripolar (straight processes not longer than 0.1 mm.) cells that appeared to arise from occasional aggregates seen in the cultures. There did not appear to be any dependant association between these cells and the identified neurons.

Dialysis and Amicon membrane filtration studies suggest that the majority of the ACM-derived activity is associated with a factor (or factors) of apparent molecular weight 20-30,000. The trophic effect is not reproduced by 2.5S β-NGF or blocked by anti-β-NGF serum. The data on characteristics and effects suggest that this may be a previously unidentified factor.

300.9 ISOLATED PLASMA MEMBRANES REGULATE NEUROTRANSMITTER EXPRESSION AND FACILITATE EFFECTS OF A SOLUBLE BRAIN CHOLINERGIC FACTOR.

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The choice of which neurotransmitter will be expressed by a neuron is influenced by a variety of environmental factors which may interact in regulating transmitter development. The present study was undertaken to characterize and begin to purify molecules that stimulate cholinergic and peptidergic development. Extracts of human, bovine, and rat brain were each found to contain a soluble factor which promoted expression of cholinergic (choline acetyl transferase-CAT) and peptidergic (substance P-SP) traits by cultured rat sympathetic neurons. The human brain factor (SBF), a 50 kDa protein, was purified 14000 fold by means of ammonium sulfate fractionation and heparin-Sepharose affinity chromatography. Treatment of sympathetic neurons with saturating levels of SBF (20 ug/ml) increased CAT activity more than 85 fold. Treatment also induced a small but significant level of SP (1 fg/neuron), whereas control cultures contained no measurable peptide. Total protein and neuron survival were unaffected by SBF treatment. The factor stimulated levels of CAT and SP in cultured striatal as well as symapthetic neurons in a dose dependent manner.

Levels of CAT and SP were also stimulated by exposure of cultured sympathetic neurons to purified plasma membranes derived either from cultured ganglion nonneuronal cells (principally Schwann cells) or from cultured sympathetic neurons. Schwann cell membranes were much more effective than neuronal membranes in stimulating SP, but were only slightly more effective in elevating CAT activity. Treatment with Schwann cell membranes (100ug/ml) elevated CAT activity more than 25 fold and SP more than 16 fold, whereas treatment with neuronal membranes (100ug/ml) increased CAT 12 fold but only tripled SP. Plasma membranes from rat cortex did not alter CAT activity suggesting specificity of the Schwann cell-neuron interaction. Finally, CAT activity after concurrent treatment with saturating levels of Schwann cell plasma membranes and SBF far exceeded the sum of the enzyme activities after the individual treatments, suggesting that membrane molecules and the soluble factor facilitated each other. Thus, cell-surface molecules, which have been shown previously to influence neuronal morphogenesis and neurite elongation, may also help determine the transmitter phenotype of the neuron. Moreover, cell-surface molecules may modulate the effects of diffusible differentiating factors. The mechanism mediating these interactions, and the effects of plasma membranes and SBF on other transmitter systems, are currently being investigated.

300.10 EFFECTS OF GANGLIOSIDES AND OTHER NEUROTROPHIC AGENTS ON HIPPOCAMPAL NEURONS IN CELL CULTURE.

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Several years ago we established in our laboratory a serum-free cell culture system of central neurons from the developing rat hippocampus (1). This system was used to investigate the trophic interaction between glial and neuronal cells (2), and the effects of certain neurotrophic agents such as pyruvate, laminin (3) and gangliosides on surival and neurite outgrowth of pyramidal neurons in culture.

Gangliosides are known to insert into the membranes upon exogenous addition to nerve cells in culture and to exhibit neurotrophic effects in neuroblastoma cells (4). Similar observations were reported for nerve cells of the PNS. In the present study we investigated this problem for neurons of the CNS using the hippocampal cell culture system.

The neurotrophic activity (NTF) which we described in conditioned medium from cerebral astrocytes (2) consists of a low-moleculare component (LMW) and a high-molecular component (HMW). LMW could be identified as pyruvate, while HMW seems to be a laminin-like protein produced by astrocytes (thesis, S.Beckh). In our bioassay the survival of hippocampal neurons in serum-free medium after 24 hrs. was poor in EM without pyruvate (10 %), better in DMEM with pyruvate (30%) and best in CDM (conditioned DMEM) with 70% cell survival. Depletion of pyruvate by LDH treatment in CDM decreased the survival rate substantially.

Gangliosides supported the survival up to 70% in all conditions in a dose-dependent manner. Higher ganglios. like GD1a and GT1b were superior to a ganglioside mixture, while GM1 had virtually no effect on the survival, as measured by trypanblue exclusion. In contrast to this survival enhancement effect, the addition of gangliosides to neurons in CDM at conc. 1 - 100 yg/ml caused inhibition of neurite outgrowth rather than stimulation. Similar results were obtained with SEM and time-lapse cinematography.

(1) W.Seifert et al.(1983) in: Neurobiol.of Hippocampus, (ed.Seifert, AcademicPr.)
(2) Müller,H.W., S.Beckh, W.Seifert (1984), Proc.Nat.Ac.Sci. 81, 1248
(3) S.Beckh, H.W.Müller, W.Seifert (1986) in: Glial-Neuronal Communic,Springer,inpress.

300.11 GANGLIOSIDE INTERACTIONS WITH THE DOPAMINERGIC SYSTEM IN RATS. H. A. Tilson, G. J. Harry, K. Nanry*, P. Hudson* and J. S. Hong. Laboratory of Behavioral and Neurological Toxicology, NIEHS, P.O. Box 12233, Research Triangle Park, NC and Biological Sciences Research Center, Univ. North Carolina, Chapel Hill, NC 2751.

Rats were given unilateral injections of 6-hydroxydopamine (6OHDA) into the substantia nigra (10 µg/0.5 µl). Some animals were dosed at the time of surgery and for 14 days after surgery with 30 mg/kg, s.c., of mixed gangliosides (GM), while others received vehicle. On the day after the last injection of GM, the rats were given 1 mg/kg apomorphine, s.c., and their motor activity measured for 60 min; 7 days later they were challenged with 0.5 mg/kg of apomorphine. Supersensitivity to apomorphine was attenuated in rats receiving GM; HPLC analysis of the neostriatum found that GM attenuated 6-OHDA-induced decreases in dopamine and DOPAC by approximately 50%. Another group of rats was implanted with chronic indwelling cannulas in the lateral cerebroventricles at the time that 6-OHDA was injected into the substantia nigra. Some rats received 25 or 50 µg of GM for 10 days after surgery, while others received 2 µl of buffered vehicle. Two weeks after surgery, the rats were challenged with 1 mg/kg of apomorphine and activity measured for 1 hr. Treatment with GM attenuated the supersensitivity to apomorphine resulting from injection of 6-OHDA. Striatal depletions of dopamine and DOPAC were also attenuated in GM-treated rats.

A separate experiment sought to determine the effects of GM1 on the the development of receptor supersensitivity produced by repeated exposure to a dopamine receptor antagonist. Rats were injected with 1 mg/kg of haloperidol, s.c., for 8 or 16 days; some rats were coadministered 20 mg/kg of GM1, s.c. Four days after the last dose, the rats were challenged with 1 mg/kg of apomorphine and activity counted for 60 min. Treatment with GM1 decreased the behavioral supersensitivity to apomorphine induced by repeated exposure to haloperidol.

These experiments suggest that treatment with GM can have a protective effect against 6-OHDA-induced depletion of dopamine if treatment with GM begins at the time of lesioning. These studies also support receptor binding data reported by other laboratories indicating that treatment with GM1 can affect processes responsible for up-regulation of dopamine receptors.

300.12 β-SUBUNIT OF CHOLERA TOXIN REVERSELY INHIBITS NGF-INDUCED REGENERATION IN PC12 CELLS. G. Ferrari*, M. Favaron* and A. Leon (SPON: M. Vitadello). Fidia Neurobiological Research Laboratories, Abano Terme, Italy.

Previous reports from our laboratory have described that the addition of monosialoganglioside (GM_1) to pheochromocytoma cells (PC12) increases the Nerve Growth Factor (NGF)-induced differentiation and regeneration (Ferrari, G. et al., Dev. Brain Res., 8:215, 1983). We have now extended our investigation by studying the possible involvement of endogenous gangliosides in NGF-induced neurite regeneration. For this purpose we added the β-subunit of cholera toxin, known to bind specifically to GM_1 on the cell surface, and evaluated both its binding and effects on neurite regeneration in primed PC12 cells. Binding studies indicated that half maximal specific binding of the choleragenoid to the cells occurred at approximately 50 ng/ml. For regeneration experiments NGF primed PC12 cells were subcultured into fresh culture dishes with medium containing NGF (50 ng/ml)with and without various concentrations of choleragenoid. With NGF alone most of the cells produced within 24 hours long neurites while in the presence of NGF together with the β-subunit (1 μg/ml) neurite regeneration was totally suppressed: the Id_{50} was approximately 30 ng/ml. Preincubation of the β-subunit with GM_1 failed to give the same response. In addition the effect of the β-subunit was reversible, i.e. wash-out after 1 day of the β-subunit followed by continued exposure to NGF resulted in the rapid appearance of long neurites within 24 hours, thereby indicating that priming had not been lost. Thus it can hypothesized that the presence of GM_1 molecules (both exogenously supplied or endogenously occurring) on the neuronal cell surface most probably play a prominent role in the regulation of NGF-induced neurite regeneration.

300.13 GLYCOSAMINOGLYCAN SYNTHESIS AND RELEASE BY EMBRYONIC SPINAL CORD NEURONS. K.E. Dow* and R.J. Riopelle (SPON: H.B. Dinsdale). Queen's University, Departments of Pediatrics and Medicine, Kingston, Ontario K7L 3N6

Glycosaminoglycans (GAGs) are important constituents of the extracellular matrix and have been implicated in neuron adhesion, process formation and axonal guidance.

Neuron-enriched dissociated cultures (neurofilament positive, galactocerebroside and glial fibrillary acidic protein negative) from 8-day chick embryo spinal cord were stained for the presence of GAGs using the Alcian blue - magnesium chloride ($MgCl_2$) method. Positive staining of neurons was seen at 0.1 M, 0.3 M, and 0.7 M $MgCl_2$. The absence of staining at 1.0 M $MgCl_2$ suggests the absence of keratan sulphate, the most strongly sulphated GAG, while staining at $MgCl_2$ concentrations up to 0.7 M suggests the presence of heparin or heparan sulphate - GAGs containing both carboxylated and sulphated groups. GAGs were extracted from $^{35}SO_4$ labeled spinal cord neurons and from neuron conditioned media after 7 days in culture by papain digestion followed by cetylpyridinium chloride precipitation. After separation by cellulose acetate electrophoresis, a single band co-migrating with a heparin standard was observed by radioautography in the GAG isolate of both neurons and neuron conditioned media.

Embryonic neurons have previously been shown to release substrate attached molecular species that promote neurite outgrowth (Riopelle and Cameron, Dev. Br. Res. 15, 1984). The present studies complement these observations by demonstrating that neurons synthesize and release to their conditioned media a GAG with electrophoretic mobility of heparin.

300.14 PROMOTION OF SURVIVAL AND NEURITE OUTGROWTH IN CULTURES OF NEWBORN RAT SYMPATHETIC NEURONS BY A cAMP ANALOG. R.E. Rydel and L.A. Greene, Department of Pharmacology, New York University School of Medicine, 550 First Avenue, New York, N.Y. 10016

We have reported that fibroblast growth factor (FGF), insulin, forskolin and various cyclic AMP (cAMP) analogs prevent the death of clonal PC12 pheochromocytoma cells in serum-free medium to a similar extent as that observed with nerve growth factor (NGF) (R.E. Rydel and L.A. Greene, Soc. Neurosci. Abst. 11:935, 1985). In the present study, rat sympathetic neurons have been analyzed for the ability of these agents to promote survival and neurite outgrowth.

Postnatal day 1 to 3 rat superior cervical sympathetic neurons were cultured on collagen-coated dishes in serum-containing medium (85% RPMI 1640, 5% fetal calf serum and 10% heat-inactivated horse serum) supplemented with one of the agents to be tested. Highly enriched neuronal cultures were obtained by treatment with 10 µM cytosine arabinoside for up to 1 week. Cultures were fed every other day and cell number per culture determined at various times up to 1 week. FGF, insulin, phorbol myristate acetate and elevated K^+ failed to maintain viability of sympathetic neurons when cultured in the presence or absence of nonneuronal cells. These agents neither enhanced nor diminished NGF-promoted cell survival or neurite outgrowth. Forskolin and dibutyryl cAMP had a moderate effect upon neuronal survival, with all surviving neurons extending neurites. In contrast, 8(4-chlorphenylthio)-cAMP (CPT-cAMP) maintained cell survival and neurite formation in cultured sympathetic neurons as effectively as NGF.

The effects of CPT-cAMP differed from those of NGF in that neurite formation was delayed 1 to 2 days and neuronal hypertrophy was significantly reduced or absent. However, the density of neuritic outgrowth appeared comparable with either treatment after 1 week. The effects of CPT-cAMP on survival and neurite formation were independent of the presence of nonneuronal cells. Removal of NGF from NGF-treated cultures along with the addition of polyclonal anti-NGF antiserum resulted in rapid loss of neuronal viability, while such treatment followed by the addition of CPT-cAMP afforded complete protection of cell viability and neurite integrity. This indicates that NGF and CPT-cAMP affect identical populations of sympathetic neurons. In addition, cultures treated with NGF and CPT-cAMP were not morphologically distinguishable from those treated with NGF alone. Our findings suggest that CPT-cAMP should prove useful for studying mechanisms involved in neuronal cell survival and neurite outgrowth. Supported by NIH grant NS16036 and NIH predoctoral training grant GM07827.

300.15 EFFECTS OF NEONATAL 6-HYDROXYDOPAMINE ON MORPHOLOGICAL ORGANIZATION IN THE POSTEROMEDIAL BARREL SUBFIELD OF MOUSE SOMATOSENSORY CORTEX. E. P. Loeb, F.-L. F. Chang, and W. T. Greenough. Depts. Psychol. and Anat. Sci., and Neur. & Behav. Biol. Prog., Univ. Illinois, Champaign, IL 61820.

While the possible roles of non-noradrenergic effects are uncertain, the consequences of neonatal 6-hydroxydopamine (6-OHDA) treatment upon development of physiological and behavioral characteristics of the visual system are profoundly disruptive (Kasamatsu et al., J. Neurophys. 45:254; Trombley et al., J. Neurosci. 6:266; Bear & Singer, Nature 320:172). Norepinephrine appears to play a modulatory role, enhancing the relative responsiveness of cells to both excitatory and inhibitory input (Yeh & Woodward, Dev. Brain Res. 11:207). If developmental effects of peripheral input are so modulated, pattern in an input-dependent structure should be less well defined after neonatal 6-OHDA treatment. We studied 160 Golgi-stained (methylene blue counterstained) spiny stellate neurons in the walls and hollows of cortical barrels of 6-OHDA lesioned mice. The 6-OHDA lesions produced at least 98% norepinephrine depletion. The mice were sacrificed on day 60.

We found that 6-OHDA had no discernable effects upon the gross parameters of barrels evident from measurements of Nissl stained cells but that it subtly disrupted the organization of dendrites in the barrel. Although gross measures of dendritic length and orientation did not differ between the two groups, neurons of the treated group reacted differently to the inner edge of the barrel wall. In 6-OHDA mice cells within the wall maintained a constant proportion of their dendritic trees there regardless of soma location, while in controls the closer these cells were to the hollow, the more dendrite they extended into it. In the hollows, control cells extended a constant small proportion of their dendritic trees into the wall regardless of soma position, while the proportion that treated cells extended into the wall decreased significantly with the distance to it. Neither group was more likely to grow dendrites out of the barrel (past the outer edge of the wall).

These results imply that the dendritic organization of 6-OHDA treated cells is not as strongly influenced by the boundary between the barrel hollow and the barrel wall as it is in control cells. Since the NE fibers terminate in the barrel hollows, it is possible that these fibers are involved in the specification of the hollow-wall boundary. Alternatively, since specific thalamic afferents also terminate in the hollows, it is possible that the noradrenergic input potentiates afferent activity effects on barrel neurons, such that barrel parameters are better defined by peripheral input. (Supported by ONR and NIMH)

300.16 TIME-DEPENDENT NEUROCHEMICAL AND NEUROBEHAVIORAL EFFECTS OF INTRA-HIPPOCAMPALLY ADMINISTERED COLCHICINE IN RATS. G.J. Harry, H.A. Tilson, L. Grimes*, B.C. Rogers*, and J.N. Peterson*. Laboratory of Behavioral and Neurological Toxicology, NIEHS, Research Triangle Park, NC 27709 and Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27512.

Previous work in this laboratory has concerned the effects of gangliosides on neurodegenerative effects produced by direct administration of colchicine into the hippocampus. This experiment investigated systematically the time-dependent changes occurring during the first 3 weeks after administration of colchicine. Rats were bilaterally infused with 2.5 µg of colchicine/site contained in a volume of 0.5 µl at dorsal and ventral sites within the hippocampus. Motor activity, acoustic startle response, latencies to respond on a hot-plate and body weights were measured weekly for 3 consecutive weeks. The rats were then sacrificed for neurochemical or histological evaluation. Dynorphin concentrations in the hippocampus were then measured using RIA. Some rats were perfused with formalin and hippocampal tissue stained with Luxol fast blue and cresyl violet.

Increased motor activity and reactivity to the tone were observed for up to 2 weeks after surgery, but the effect diminished by the third week. Colchicine also decreased the rate of body weight gain. Reactivity to the aversive thermal stimulus was not affected. The wet weight of the hippocampus was decreased by 20% in colchicine-treated rats; weights of the caudate or hypothalamus were not affected. Concentrations of dynorphin, which is believed to be preferentially located in the granule cells and mossy fibers, were decreased (45-62%) in colchicine-treated rats. Histopathological assessment of the hippocampus found that colchicine decreased granule cells by approximately 80% with minimal changes in pyramidal cells (10-15% decrease). These data suggest that colchicine-induced degeneration of this pathway is responsible for the observed behavioral effects.

In a separate experiment, rats were given 20 mg/kg of GM1, s.c., 24 hrs prior to receiving colchicine. Injections of GM1 or saline continued for 21 days, at which time the rats were assessed for hypermotility. In contrast to the previous study, colchicine-treated rats handled daily for injections did not show signs of increased motor activity. Under the conditions of this study, GM1 had no significant effect on behavioral alterations produced by colchicine.

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300.17 TPA AND RETINOIC ACID IN SERUM-FREE MEDIA INHIBIT ADHESION AND NEURITE OUTGROWTH IN A HUMAN NEUROBLASTOMA CELL LINE THAT SECRETES FIBRONECTIN

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Neurite outgrowth by the neuroblastoma cell line SH-SY5Y is dependent on substrate adhesion. Extracellular components are required to mediate attachment and motility. SH-SY5Y successfully adhere to culture surfaces and extend neurites in the presence of fetal bovine serum (FBS); NGF enhances the outgrowth response. The tumor promoter 12-O-tetradecanoyl phorbol-13-acetate (TPA) in the presence of FBS induces morphologic differentiation in these cells, as evidenced by the formation of long, straight neurites with broad growing tips. Retinoic acid also induces an outgrowth response in these adherent cells.

In serum-free growth medium (DMEM, 7ug/ml insulin, 7ug/ml transferrin, 100uM putrescine, 20nM progesterone, 45nM selenate) freshly trypsin-dissociated SH-SY5Y cells were able to adhere to tissue culture plates and, as they released protein into the media, these cells extended long neurites. Neurite outgrowth was enhanced by the addition of NGF (200ng/ml), which had similar effects whether added immediately or two days after cell adhesion had begun. In contrast, the addition of TPA (17nM) to the serum-free medium hindered attachment and subsequent neurite outgrowth. When the substrate was pretreated with serum, the negative effect of TPA on adhesion and outgrowth could be diminished. Pretreatment with fibronectin-stripped FBS was less effective in this regard, while readdition of purified fibronectin (50ug/ml) restored completely the properties of FBS pretreatment. Retinoic acid (1uM) also disrupted SH-SY5Y adhesion and outgrowth in serum-free medium; however cells first successfully adhered and demonstrated neurite outgrowth, but retracted and lost adhesion after 6 days. Again, the progressive loss of the ability to adhere was reversed by pretreating the substrate with fibronectin containing serum.

The addition of radioactive amino acids to media containing FBS or FBS stripped of fibronectin showed that SH-SY5Y cells synthesized and secreted a radiolabelled protein which was immunoprecipitated by anti-fibronectin IgG; it migrated on SDS-PAGE with an Mr=220-240 kD. De novo fibronectin was demonstrated in conditioned serum-free media and in cells by immunostaining electroblots with anti-fibronectin IgG. An ELISA was successful in quantifying fibronectin (100ng/ml sensitivity) present in the conditioned media. Also, the cellular localization of fibronectin associated with the neurite outgrowth of SH-SY5Y cells was demonstrated by immunocytochemistry. Presently, these techniques are being used to explore the role of secreted fibronectin in cellular and growth cone adhesion in SH-SY5Y cells treated with TPA and retinoic acid under serum-free conditions.

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300.18 BRAIN NEURON DEVELOPMENT IN A HORMONALLY DEFINED GLIAL-FREE ENVIRONMENT: EFFECTS OF TRANSFERRIN, INSULIN, INSULIN-LIKE GROWTH FACTOR-I AND THYROID HORMONE. Y. Aizenman and J. de Vellis. Lab. of Biomedical and Environmental Sciences, Mental Retard. Res. Center, Depts. of Anatomy and Psychiatry, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

We have developed a pure cortical neuronal culture free of glial cells, grown in a serum-free environment. The cultured cells immunostained positively with neurofilament antibody while they displayed virtually no glial cell characteristics, such as glial fibrillary acidic protein, glycerol phosphate dehydrogenase or glutamine synthetase. Insulin and transferrin were necessary and sufficient for neuronal survival, neurite extension and glutamic acid decarboxylase (GAD) expression. Insulin-like growth factor-I (IGF) was able to replace insulin and was active close to its physiological levels, suggesting it might be the in vivo factor influencing neuronal growth in the brain. The dynamics of the developmental process were striking. The neurons moved on the poly-D-lysine covered plastic dish, and rearrangements in contacts between cells were observed. At first the neurons underwent a general cellular growth manifested by a large increase in the culture total protein content and by the initiation of neurites. A more specific differentiation, as indicated by the sharp increase in GAD levels which was concurrent with an increase in interneuronal contacts, lagged behind the initial growth. Thyroid hormone (TH), unlike insulin and transferrin, affected this differentiation process without affecting neuronal survival. TH caused a further increase in GAD levels, and its effect also started at the onset of contact formation. This neuronal culture, glial and serum-free, provides a new model system for investigating neuronal development and function in the mammalian central nervous system. (This work was supported by DOE and NICHD).

300.19 ESTABLISHMENT OF LONG-TERM CULTURES OF EMBRYONIC AND POST-NATAL CENTRAL NERVOUS SYSTEM NEURONS. Stephen Rapp*, Wayne Engleman* and Dikla Roufa (SPON:P.Manning). Searle R&D St. Louis, MO 63198.

Studies on cellular interactions and the effect of environmental factors on the developing and regenerating CNS are greatly facilitated by the availability of tissue culture systems that contain neuronal cells alone or in combination with glial cells. We report here the tissue culture methods that enabled us to grow cultures of septal area, hippocampal and cortical neurons obtained from embryonic (E15-E20) or post-natal (P2-P5) rat brain and maintained in culture as "pure" neuronal or "mixed" neuronal-glial cultures. To study the effect of various exogenous agents on these cells, we routinely maintain these cultures for 3-5 weeks and in some cases up to 8-10 weeks in vitro. All brain tissues were dissected from Sprague Dawley rats, cleaned of connective tissue, minced into small pieces and dissociated into a single cell suspension using a 30min incubation at 37°C in 0.25% trypsin/2% DNAase solution followed by gentle trituration with a pasteur pipet. The cells are plated in poly-lysine coated 24-well or 96-well microtiter plates. Optimal cell density for survival had to be established for each tissue type from embryonic and post-natal animals. Plating densities of 25,000-150,000 cells/well in 96-well microtiter plates were used depending on the tissue type, age of the animal from which it was obtained and the culture medium used. We found Leibovitz's L-15 based medium to be best suited for long-term survival of embryonic septal area neurons while Eagle's minimum essential medium (MEM) based medium was better for post-natal septal, and hippocampal and cortical neurons. The culture medium contained in addition to MEM, 10% human serum or fetal calf serum, 0.6% glucose and 1% L-glutamine (higher concentration of L-glu will selectively kill hippocampal neurons). All three tissue types, obtained from embryonic and post-natal animals, can be maintained in the chemically defined medium N2 (Bottenstein and Sato, PNAS, 76:514, 1979). In order to selectively eliminate all dividing non-neuronal cells, 10-50μM uridine/fluorodeoxyuridine was added to the culture medium either at the time of plating (post-natal) or after 24hr in vitro (embryonic). Better than 90% and in some cases all astrocytes were eliminated as confirmed by GFAP immunocytochemistry. We obtained optimal long-term survival when the culture medium was changed 24hr after plating and was followed by no further medium changes. In cultures where non-neuronal cell proliferation was not controlled, most neuronal cells were eliminated within 2-3 weeks leaving a carpet of glial cells. The above protocols present considerable improvement in the ability to maintain embryonic and post-natal CNS neurons in vitro for extended periods of time. Immunocytochemical characterization of these cultures is described in a companion abstract by Roufa et al.

TROPHIC INTERACTIONS II

301.1 THE QUANTITATIVE ASSESSMENT OF NERVE FIBER DISTRIBUTION IN MUSCLES DURING THE NORMAL CELL DEATH PERIOD AND FOLLOWING PREVENTION OF NEURONAL CELL DEATH. L.M. Dahm and L.T. Landmesser. Dept. of Physiology and Neurobiology, Univ. of CT, Storrs, CT 06268.

Naturally occurring cell death has been proposed as a mechanism to match the size of a neuronal population with its target. To survive, neurons are thought to require uptake of a target-supplied trophic factor. In chick, quail, and chick-quail chimeras, the number of lumbosacral (LS) motoneurons surviving the cell death period is tightly correlated with the number of myotube clusters at its onset, suggesting that some aspect of the target, such as the number of potential synaptic sites, may regulate survival. Neuromuscular blockade has also been shown to prevent motoneuron cell death. However, neither during normal development nor during treatments that prevent cell death has the actual relationship of nerve fibers to target myotubes been assessed during the actual cell death period (st 30-36 for chick LS motoneurons). Since this information is essential for formulating hypotheses regarding the mechanism of the proposed trophic interaction, we have assessed the temporal pattern of axonal invasion and ramification within two chick hindlimb muscles, the predominately slow iliofibularis (IF) and the fast posterior iliotibialis (PIT). The distribution of nerve fibers within these muscles between st 30-34 was quantitatively assessed from serial frozen sections stained with a monoclonal antibody (MAB) to nerve fibers while myotubes were visualized with either MAB to NCAM or myosin ATPase staining.

Although nerve trunks are adjacent to the presumptive muscles by st 26, muscle nerves only form at st 28 co-incident with the appearance of mature myotube clusters. Between st 30-32 axons rapidly invade the developing muscles to form highly fasciculated intramuscular nerve branches distributed in an organized array throughout the muscles. By st 32 there is also an increase in the number of ramifying single axonal profiles, bringing them into potential synaptic contact with most of the available target. Throughout the cell death period, the innervation density of the fast PIT was half that of the slow IF.

To determine if treatments which prevent cell death alter the density and/or pattern of innervation, embryos were treated with aBTX from st 26-33 and analyzed as described. We observed a large increase in the density of innervation (about twofold). In addition, the nerve branch profiles were much more irregularly distributed and loosely fasciculated. The altered appearance of these profiles suggests that aBTX treatment may rescue cells via a mechanism different from that which normally regulates cell death. It will now be important to determine the extent to which these neuronal profiles actually make synaptic contacts with myotubes, both during normal development and following aBTX treatment.
Supported by NIH grant NS 19640.

301.2 GEOMETRICAL CHANGES OF MOUSE SYMPATHETIC GANGLION CELLS AFTER AXOTOMY. H. YAWO* (SPON: R.D. HADLEY). Dept. of Anat. and Neurobiol., Washington Univ. Sch. Med., St. Louis, MO. 63110.

The relationship with peripheral target tissue is known to be important for the maintenance of preganglionic synaptic contacts on mammalian sympathetic ganglion cells (D. Purves, J. Physiol. 252:429, 1975). Here I report studies of the influence of peripheral connectivity on dendritic geometry in the mouse superior cervical ganglion (SCG).

Adult mice (25 - 30 g) were anesthetized and one of the postganglionic branches of the left SCG (the external carotid) was crushed 0.5 - 1.0 mm from the ganglion. One to 4 weeks after recovery from this operation the animals were reanesthetized by an overdose of pentobarbital and one or both SCGs were excised. The SCGs were placed in a Plexiglas chamber and superfused with oxygenated mammalian Ringer solution. Ganglion cells were impaled at random with a pipette containing horseradish peroxidase (HRP; R = 120 - 160 Mohm); the neurons were then labeled by iontophoresis (D. Purves & R. I. Hume, J. Neurosci. 1:441, 1981). The identity of axotomized neurons was determined by the presence of labeled axons extending into the crushed branch; some axons, however, terminated in the ganglion with varicosities or growth cone-like structures. These neurons were also counted as having been axotomized. The neurons in the intact right SCG were used as controls.

The total average length of dendritic arbors measured from camera lucida drawings of 58 control neurons was 850.7 ± 37.6 μm (mean ± S.E.M.). This value is similar to that measured in nonoperated animals (D. Purves & J. W. Lichtman, Science 228:298, 1985). After 7 days, some axotomized neurons showed marked shrinkage of dendrites; thus about 30% of the cells had a total dendritic length of less than 400 μm. The corresponding fraction was only 3% in the controls. By 14 days after nerve crush, the dendrites of most affected cells had retracted to at least some degree; these neurons had shorter primary dendrites, fewer terminal branches, and smaller overall dendritic areas. The total dendritic length of axotomized neurons 14 days after nerve crush was reduced to 478.6 ± 51.4 μm (mean ± S.E.M.; N = 41). The dendrites showed a tendency to recover their original lengths by one month, about the time peripheral reconnection might be expected; whether recovery is eventually complete is under investigation.

These results indicate that the dendritic morphology of SCG neurons is dependent on the normal axonal extention to postganglionic targets. A possible explanation of this dependence is that trophic substances from the target tissues are necessary for the maintenence of the ganglion cell dendrites.
Supported by NIH Grants NS11699 and NS18629 to D. Purves.

301.3 THE EFFECT OF VESTIBULAR NERVE DEGENERATION AND REGENERATION ON THE MORPHOLOGY OF THE MAUTHNER CELL. L.A. Goodman and P.G. Model. Dept. Neurosci., Albert Einstein Coll. of Med., Bronx, NY 10461

Afferent innervation affects the properties of target neurons: for example, during development, ingrowing axons are important in regulating the elaboration of normal dendritic arborizations by postsynaptic neurons (Goodman and Model, Soc. Neurosci. Abs., 11:946, 1985). Here we report on the long-term effects that neurons have on the cells that they innervate. We have examined whether afferent contacts are required for the maintenance of the normal morphology of a single target neuron, the Mauthner cell (M-cell). In the medulla of the axolotl (Ambystoma mexicanum), M-cells occur as a pair of large, uniquely identifiable neurons at the level of entry of the vestibular nerve (nVIII). Each M-cell receives synapses from the ipsilateral nVIII. These terminals can be identified morphologically as club endings and are restricted to an extensively branched region of the M-cell lateral dendrite.

We looked at the response of the M-cell to deafferentation in the presence or absence of regenerated afferent contacts. Following the unilateral severence of nVIII in ten young axolotl larvae, nVIII was allowed to regenerate in five. In the remaining larvae, nVIII ganglion cells were removed to prevent nerve regeneration. The contralateral sides served as controls. After three months survival, the animals were prepared for microscopy. Four of the five animals with intact nVIII ganglia had successfully regenerated nerves. The fifth animal in that group and the five animals with ablated nVIII ganglia had a small number of regenerated axons. Control and experimental M-cells were reconstructed and compared. Three M-cells deprived of nVIII afferents showed a substantial loss of dendritic branches in the region that normally receives nVIII connections. Two other deprived M-cells showed a moderate, but significant loss of branches and one only a slight loss. The extent of dendritic branching in all four of the M-cells that received regenerated vestibular nerves approximated that of the contralateral control cell. An unexpected finding was that the distribution of dendritic branches on many experimental M-cells was broader than that on control cells. While branches were restricted to the nVIII tract in the control sides, they extended far into adjacent nerve tracts in the experimental sides. We are examining the displaced dendritic branches to determine whether they receive vestibular synapses.

Deafferentation of the M-cell results in a reduction of dendritic branches in the region deprived of vestibular contacts. Restoration of the branches occurs when nVIII terminals re-establish contact on the M-cell. These data indicate that afferent innervation is necessary for the maintenance of dendritic branching patterns in postsynaptic neurons.

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301.4 THE EXTENT OF SUPERINNERVATION PRODUCING TRANSSYNAPTIC STIMULATING EFFECTS ON MAUTHNER CELL MORPHOGENESIS. P.G.Model and L.A.Goodman. Dept. Neurosci., Albert Einstein Coll. Med., Bronx, NY 10461

The formation of afferent contacts is important in regulating the morphogenesis of developing target neurons (Goodman and Model, Soc. Neurosci. Abs., 11:946, 1985). We showed that in the axolotl (Ambystoma mexicanum), superinnervation of a uniquely identified target neuron, the Mauthner cell (M-cell), produces an exuberant postsynaptic dendritic branching pattern as compared to the control. M-cells occur as a pair of large interneurons in the medulla of the axolotl. A restricted portion of the M-cell lateral dendrite receives synapses from the ipsilateral vestibular nerve. M-cells were superinnervated in embryos that had developed with an extra vestibular primordium implanted rostral to the in situ one. In several larvae, the ectopic nerve was identified by labeling it with horseradish peroxidase (HRP). Subsequent EM examination revealed that labeled axons made synaptic contact on the appropriate region of the M-cell. The resulting increase in dendritic growth was restricted to this region. We have now quantified the degree of superinnervation by counting vestibular synapses on experimental and control M-cells.

M-cells were reconstructed from micrographs of serial LM sections. Each thick section was re-embedded and sectioned for EM. We examined a representative thin section from each thick section to determine the number of vestibular synapses. Club terminals were easily identified because they are very large (3-12u in diameter), they make morphologically mixed (chemical and electrical) synaptic contacts, they have mitochondria grouped near the contacts, and they contain densely packed and highly organized neurofilaments. Experimental and contralateral control sides of four animals were evaluated and the following results obtained:

	NUMBER OF VESTIBULAR SYNAPSES		
ANIMAL	CONTROL	EXPERIMENTAL	% INCREASE
1	124	165	33
2	127	212	67
3	115	150	30
4	115	163	42
$\bar{x}$:	120	173	43

The mean number of vestibular synapses on experimental M-cells was significantly greater than that on control cells ($p<0.02$, paired-sample t-test). The ectopic nerve in animal 4 was labeled with HRP. The superinnervated M-cell in this animal had 48 vestibular synapses in excess of the control; 37 of these were labeled. Thus, our technique labeled most of the axons in the ectopic nerve.

We are currently attempting to correlate the extent of superinnervation with that of dendritic growth as a measure of the transsynaptic stimulation imposed by afferent contacts on postsynaptic neurons. (Supported by NIH grants 5T32GM7288 and NS-18823)

301.5 PRE- AND POSTSYNAPTIC ALTERATIONS AT OUTPUT SYNAPSES SPARED BY AXOTOMY. M.Titmus and D.S. Faber. Dept. Physiology, Div. Neurobiology; SUNY, Buffalo, NY 14214.

We previously reported indirect evidence suggesting that axotomy of the goldfish Mauthner (M-) cell produces a long term destabilization of its output synapses 4mm or more proximal to the lesion site (Titmus & Faber, Neurosci. Abst. 9:603, 1983). This suggestion has been confirmed with simultaneous pre- and postsynaptic intracellular recordings. The results indicate there is both impaired transmitter release and altered postsynaptic responsiveness at these spared contacts.

Recordings were made from single M-axons and identified cranial relay neurons (CRNs) postsynaptic to tham. Data are presented for 5 control and 5 experimental fish. Both spontaneous miniature excitatory postsynaptic potentials (mEPSPs) and evoked EPSPs were recorded and analyzed. Following data collection, both cells were injected with HRP to confirm that contacts between the M-axons and the CRNs were still intact, at the light microscopic level.

In controls, the mean amplitude, peak time (20-80%) and half decay times of the mEPSPs were, respectively, 323±145uV (range: 207-570), 185±62us (range: 141-203) and 647±190us (range: 407-872). The normal evoked EPSPs ranged from 7.5 to 31mV and always triggered spikes in CRNs with stimulation frequencies ≤ 0.5Hz. In contrast, when axotomized M-axons were stimulated at similar rates, mean EPSP amplitude averaged 0.45±0.25mV. This difference is not due to a change in the mEPSP amplitudes, which averaged 277±33uV (range: 241-326) in the axotomized preparations. Thus, quantal size was unchanged following axotomy, and quantal content (EPSP/mEPSP) was reduced from 35.8±24.3 (range: 13-69) to 1.64±1.0 (range: 0.77-4.0).

In controls the peak and half decay times of subthreshold EPSPs evoked at stimulation rates from 1-4Hz, averaged 163±54us and 602±205us, respectively--values comparable to those obtained for the mEPSPs. Following axotomy, both parameters of the evoked responses were significantly increased (mean peak time: 339±80usec, mean decay time: 1.29 ±0.4ms). This effect might be due to a greater temporal dispersion of release at these synapses, given that quantal content is reduced. However, while there was no change in the peak time of the mEPSPs (mean: 196±19us) following axotomy, the decay times of these quantal events were increased (879±190us). This result suggests that in addition to impaired release at the spared synapses, long-term axotomy is also associated with either a change in the structural organization of the M-axon's output synapses or with alteration in postsynaptic receptor properties.

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301.6 MECHANISMS OF POSTSYNAPTIC PLASTICITY: MODIFICATION OF JUNCTIONAL ACETYLCHOLINE RECEPTOR CLUSTERS INDUCED BY MOTOR NERVE TERMINAL OUTGROWTH. W-C. Yee* and A. Pestronk, Neuromuscular Division, Johns Hopkins Sch. of Med., Baltimore, MD 21205

Motor nerve terminal outgrowth (NTO) at neuromuscular junctions (NMJs) occurs rapidly in response to denervation changes in muscle. Using a combined cholinesterase-silver stain, NTO is manifest as an elongation of the NMJ (cholinesterase-staining area) with an increase in nerve terminal branching. We have now examined the NTO-induced changes in the stable cluster of junctional acetylcholine receptors (J-AChRs) and the mechanisms underlying these changes.

NTO was evoked using botulinum toxin to block neuromuscular transmission and cause denervation changes in the soleus muscles of adult rats. J-AChRs were visualized using an α-bungarotoxin (α-BuTx) peroxidase anti-peroxidase (PAP) technique. In a previous study, we showed that J-AChR clusters were significantly elongated after a 7 day period of NTO. The number of AChRs per NMJ, measured using ^{125}I-α-BuTx, increased by 62% in muscles with elongated NMJs. No elongation of the J-AChR cluster was seen after surgical denervation, suggesting that NTO itself, and not denervation changes in muscle, is responsible for J-AChR changes.

In the present study, we examined whether NTO-induced elongation of J-AChR clusters occured by 1) redistribution of pre-existing J-AChRs, or 2) insertion of new J-AChRs, derived from either newly synthesized AChRs or those migrating from extra-junctional regions of muscle. To study pre-existing J-AChRs, they were labeled with α-BuTx before NTO. After a 7 day period of NTO the remaining prelabeled J-AChRs were visualized using an α-BuTx PAP technique. We found that the distribution of the prelabeled J-AChRs became significantly elongated - mirroring the changes in the whole population of J-AChRs and in cholinesterase. The contribution of new J-AChRs to NMJ elongation was then examined by a) injecting α-BuTx to block pre-existing AChRs, and b) after allowing time for insertion into the membrane of new AChRs, labeling them with ^{125}I-α-BuTx. We found large accumulations of new J-AChRs at NMJs. Autoradiographs showed that these J-AChRs were diffusely distributed over elongated NMJs and not selectively inserted at the ends as might be expected if extrajunctional AChRs migrated to the NMJ. Thus, most new AChRs at elongated NMJs seem to be derived from newly synthesized AChRs inserted over the new length of NMJ membrane.

Our results show that NTO induces rapid modification of the otherwise stable J-AChR cluster, causing elongation of the cluster length with an increase in the number of J-AChRs. These changes are brought about mainly by redistribution of pre-existing AChRs and insertion of newly synthesized AChRs.

301.7 INCREASED AChE mRNA IS CORRELATED WITH INCREASED AChE ACTIVITY IN FIBRILLATING CULTURED EMBRYONIC RAT MYOTUBES L.H. Younkin, S.K. Brockman*, M.F. Usiak*, C.F. McTiernan*, and S.G. Younkin, Depts. of Pharmacology and Pathology, Case Western Reserve University, Cleveland, Ohio 44106

When cultured embryonic rat myotubes are treated with tetrodotoxin (TTX) on day 4, the increase in acetylcholinesterase (AChE) activity that normally begins with spontaneous fibrillation on day 4-5 is prevented. We have previously shown (J. Neurosci. 4:131-140, 1984) that this increase in AChE activity with fibrillation is the result of increased synthesis of AChE. We have now extracted mRNA from parallel dishes of 8-day cultured myotubes grown with or without TTX. RNA was extracted with guanidinium thiocyanate and guanidine hydrocholoride washes (Chirgwin, et al, Biochem.,18:5294-5299, 1979); poly(A)+ mRNA was purified on an oligo dT cellulose column. Fibrillating and non-fibrillating cultures were processed as pairs; the poly(A)+ mRNA of each was taken up to identical final concentrations. There was no significant difference in the amount of mRNA per dish of fibrillating (27.4 + 3.3 ug/dish) and non-fibrillating (25.4 ug/dish) myotubes. The rare AChE mRNA was assayed by translation in the Xenopus oocyte. The same concentration of mRNA (2 mg/ml in 10 mM HEPES) from fibrillating and non-fibrillating myotubes was injected in 50 nl into each oocyte; 50 nl of 10 mM HEPES was injected as control. 20 oocytes were injected with each sample; they were incubated at 20 ° C for about 22 hours in 0.3 ml Barth's medium; then the medium was removed and the oocytes homogenized in 0.3 ml 0.1 M PO4 buffer plus 1% Triton X-100. After addition of p-chloromercuriphenylsulphonic acid (pCMPSA) to a final concentration of 1 mM, tripliates were assayed with 3H acetylcholine. The AChE produced in oocytes as a result of mRNA injection is calculated by first subtracting non-enzymatic background from all values, then subtracting the HEPES-injected values from the mRNA-injected values. Six pairs of cultures were assayed. There is a significant difference in the AChE activity/dish between fibrillating (5332 + 688 pm ACh hydrolyzed/min-1 dish-1) and non-fibrillating (1861 + 224) cultures; and, there is a significant difference in the AChE activity produced in oocytes as a result of microinjection of the mRNA extracted from fibrillating (0.46 + 0.09 pm ACh hydrolyzed/min-1 oocyte-1) and non-fibrillating (0.10 + 0.05) myotube cultures. Thus, there is increased AChE mRNA in fibrillating cultures that have increased AChE activity.

301.8 **EVIDENCE THAT CGRP IS A MOTONEURON-DERIVED MUSCLE TROPHIC FACTOR.** H V NEW* and A W Mudge* (SPON J G Jefferys) MRC Developmental Neurobiology Programme, Department of Zoology, University College London, Gower Street, LONDON WC1E 6BT

During muscle development it is thought that motoneurons produce trophic factors that are involved in the formation of clustered acetylcholine receptors (AChR) at the neuromuscular junction, and that are required, together with muscle activity, for division of secondary myoblasts.

By immunohistochemistry we have found calcitonin gene-related peptide (CGRP) in a subpopulation of chick motoneurons from embryonic day 6 (E6) onwards. CGRP is also present in some nerve terminals at neuromuscular junctions identified by acetycholinesterase staining. When added for 24 hours to E11 chick myotubes in vitro, 10^{-8}M CGRP causes an increase in the number of AChRs as measured by ^{125}I-αBTx binding. This increase was found to be due to an increased rate of incorporation of new receptors rather than stabilisation of pre-existing ones. An increase in AChR number is apparent 8 hours after CGRP addition; this, together with the fact that there was no increase in ^{3}H-thymidine incorporation in parallel cultures, indicates that CGRP affects AChR number in the absence of any effect on mitosis.

Under certain conditions, however, we also find that CGRP acts as a mitogen. In these experiments, muscles were grown in a defined medium to eliminate the effects of mitogens present in serum and embryo extract. There was no effect of CGRP on mitotic rate when added in defined medium alone. However, when CGRP was added together with the calcium ionophore A23187, there was a marked increase in mitosis compared to the effect of A23187 alone. Since the division of secondary myoblasts is thought to depend on a combination of muscle activity and nerve-derived trophic factor, the ionophore may be substituting for the effects of activity in these experiments.

Since CGRP is present in motoneurons early in development and has demonstrable effects on muscle cell development in vitro, it seems likely that it functions as a motorneuron-derived muscle trophic factor in vivo.

301.9 **DEVELOPMENTAL AND NEUROTROPHIC REGULATION OF MUSCLE GLUCOCORTICOID RECEPTORS.** C.L. Weill. Depts. of Neurology and Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

This study characterizes glucocorticoid binding sites in embryonic skeletal muscle myoblasts, in myotubes at fusion and after 3 and 6 days in culture, and in myotubes treated with conditioned medium from cultured spinal cord neurons (SC-CM).

Myoblasts were obtained from 12 day chick embryo leg muscle. Cultures were prepared by plating 8.5-15 x 10^{6} cells on collagen coated 100mm culture dishes in Earle's Minimum Essential Medium containing 10% heat inactivated horse serum, 5% chick embryo extract, and antibiotics. Cultures were fed 24 hr after plating and every 2 days thereafter. When fusion was > 90% complete, cultures were treated with 10uM araC for 48 hr.

Binding sites were assayed using (^{3}H)dexamethasone and cytosols prepared from freshly dissociated myoblasts or from freshly harvested cultures. Binding was linear with respect to protein and saturable with respect to time. Standard assay conditions were 0.05-2.0 mg protein per assay point and 20 hr at 4°C.

Saturable binding was observed for all tissues. Binding constants determined by Scatchard analysis were: myoblasts, K_D=2.6 +/- 0.3 nM (n=5) and B_{max}=230.3 +/- 38.1 femtomoles/mg, myotubes at fusion, 5.6 +/- 0.8 nM and 147.9 +/- 5.5 fm/mg (n=3), 3 day myotubes, 4.6 +/- 1.8 nM and 48.2 +/- 1.9 fm/mg (n=3), 6 day myotubes, 3.8 +/- 0.4 nM and 40.1 +/- 12.4 fm/mg (n=3).

The specificity of ligand binding was determined by competition assay. The order of inhibitory potency was; myoblasts, Dex>Cort>R5020>R1881>Ald>6αMHP>βE>Pro>DHT, myotubes at fusion, Dex>R1881>R5020>Cort>βE>Ald>6αMHP=Pro>DHT, 3 day myotubes, Dex>Cort>R1881>R5020>Ald>βE>6αMHP=Pro>DHT, 6 day myotubes, Dex>Cort>R5020>R1881>Ald>βE>6αMHP=Pro>DHT.

Sedimentation analysis in linear 5-20% sucrose gradients demonstrated the presence of a single peak of bound (^{3}H)Dex in the 7S region of the gradient. Incubation and sedimentation in the presence of 0.4M KCl resulted in the anticipated shift in S value to 3S.

Cultures treated for 6 days with 50% SC-CM displayed binding constants of 1.7 nM and 54.7 fm/mg. The order of ligand inhibitory potency was Dex>Cort>R5020>R1881>βE>Pro>6αMHP>DHT.

These data demonstrate that glucocorticoid receptors are present in myoblasts and in newly fused and young myotubes. Upon fusion there is a 36% decrease in the number of binding sites and a 2-fold decrease in their affinity. Maturation of myotubes in culture for 6 days results in a further decrease in the number of sites to 27% of those observed in newly fused myotubes with a concomitant 1.5-fold increase in binding affinity. Treatment of muscle cultures with SC-CM results in a comparable number of binding sites of 2-fold greater affinity. Supported by NIH grant NS18642.

301.10 NEURAL CONTROL OF GLUTAMINE SYNTHETASE ACTIVITY IN RAT SKELETAL MUSCLE. M. Konagaya*, Y. Konagaya* and S.R. Max. Dept. of Neurology, University of Maryland School of Medicine, Baltimore, MD 21201.

Skeletal muscle produces and releases glutamine, which may serve as a substrate for energy metabolism of a number of tissues, including intestine and brain. The production of amino acids is enhanced in situations of energy deficiency, such as fasting and during muscle wasting. Most of the glutamine is synthesized de novo, and glutamine synthetase, the enzyme responsible for its synthesis, plays an important role in this process. Therefore, we studied glutamine synthetase in rat skeletal muscle following either denervation or limb immobilization as a step toward understanding the role of glutamine synthetase in muscle atrophy. Adult male rats were subjected to mid-thigh section of the sciatic nerve. At 1, 2 and 5 hours and 1, 2 and 7 days after denervation, rats were decapitated, and denervated and contralateral control soleus, plantaris and gastrocnemius muscles were excised, weighed, homogenized and assayed for glutamine synthetase and glutaminase, which is responsible for degradation of glutamine. Glutamine synthetase activity increased 2-3 fold 1 hour after denervation in all 3 muscles. This increase in enzyme activity was maintained for up to 7 days post-denervation. Enhanced glutamine synthetase activity probably does not reflect increased production of glutamine synthetase molecules because cycloheximide did not block the denervation-mediated increase in activity. Increased activity was associated with increased apparent Vmax with no change in apparent Km. However, a mixing experiment failed to yield evidence for the presence of an enzyme activator in denervated muscle. Limb immobilization for 7 days also resulted in an increase in glutamine synthetase activity, albeit smaller than that caused by denervation, suggesting that muscle usage is involved in regulating the activity of this enzyme. Glutaminase activity was not altered by denervation or by limb immobilization. These data suggest a role for glutamine synthetase in muscle wasting secondary to disuse and denervation. [This work was supported in part by grants from NASA (NAG 2-100) and NIH (HD-16596)].

301.11 EFFECT OF NERVE EXTRACT ON ULTRASTRUCTURAL CHANGES IN DENERVATED SKELETAL MUSCLE IN VIVO. C.S.Heck* and H.L. Davis. Department of Anatomy, McGill University, Montreal, Canada, H3A 2B2.

Denervation of a skeletal muscle results in many changes including atrophy, loss of protein and quantitative and qualitative ultrastructural alterations of component fibers. It is generally agreed that changes in denervated muscle fibers are due to both the disuse caused by paralysis of the muscle and to the loss of myotrophic substances.

It has previously been shown that systemic administration of nerve extract to mice diminished the atrophy of denervated muscle fibers and ameliorated the physiological twitch characteristics of denervated fast-twitch muscles. The present investigation asses sed the effects of nerve extract on ultrastructural changes which accompany denervation, in different types of skeletal muscle fibers.

Eight week old male C57Bl mice were subjected to unilateral hindlimb denervation for seven days and were either untreated or received daily intraperitoneal injections of soluble proteins extracted from rats' sciatic nerves (250 mg protein/kg body weight). Normal (NOR) and denervated (DN) muscles from untreated mice were compared to those from injected mice (NOR-Rx and DN-Rx, respectively). Various morphological parameters were examined by electron microscopy and quantitated by stereological techniques. Types I and IIA fibers of soleus and type IIB fibers of gastrocnemius muscles were assessed.

Denervation of muscle fibers for seven days resulted in losses of absolute size and proportions of total fiber volume for sarcoplasmic reticulum, t-tubules and mitochondria (both intermyofibrillar and perinuclear). The mitochondrial changes were greater for type I fibers whereas changes in the membranous systems were greater for type II fibers.

Denervated muscles of mice treated with nerve extract exhibited significantly smaller changes in mitochondria, sarcoplasmic reticulum and t-tubules than those in untreated animals. In general, 80 to 90% of the post-denervation changes were prevented by the extract, and in some cases DN-Rx muscles were not significantly different from normal muscles.

Thus, the findings of this study indicated that treatment of denervated muscle with nerve extract ameliorated, almost completely, many ultrastructural changes of mitochondria and membranous systems. Treatment with extract was also found to influence atrophy, content of protein and Z-line thicknesses in denervated fibers.

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301.12 NEURAL REGULATION OF MUSCLE REGENERATION. G.T. Patterson and W-D. Dettbarn. Pharmacology Dept., Vanderbilt Univ. Med. Ctr., Nashville, TN 37232.

The influence of nerve on muscle fiber characteristics during development, after denervation and upon reinnervation has been well studied. Less is known about the influences of nerve on regenerating muscle fibers. In this study we have investigated the role of neural influences on muscle fiber types and acetylcholinesterase (AChE) regulation during regeneration of the fast extensor digitorum longus (EDL) and the slow soleus (SOL) muscles of the rat. Innervated and denervated EDL and SOL were injected in situ with 1 ml of 0.5% Bupivacaine (BPVC, Marcaine) solution to produce complete muscle fiber degeneration. Histochemical ATPase activity, AChE activity and its molecular forms were determined on the regenerating muscles for up to 8 weeks. Complete fiber destruction was found within 3 days after the Bupivacaine injection in both innervated and denervated muscles. By day 7 the onset of muscle regeneration was evident in all treated muscles. In the innervated regenerating muscles, both the EDL and SOL were initially populated mainly by type II (fast) fibers. With time the population of type I (slow) fibers increased in SOL to that found in normal SOL muscles and the EDL remained populated mainly by type II fibers similar to that found in normal EDL. AChE activity in both the innervated EDL and SOL initially decreased with fiber degeneration followed by an increase to above that of control and return towards normal levels. No qualitative differences in the AChE molecular forms pattern in the innervated muscles, compared to controls, were found during regeneration. In the denervated muscles, both the EDL and SOL were initially repopulated with type II fibers but in the SOL no increase in type I fibers was found with time. AChE activity of these denervated regenerating muscles remained below the levels for controls. Qualitatively, the AChE molecular forms pattern in the regenerating denervated SOL changed towards the normal EDL pattern. These results support the views that developing non-innervated rat myotubes have the characteristic of fast muscle, at least for ATPase and AChE regulation as determined by histochemical and subcellular gradient techniques. With innervation and use normal characteristics of mature fast and slow muscles are established. Supported by NASA Grant #NAG 2-301.

301.13 MOTOR AXON REINNERVATION IS ACCELERATED BY SKELETAL MUSCLE REGENERATION FOLLOWING NECROSIS. K.E. Misulis, W-D. Dettbarn, and M.E. Clinton*. Neurology and Pharmacology Depts., Vanderbilt Univ., Nashville, TN 37212.

Recent evidence has supported a trophic effect of denervated muscle on motor axons, suggesting that muscle may play a role in promoting and perhaps guiding reinnervation. This trophic effect may be active not only in reinnervation following denervation, but also in fetal development, where there is axonal extension to skeletal muscle. In a study of muscle necrosis and regeneration due to Marcaine (bupivacaine) injection, we found that muscles developed the biochemical characteristics of early reinnervation at a time when non-regenerating denervated muscles did not. This suggested that regenerating muscle may promote axonal extension even more strongly than denervated muscle. This question was directly addressed in this study.

The sciatic nerves of young adult rats were lesioned under ether anesthesia by section or calibrated crush. Experimental animals had either the soleus or extensor digitorum longus (EDL) muscle dissected free from surrounding connective tissue and injected throughout its length with 0.5% Marcaine. Control animals had the soleus and EDL exposed but received no injection. Wounds were closed and the animals allowed to recover. At weekly intervals the animals were re-anesthetized with pentobarbital sodium for electrophysiological assessment of reinnervation.

At 2 weeks after nerve crush there was incipient reinnervation in control animals with twitch tensions in the soleus and EDL being 7.6% and 1.6% respectively of non-denervated muscles from age-matched animals. In animals which had received injection of Marcaine in the soleus, the soleus had recovered to 23.5% of normal but the EDL was 1.5%. With injection of the EDL, EDL tension was 11.1% of normal with soleus 19.9%. AT 2 weeks after nerve section there was no activation of either muscle in control animals but some activation of the soleus and EDL in injected animals. At 4 weeks after nerve section there was beginning reinnervation in controls but both the soleus and EDL in injected animals had significantly more advanced functional reinnervation.

The results of this study indicate that Marcaine-induced skeletal muscle necrosis and regeneration results in a facilitation of reinnervation not only in the injected muscle, but also in muscles in close proximity. This suggests that regeneration results in the production of factors which stimulate motor axon extension and/or end-plate formation, and that this effect is stronger than that present in denervated, but otherwise intact skeletal muscle. This may be related to the mechanisms of axonal extension in development.

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301.14 SOMATOFUGAL AXONAL ATROPHY PRODUCED BY BOTULINUM TOXIN. B.G. Gold*§, J.W. Griffin, A. Pestronk, P.N. Hoffman, E.F. Stanley and D.L. Price. (SPON: H.E. Lowndes) §Rutgers University College of Pharmacy, Piscataway, NJ 08854 and The Johns Hopkins University School of Medicine, Baltimore, MD 21239

Botulinum toxin prevents vesicular release from synaptic terminals at the neuromuscular junction resulting in an increase in extrajunctional acetylcholine receptors and nerve terminal sprouting. However, the responses of the neuronal perikaryon to vesicular blockade are poorly understood. In the present study, we have examined axonal calibers in the proximal portion of the hypoglossal nerve following injection of botulinum toxin.

Three rats were given a single unilateral injection of botulinum type A (1.2 ng) in the tongue. Two weeks later, botulinum toxin-injected rats, along with 3 age-matched controls, were perfused with 5% glutaraldehyde. The hypoglossal roots, exiting from the ventral portion of the brain stem, were removed and prepared for light and electron microscopy. As an internal control, the V cranial nerve was taken to examine the sensory fibers from the tongue. In a second series of experiments, the hypoglossal nerve of 3 rats were unilaterally sectioned and the animals perfused 2-4 weeks later. Morphometric analysis was performed using light microscopic prints (600 fibers/animal, selected from grid overlays due to the non-random distribution of fiber sizes within each rootlet) to determine axonal diameters, areas, perimeters, and myelin sheath thickness (MST). Shape factors, an indicator of deviation from circularity, were also calculated. Electron microscopic prints of randomly photographed fibers (14/animal) were used to determine numbers of neurofilaments.

Histograms constructed from axonal diameters showed a significant ($p<.05$) shift towards smaller diameters fibers in botulinum-intoxicated animals. Mean axonal areas were significantly ($p<.05$) reduced by 38%. In contrast, axonal perimeters and MST were not significantly changed. Shape factors were very significantly ($p<.005$) reduced from 0.78 in controls to 0.66 in botulinum-intoxicated animals. Electron microscopy revealed a paucity of neurofilaments within atrophic axons; total numbers of neurofilaments and neurofilament densities were significantly ($p<.05$) reduced compared to controls with similar axonal perimeters. Fibers from hypoglossectomized rats demonstrated similar alterations in caliber. In contrast, sensory fibers in the V nerve showed no alterations.

Proximal, somatofugally spreading axonal atrophy (beginning near the perikaryon and extending distally along the axon with time) is also observed following axotomy. It is suggested that, following injection of botulinum toxin, altered delivery of a trophic substance from the periphery to the perikaryon results in the development of this stereotypic response in the neuron.

301.15 ENHANCED SYNAPTIC ACTIVITY REGULATES A SYNAPTIC VESICLE ANTIGEN DURING POSTNATAL DEVELOPMENT IN THE RAT SUPERIOR CERVICAL GANGLION H. Trenchard* and K.F. Greif (SPON.: M. Cohen) Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010

The role of increased synaptic activity as a factor regulating normal postnatal development in the sympathetic nervous system was studied using male spontaneously hypertensive (SHR) rats as a model for enhanced sympathetic activity. A monoclonal antibody (SV) directed against a 65 kdal integral membrane protein of synaptic vesicles (Matthew et al, J. Cell Biol. 91:257, 1981) was used as a marker of synaptic vesicle levels. Synaptic activity is known to regulate neurotransmitter synthesis in the SCG (Kessler et al, Science 214:335, 1981). We have shown in previous work that SV levels are also subject to regulation by synaptic activity (Greif and Trenchard, Soc. Neurosci. Abstr. 10:1056, 1984). Neonatal deafferentation causes a decrease in SV levels. Similar results are obtained by the use of ganglionic blocking agents.

In the superior cervical ganglion (SCG), antigen levels were significantly increased (40%) over the normotensive control strain, Wistar-Kyoto (WKY) at 12 weeks postnatal. The iris and pineal, target organs of the SCG, showed no significant differences in SV level compared to WKY rats at 12 weeks. At 6 and 8 weeks postnatal, SV levels in the SCG of SHR did not significantly differ from levels in WKY rats of the same age, while antigen levels in the iris and pineal were reduced. Morrison and Whitehorn (Brain Res. 296:152, 1984) reported enhanced sympathetic activity in SHR rats resulting in an increase in blood pressure. The increase in SV levels corresponds to the time of onset of hypertension in SHR rats, 6 to 20 weeks postnatal. These results provide further evidence that impulse activity exerts a general regulatory effect on the postnatal development of the sympathetic nervous system.

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301.16 ROLE OF IMPULSE ACTIVITY IN REGULATION OF EXPRESSION OF A SYNAPTIC VESICLE ANTIGEN IN THE RAT SUPERIOR CERVICAL GANGLION K.F. Greif and K.M. Linderman* Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010

We have demonstrated previously that deafferentation of the superior cervical ganglion (SCG) alters the levels of a synaptic vesicle-associated antigen in the SCG of Sprague-Dawley rats (Greif and Trenchard, Soc. neurosci, abstr. 10: 1056, 1984). The present study was designed to investigate directly the role of presynaptic activity in mediating changes in synaptic vesicle pools. Changes were monitored using monoclonal antibodies directed against a 65 kD integral membrane protein associated with synaptic vesicles (Matthew et al, J. cell biol. 91: 257, 1981). Antigen levels were quantified using radioimmunoassay.

Blockade of synaptic activity in the SCG was achieved by treatment with chlorisondamine, a ganglionic blocker. Neonatal rats were treated for 14 days, beginning the day after birth (5 mg/kg, s.c., b.i.d.). Control littermates were treated with saline. After 14 days, SV levels were reduced to 60% of control levels in the SCG. This finding is consistent with the levels of SV observed in the SCG 14 days after neonatal deafferentation.

Previous studies of plasticity of SV in adult ganglia (Greif, Soc. neurosci. abstr. 11: 1103, 1985) showed that both deafferentation and ganglionic blockade produced an increase in SV in the SCG after seven days. However, with longer survival periods after deafferentation, SV levels dropped, reaching 40% of control levels by 30 days after surgery. We wished to determine whether reductions in activity could account for the transient nature of the increase in SV, or whether other factors associated with deafferentation might play a role. Adult rats were implanted with osmotic pumps (Alza) containing either chlorisondamine (5 mg/ml, equivalent to 1.2 mg/day) or saline and were allowed to survive for either 14 or 28 days of drug treatment. After 14 days, SV levels in drug-treated animals did not differ from controls, consistent with observations observed following deafferentation. After 30 days, SV levels in treated SCG had dropped still further to 77% of control values. These results confirm the transient nature of the increase in SV levels following deafferentation, and indicate that reduced presynaptic activity plays a significant role in mediating the observed effects. The smaller decrease in SV levels is likely the result of surviving vesicles in intact presynaptic terminals.

These studies provide further evidence that impulse activity plays a significant role in the regulation of neural antigens in the rat sympathetic nervous system.

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TROPHIC INTERACTIONS III

302.1 SPECIFIC BINDING OF ^{125}I-LAMININ TO NEURAL CELLS IN CULTURE. W. Harvey* and S. Carbonetto. Neurosciences Unit, The Montreal General Hospital Research Institute, McGill University, Montreal, Quebec, Canada H3G 1A4.

Several observations in vivo and in culture indicate that the extracellular matrix (ECM) of the nervous system strongly influences nerve fibre growth. Laminin, a glycoprotein component of the ECM, is present in mammalian PNS and developing CNS as well as in amphibian optic nerves undergoing regeneration but is absent in adult mammalian CNS where there is little regeneration following trauma (Liesi, EMBO.J. 4, 2505; 1985). Moreover, laminin is conspicuous among ECM adhesive proteins in stimulating nerve fiber growth in culture. However, the molecular mechanisms of laminin-neuron interactions have yet to be detailed. Using ^{125}I-laminin, we have begun to characterize the binding of laminin to neural cells in culture. Laminin binds specifically to chick embryo DRG and spinal cord cells, rat embryonic astrocytes as well as neuroblastoma cell line NG 108. Scatchard analysis indicates a single dissociation constant (K_d) of 2-4 nM for DRG, spinal cord cells, rat astrocytes and NG 108 with 20,000-40,000 specific binding sites per cell. PC-12 cells adhere avidly to laminin but they have only 1000-2000 laminin binding sites per cell. Light microscope autoradiography of primary neuronal cultures DRG and NG 108 indicates that ^{125}I-laminin binds specifically to nerve fibers and cell bodies of neurons, achieving its highest densities on growth cones.

These findings demonstrate the presence of specific, high-affinity binding sites for laminin on neurons, glia and neuroblastoma. Future studies will be aimed at further structural and functional characterization of these sites with regard to their role in neuron-substratum adhesion during nerve fiber growth.

302.2 ASSAY OF NEURITE EXTENSION FACTOR USING NEURO-2A CELLS IN DEFINED MEDIUM. D. Kligman* and L.-S. Hsieh* (SPON: H. Chin). Lab. of Cell Biology, NIMH and Lab. of Biochemical Genetics, NHLBI, Bethesda, MD 20892.

Using a sensitive, in vitro bioassay, a soluble neurite extension factor (NEF) from bovine brain was partially purified.[1] This assay quantitates the extension of neurites from seven-day old chick embryo cerebral cortical neurons grown at low cell density in a defined, serum-free medium on a poly-lysine substratum. Subsequently, NEF was purified to apparent chemical homogeneity by reversed-phase high performance liquid chromatography.[2] Characterization by SDS gel electrophoresis, amino acid analysis, and microsequencing revealed that NEF was a disulfide-bonded oligomer of S100β.

To identify and characterize the cell surface receptor for NEF, and to study its mechanism of action, it would be useful to have a neuronal cell line which is responsive to NEF. Using an adaptation of the assay with primary neurons, we now report that Neuro-2A cells (a mouse neuroblastoma cell line) when grown in a defined medium[3] at low cell density (5,000 cells/cm^2), respond to NEF by a change in morphology: the cell body becomes rounded and processes are elongated. In the absence of NEF, the cells are flattened and do not extend processes. This response is rapid, occurring within 4-6 hours. The response is dose-dependent, with half-maximal stimulation at an NEF concentration of about 300 ng/ml.

The assay of NEF with Neuro-2A cells should be useful for the study of the mechanism of action of NEF, and offers a more rapid and easier to perform bioassay.

[1] Kligman, D. (1982): Brain Research, 250, 93-100.
[2] Kligman, D. and Marshak, D. (1985): PNAS, 82, 7136-7139.
[3] van Zoelen, E.J.J. et al. (1984): PNAS, 81, 4085-4089.

302.3 THE EFFECT OF MUSCLE EXTRACT FROM ADULT AND AGED RATS ON NEURITE OUTGROWTH FROM EMBRYONIC CHICK MOTONEURON-ENRICHED FRACTIONS IN VITRO. J.L.Rosenheimer, K.M.Bork* and D.O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

During the aging process, there is a decline in the occurrence of new nerve terminal growth, or sprouting, at the neuromuscular junction. Likewise, terminal sprouting in response to muscle denervation, is virtually absent at the neuromuscular junctions of old animals.

To see if there is a concurrent age-related decline in the synthesis by the muscle of neuronotrophic factor(s), the effect of muscle extract on neurite outgrowth from dissociated embryonic chick motoneurons was examined in vitro. Motoneurons from the lumbar regions of 7-day embryonic chick spinal cords were isolated based on their buoyant densities (Schnaar and Schaffner, J. Neurosci.1:204, 1981). Cells were plated at a density of 200 cells mm^{-2} and maintained for 72 hours on polylysine-coated plates in Dulbecco's modified medium supplemented with 10% horse serum, glucose (33mM), penicillin (100 Uml^{-1}), streptomycin (50ug ml^{-1}) and muscle extract ranging in concentration from 10 to 500ug protein ml^{-1} culture medium or phosphate-buffered saline (PBS) as a control. Muscle extract was prepared according to procedures described by Smith et al.(J. Cell Biol.101:1608, 1985) from 5 adult (10-mos) and 5 aged (25-mos) F344 male rat extensor digitorum longus muscles that had been unilaterally denervated for 5 days. Extract from either the contralateral innervated or denervated muscles was examined for its effect on neurite outgrowth.

After 72 hours in culture, 72.8% ± 2.1% of the cells remained attached to the tissue culture wells. As judged by exclusion of trypan blue, 65.0% ± 1.0% of these cells appeared viable in wells supplemented with concentrations of muscle extract less than 250ug protein ml^{-1} medium. The concentration of extract derived from innervated and denervated muscles from 10-mos animals which was necessary to produce a half-maximal increase in neurite outgrowth over that observed in control wells was 19 and 30ug protein ml^{-1} medium, respectively. Concentrations of 186 and 195ug ml^{-1} were necessary to produce similar half-maximal responses from extract derived from innervated and denervated muscles, respectively, from 25-mos rats. Therefore, it appears that although the potency of extract from the younger animals is greater than that from the aged, denervation of the muscle does not result in a significant increase in that potency in rats 10-mos of age or older. Supported by NIH grants AG05340 (JLR) and AG01572 (DOS).

302.4 THE EFFECTS OF NGF ON THE GROWTH OF EMBRYONIC CHICK METENCEPHALIC TRIGEMINAL NEURONS IN CULTURE. M.B. Heaton and D.B. Wayne*. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

Nerve growth factor (NGF) has recently been reported to influence the differentiation of central cholinergic populations. In addition, Raivich et al. (EMBO, 4 (3): 637, 1985) found transient receptor binding of NGF in the lateral motor column of the chick during development. This appeared to relate to our studies on the effects of the trigeminal ganglion on the development of the trigeminal motoneuron population. In vivo study (Moody and Heaton, J. Comp. Neur., 213: 350, 1983) had shown that process outgrowth from the trigeminal motoneurons was dependent on, and directed towards, the trigeminal ganglion cells. The trigeminal ganglion, partially neural crest derived, is responsive to NGF at an early stage. Subsequent in vitro study of the effects of ganglion-conditioned medium included NGF and suggested that NGF might act directly on the trigeminal motoneuron population. The purpose of the present experiment was to clarify the direct effects of NGF on trigeminal motoneurons. The metencephalic basal plate was excised from stage 11 chick embryos. The explants were cultured on a combined substratum of collagen and poly-DL-ornithine in either control medium (F-12, 10% FBS) or the same medium supplemented with 15ng/ml NGF (2.5S, Sigma). Ara-C added after 36hr prevented further cell proliferation and limited the neurons present to previously established populations, almost exclusively the trigeminal motoneurons. Neurite outgrowth was quantified after 6 days in culture. The outgrowth index in the NGF group averaged 526.0 compared to 333.4 in the control group. NGF significantly enhanced outgrowth. In a second study explants were grown in control medium alone or supplemented with 1) NGF, 2) NGF and anti-NGF, and 3) anti-NGF alone. The mean outgrowth index was 248.8 for control explants compared to 414.3 in the presence of NGF, and 219.5 with both NGF and anti-NGF present. Explants grown in anti-NGF alone had an average index of 195.8, compared to 173.0 for their controls. Thus, early trigeminal explants demonstrate a specific responsiveness to NGF. Preliminary work on dissociated cells indicates that NGF does not affect the numbers of cells which elaborate processes but may affect the length and branching of a process once it is initiated. (Supported by NIH grant NS20387 to M.B.H.)

302.5 REGIONALLY SPECIFIED NEURO-ASTROGLIAL INTERACTION IN THE DEVELOPING NERVOUS SYSTEM. A. Prochiantz, B. Chamak*, A. Autillo-Touati*, A. Fellous*, L. Fetler* and A. Rousselet*. INSERM U.114, Chaire de Neuropharmacologie, Collège de France, 75231 Paris cedex 05, France.

The morphological maturation of mesencephalic and striatal neurons was analysed in different co-culture conditions with astrocytes. Striatal or mesencephalic astrocyte monolayers were prepared and embryonic neurons from the same regions plated on them in the four possible combinations. Neuronal morphology was observed after 2 and 4 days with a special focus on dopaminergic neurons (for the mesencephalon) and GABAergic neurons (for the mesencephalon and the striatum). It appears that more complex morphological patterns of the neuritic arborization are produced when neurons and astrocytes are derived from the same regions of the brain (homotopic co-cultures). The main feature of this arborization is the high number of departures from the soma and the occurrence of numerous branching points. On the contrary in heterotopic co-cultures most neurons grow one main long and linear neurite with few branches. In order to elucidate the physiological significance of this phenomenon the ultrastructure of the neurons was examined in electron microscopy. In homotopic co-cultures we can observe a large amount of neurites with dendritic characteristics, such as fast tappering of the fiber diameter, presence of numerous ribosomes and proximal branching. In addition the cell bodies look very mature with a low nucleo-cytoplasmic ratio and a well developed Golgi zone. In heterotopic conditions neurons are less mature and few dendrites are present. Most neurites are long, thin and linear with no ribosomes and a constant diameter on their entire length. They evoke immature axons. The possible specific outgrowth of dendrites in homotopic conditions was confirmed by immunostaining with MAP-2 antibodies. The proportion of neurons with MAP-2 positive fibers is higher in homotopic than in heterotopic conditions. The association of an enhanced neuronal maturation and of a specific dendritic development in homotopic neuro-astroglial co-cultures leads us to propose that astrocytes synthetize regionally specified molecules which act as growth factors and/or dendrite growth signals for co-localized neurons.

302.6 BIOCHEMICAL HETEROGENEITY OF CULTURED ASTROCYTES FROM DIFFERENT PARTS OF THE BRAIN. G. Barbin, B. Chamak*, A. Rousselet* and A. Prochiantz. INSERM U.114, Chaire de Neuropharmacologie, Collège de France, 75231 Paris cedex 05, France.

Previous studies performed in our laboratory have shown that cultured astrocytes issued from different parts of the rodent central nervous system exhibit different functional properties: mesencephalic dopaminergic neurons have a different morphology whether they are seeded on mesencephalic or striatal glial cells (1).

The biochemical counterpart of such a functional heterogeneity has been investigated using confluent monolayers of mesencephalic and striatal glial cells; in both cases the majority of the cells were astrocytes as evidenced by their glial fibrillary acidic protein content. Cells were radiolabeled by external iodination with Na-125-I in the presence of lactoperoxydase and glucose oxidase. Analysis of the iodinated proteins by one- or two-dimensional electrophoresis revealed qualitative differences for proteins having a molecular weight range between 100 and 200 kilodaltons (Kd) and around 45 Kd.

Glycoproteins of these cultured cells were also examined using two protocols. First of all cells were labeled with 35-S-methionine and their glycoproteins isolated by biotinylated lectins conjugated to streptavidin agarose. Alternatively non labeled cell extracts were processed through two-dimensional electrophoresis and western blotting followed by detection with biotinylated lectins and a streptavidin-biotin-peroxidase complex. Among several lectins tested, Concanavalin A (ConA), Lens Culinaris (LcA) Ulex Europaeus F (UEA-F) and to a lesser extent Wheat Germ Agglutinin (WGA) revealed a large number of glycoproteins whereas Peanut Agglutinin detected only a few ones. With ConA, LcA and UEA-F, many glycoproteins ranging from 45 to 200 Kd were detected in striatal glial cell extracts that were absent from mesencephalic ones.

These data evidence a biochemical heterogeneity of cultured astrocytes according to their origin within the brain, thus supporting their functional heterogeneity. Analysis of the molecules secreted by these cells in the culture medium will be also presented.

(1) Denis-Donini et al., Nature 30 (1983) 641-643.

302.7 GROWTH OF A PURIFIED POPULATION OF POSTNATAL RAT CORTICAL MOTORNEURONS IN CULTURE. B. Swearingen* and L.L.Y. Chun* (SPON: N.T. Zervas). Depts. of Neurosurg. and Neurol., Mass. General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, MA 02114.

Studies on the postnatal development of central nervous system neurons in culture have been difficult because the cells obtained from cortical dissociates are extremely heterogeneous. Fluorescence-activated cell sorting has been applied to this problem in studies of embryonic spinal cord and cerebellum. We have used this technique to purify a population of cortical motorneurons from the postnatal rat, where the number of non-neuronal cells greatly exceeds the number of primary motorneurons. Rhodamine-conjugated latex microspheres (.5-1 ul) are injected into the corticospinal tract of three day old rat pups. The beads are transported retrogradely and label the pyramidal cells in layer V of sensorimotor cortex. Cortical cells are prepared from P5 animals by enzymatic and mechanical dissociation using a modification of the technique of Huettner and Baughman (J. Neurosci., in press). Approximately one percent of the dissociated cells contain label. These cells are separated by passage through a flow cytometer and purities of 80-90% are routinely obtained. Approximately 10^4 labelled cells are present in a dissociate of 10^6 cells; sorting with the parameters we use recovers about 1/3 to 1/2 of these. This purification technique allows in vitro analysis of a pure population of CNS projection neurons. We are now able to selectively reconstitute the normal cellular environment and investigate the interactions between these projection neurons and their target spinal cord and glial support. Preliminary results indicate that the sorted cells will survive in culture for at least one week when plated onto a monolayer of astrocytes or embryonic spinal cord.

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302.8 EXPRESSION OF SEROTONIN, TYROSINE HYDROXYLASE, AND GABA IN CULTURES OF NEUROGENIC CELLS FROM NEURAL CREST AND BRANCHIAL ARCHES: EFFECT OF THE ADDITION OF BOWEL. H. M. Mackey*, R. F. Payette* and M.D. Gershon. (SPON: L. Cote) Dept. of Anat. and Cell Biol., New York, N.Y. 10032

The phenotypically diverse neurons of the enteric nervous system are developmentally derived from precursors that migrate to the bowel from the "vagal" and sacral regions of the neural crest. In order to gain insight into the generation of enteric neuronal diversity we examined the expression of serotonin (5-HT), tyrosine hydroxylase (TH), and GABA *in vitro*. In the mature avian gut intrinsic neurons contain 5-HT or GABA but not TH. These markers were demonstrated immunocytochemically, singly or simultaneously, using species-specific secondary antisera labeled appropriately with fluorescein or rhodamine (or Texas red). Isolated neural crest cells (from cranial, vagal, and truncal levels), neurogenic cells from the 3rd and 4th branchial arches, dissociated intestine (of 7 days of incubation, following colonization by crest) and pancreas were cultured. In addition, crest cells and branchial arches were also grown in co-culture with aneuronal hindgut. Since catecholaminergic cells non-specifically take up 5-HT, we grew the cultures in a medium that was 5-HT-poor. Crest cells from each axial level, cultured by themselves, expressed 5-HT, TH, and GABA. The morphologies of the cells expressing each of these markers were distinct, suggesting that they constitute separate populations. Moreover, the absence of co-localization, when cultures were doubly stained with anti-TH and anti-5-HT sera, indicated that the 5-HT-cells were not catecholaminergic. Co-culture of crest cells with aneuronal hindgut increased the number of 5-HT-immunoreactive cells in the immediate proximity of, but not at a distance from, the explant of bowel. TH-immunoreactive, but not 5-HT or GABA-containing cells developed in cultures of the branchial arches when these structures were grown by themselves; however, 5-HT-immunoreactive neurons appeared when the branchial arches were co-cultured with aneuronal hindgut. Cultures of dissociated bowel contained TH- and GABA-immunoreactive neurons, but not 5-HT. Cultures of dissociated pancreas contained none of the markers. These observations indicate that the neural crest contains precursor cells able to express 5-HT, TH, and GABA. The branchial arches, which are filled with crest cells and contain "vagal" crest migrating to the gut, must also contain serotonergic precursors; however, 5-HT is apparently not expressed within the branchial arches unless bowel is added. This addition also enhances the expression of 5-HT by crest cells in the vicinity of enteric explants; therefore, the enteric microenvironment facilitates the expression of at least one type of enteric neuron. Dissociation of the gut appears to vitiate this effect of the enteric microenvironment. This is seen in the failure of 5-HT-immunoreactive cells to develop in cultures of dissociated bowel and in the appearance of TH-containing cells in such cultures. The expression of TH, manifest in crest cells and in branchial arches *in vitro*, is normally suppressed and not seen in the avian gut *in situ*.

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302.9 FAILURE OF VASCULAR SMOOTH MUSCLE TO SUPPORT THE SURVIVAL OF CULTURED CILIARY GANGLION NEURONS. Douglas J. Creedon and Jeremy B. Tuttle, Dept. of Physiology, Univ of Virginia Sch. of Medicine, Charlottesville, VA 22908.

In vitro, neuronal survival depends upon soluble and membrane factors, but the specificity of this trophic effect has not been thoroughly investigated. In dissociated cell culture, the entire population of neurons in the ciliary ganglion can derive support from striated muscle. This occurs despite the fact that choroid cells, which account for approximately half of the neurons in the ganglion, selectively innervate vascular smooth muscle (VSM) in vivo. This study was undertaken to determine if a trophic relationship exists between ganglionic neurons and a vascular smooth muscle target.

Dissociated ciliary ganglion cells from 9-day chick embryos were added to microwells containing one of the following: collagen, pectoral muscle myotubes, chicken aortic VSM, or rat aortic VSM. Pectoral muscle was from 11-day chick embryos. Chicken VSM was cultured from aortas of 2-3 week old chickens by combining enzymatic and mechanical dissociation. Rat VSM cells were obtained from the third or fourth passage of a primary aortic VSM cell line. Both types of VSM were plated at $1x10^4$ cells per cm^2 and allowed to reach confluency prior to the addition of the neurons. Electron microscopy confirmed the identy of the VSM. Ciliary ganglia were dissociated and plated in the absence of chick embryo extract or other survival protein. Counts of surviving neurons were taken after one, two, five, and seven days. On the second day, 55.9% of the neurons remained in the the chick VSM co-cultures. By five days only 2% remained and 0% remained at seven days. A similar sequence occurred in the mirowells containing neurons and rat VSM: 57.9% of the neurons remained on day 2, 10.4% on day 5, and 3.3% at one week. This is in contrast to pectoral muscle cultures in which no loss of neurons was detected. Neurons plated on collagen were reduced to 9.3% of their original numbers by day 2. Neurons were also plated on collagen, but fed medium conditioned by each of the three muscle sources. Both pectoral muscle and chick-VSM were partially effective, with 52.5% and 21.5% neuronal survival, respectively, at seven days.

These results contrast with the ability of even foreign striated muscle to support the survival of ganglionic neurons destined to innervate both smooth and skeletal muscles. The failure of VSM to support survival raises the possibilty that no trophic relationship exists with this target. Alternatively, survival could depend upon factors derived from other elements of the target tissue, or smooth muscle may demonstrate greater specificity in trophic interactions than its striated counterpart.

Supported by grants from the NSF and NIH.

302.10 PC-12 CELLS AND ADRENAL MEDULLARY ENDOTHELIAL CELLS FORM COMPLEXES IN CO-CULTURE ANALOGOUS TO CHROMAFFIN CELL/ENDOTHELIAL INTERACTIONS SEEN IN MATURE ADRENAL MEDULLA TISSUE. Y. Mizrachi[1]*, P.I. Lelkes[2]*, _____ and H.B. Pollard[2]. (SPON: K.L. Kelner) NICHD (1), NIADDK (2), National Institutes of Health, Bethesda, MD 20892.

Chromaffin cells form close associations with endothelial cells in the intact adrenal medulla, thus ensuring prompt introduction of secreted substances into the blood stream. The genesis of this association and its maintenance is obviously important, but not presently understood. We have attempted to mimic this phenotypic organization with co-cultures of PC-12 cells and bovine adrenal medullary endothelial (BAME) cells. Under these conditions PC-12 cells were found to adhere rapidly and specifically to BAME cells, as defined by a kinetic assay. The PC-12 cells firmly adhered to the endothelial cells rather to the matrix, and this process approached completion within 60 minutes. The PC-12 endothelial cell complex was also stable upon prolonged culture, the PC-12 cells forming clusters upon the endothelial cells reminiscent of the chromaffin cell endothelial cell interactions in the mature adrenal medulla.

The specificity of this contact process was further manifest by the fact that PC-12 cells showed little adherence to some other cell types. The adherence of PC-12 cells to bovine corneal endothelial cell matrix was also qualitatively different. The rapidity and specificity of complex formation between PC-12 cells and endothelial cells lead us to expect that preformed membrane molecules might be responsible for the interaction. We therefore tested urea extracts containing plasma membrane particles from both cell types for their capacity to interfere with the formation of PC-12 endothelial complexes. Indeed we found that this complex formation could be blocked by these plasma membrane particles, but not with plasma membrane particles derived from other cells. We also found that the membrane particles from PC-12 and endothelial cells respectively aggregated when mixed together in a cell-free system when measured by changes in light scattering.

We conclude that PC-12 cells and endothelial cells interact specifically, and the interaction is mediated by specific membrane components on each cell. This interaction may also be important in growth and differentiation of the intact adrenal medulla.

302.11 NEONATAL MONOCULAR ENUCLEATION ALTERS THE NEURONAL DEVELOPMENT OF ACETYLCHOLINESTERASE, SOMATOSTATIN AND CONNECTIONS IN VISUAL CORTEX. L.A. Benevento, J.E. Camel and S. K. Itaya. Dept. of Anatomy, University of Illinois College of Medicine, Chicago, IL 60612.

We have studied the development of somatostatin (SS) and acetylcholinesterase (AChE) containing cells and neuronal connections in the visual cortices of normal and monocularly enucleated rats. All enucleations were done on postnatal day zero (PND=0). Each brain was stained for SS and AChE with immunocytochemical (ABC-peroxidase) and histochemical (Hedreen et al. '83-silver) methods.

In normal PND=15-17 pups, areas 17, 18a and 18 contain AChE and SS positive cell bodies concentrated in layers II, III and V, VI. The AChE cell population includes polymorphic, medium-sized pyramidal and fusiform cells, while SS containing cells include a variety of morphological types. In PND=15-17 rats which were monocularly enucleated, a different picture exists. In the contralateral cortex there is a large reduction of AChE positive cells in contrast to litter controls or ipsilateral cortex. For SS the reverse is true. The number of SS containing cells in the cortex contralateral to the enucleation increased about 20%.

In the same animals, HRP injections made 1 to 2 days before sacrifice, show that in normal PND=15-17 pups the distribution of callosally projecting cells (CC-Cells) is concentrated in columns, mainly in layers III and V at the borders of areas 17/18a and 17/18. In rats which were enucleated a different HRP picture exists. At PND=15-17 the cortex contralateral to the enucleation contains retrogradely labeled CC-Cells in layers III and V (with some in II and VI) in areas 18a and 18 and throughout area 17 thus demonstrating thwarted collateral elimination. That is, the callosal connections remain in an undifferentiated neonatal-like state.

Thus, as a result of enucleation, CC-Cells in the contralateral cortex fail to undergo collateral elimination and there is also a precipitous decrease of AChE stained cells in the same layers. At the same time, there is an increase in the number of SS cells in the same cortical areas and layers. Since both chemical changes and HRP labeling of CC-Cells take place in the same cortical areas, layers, and in some cases, the same cell types, it is intriguing to hypothesize that the decrease in AChE and increase in SS indicates that they are related to regulation of synaptic connections either as a constituent of CC-Cells, or indirectly via intrinsic connections. (Supported by NS 21948)

302.12 LIFESPAN CHANGES IN THE STIMULATORY EFFECT OF POLYAMINES ON MITOCHONDRIAL CALCIUM TRANSPORT. J. R. Jensen, G. Lynch and M. Baudry. Center for the Neurobiology of Learning and Memory, Univ. Calif. - Irvine, CA 92717.

Mitochondria are thought to play an important role in the regulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), particularly during periods of increased $[Ca^{2+}]_i$. Disturbances in mitochondrial Ca^{2+} buffering have been postulated to occur with aging in various tissues including brain. Recently it has been reported that the polyamines, spermine and spermidine, might have a physiological role as regulators of mitochondrial Ca^{2+} cycling in hepatocytes. In the present study, we investigated the effect of those polyamines on rat brain mitochondrial Ca^{2+} transport. We report that polyamines stimulate transport and that this effect decreases as a function of age of the animals.

Spermine both increased the rate of Ca^{2+} accumulation and decreased the set-point at which mitochondria buffer free $[Ca^{2+}]$ in adult rat; in the presence of spermine extramitochondrial free Ca^{2+} ($[Ca^{2+}]_e$) was lowered to values around the resting $[Ca^{2+}]_i$. Analysis of the kinetics of calcium accumulation revealed that maximal spermine stimulation occurs at about 1 uM $[Ca^{2+}]_e$; beyond this level the stimulatory effect decreases and spermine can even inhibit net Ca^{2+} uptake. The effect of polyamines was concentration-dependent with a half-maximal effect observed at 0.1-0.4 mM for spermine while spermidine was about 10 times less potent. The stimulating effect of spermine was not due to an increase in the transport of respiratory substrates inside the mitochondria nor to an effect on the enzymes using these respiratory substrates.

We determined the effect of spermine on Ca^{2+} transport in hippocampal mitochondria from animals of different ages and found a dramatic decrease in the stimulatory effect of the polyamines on NAD-dependent Ca^{2+} accumulation as a function of age. Spermine maximally stimulated mitochondrial Ca^{2+} accumulation at postnatal day 12 (4-5 fold stimulation); the stimulation was 2-3 fold in adult rats and was only about 50% above basal in rats above 2 years of age.

These results suggest a critical role for polyamines in the regulation of $[Ca^{2+}]_i$ in brain, especially since polyamine levels exhibit large variations under different conditions. Moreover, the decrease in the stimulating effect of polyamines on mitochondrial Ca^{2+} transport with age might be related to a decreased ability of mitochondria to buffer $[Ca^{2+}]_i$, a condition that might well be expected to produce neuropathologies of the type associated with aging.
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DEVELOPMENT AND PLASTICITY: PROCESS OUTGROWTH III

303.1 DIFFERENTIAL PROMOTION OF RETINAL NEURITE OUTGROWTH BY ISOLATED EXTRACELLULAR MATRIX COMPONENTS. R. Perris*, N.G. Carri and T. Ebendal*. Dept. of Zoology, Uppsala University, Uppsala, Sweden.

The role of the extracellular matrix (ECM) in the control of neurite elongation and guidance during development of the visual system remains undetermined. In order to explore this and to identify ECM constituents possibly involved in this process, we tested the capacity of discrete ECM molecules to stimulate retinal neurite outgrowth in vitro. A previously described bioassay based on organotypic culture of embryonic chicken retina, growth-stimulated by optic lobe-extract, was utilized (Carri, N.G. & Ebendal, T. Dev. Brain Res. 6, 219-229, 1983). Neurite extension was highly promoted in explants grown on hydrated collagen gels, the fibers projecting radially and showing pronounced bundle formation. Retinal explants on laminin extended shorter, more numerous fibers exhibiting little fasciculation. Neurites invariably avoided intact plasma fibronectin (FN), vitronectin and chondroitin sulphate proteoglycans. The same behavior was shown on tissue culture plastic and bovine serum albumin.

Proteolytic fragments of the FN molecule, possessing functionally distinct activities, diverged in their ability to support neurite sprouting. A strong outgrowth response was evoked by substrata containing a 31,000-Dalton FN fragment, representing the heparin binding domain. On this fragment, axonal growth was more luxurious than on laminin, with density and length of projecting fibers as well as neurite bundling clearly augmented. Conversely, no outgrowth was observed on substrata of FN fragments constituting the cell binding (105,000-Dalton), the collagen binding (40,000-Dalton) and a combination of the collagen and heparin binding regions of the molecule. These results contrast observations made on neural crest cell migration, the latter being profoundly promoted by FN, vitronectin and the 105,000-Dalton FN fragment as substrata in vitro.

We propose that multivalent neuron-ECM interactions may be crucial for neurite growth during development of the visual system. Conceivably, collagens may predominantly enhance elongation of growing neurite bundles, whereas laminin and specific portions of the FN molecule could locally influence sprouting in terminal areas.

303.2 OLFACTORY BULB NEURITE EXTENSION IN CULTURE IS AGE AND SUBSTRATE DEPENDENT. N. Kleitman and M.I. Johnson. Dept. Anat. & Neurobiol. Washington University School of Medicine, St. Louis, MO, 63110.

Previous studies have described a correlation between neurite extension rate, age of donor tissue, and growth cone morphology in superior cervical ganglion cultures (Argiro et al. 1984 J. Neurosci. 4:3051). In preparation for developmental studies of growth cones from CNS tissue, we have cultured embryonic and perinatal rat olfactory bulb (OB) tissue on a variety of substrates and compared neurite extension rates. OBs from day 15 embryos (E15) to 7 day old pups (P7) were studied. Culture media contained MEM, 10% human placental serum, 2% chick embryo extract and NGF.

E15-E17 OB outgrowth was compared on a variety of substrates. On air-dried type I collagen (ADC), neurite outgrowth occurred within 24 hr (ave. extent 188 ± 31 μm, n=12 explants). Neurites were unfasciculated and free of non-neuronal cells (NNC). Growth continued for over 2 wk in culture. On diluted ADC (20%; a flat substrate), neurites extended in fascicles populated with NNC. Explants attached poorly to ammoniated type I collagen, type IV collagen, fibronectin, and uncoated glass coverslips. Early neuritic outgrowth detached and retracted; later outgrowth was associated with NNC. On poly-ornithine, OBs attached and extended short neurites associated entirely with NNC. On coverslips treated with laminin, or laminin over poly-ornithine, dilute ADC or type IV collagen, OBs attached and extended neurites in association with flattened NNC. Neurite extension rate approached that seen on ADC only on laminin alone. On a substrate of noncellular extracellular matrix [ECM, prepared from myelinating dorsal root ganglion cultures on ammoniated collagen (M. Ard et al., submitted), extracted with detergents (Carey et al. 1983 J. Cell Biol. 97:473)] OB neurites were relatively free of NNC and followed the pattern of the ECM laid down by previous Schwann cell outgrowth. Finally, OBs on ADC grown in defined serum-free medium survived and extended neurites comparable to those seen in serum-containing medium.

As neurite outgrowth was maximal on ADC and unobscured by NNC, outgrowth rate on ADC was studied as a function of age. Outgrowth diminished significantly between E15 and P1 in density and rate. Neurite extension was not observed from older explants. Relative to E16 outgrowth, neurite length was 45% less in E20 cultures after 1-2d, 23-41% less after 7d and 24% less after 13d in culture.

We conclude that like neurite outgrowth from sympathetic neurons and several other CNS tissues, OB neurite extension is age-dependent. The chemical and 3-dimensional structure of the substrate are also critical factors. (Supported by NIH grants NS 07071, NS 21771 and NSF grant BNS 8508148.)

303.3 STIMULATION OF SPINAL CORD NEURITOGENESIS BY A LAMININ SUBSTRATE
J.M. Thompson and S.t. Pierce* Department of Anatomical Sciences, College of Medicine, University of Illinois, Urbana, IL 61801.

Neurite outgrowth studies in vitro are important in understanding normal neuronal development and in providing insight into correcting developmental abnormalities. It has been previously found that neurite outgrowth on collagen substrates decreases with increasing gestational age of chick embryo spinal cord neurons in tissue culture (Thompson and Rapoport, Devel. Biol., 84:244, 1981). In the current study, we examined the ability of a laminin substrate to promote neurite outgrowth from spinal cord neurons and to reverse the developmental decline which occurs on collagen substrates. Laminin's neurite extension promoting ability has been verified in other systems (Lander, Fujii and Reichardt, Proc. Natl. Acad. Sci. USA, 82:2183, 1985). Laminin, polylysine and collagen were compared in their efficacy in promoting neurite extension from spinal cord neurons from chick embyros aged 6 to 16 days. The percent of neurons with processes and the length of the processes were determined at 1 and 3 days of culture. The order of the ability of the substrates to promote neurite outgrowth was laminin > polylysine> collagen at 1 day of culture. An analysis of variance demonstrated a significant increase in neuritogenesis by laminin compared to either polylysine or collagen. In 3 day cultures, the order of promotion of neurite outgrowth was laminin = polylysine> collagen with increased percentage of neurons producing neurites on collagen and polylysine compared to the 1 day cultures. Further, in those cultures grown on a laminin substrate, there was a significant reversal of the decline in neurite outgrowth on collagen and polylysine which occurs with increasing developmental age. The differences observed between the substrates were not due to differential survival rates of the spinal cord cells. Neurite length measurements also demonstrated a significant stimulation of neuritogenesis by laminin compared to polylysine or collagen in 1 day cultures. In 3 day cultures, there were longer neurites in cultures from younger embryos, but no difference between the three substrates. These results indicate the superior ability of laminin to promote neuritogenesis as compared to either polylysine or collagen at two incubation periods, and the enhancement of neurite growth at all developmental ages. These results suggest that the developmental decline in neurite extension previously observed is regulated by extrinsic factors, rather than intrinsic changes in the older neurons.

303.4 SUBSTRATES FOR CENTRAL NEURONAL AXON EXTENSION.
C. Lagenaur. Dept. of Neurobiology, Anatomy and Cell Science, Center for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Although central neurons are known to be able to extend neurites on substrates of poly-l-lysine (pll), collagen and laminin, these are unlikely to represent natural substrates since they are absent or present at very low levels in the brain. Two new brain derived substances that support the extension of neurites by mouse cerebellar neurons have been identified. M6 antigen is a 35 kilodalton glycoprotein that has been purified from mouse brain by immunoaffinity chromatography. Earlier work has shown that M6 antibody inhibits neurite extension (Lagenaur et al., submitted for publication). M6 antigen coated petri plates support rapid attachment of postnatal mouse cerebellar cells including neuronal and nonneuronal cells. The cell substrate attachment activity associated with M6 is resistant to boiling. Neurons on M6 extend mostly unfasciculated neurites, in contrast to neurons on pll, collagen or laminin. A second, distinct brain derived substrate designated NEF (neurite extension factor) has also been partially purified. NEF is obtained from mouse brain membranes solubilized in deoxycholate. Soluble membrane components are adjusted to 30% saturation with ammonium sulfate and centrifuged. The supernatant is then chromatographed on Sephacryl S 400. NEF activity is detected in the void volume suggesting a high molecular weight. Cell attachment is poor on NEF but the cells that successfully attach, rapidly extend long unfasciculated neurites that are often branched. Unlike M6, NEF activity is destroyed by boiling. These two substances may represent substrates that play important roles in CNS axon extension.

303.5 MODULATION OF NEURITE FORMATION ON DEFINED SUBSTRATES BY THE MONOCLONAL ANTIBODY HNK-1.
R.J. Riopelle, S. Mirski* and J.C. Roder. Dept. of Medicine (Neurology), Queen's University, Kingston, Ont., Canada K7L 3N6.

The monoclonal antibody HNK-1 (Leu 7) recognizes a carbohydrate epitope on a family of glycoproteins synthesized de novo by peripheral and central neurons (McGarry, R.C. et al., J. Neuroimmunol., 10:101, 1985). This epitope has been detected on antigenically-related (Grumet, M. et al., PNAS U.S.A., 81:267, 1984) cell-adhesion molecules (CAM's) that subserve neuron-neuron and neuron-glia interactions (Kruse, J. et al., Nature, 311:153, 1984), and recently has been found to be involved in neurite formation on immobilized substrates contained in conditioned medium from neuron-enriched cultures (Riopelle, R.J. et al., Brain Res., 367:20, 1986).

The latter studies have been extended to determine if the HNK-1 epitope is involved in interactions of neurons with the defined substrates laminin, fibronectin, and poly-D-lysine. On laminin but not on fibronectin or poly-D-lysine, the monoclonal antibody HNK-1 (Leu 7) inhibited neurite formation by dissociated chick embryo sensory neurons in a dose-dependent manner. This effect was NGF-independent, partially reversed by increased substrate-bound laminin, and could not be accounted for by altered cell attachment, cell death, or mitochondrial dysfunction. Immunoblotting studies determined that epitopes on laminin were not recognized by the monoclonal antibody.

The neuronal HNK-1 epitope may be a receptor site for a laminin domain involved with neurite outgrowth. The presence of an epitope for neuron substrate interactions on antigenically related neuron-neuron and neuron-glia CAM's lends further support to the idea that these neuronal glycoproteins subserve multiple adhesion functions.

This work was supported by MRC Canada and the MS Society of Canada.

303.6 INTERACTION OF CENTRAL AND PERIPHERAL NEURONS WITH PROTEOLYTIC FRAGMENTS OF LAMININ.
S.L. Rogers, S.L. Palm*, J.B. McCarthy*, L.T. Furcht, K. Hanlon*, and P.C. Letourneau. Dept. of Anat., Univ. of New Mexico, Albuquerque, NM 87131 and Dept. of Anat. and Lab. Med. and Pathol., Univ. of Minnesota, Minneapolis, MN 55455.

Laminin (LAM) is a key component of extracellular matrices and has been implicated in adhesion, migration and/or differentiation of a variety of cell types. Embryonic neurons may contact LAM during certain stages of differentiation and extend neurites in response to the substratum-bound molecule in vitro. We have begun to dissect the molecular nature of LAM-mediated neuronal behavior by examining interactions of these cells with proteolytic fragments of LAM. Laminin is a cross-shaped molecule with one long arm and three short arms, all of which contain globular domains at their distal ends. The binding site for non-neuronal cells is thought to reside at, or near, the intersection of the four arms (Terranova et al., PNAS 80:444), while one report suggests that neurons bind to a site near the end of the long arm of the molecule (Edgar et al., EMBO J. 3:1468). We have used three LAM fragments in the present study (see Palm et al., Biochemistry 24:7753): a 600 kDa thrombin fragment containing the two opposing short arms and a portion of the long arm, a 440 kDa chymotrypsin fragment which contains the central region of the 600 kDa fragment minus its outer globular domains, and a small (approx. 30 kDa) thrombin fragment that appears to arise from the distal portion of the long arm of LAM. Tissue culture plastic was treated with various concentrations of these peptides and dissociated neurons from the central (CNS) and peripheral nervous systems (PNS) of embryonic chickens were plated on these substrata in serum-free medium. Both cell types attach to and extend neurites on intact LAM, as we have shown previously. In response to the 600 kDa fragment, neurites of both CNS and PNS neurons were as long or longer than those on intact LAM. Although these cells extended neurites on the small thrombin fragment, in all cases they were significantly shorter than on intact LAM. The 440 kDa chymotrypsin fragment elicited different responses from the two types of neuron: while PNS neurons extended neurites (32% neurite length on intact LAM), CNS neurons did not. The absence of CNS neurites on this fragment is probably due in part to faulty initial adhesion of the cells to the substratum. These observations support the hypothesis that cells can have multivalent and/or cell-specific interactions with extracellular matrix molecules like laminin. It appears that CNS neurons either utilize a site within the 600 kDa fragment that is distinct from, or is required in addition to, that used by PNS cells. We will now further analyze the adhesive properties of these domains of laminin and determine how they relate to cell binding sites described by other laboratories.

303.7 AXON OUTGROWTH OF EMBRYONIC ZEBRAFISH NEURONS IS PROMOTED BY LAMININ AND INHIBITED BY FIBRONECTIN. Deanna Frost* and Monte Westerfield (SPON: Judith S. Eisen). Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Growth cones of identified motoneurons in the zebrafish navigate along specific pathways and establish stereotyped peripheral arbors which are segmentally restricted. What molecular mechanisms are used by motor growth cones to establish these patterns? We have found that two macromolecular components of the extracellular matrix, laminin and fibronectin, may play important roles in neurite outgrowth.

To study the distribution of laminin and fibronectin in the embryo we used polyclonal antibodies generated against mouse laminin and human fibronectin. Throughout the time of axon outgrowth by motoneurons, laminin appears to be uniformly distributed on the surfaces upon which growth cones navigate. In contrast, during this time fibronectin is found in regions that growth cones avoid--the lateral surfaces of the somites and the septa separating adjacent segments. Fibronectin staining disappears at a later time when the growth cones enter these regions. These results suggested that laminin, rather than fibronectin, may be an important substrate for motor neurite outgrowth.

We tested this hypothesis by comparing the effectiveness of laminin and fibronectin in promoting neurite outgrowth in vitro. Embryonic tissues were dissociated at the time of initial outgrowth of primary motoneurons and were plated in dishes coated with purified laminin or fibronectin. Neurons responded to increased concentrations of laminin by growing longer neurites. This effect could be blocked in a dose-dependent manner by addition of laminin antibodies. In contrast, increased concentrations of fibronectin decreased the length of neurites. We suggest that laminin supports and may be required for axogenesis by motor growth cones whereas fibronectin may function to exclude these growth cones from particular regions. We thank Jim McCarthy for the antibodies. Supported by NIH NS21132, GM07257 and NSI7963.

303.8 DEVELOPMENT OF EARLY SENSORY SYSTEMS IN THE ZEBRAFISH. W.K.Metcalfe, P.Z.Myers*, and B.Trevarrow Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

In the larval zebrafish, the Mauthner cell is known to receive sensory input from trigeminal, Rohon-Beard, auditory, and lateral line neurons, and to make excitatory outputs to primary motoneurons of the spinal cord. Both the Mauthner cell and primary motoneurons begin axogenesis in the early embryo and form part of a 'primary' neuronal circuit that appears to underlie early motile behaviors. We have now characterized the development of the sensory components of this primary circuit using HRP as a retrograde axonal tracer, monoclonal antibodies directed against young zebrafish sensory neurons, and fluorescent labeling of sensory neuron precursors with lineage tracer dyes.

The first developing sensory systems are thought to respond to tactile (Rohon-Beard and trigeminal) and vibratory (auditory and lateral line) stimuli, and all project central axons into the developing hindbrain. We have found that although the times of development of these systems overlap, the tactile senses are probably functional first. Rohon-Beard cells, which innervate the trunk, and trigeminal sensory neurons, which innervate the head, both begin to grow axons about 18 hours after fertilization. The peripheral arbors of these cells quickly form dense networks and at the same time their central axons grow rapidly through the hindbrain.

Posterior lateral line sensory neurons are the first of the vibratory sensory neurons to grow axons. Axons emerge about 18 hours after fertilization, but their central axons grow relatively slowly so that the central axons of the tactile senses precede them in the hindbrain. Several hours later, axons of the auditory ganglion enter the brain, followed by the ingrowth of axons of the anterior lateral line ganglion. The central projections of the tactile, auditory, and lateral line senses form discrete longitudinal fascicles in the hindbrain.

The axons of all of these sensory neurons grow concurrently with primary motor axons and the Mauthner axon. Thus, the nervous system of the embryonic zebrafish includes several populations of large cells that begin axogenesis early in development and that rapidly interconnect to form a complete neuronal circuit containing sensory neurons, interneurons, and motoneurons.

303.9 DISTINCT MECHANISMS UNDERLIE NEURITE OUTGROWTH ON CELL SURFACES VS. EXTRACELLULAR MATRIX. J.L. Bixby, K.J. Tomaselli, and L.F. Reichardt. Dept. of Physiology, U.C.S.F., San Francisco, CA. 94143.

During growth in vivo, axonal growth cones interact with complex substrates, including both the extracellular matrix(ECM), and non-neuronal cell surfaces. Molecules of the ECM, including laminin(LN) and fibronectin(FN), are potent promoters of neurite outgrowth in vitro. The ability of LN to promote outgrowth is mimicked by LN-containing conditioned medium factors from a variety of cell types. In addition, profuse neurite outgrowth is stimulated when neurons are cultured on monolayers of specialized non-neuronal cells such as astrocytes, Schwann cells, and myotubes.

Does the ability of non-neuronal cells to induce neurite outgrowth depend on their synthesis and secretion of ECM molecules? To address this question, we have compared neurite outgrowth on complex ECM substrates vs. that on non-neuronal cell surfaces, using neurons of the chick ciliary ganglion(CG). We found that neurite outgrowth on LN, FN, and conditioned medium factors from a number of cell types depends on interactions of these ECM molecules with a glycoprotein complex called the CSAT antigen, a putative LN and FN receptor(Horwitz et al J. Cell Biol. 101: 2134, 1985). This was demonstrated by the ability of two antibodies which recognize this complex(CSAT and JG22) to block neurite outgrowth on these substrates. The CSAT and JG22 antibodies, therefore, can be used to determine whether growth on cell surfaces depends on neuronal interactions with LN and FN, other cell-surface associated molecules, or both. We have grown CG neurons on monolayers of muscle, Schwann cells, astrocytes, and fibroblasts, in the presence and absence of the CSAT and JG22 antibodies. In addition, non-neuronal cells were extracted with non-ionic detergent and used as substrates to ask whether cell membranes were necessary for induction of neurite outgrowth by the cells. The results indicate that non-neuronal cells possess multiple mechanisms for stimulation of neurite outgrowth by these peripheral neurons.

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303.10 INDUCTION OF NEURITE OUTGROWTH FROM GANGLIA EXPLANTS BY ACTIVATORS OF PROTEIN KINASE C. L. Hsu and K.Y. Chen, Department of Chemistry, Rutgers University, Piscataway, N.J. 08854

We have demonstrated that the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA) was effective in eliciting a dense outgrowth of neurites from peripheral ganglia explants (Hsu et al, Cancer Res. 44, 1984). The native receptor for phorbol esters has been shown to be the calcium-activated, phospholipid dependent protein kinase (protein kinase C) (Castagna et al, J. Biol. Chem. 257, 1982). Protein kinase C is in turn activated by products of phosphoinositol degradation including diacylglycerols (Kaibuchi et al, Cell Calcium 3, 1982). We now report that the addition of synthetic diacylglycerols to defined, serum-free growth medium induced the rapid outgrowth of neurites from 9 day old embryonic chick sensory ganglia explants. We have tested 1-oleoyl -2-acetyl-glycerol, 1,2-dioleoyl-rac-glycerol and 1,2-dicapryloyl-rac-glycerol and found these to be effective in promoting neurite development at concentrations of 10-500 μM in Ham's F12 medium supplemented with insulin, transferrin, selenium and progesterone. The dense outgrowth of radial fibers from the explant was comparable to that observed with treatment of high concentrations (50-100 ng/ml) of TPA. However, the diacylglycerols at the concentrations tested did not produce the short and thick nerve bundles which are characteristic of explants maintained in high concentrations of TPA. Phospholipase C, which increases intracellular diacylglycerol was also effective in inducing neurite outgrowth at concentrations of 0.01-0.05 enzyme units/ml although the effects are less dramatic. These results indicate that exogenous diacylglycerol and phospholipase C can mimick the neurite-promoting effects of TPA and support our hypothesis that neurite differentiation by phorbol esters involves the activation of protein kinase C.

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303.11 MOUSE SALIVA AND SUBMANDIBULAR GLAND EXTRACTS PREVENT NEURITE FORMATION IN PC-12 CELLS. J. Lakshmanan* (SPON: M.E. Weichsel, Jr.), Growth Factor Laboratory, Center for Neurologic Study, San Diego, CA 92121

A recent study (Am. Soc. for Neurochem., 1986. Abstr. 208) in our laboratory indicates that epinephrine-provoked adult male mouse saliva contains biologically interfering substances for nerve growth factor (NGF) dependent neurite formation in rat PC-12 pheochromocytoma cells. PC-12 cells are also known to extend neurites in response to N^6, O^2 dibutyryl adenosine 3' 5' cyclic monophosphate (Bt_2 cAMP), but the mechanisms of neurite induction by NGF and Bt_2 cAMP were reported to be different (Gunning et al, J. Cell Biol. 29, 240-245, 1981). In the present study we have examined the influences of adult mouse saliva (S) and SMG extracts (SMGE) on the neurite outgrowth responses provoked by NGF and/or Bt_2 cAMP.

Cells were cultured at a density of 1×10^4 cells/ml in the incubation medium containing RPMI-1640 buffer supplemented with 3% horse serum. Saliva (5 µl/dish), SMGE (equivalent to 16 mg wet weight/dish), SMG-NGF (600 ng/dish) and Bt_2 cAMP (final concentration 1 mM) were added to culture dishes containing 2 ml cell suspensions. Saliva and SMGE of both male and female mice were added at similar concentrations. Cells were incubated in 6-well plastic culture dishes at 37°C and the neurite responses were recorded at 48 hr. RESULTS:

	PC-12 NEURITE RESPONSES IN THE	
TREATMENT	ABSENCE OF S OR SMGE	PRESENCE OF S OR SMGE
NONE	NEGATIVE	NEGATIVE
SMG-NGF	POSITIVE	NEGATIVE
Bt_2 cAMP	POSITIVE	NEGATIVE
SMG-NGF and Bt_2 cAMP	POSITIVE	NEGATIVE

Neither saliva or SMG extracts of adult male and female mice stimulated neurite formation in PC-12 cells. In addition, they prevented the neurite formation induced by SMG-NGF and Bt_2 cAMP. However, at hundred-fold lower concentrations neither of them prevented the neurite formation. CONCLUSION: The results of this study indicate that mouse saliva and SMG extract contain factors that prevent neurite formation by both transcription-dependent (NGF) and transcription-independent (Bt_2 cAMP) mechanisms.

303.12 HUMAN AMNION MEMBRANE PROMOTES AXONAL GROWTH IN THE ADULT RAT CENTRAL NERVOUS SYSTEM. M. Manthorpe, G.E. Davis*[1], E. Engvall*[3], S. Varon[1] and F.H. Gage[2]. Depts. of [1]Biology and [2]Neuroscience, Univ. of California San Diego, La Jolla, CA 92093 and [2]La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The acellular human amnion basement membrane surface is a potent promoter of axonal outgrowth from cultured peripheral and central nervous system neurons (see adjacent abstract by G.E. Davis et al). Here we have explored the potential usefulness of this membrane as a bridging material for the regeneration of axotomized adult rat central nervous system neurons.

The septo-hippocampal system was employed as an in vivo model for assessing the growth on the amnion membrane of regenerating or sprouting axons. Prior to grafting in brain, the membrane was coiled and cut into short segments. Following complete unilateral aspirative transection of the fimbria-fornix, the amnion graft was placed directly into the transection cavity. At 2, 4, 6 and 8 weeks post-transection and -grafting, the brains of the animals were evaluated using a variety of histological methods.

The results indicate that i) the grafts apparently remained intact and there was no histological indications of immunological rejection in the host brain, ii) acetylcholinesterase (AChE) and neurofilament antibody-stained fibers were present on the amnion membrane and could be identified as arising from the septal side of the fimbria-fornix lesion, iii) many more AChE-positive fibers were observed in the denervated hippocampus of those animals receiving amnion grafts than of control animals and iv) glial cells from the host brain migrated extensively upon the amnion membranes. These results suggest that the grafted acellular human amnion membrane can serve as a terrain for regenerating or sprouting central nervous system axons.

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303.13 HUMAN AMNION BASEMENT MEMBRANE PROMOTES AND GUIDES NEURITE OUTGROWTH IN VITRO. G.E. Davis*, E. Engvall*[1], S. Varon and M. Manthorpe. Dept. of Biology, Univ. of Calif. San Diego, La Jolla, CA 92093 and [1]La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Basement membranes are known to contain signals which affect cell behaviors such as adhesion, migration, differentiation and proliferation. The major identified proteins of basement membranes are laminin, collagen type IV, heparan sulfate proteoglycans and entactin. Our interest has been whether such proteins influence neuronal cell behaviors. Indeed, we and others have reported that purified laminin and laminin-proteoglycan complexes promote robust neurite extension from cultured peripheral and central neurons. In this study, we have addressed the question whether an in vivo-constructed basement membrane possesses neurite promoting activity. The model basement membrane utilized is a previously described acellular human amnion membrane preparation (Liotta et al., Cancer Lett. 11: 141-152, 1980) which contains an intact basement membrane surface and a collagenous stromal surface.

Amnion membranes were rendered acellular by removing the columnar epithelial cell layer with ammonium hydroxide and gentle brushing. The membranes were then cut into small squares and each square anchored to nitrocellulose paper such that either the basement membrane surface (the side to which the epithelial cells were orginally attached) or stromal surface was presented up. Alternatively, the membrane was folded, frozen, cross-sectioned and the sections anchored to nitrocellulose paper. Dissociated neurons from 8-day embryonic ciliary (CG) or dorsal root (DRG) ganglia or 18-day fetal rat hippocampus (HIPP) were seeded onto the membranes for up to 4 days in vitro in a serum-free or serum-supplemented media.

In serum-free media all three neuronal populations attached to either the basement membrane or stromal surface but grew extensive neurites only on the basement membrane side (neurons were visualized using an immunoperoxidase stain with a monoclonal antibody to neurofilaments). In the presence of 10% fetal calf serum, neurite growth was found to be inhibited from CG but not from DRG or HIPP neurons. When cross-sectioned amnion membranes were seeded with CG neurons in serum-free medium, the neurons attached uniformly to both the paper and the sections but grew extensive neurites preferentially along pathways which stain using anti-laminin immunofluorescence histochemistry. These data demonstrate that the human amnion basement membrane can provide both neurite promoting signals and neuritic guidance cues. Thus, the acellular human amnion membrane represents a novel and interesting substratum for the culture of neural cells and potentially could be used as neurite promoting bridging material for in vivo regeneration studies. Supported by NSF grant BNS 85-02198 and NIH grant AM 30051.

303.14 MULTIPLE NEURITE-PROMOTING FACTORS IN CONDITIONED MEDIA DERIVED FROM DIFFERENT CNS GLIAL CELL POPULATIONS. L.K. McLoon and S.C. McLoon. Depts. of Ophthal. and Cell Biology and Neuroanat., Univ. of Minn., Minneapolis, MN 55455.

Conditioned media derived from a variety of different vertebrate tissues have been described as having neurite promoting activity from a number of neuronal types (Adler et al.,'81,Dev. Biol.; Lander et al.,'82,J.Cell Biol.). We have examined the ability of conditioned media prepared from glial monolayers derived from either postnatal day 7 (P7) optic nerve (ONCM) or P7 cerebral cortex (CCCM) to promote neurite outgrowth from embryonic tectum in vitro. Monolayer cultures of glia from P7 cerebral cortex or P7 optic nerve were prepared according to standard procedures. Two days prior to use conditioned media were prepared by incubating the monolayer cultures in a defined medium. Tecta were dissected from rat fetuses on embryonic day 14 or 15 and placed in explant culture on polylysine coated dishes. Explant cultures were incubated in defined media, ONCM or CCCM, from both short-term and long-term cultures. Media was replaced with fresh conditioned media every two days. Neurite outgrowth was assessed every day for one week. When explants were grown in the presence of non-conditioned defined medium, neurites formed but the neurites were relatively short. Tectal explants treated with ONCM responded with rapid and extensive neurite outgrowth. Tectal explants did not, however, adhere to monolayers of glial cells derived from P7 optic nerve. When the ONCM was serially passaged to remove any substrate-adsorbable factor, the neurite outgrowth promoting activity was unchanged; neurite outgrowth was still rapid and extensive. Tectal explants cultured in the presence of CCCM responded rapidly with extensive neurite outgrowth. Neurite outgrowth from the tectal explants in the presence of CCCM from short-term glial cultures was markedly different from that seen in the presence of CCCM from long-term cultures, with the former resulting in more convoluted and dense networks of neurites. Tectal explants adhered well to monolayers of glial cells from P7 cerebral cortex. After the serial passaging of the CCCM, the neurite promoting activity was no longer present in the media. Neurite outgrowth from the explants resembled that seen in the presence of defined media alone. The neurite promoting activity of both the ONCM and the CCCM was inactivated by treatment with trypsin and by heat treatment to 80 °C. It appears that there are factors present in glial conditioned media that have the ability to promote neurite outgrowth from embryonic tectal explants in vitro. It is also clear from the serial passaging results that differences exist in the neurite-promoting factors contained in the conditioned media produced by the two different glial populations. (Supported by NIH grant EY05432.)

303.15 AN *IN VITRO* BIOASSAY FOR NEURITE-PROMOTING MOLECULES THAT MAY BE ACTIVE DURING EMBRYONIC DEVELOPMENT *IN VIVO* R. Nishi. Dept. of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115

Observations of neurite outgrowth during embryonic development *in vivo* indicate that local cues have a powerful influence in directing neurite outgrowth. Studies *in vitro* have helped to elucidate what mechanisms may be used by neurons to recognize these local cues. For example, regenerating neurites have the capacity to follow artificially produced patterns of substrate molecules. Furthermore, potential candidates for substrate molecules *in vivo* have been identified through systematically assaying the ability of purified molecules to induce neurite outgrowth when immobilized to plastic or glass. However, it is not clear what relevance the cues identified *in vitro* have to the actual cues *in vivo* that neurites utilize. In order to test whether such candidate molecules may be active *in vivo*, and to identify others with similar activities I designed an *in vitro* bioassay that tests the ability of antibodies to block neurite-promoting molecules associated with the natural substrate of embryonic mouse submandibular ganglion neurons, the salivary gland epithelium. The bioassay is based upon the observation first reported by M. Coughlin (1975, Dev. Biol. 43: 140) that patterned neurite outgrowth of submandibular ganglion neurons is induced by the developing submandibular salivary gland epithelium and associated basal lamina, even when they are lightly fixed and hence metabolically dead (Coughlin & Rathbone, 1977, Dev. Biol. 61:131).

Conventional tissue culture techniques were modified to facilitate the routine manipulation of large numbers of embryonic explants. The primary modification was to eliminate the need for small rafts or hanging drops by placing explants in the center of 35 mm tissue culture dishes in a very small amount of medium (.25 ml) to ensure that the developing rudiments were always near an air-water interface. Three to 4 explants were typically placed in one dish. The growth of explants was readily monitored with an inverted microscope. Salivary gland rudiments were removed from E.13.5 day CD-1 mice. To exclude the submandibular ganglion from the explant only the distal tip of the rudiment including the cap mesenchyme and one epithelial lobe was removed and cultured (the ganglion normally sits close to the stalk of the epithelial structure). Within 5 days the aganglionic epithelia had undergone extensive morphogenesis and generated a complex budded structure. After 6 days test epithelia were incubated with antibodies for 2-3 hrs at 37°C, washed, and fixed for 15 min with 2% paraformaldehyde. After extensive washing the fixed epithelia were recombined with freshly isolated submandibular ganglia from E13.25 day embryos. Combined explant cultures were fixed 2 days later and processed for the Karnovsky-Roots histochemical technique for visualizing AChE in order to assess the extent of fiber outgrowth.

Under these conditions: 1). the extent of neurite outgrowth on fixed epithelia was comparable to that on living epithelia; 2) another epithelium, that of the embryonic lung, also stimulated neurite outgrowth if the overlying mesenchyme was removed; 3) a rabbit antiserum directed against mouse laminin had no effect at dilutions up to 1:50. The ability of other anitbodies to block neurite outgrowth in this assay is currently being examined.

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303.16 CYCLIC AMP INDUCES RAPID CHANGES IN ORGANELLE DISTRIBUTION AND TRANSPORT IN GROWTH CONES OF APLYSIA BAG CELL NEURONS. Paul Forscher, Leonard K. Kaczmarek, JoAnn Buchanan* and Stephen J Smith*. Section of Molecular Neurobiology and Dept. of Pharmacology, Yale School of Medicine, New Haven, CT 06501

We have used bag cell neurons to examine structural and molecular events involved in neurite outgrowth and secretory granule transport with video DIC microscopy and digital image analysis techniques. Release of neuroactive peptides from bag cells is accompanied by elevation of cAMP which has been shown to modulate voltage-dependent K channels, increase the Ca component of the action potential and stimulate peptide synthesis. We have studied dissociated bag cells in vitro to test whether acute changes in cAMP induce changes in growth cone motility or structure. Under control conditions axoplasmic transport is observable in real time. Spherical organelles likely to be 200 nM neurosecretory granules are transported on linear trajectories at rates of 1-2 uM/sec from the axon shaft into the base of extending growth cones. Microtubules probably provide the substrate for this organelle transport (Schnapp et al (1985),Cell 40:455-462). The distal edge of a growing cone is typically a granule-free lamella displaying a distinctive retrograde wavelike motion as the cone advances. Application of forskolin (20-50 uM) and a phosphodiesterase (PDE) inhibitor (20-50uM RO-20-1724 or 1-2mM theophylline) at concentrations known to induce changes in bag cell protein phoshorylation resulted in: 1) rapid extension of granule transport into lamellae, 2) apparent increased rate of granule transport and 3) cessation of growth cone extension and retrograde wave activity. These changes effected transformation of lamellae into neurite endings packed with microtubules and granules. Such transformations were reversible, dose dependent, potentiated by PDE inhibitors and mimicked by 6-N-butyl-8-benzylthio-cAMP. Preliminary results suggest these effects do not involve influx of external Ca as they persist in Ca-free/EGTA solutions. In contrast, acute exposure to depolarizing high K/Ca solutions resulted in several rapid Ca-dependent effects on growth cones distinct from those mediated by cAMP: 1) rapid extension of filopodia, 2) rapid disappearance of granules and 3) disassembly of putative microtubule arrays. The induced changes in growth cone morphology we report here suggest a possible role for cAMP dependent protein phosphorylation in promoting differentiation of growth cones into neurosecretory endings. In addition, increased cAMP levels during bag cell activation may facilitate peptide release by causing secretory granules to be transported physically closer to their release sites at faster rates.

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303.17 FILOPODIA ADHESION MEDIATED BY EXTENDED FILAMENTOUS STRUCTURES OF ADHERON MOLECULES. H-C. T. Tsui and W.L. Klein. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 and Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Adhesive contacts made by filopodia of neuronal growth cones are very likely to be important in neurite growth and in the formation of synaptic junctions. Previous examination of cultured chick retina neurons with video-enhanced contrast differential interference contrast light microscopy has shown that filopodia frequently attach to one another at their tips and remain attached despite great motile activity. The linked motility of the attached filopodia occurs even when apparent gaps exist between filopodia. Examination of these gaps with the electron microscope revealed that the apposed membranes are connected by filamentous structures 10 to 13 nm in diameter and 30 to 100 nm in length (PNAS 82:8256-8260).

In order to understand the adhesive properties of filopodia at the molecular level, we have studied the distribution of N-CAM and adheron molecules on growth cones and filopodia. Live or fixed cultured chick retina neurons were labeled with the immunogold method, prepared with the critical point dried procedure and examined as whole mounts with the electron microscope.

Labeling done on fixed cells showed that both N-CAM and adheron molecules were present on growth cones and filopodia. However, there were two major differences in the labeling patterns of the two antibodies. First, in live cell labeling conditions, N-CAM molecules were frequently found to patch at the neurites or at the middle of growth cones. In contrast, adheron molecules were found to concentrate at the edges of growth cones, at filopodia where adhesion to the substrate occurred, and at areas where filopodia contacted each other. Second, filamentous structures that connected filopodia membranes to the substrate or to other filopodia were labeled with antibodies against adheron, but not with antibodies against N-CAM. Our observations suggest that adheron molecules may be present as filamentous structures extending from the membrane and that they are involved in filopodia adhesion mechanisms.

(Supported by NIH grant NS21088)

303.18 TRANSIENT D1 RECEPTOR MEDIATES DOPAMINE INHIBITION OF GROWTH CONE MOTILITY AND NEURITE OUTGROWTH IN A SUBSET OF VERTEBRATE CNS NEURONS. K. L. Lankford*, F. G. DeMello* and W. L. Klein. (Spon: B. Menco) Dept. Neurobiology & Physiology, Northwestern University, Evanston Il. 60201.

Although dopamine and serotonin have been shown to selectively inhibit growth cone motility in specific neurons of the snail Helisoma, (McCobb et al, Soc. Neurosci. Abstr. 11: 761 1985, Haydon et al, Science 226: 561, 1984), little is known about the effects of neurotransmitters on the development of neurons in higher animals. To investigate the possible developmental significance of a transiently expressed embryonic form of the D1 dopamine receptor in the chick retina (Ventura et al, Dev. Brain Res. 12: 217, 1984), we exposed cultured neurons to dopamine and examined both long term and short term effects on morphological differentiation. Neurite numbers were reduced in cultures exposed to 1 uM dopamine for one day, but not in control cultures exposed to dopamine and 1 uM haloperidol. To investigate the phenomenon further, neurons were cultured for 1-5 days on glass coverslips, which were then attached to special chambers, and monitored continuously with video microscopy through several changes of control and experimental media. At each age tested, a subset of neurons responded to dopamine with a reduction in filopodial activity and/or neurite retraction. Changes in filopodial activity were apparent almost immediately after dopamine addition, and neurites could shorten as much as 75% within 15 minutes. The response to dopamine appeared to vary somewhat with age, and neurite retraction was observed predominantly in younger cultures. The effects of dopamine were reversed by haloperidol. Recovery from dopamine stimulation was typically slower than the onset of dopamine response and the rate of recovery decreased with longer dopamine exposures. Experiments to assess the mechanism of dopamine action have shown that forskolin mimicked the effects of dopamine, indicating that the dopamine effect may be mediated by cAMP elevation. Our data show that dopamine exerts a powerful inhibitory effect on growth cone activity and neurite outgrowth in a subset of retina neurons, and suggest that this and other neurotransmitters may play important roles in the morphological differentiation of CNS neurons.

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303.19 PROTEASE INHIBITION CAN INFLUENCE THE DIRECTION OF NEURITE OUTGROWTH BY NEONATAL MOUSE SENSORY GANGLIA. R.L. Hawkins* and N.W. Seeds. Department of Biochemistry, Biophysics & Genetics, University of Colorado Medical Center, Denver, CO 80262

We have previously observed that neonatal mouse dorsal root ganglia (DRG) in vitro secrete proteases and that addition of soybean trypsin inhibitor (STI) or other protease inhibitors to the medium can enhance the degree of neurite outgrowth from DRG explants. The current work examines whether STI can preferentially determine the direction of outgrowth. Two methods have been used that allow DRG a choice between growing towards or away from a source of STI. In the first method, polyacrylamide gels are cast to form inner and outer chambers from which test substances can diffuse towards ganglia placed inbetween the wells. We have observed a greater extent of neurite outgrowth towards a well containing STI at 10 ug/ml in serum-free medium (N2) than towards a well containing N2 alone. In the second method, STI is immobilized on the substratum. Glass coverslips are treated with 3-aminopropyl-triethoxy silane, bovine serum albumin (BSA) is then bound to the slip via glutaraldehyde, and then STI is gently coupled to one-half of the slip with carbodiimide while the other half is treated further with BSA. We have demonstrated that the STI retains its protease inhibitory activity and that neurite outgrowth from DRG placed in the center of the derivatized coverslip is preferentially oriented towards the STI-treated side. These results provide further confirmation that proteases and protease inhibitors can regulate neurite outgrowth.

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303.20 **SEROTONIN INHIBITION OF GROWTH CONE MOTILITY IS BLOCKED BY ACETYLCHOLINE.** D.P.McCobb and S.B.Kater. Dept. of Biology, University of Iowa, Iowa City, IA 52242 and Program in Neuronal Growth and Development, Colorado State Univ., Fort Collins, CO 80523.

The electrical activity of a neuron is under the control of a combination of excitatory, inhibitory, and modulatory inputs. The present study suggests an analogous combinatorial control of the motile activity of neuronal growth cones. The classical neurotransmitter serotonin (5-HT) was recently shown to inhibit the motility of growth cones of specific identified *Helisoma* neurons in cell culture. (Haydon et al, Science 226:561, 1984). We now report that the presence of a second neurotransmitter, acetylcholine (Ach) negates the inhibitory action of 5-HT on the motility of growth cones.

Cell bodies of neuron 19 were dissected from buccal ganglia and placed individually into cell culture. Growth cones were photographed at 20 minute intervals for 2 h. before and 2 h. after the addition of either 5-HT alone (final concentration = 1μM), Ach alone (final concentration = 50μM), or Ach followed in four minutes by 5-HT. Advance of growth cones was measured in reference to fixed points on the substratum.

Consistent with previous studies, the progress of motile growth cones of neuron 19 was arrested upon addition of 5-HT to the dish, and filopodia and lamellipodia were retracted into a club-like terminal. By contrast, growth cones treated with Ach just prior to addition of 5-HT were not arrested, but continued to grow, and maintained their growth cone-like structure. Mean post-treatment rates of neurite elongation, expressed as a percentage of pre-treatment rates (± S.E.), were : -2.6 ± 10.6 % for 5-HT treated, and 103.7 ± 12.0 % for Ach + 5-HT treated growth cones . Ach alone had no effect on growth cone motility.

The study of the combined actions of 5-HT and Ach on growth cone motility was motivated by the finding that the electrophysiological actions of these transmitters on neuron 19 are opposing. 5-HT alone has a sustained excitatory effect, while Ach has an inhibitory effect on electrical activity. In the presence of Ach, the excitatory effect of 5-HT is much reduced. While there are many possible mechanisms whereby Ach might oppose the inhibitory action of 5-HT on neurite outgrowth, e.g. they might have opposing actions on a second messenger system, the possibility that the electrophysiological actions of these two transmitters are causally related to their actions on outgrowth is intriguing. Action potential generation has been directly shown to inhibit the motility of growth cones of *Helisoma* neurons in cell culture (Cohan and Kater, Science, 1986 ,in press). Thus the nature of the actions of 5-HT and Ach on neurite outgrowth is consistent with the hypothesis that these actions derive from the electrophysiological effects of these transmitters. A more quantitative study of the relationship is in progress.

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DEVELOPMENT AND PLASTICITY: MOTOR SYSTEMS

304.1 DEVELOPMENT OF HINDLIMB NEUROMUSCULAR PATTERNS IN KITTENS: SCRATCHING IN NORMALS, PAW SHAKING IN NORMALS AND SPINALS. N.S. Bradley and J.L. Smith, Dept. Kinesiology, UCLA, Los Angeles, CA 90024

Although, the onset of various motor behaviors in neonatal kittens has been described, little is known about the development of neuromuscular patterns. Last year, we described the development of hindlimb locomotor patterns in normal kittens and kittens spinalized (T-12) at birth (1-d) or 2 weeks (2-wk) of age (*Soc. Neurosci. Abstr.* 11:1985). We now describe the development of neuromuscular patterns for scratching and the paw-shake response (PSR) in the hindlimb of these animals. Muscles were chronically implanted at birth and the implants maintained throughout the 2-mo testing period. Results were based on 12 kittens, 4 per group. Scratching was elicited by placing tape in the ear canal in normals, but was not tested for in spinals as the stimulus is ineffective in eliciting a response caudal to the transection. To elicit PSRs, tape was applied to the plantar pad. Scratching onset in normal kittens was 21±2 days, while PSR onset in spinals (1-d=14±3 days; 2-wk =16±2 days) preceded onset in normals (26±4 days).

Normals. While maturation of scratching and the PSR in normal kittens was characterized by changes in posture and integration with other behaviors, neuromuscular patterns varied little during the 2 mo and were similar to those in normal adult cats. Scratching bouts averaged 20±4 cycles at a frequency of 5 Hz and was characterized by coactivation of the ankle extensor, lateral gastrocnemius (LG), and knee extensor, vastus lateralis (VL), reciprocally active with the ankle flexor, tibialis anterior (TA). Average burst durations (53-61% cycle) were similar for all muscles. Initial PSRs averaged 4±1 cycles (increasing to 5±1 at 2 mo) at a frequency of 10 Hz and consecutive cycle durations were linearly correlated. The TA and VL were coactive near 60% of the LG cycle, and burst durations were shorter for extensors (VL=34±13; LG=29±6 ms) than the flexor (TA=60±7 ms). Initially, there was also a VL burst coactive with TA activity.

Spinals. Although the hindlimb PSR was elicited at an earlier age in spinal kittens, initial PSRs were shorter (3±1 cycles) and slower (9 Hz) than initial PSRs in normal kittens. Also, a single VL burst was frequently coactive with LG rather than TA activity. By the end of the 2-mo period, cycle frequency increased to 10 Hz, but consecutive cycle periods were unrelated, suggesting that the temporal structure of the response was not mature. Further, the pairing of TA and VL activity during PSRs did not develop, and VL activity was particularly erratic.

Conclusions. The data suggest earliest scratching and PSRs in normal kittens were characterized by adult-like neuromuscular patterns. While spinalization accelerated PSR onset in spinal kittens, some features of the pattern were abnormal and failed to mature during the 2-mo period. It is not known whether the altered neuromuscular patterns resulted from spinalization-induced changes in the lumbosacral networks that produce PSRs, *or* altered limb dynamics during the response. Perturbation of normal limb afference during PSR in adult spinal cats (Cox & Smith, *Soc. Neurosci. Abstr.* 10:1983) specifically disrupts VL recruitment. While the limb dynamics of PSRs in kittens are not known, the pendent hindlimb posture during PSRs in spinal kittens may contribute to altered limb dynamics and warrants further study. Supported by NIH grant NS 19864.

304.2 INTRACELLULAR RECORDINGS FROM EMBRYONIC CHICK MOTONEURONS DURING SPONTANEOUS AND EVOKED MOTOR ACTIVITY. M.J. O'Donovan. Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242

The isolated spinal cord of the chick embryo generates spontaneous motor activity in vitro. Extensor motoneurons discharge early during each cycle whereas flexor motoneurons fire at the beginning of the cycle and again later in the cycle. Intracellular recordings were obtained from antidromically identified motoneurons during motor activity in 15 day embryos to analyze the mechanisms controlling motoneuron firing. We also used high gain DC recordings from the ventral roots and muscle nerves to monitor the time course of membrane potential changes from populations of motoneurons. Stable penetrations revealed spikes with an amplitude between 30–40 mV and duration 2–5 ms. In some cells the action potential was made up of two components, a fast initial component and a slower component that disappeared during perfusion with low calcium solutions. During each cycle the membrane potential was depolarized by up to 30 mV for a varying fraction of the cycle. Motoneurons discharged on the rising phase of the step and again after a delay (which was short in extensors). The amplitude of the step increased with hyperpolarizing current injection and was reduced by depolarizing currents, suggesting it was mediated by excitatory synapses. The membrane trajectory during the delay remained flat without evident hyperpolarization. We are currently establishing if the delay is the result of synaptic or intrinsic motoneuron properties. DC recordings from flexor and extensor muscle nerves during motor activity were positive going and similar in form suggesting that antagonist motoneurons may share a common excitatory drive.

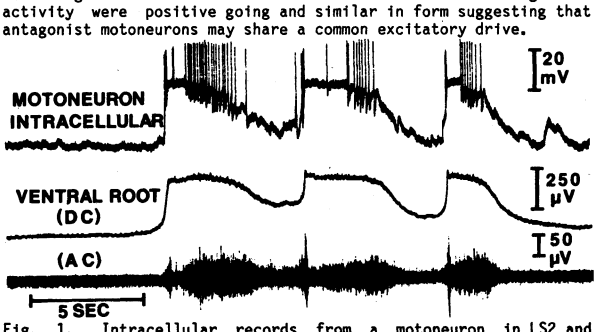

Fig. 1. Intracellular records from a motoneuron in LS2 and simultaneous DC recording from LS2 ventral root during spontaneous activity. Supported by the NSF (BNS-8402838).

304.3 DOES SYNAPSE ELIMINATION SHAPE NEUROMUSCULAR COMPARTMENTS?
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Mammalian skeletal muscles are partitioned into smaller elements which consist of collections of motor units. In the ankle extensor muscles the elements are organized about the primary branches of the muscle nerves and are known as neuromuscular compartments. At birth, the well-known polyneuronal innervation of muscle fibers dictates that individual motor units are relatively larger than adults. The subsequent loss of synapses might serve to define neuromuscular compartments if the original motor unit innervation territories included more than one such partition. In the rat lateral gastrocnemius (Donahue and English, Amer. Zool. 25:107A, 1985) and extensor digitorum longus (Balice-Gordon and Thompson, Soc. Neurosci. Abstr. 11:101, 1985) the available evidence has been against this hypothesis, but in the rat gluteus maximus muscle (GM), Brown and Booth (Nature 304:741-742, 1983) have described a marked postnatal decrease in the size of the innervation territories of individual motor units. The present study has investigated the innervation patterns of the compartments of GM in adult (>P17) and neonatal (P0-P7) rats to determine whether this reduction in motor unit size serves to define its compartments. Both the superior gluteal nerve (SGN) and the inferior gluteal nerve (IGN) innervate GM. Each nerve contains three primary branches which innervate the six elements or neuromuscular compartments found in GM. Using evoked EMG activity, the innervation patterns of these compartments was mapped onto the surface of the muscle. Each compartment occupies 30-40% of the innervation territory of its respective muscle nerve, a value in the same range as reported for single motor units in neonatal rats by Brown and Booth. The caudal-most compartment of the SGN is almost entirely overlapped by the rostral-most element of the IGN. Analysis of the distribution of muscle fibers of different histochemical types and of muscle spindles along with reconstruction of the SGN and IGN branching territories from serial histological sections of the muscle confirm these physiological findings. In neonatal rats, largely the same compartmental innervation patterns were noted, using both anatomical and physiological techniques. Thus the boundaries of the neuromuscular compartments of GM are well established at birth. Synapse elimination may change the distribution of innervation of some motor units within compartments, but it is not a process which forms the compartments. Supported by grant NS20545 from the USPHS.

304.4 ONTOGENY AND SEXUAL DIMORPHISM OF FAST TOADFISH SONIC MUSCLE.
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The Oyster toadfish (Opsanus tau) produces sounds by simultaneously contracting fast muscles on either side of its swimbladder. Previous work has demonstrated that the combined swimbladder sonic-muscle complex grows larger in males than in females and that growth is primarily under androgenic control. This study quantified growth of the sonic muscle and examined its morphological basis by counting and measuring muscle fibers.

Muscles grew larger in males than in females and comprised approximately 60% of the organ weight in all sized individuals of both sexes. Muscles in large males weighed 7-9 gm compared to 3-4 gm in large females. Muscle fibers, examined in grids on a lateral transect, increased in number and decreased in size toward the outside, suggesting the addition of new cells. Also, fibers at the periphery of fascicles were generally smaller than ones toward the center. The number of muscle fibers increased almost four fold (132,000 to 489,000) as fish grew. There was an average of 38.6% more fibers in males than females, which agreed with a 39.6% greater cross-sectional area in males. Fiber density was similar between the sexes. Average minimum diameter of fibers increased from 16 um to 28.6 um. In fish of comparable sizes, females had larger muscle fibers than males, and androgen implants decreased fiber diameter in females. Individual fibers contained sarcoplasmic pockets, and some fibers had myofibrils separated into fragments. Within a fish, larger fibers tended to have more pockets and fragments. Yet the mean number of pockets and fragments per cell (1.9 and 1.6 respectively) did not increase in larger fish which had larger fibers. This finding and microscopic examination of muscles suggest that fragmentation results in cell division. Further, multiple pockets and fragments were evident in smaller sized cells in the actively growing outside portion of the muscle.

In summary sonic muscles grew by adding new fibers and fiber hypertrophy. Muscles in males were larger and had more fibers than in females although their cells were smaller. We propose fragmentation as one mechanism of fiber addition. The smaller cells of males and the existence of multiple pockets are interpreted as a mechanism for increased contraction speed and fatigue resistance. Supported by NIH grant MH38921

304.5 DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF MOTOR CORTICAL TERMINATIONS IN THE KITTEN SPINAL CORD.
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Electrophysiological studies have shown a lead-lag relationship of approximately 20 days between the appearance of microstimulation-evoked motor cortical (MC) output to limb musculature [at 40 days postnatal (dPN)], and the development of mechanoreceptor input to the MC (at 64 dPN) and raise the question of whether the sequential occurrence of these events is dependent upon the completion of spinal synapses (Bruce & Tatton, 1980a,b; 1981). In this regard, morphometric analysis of developing α-motoneurons has shown that dendritic processes only begin to increase to adult calibers by about 40 dPN despite the fact that the distribution of somal diameters is already within the adult range by 20 dPN (Tatton et al., 1983; 1984). In the present study we examined the anatomical distribution of MC axons in the dorsolateral columns (DLC) and their pattern of terminations in the grey matter of the developing kitten spinal cord. Discrete unilateral injections of WGA-HRP were made into the fore- and hindlimb areas of the pericruciate MC in 14 time-mated kittens aged 16 to 74 dPN (81-139 gestational days) and 3 adult controls. The animals were sacrified and the injection site and portions of the cervical, thoracic and lumbar cord were serially sectioned and reacted for HRP histochemistry (Itoh, 1979). Camera lucida drawings were made using a standardized 2-D grid, and the number and distribution of labelled axons in the DLC and gray matter were calculated for all three segmental levels in each animal. Total MC axon populations for each animal, determined by counts of the DLC in segments just anterior to the brachial and lumbar enlargements, provided baseline values for intersegmental and inter-animal comparisons. These distributions were presented in individual 3-D histograms superimposed onto camera lucida grids representing the spinal segments. The results show that during the first 6 weeks of postnatal life there appears to be an overall reduction (about 20%) of MC axons in the contralateral DLC. Importantly, there is a remarkable redistribution of the initially widespread and bilateral pattern of MC terminations in the grey matter. In young kittens MC axons appeared to project bilaterally within a given segment of the spinal cord, occupying approximately 48% of the ipsilateral gray matter and 56% of the contralateral gray. By 40-45 dPN an adult-like distribution was apparent in all levels of the spinal cord where MC terminations became largely restricted to the medial portion of laminae IV through VII. This period of MC terminal redistribution coincides with the dramatic maturation of the α-motoneuron dendritic tree in the same spinal segments and with the onset of effective MC microstimulation in eliciting limb muscle responses. These observations suggest that the establishment and redistribution of MC input to the cervical and lumbar segments and the shaping of the mature pattern of dendritic arborization of α-motoneurons may be involved in the refined control of limb movements observed at that time. Supported by MRC grant MT5 218.

304.6 ROLES OF CELL DEATH AND AXONAL PRUNING IN POSTNATAL SHAPING OF CORTICOSPINAL CELL DISTRIBUTIONS IN THE RAT.
D.R. Humphrey, D. Bulyalert*, and D.J. Reed. Lab. of Neurophysiology, Emory Univ. Sch. Med., Atlanta, GA 30322.

On the seventh day postnatally, corticospinal (CS) neurons that are labeled by cervical cord injections of retrogradely transported tracers are distributed as a continuous sheet within lamina V of the frontal, parietal and cingulate areas of the rat cerebral cortex. By postnatal day 21, however, injections of tracer reveal three 'islands' of cells, located within frontal, dorsal parietal and ventrolateral parietal regions, respectively (Schreyer and Jones, Neurosci. Abstr., 11: 988). Thus, the corticospinal neurons that were within the gaps which separate these islands have died, their spinal axons have been pruned, or, for some reason, they cannot be labeled.

To investigate the role of these factors in shaping the early postnatal distribution of CS cells, we placed small gelfoam pledgelets saturated with a fluorescent dye (2 % True Blue or Fast Blue) into the left, transected corticospinal tract at the C_4 level of the cord in 5 to 6 day old rats. The animals were then sacrificed on postnatal days (PNDs) 9, 14 or 21, and coronal or parasagittal sections of the cerebral hemispheres were prepared for visualization of retrogradely labeled neurons. Our major results to date, from 14 animals, are as follows.

(1) On PND 9, the labeled cells form a nearly continuous distribution, as described above. (2) By PND 14, however, a clear gap containing no labeled neurons is seen in the lateral parietal cortex, between a dorsal frontoparietal island of cells and a ventrolateral island. (3) No further gaps in the cell distribution were seen in animals surviving to PND 21.

These results suggest that the lateral parietal gap occurs because of the death of corticospinal neurons in that region, whereas the gap which is seen with injections of tracer after PND 20 at the dorsal border between frontal and parietal areas results from axonal pruning or some other mechanism. Thus, the distribution of corticospinal neurons in the adult rat appears to be formed by both cell death and axonal pruning, with each factor operating principally in a different cortical region. (Supported by NIH Grant NS 20146).

304.7 DEVELOPMENT OF MOTOR CORTEX EXCITABILITY IN NORMAL AND IN NEONATALLY BRAIN INJURED RATS. M. Cox* and D.R. Humphrey, Lab. of Neurophysiology, Emory Sch. Medicine, Atlanta, GA 30322.

In the developing rat, corticospinal tract (CST) axons can be seen at the light microscopic level to enter the spinal gray matter at two weeks of age, though no CST synapses are seen at that time at the electron microscopic level (Schreyer and Jones, J. Neurosci., 7: 1837, 1982). Very little is known, however, about the development of functional transmission through the corticospinal-muscle pathway in the developing animal. To examine this process, we used intracortical microstimulation (ICMS) methods to examine the development of excitability within the general forelimb region of the motor cortex in rats 2 to 10 weeks of age. Maps were obtained under ketamine anesthesia (60 mg/Kg) from both normal animals (N=13) and from the surviving cortex of animals subjected to unilateral motor cortex ablation on postnatal day 1 (N=12). Maps were obtained with both microelectrodes (20-35 um tip length) and macroelectrodes (200 um tip length), using the following current parameters: 0.2 msec current pulses, 30 pulse train, 300 pps, with intensities up to 80 uA (microelectrode) or 150 uA (macroelectrode).

Our major results were as follows. (1) No evoked movements were observed at two weeks of age. (2) By three weeks of age, some movements were evoked in both normal and lesioned animals with the macroelectrode. (3) By four weeks of age, some evoked movements were obtained with the microelectrode, and a larger set with the macroelectrode. (4) By six weeks of age, the forelimb motor map was as extensive as that seen in the adult, in both normal and lesioned animals, and thresholds were in the range that is effective in adults. (5) At six weeks, the map has an adult-like somatotopy, with two exceptions. First, not all normal animals developed the two forelimb zones seen in many adult rats, and the neonatally lesioned animals never developed two such zones. Second, the development of these two zones occurred in some cases after 6 weeks of age. (6) Ipsilateral movements were evoked in both normal and neonatally lesioned animals, but they did not appear before 8 weeks of age in the normal animal and 6 weeks of age in the lesioned animal.

Thus, the motor cortex map first 'appears' in the developing rat at 3 weeks postnatally, and resembles the adult map by 6 weeks. Other research in our laboratory has shown that all such evoked responses are mediated by CST axons. Thus, the ontogenetic increase in the size of the map and the decreasing current thresholds for evoked movement suggest that CST synapses are forming during this period, or increasing their functional efficiency. (Supported by NIH Grant NS20146 to DH, and an NSF Graduate Fellowship to MC).

304.8 THE DEVELOPMENT OF BULBOSPINAL PROJECTIONS IN THE CHICKEN EMBRYO. J.C. Glover, G. Petursdottir* and J.K.S. Jansen*. Physiology Institute, University of Oslo, Oslo, Norway.

We used retrograde axonal tracing in in vitro preparations of the chicken embryo brainstem to chronicle the formation of bulbospinal tracts and define their parent cell groups at different embryonic stages. The in vitro preparations allowed us to very precisely localize injections of tracers. We used two types of experiments to visualize the projection patterns of the bulbospinal groups: 1) injections of HRP into specific tracts on one side of the cervical spinal cord, and 2) injections of fluorescein- and rhodamine-conjugated dextran-amines into opposite sides of the cervical spinal cord.

We used embryonic day 11 (E11) as an arbitrary developmental endpoint. At this stage, which is midway through embryogenesis, the pattern of vestibulo- and reticulospinal projections is similar to that in the adult chicken. These projections run in two different tracts, the lateral vestibulospinal tract (LVST) and the medial longitudinal fasciculus (MLF). We found a pontine reticular group projecting in the ipsilateral MLF; 3 vestibular groups projecting, respectively, in the ipsilateral LVST, the ipsilateral MLF, and the contralateral MLF; and several medullary reticular groups projecting in the MLF, some ipsi-, some contra-, and some bilaterally. We conclude from the double-labelling experiments that 1) although the 3 vestibulospinal groups are clustered together, they are absolutely discrete, and 2) bilaterally-projecting groups are not composed of bilaterally-projecting neurons, but rather of intermingled ipsi- and contralaterally-projecting neurons.

The development of these projections begins on E3, when a handful of brainstem neurons project to the cervical cord. By E4 the MLF and a lateral tract are evident, and the basic E11 pattern of the laterality of bulbospinal neurons is established. At this stage the identity of the lateral tract is ambiguous. By E6 the LVST and its parent cell group can be unambiguously identified, but the 2 MLF-projecting vestibular groups are not evident until E8-9. At E9 the pattern is essentially identical to that at E11.

Although retrograde tracing studies are not conclusive, the results suggest that the projection of bulbospinal axons is directed from the outset, with the choice of side and tract being made with few or no errors. Different projections reach the cervical spinal cord at different times. Now that we know the locations of these cell groups and their axons, we plan to begin orthograde tracing studies to further characterize the process of pathway choice in this system.

304.9 EARLY POSTNATAL DEVELOPMENT OF MONKEY SUBTHALAMIC NUCLEUS. Jean E. Fisher*, Pedro Pasik and Tauba Pasik. Depts. of Neurology & Anatomy, Mount Sinai Sch. Med., CUNY, New York, NY 10029.

The subthalamic nuclei of 11 rhesus monkeys, ranging in age from newborn to 17 weeks, were examined at the light (coronal sections) and/or electron microscopic levels, using computer-assisted quantitative methods. The volume of the structure does not change significantly over the period of study. The mean cross-sectional area of neuronal somata, however, decreases by 33%, and most markedly during the first month. This is paralleled by a similar change in the mean area of cell nuclei although the perikaryon/nucleus ratio increases steadily after the first week. There is an overall decline in total cell numbers from newborn to 17 weeks. Cluster analyses confirm the qualitative observations of regional differences in cell size at all ages examined. A parvocellular segment is located in the medial regions and close to the borders of the nucleus, possibly forming a cap on the rostral and caudal poles. A magnocellular division occupies mostly the core and ventral areas. Further analyses show a significantly higher percentage of small cells in the medial and dorsal halves. The relative extent of the two regions does not change during the period of study. The possible influence of the plane of section on these results is ruled out by similar findings obtained from coronal, horizontal, and sagittal series of two additional adolescent monkeys. The neuronal density is consistently greater in the parvocellular region.

Ultrastructural features include dendritic growth cones in the neonatal monkey, and signs of axonal degeneration during the entire period. In addition to conventional axosomatic and axodendritic synapses, there are also synaptic junctions between vesicle-containing profiles which are seen only after the first month. Using the Coupland stereologic procedure, the distributions of synaptic plaque diameters were reconstructed from linear measurements of synaptic profiles identified in electron micrographs taken without bias. These distributions were used to calculate mean synaptic plaque diameters and synaptic plaque densities. Finally, since the samples were obtained without consideration of the possible subdivision of the structure into magnocellular and parvocellular regions, the density values were adjusted to the volume of the entire nucleus resulting in the absolute number of synaptic plaques. This number is stable during the first postnatal month, but it declines markedly in the second month, and to a lesser degree thereafter, so that by 4 months the value is only 55% of that at birth.

Findings indicate the occurrence of substantial changes in the subthalamic nucleus during the first 4 postnatal months which include a marked synapse elimination process. Additionally, the consistent and significant differences in the size and location of two neuronal populations found at all ages may correlate with the topology of the extrinsic connections of this structure.

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304.10 SELECTIVITY IN RE-INNERVATION OF NEUROMUSCULAR COMPARTMENTS. O. I. Weeks* and A. W. English (Spon: J. W. Scott), Dept. of Anat. and Cell Biol., Emory Univ., Sch. of Med., Atlanta, Ga. 30322.

This is the first in a series of studies aimed at examining the specificity of re-innervation of a compartmentalized muscle. The rat lateral gastrocnemius (LG) muscle, like the cat, is compartmentalized via its primary muscle nerve branches (PMNB). Each compartment is arranged in a consistant pattern with reference to the tendons of origin and insertion which divides it into three heads. Each compartment consists of unique populations of motor units whose motoneurons are arranged in a topographic-like manner in the spinal cord.

The lateral gastrocnemius-soleus (LG-S) nerve of deeply anesthetized rats (180-330gms) was crushed with fine forceps at its branching point from the sciatic nerve. A small silastic ring was then placed around the crushed region. After periods of 4 and 14 days, the animals were re-anesthetized, a specified PMNB cut at its entry point into the muscle and soaked in horseradish peroxidase (HRP) for 2 1/2-4 hrs. As a control, the corresponding branch on the contralateral side was similarly cut and soaked. After a 48 hr survival period, the parent nerve or a selected PMNB was stimulated electrically to deplete the re-innervated muscle of stored glycogen. The entire LG muscle was excised, quickly frozen and histologically processed and stained for glycogen. The animal was then perfused and spinal segments L3-S1 removed and processed for the demonstration of HRP.

Results indicate no evidence of retrogradely labeled HRP neurons at 4 days after the nerve crush. There is no electrical or mechanical response of the nerve to nerve stimulation distal to the crush. Fourteen days after the crush, HRP labeled motoneurons innervating a given compartment constitute 60-75% of the number on the control side. The ratio of large (α) to small ( ) cells re-innervating a compartment remains relatively the same. Rostrocaudal distributions of motoneurons innervating different PMNB's are quite similar to controls, but there are subtle differences. Analyses of intercellular spacings indicate that the motoneurons re-innervating different LG compartments are spaced further apart than their comparable controls. This suggests that the motoneurons re-innervating a PMNB are not entirely from the same population which innervated the branch before crushing, and may reflect a type of segmentally selective re-innervation similar to that suggested by Wigston and Sanes (Nature, 299:464-467, 1982). Supported by grant NS20545.

304.11 ACETYLCHOLINESTERASE MOLECULAR FORMS IN DEVELOPING RAT SKELETAL MUSCLE: EFFECTS OF DENERVATION. E.J. Gregory,*[1] J.A. Donoso[1,2] and H.L. Fernandez.[1,3] Neuroscience Research Laboratory, Veterans Administration Medical Center, Kansas City, MO 64128,[1] and Department of Neurology,[2] and Physiology,[3] University of Kansas Medical Center, Kansas City, KS 66103 (U.S.A.)

The existence of multiple acetylcholinesterase (AChE) molecular forms in mammalian neuromuscular synapses is well documented. As an early step toward understanding the developmental regulation of these molecules we studied how they are affected by muscle denervation in neonate Sprague-Dawley rats. One of the gracilis muscles was denervated (experimental), while the contralateral muscle was left intact (control). At various times, thereafter, AChE forms were analyzed by velocity sedimentation on sucrose gradients. In one day old rat muscles, there was a dramatic decrease in the activity of all AChE forms after 2-to-4 days of denervation. Following longer periods (4 through 27 days), 16S AChE continued to gradually decrease, whereas the 4S and 10S AChE forms showed either no further change or a very slight increase. Similar results were obtained for 3 and 7 day old rat muscles. However, in 14 and 90 day old animals, while the expected 4S and 16S decay was observed after 2 days of denervation, there was a significant (100% above control levels) increase in 10S AChE. Whether this increase of 10S AChE is triggered by muscular activity itself (e.g., denervation induced fibrillations) or by the lack of an inhibitory action normally exerted by nerve-evoked muscle activity and/or putative "trophic" substances released by nerve, is currently under investigation. Whatever the case may be, our findings not only confirm that the metabolism of muscle AChE forms depends on an intact motor innervation,but also show that this dependence changes during postnatal development, i.e. immature muscles (less than 14 days old) do not respond to the experimental elimination of motor nerve influences in the same manner as more mature muscles (older than 14 days). (Supported by the Medical Research Service of the Veterans Administration and the KUMC-ALS Research Service)

304.12 **AMPHETAMINE AND RECOVERY OF FUNCTION: USE OF AN ALPHA-2 ANTAGONIST PROVIDES FURTHER EVIDENCE THAT NOREPINEPHRINE MEDIATES THE AMPHETAMINE-INDUCED ACCELERATION OF RECOVERY OF MOTOR FUNCTION AFTER MOTOR CORTEX LESIONS.** A. Tyson*, G. Miller*, D. Feeney and J. Davis (SPON: D. Lorton). VA Medical Center and Depts. of Medicine, Psychiatry, and Pharmacology, Duke Univ. Medical Center, Durham, NC 27705.

Amphetamine has been shown to accelerate recovery of motor function after unilateral somatomotor cortex ablations in rats and cats. To evaluate the hypothesis that this effect of amphetamine is mediated by norepinephrine, we examined the effect of the selective alpha$_2$ antagonist idazoxan on recovery of motor function in the presence and absence of amphetamine.

Seventy-two male Sprague-Dawley rats were trained to walk a narrow beam without slipping. After reaching criterion, rats underwent right somatomotor cortex suction ablation under nembutal anesthesia. Twenty-four hours later animals were given: 1) saline alone, 2) amphetamine (2mg/kg) alone, 3) idazoxan (1 mg/kg) alone, or 4) amphetamine (2mg/kg) plus idazoxan (1mg/kg). Following drug administration all animals were tested hourly for six hours with a scoring system which reflected utilization of the left hindlimb in traversing the beam.

Amphetamine accelerated the recovery of beam walking ability significantly compared to saline. Recovery was not affected by amphetamine plus idazoxan, nor by idazoxan alone. Thus, idazoxan blocked the effect of amphetamine on recovery of motor function.

These data (1) provide further evidence that NE mediates the acceleration of recovery of function seen with amphetamine, and (2) suggest that NE may be working through an alpha-2 receptor.

304.13 NOREPINEPHRINE INFUSIONS INTO CEREBELLUM FACILITATE RECOVERY FROM SENSORIMOTOR CORTEX INJURY IN THE RAT. M.G. Boyeson , K.A. Krobert and J.M.Hughes. Dept. Rehab. Med., Univ. Wisconsin Med. Sch., Madison, WI 53706.

Following the initial findings of amphetamine-induced recovery of function after unilateral sensorimotor cortex (SMX) injury (Feeney et al. Science 217, 1982), efforts were directed to determine the role of catecholamines in recovery from SMX injury. It was found that intraventricular infusions of norepinephrine (NE), rather than dopamine, closely mimicked the amphetamine-induced recovery of function on a beam walking task (Boyeson and Feeney, Neurosci. Abstr. 10, 1984). In the present study, we have made an attempt to localize the NE-induced recovery of function. Animals were trained to run on a narrow, elevated beam, then either received sham or unilateral 6-OHDA lesions of the right Locus Ceruleus (LC). No deficits were noted on the beam walking task for the following 2 weeks. At that time, animals received a right unilateral SMX injury and had a cannula implanted into the contralateral cerebellum. At 24 h postsurgery, animals were infused with NE or vehicle through the cannula one time and tested on the beam walking task at .5 h intervals through 1.5 h, then at 3, 6, and 24 h postinfusion, and subsequently every other day. Animals infused with NE in the absence of the LC recovered beam walking ability (normally observed in 9 days postinjury) within 6 h, although the effect was drug-dependent. In the presence of the LC, NE infusion into the cerebellum permanently accelerated recovery of function compared to vehicle controls on the beam walking task. The results substantiate the effect of a remote functional depression (in the cerebellum) that is intimately involved in the recovery of function from SMX injury, and additionally provides evidence that the restitution of behavioral function is modulated in large part by the LC.

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304.14 SIMPLE DISTURBANCES IN THE NEONATAL PERIOD CAN ALTER CATALEPSY IN ADULTHOOD. H. Schreiber III, G. Gallegos*, M. Ortiz*, B. Gonzalez*, N. Jeanniton*. Psychobiol. Lab., New Mexico Highlands University, Las Vegas, NM 87701

A guiding principle in developmental psychobiology is that seemingly minor events early in life can have profound consequences in later life. In the two experiments presented here, we investigated the consequences of ordinary aspects of experimentation in the neonatal period on catalepsy induced by haloperidol and catatonia induced by morphine, and on haloperidol-induced catalepsy following repeated haloperidol injections.

In Experiment 1, twenty-four litters of hooded rats (Rattus norvegicus) were distributed to 4 treatments: Unobserved Control (Unobs C) litters and dams were left undisturbed. Observed Control (Obs C) had 10 min twice daily observations of maternal behavior for 7 Ds postpartum. Early Handled (Early Han) litters were removed from nest and dam for 3 min/D, 21° C plus twice daily observations. The fourth group (Mat Sep) had the mothers removed for 1 hr daily plus twice daily observations. At 115 Ds, male littermates were assigned to receive saline, haloperidol (2 mg/kg) or morphine sulfate (20 mg/kg) and forepaw-on-dowel testing at 20 min intervals from 20 to 180 min after injections, averaged per hour.

A Drug by Time interaction was obtained, $F(4, 120)=37.34$, $p<.01$. Haloperidol treated subjects showed increasing catalepsy over time and morphine treated subjects showed decreasing catalepsy over time, as expected. Also, an Early Treatment by Drug interaction, $F(6, 60)=3.25$, $p<.01$ was found. Among haloperidol treated Ss, Unobs C showed significantly more catalepsy than Obs C; also Early Han showed significantly more catalepsy than Obs C. Among morphine treated rats, Obs C showed significantly more catalepsy than Unobs C, significantly more catalepsy than Early Han and significantly more catalepsy than Mat Sep. Thus, catalepsy and catatonia were distinguishably influenced by manipulations impinging on the pup and dam as well as by mere observation.

In Experiment 2, dams and litters were left undisturbed or were removed (while untouched in their dish-type homecage) to an adjoining room for 30 min/day for 10 days after birth. In adulthood, rats received haloperidol or saline for 3 weeks and then were tested for latency to remove forepaw from dowel following haloperidol (1.5 mg/kg). Simple disturbance of cages diminished haloperidol-induced catalepsy, and a history of haloperidol treatment obviated this early experience effect.

We conclude that ordinary aspects of experimentation and animal care in the neonatal period can influence adult catalepsy. (Supported by NIH-MBRS Grant #RR-08066-13).

305.1 THE INFLUENCE OF NEIGHBORING BLASTOMERES ON THE LINEAGE OF THE V 1.2 BLASTOMERE OF THE 16-CELL XENOPUS EMBRYO AS DETERMINED BY ABLATION EXPERIMENTS. B.C. Gallagher and S.A Moody. Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, Va. 22908.

One of the goals of developmental neurobiology is to identify the factors that lead to cell differentiation. During cleavage, the subdivision of the cytoplasm among daughter cells may lead to a localized distribution of determinants which are thus inherited by the progeny of ancestral cells. Another factor may result from a cell's interactions with its neighboring cells. One way of gaining insight into this question is to compare the progeny of cells which result when a neighboring cell has been ablated with that of cells generated in the unoperated embryo, using cell lineage analysis with HRP.

Blastomeres at the animal pole of the 16-cell embryo that give rise to different numbers of Rohon-Beard neurons and primary motoneurons were injected with HRP, the vitelline membrane of the embryo was removed, and a neighboring blastomere was detached manually. The cells, being approximately 600 - 800 um in diameter, could be peeled away from the rest of the embryo without leaving any cellular debris within the embryo and without apparent damage to the remaining cells. In nearly all cases, the injected blastomere produced fewer Rohon-Beard neurons after a neighboring cell was ablated than it did in the normal, intact embryo (36% - 55% of the normal numbers). In two cases, the injected blastomeres produced the normal number of Rohon-Beard neurons, but produced fewer primary motoneurons. In embryos in which the designated cell was removed and then reinserted (sham operation), the total numbers of Rohon-Beard neurons and primary motoneurons were the same as in the normal, intact embryos. Supported by NIH grant NS20604 to S.A.M. and N.I.H. postdoctoral fellowship IF32NS07868-01 to B.C.G.

305.2 FATE MAPPING OF BLASTOMERES INVOLVED IN XENOPUS NEURAL DEVELOPMENT: SLOW INTERMIXING OF CELLS. Richard Wetts & Scott E. Fraser. Developmental Biology Center and Dept of Physiology & Biophysics. University of Calif, Irvine, CA 92717.

After a single cell is injected with a vital dye, the resulting pattern of labelled cells is a fate map of the original cell. Regardless of how modern the dye or how sophisticated the analysis, a fate map alone cannot reveal the state of determination or commitment of the injected cell. For example, sea urchin cleavage is stereotyped, and because there is no intermixing, individual blastomeres always give rise to the same spectrum of cell types. This impression of clonal restriction is false because single blastomeres, when isolated, can develop into whole embryos. Similarly, apparent clonal restrictions in the CNS of Xenopus laevis could be due to a lack of cell mixing. To determine if there is limited cell mixing in Xenopus, we filled single blastomeres with a vital dye and examined the fate map at various stages of development.

Lysinated fluorescein dextran was pressure injected into single blastomeres at the dorsal/animal pole of the 16-cell stage embryo. These cells give rise to a large number of different tissues of all three germ layers, including the bulk of the CNS. At various stages after injection, the embryos were fixed with 4% paraformaldehyde, embedded in glycol methacrylate and serially sectioned at 6 microns.

Prior to gastrulation (St 9), the labelled cells form a single coherent group, but the boundary is not absolute: single labelled cells can be seen among unlabelled cells, and vice versa. At the end of gastrulation (St 16), individual cells have spread further into the unlabelled area, though the majority of cells are still clustered within a single area. This area is elongated along the rostral-caudal axis and many times shows a co-distribution of labelled cells in all three germ layers. After neurulation (St 23), a little more cell mixing has occurred. In the CNS, the coherence is still quite striking. Completely labelled areas are separated from unlabelled areas by a narrow zone of intermixed cells. By St 39, labelled cells are located throughout the free-swimming larva, including all regions of the CNS. Boundaries between labelled and unlabelled regions can no longer be seen, suggesting that the slow, progressive mixing seen at earlier stages has finally become complete.

Thus, cell mixing in Xenopus is slow, beginning at gastrulation, continuing throughout embryogenesis, and becoming noticeable only after hatching stages. In contrast, the intermixing which occurs in mice and zebrafish is rapid and dramatic. Because of the slow intermixing in Xenopus, labelled cells are likely to remain within any arbitrarily drawn boundary, especially if blastomeres are injected late or if the larvae are examined early since this allows less time for cell mixing. Thus conclusions about clonal restrictions in the developing CNS of Xenopus must await experiments in which the fate map is challenged by ablations or transplantations.

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305.3 RELATIONSHIP BETWEEN DIFFERENTIATION AND TERMINAL MITOSIS OF NEURONAL PRECURSOR CELLS IN THE CHICK PERIPHERAL NERVOUS SYSTEM. H. Rohrer* and H. Thoenen. (SPON: Y.-A. Barde) Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033 Martinsried, F.R.G.

A population of undifferentiated cells has been detected early during development in dorsal root ganglia (drg), nodose ganglia (nod) and ciliary ganglia (cil). This population is characterized by the absence of surface markers specific for neurons (tetanus toxin receptor, Q211 antigen) and for glial cells (04 antigen). The undifferentiated cell populations were isolated from these ganglia and were shown to contain neuronal precursor cells which were able to differentiate in vitro to neurons, as characterized by morphology and surface antigens. Undifferentiated cells were detected only during the period of neuronal birth in these ganglia, indicating that dividing neuronal precursor cells do not express neuron-specific surface markers. This was directly shown by ^{3}H-thymidine labeling studies. In addition it was demonstrated that the neuron-specific surface antigens start to be expressed about 12 hrs after the terminal S-phase of sensory neuron precursors in drg.

In sympathetic ganglia, however, no undifferentiated neuronal precursor cells were detectable at developmental stages when sympathetic neurons are being born (E7). ^{3}H-thymidine injected at that stage was incorporated into differentiated neurons identified by tetanus toxin and the monoclonal antibody Q211. Quantitative fluorometric determination of DNA-content of dissociated sympathetic ganglion cells also demonstrated the presence of a population of Q211-positive sympathetic neurons which is in the G2 phase of the cell cycle. These results extend previous findings that sympathetic neuron precursors continue to divide after acquisition of some adrenergic characteristics (Rothman et al. (1978) Dev. Biol. 65, 322-341).

It is concluded that the relationship between terminal mitosis and onset of differentiation varies according to the type of ganglion in the chick peripheral nervous system: Whereas sympathetic neurons continue to divide after the acquisition of neuronal properties other autonomic and sensory neurons start to differentiate after a terminal mitosis.

305.4 DETERMINING NEURONAL BIRTHDATES IN THE FACIAL MOTOR NUCLEUS USING A MONOCLONAL ANTIBODY TO 5-BROMODEOXYURIDINE. A.J. Alles* and R.M. Dom* (SPON: R.A. Schmiedt). Department of Anatomy and Cell Biology, Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina 29425.

Pregnant Long-Evans rats were injected with 5-bromodeoxyuridine (BrdU) at day 12 (E12) or day 13 (E13) of gestation (day 1 being the day of sperm positivity). At E20 the rats were sacrificed and the fetuses removed for processing. The fetuses were decapitated and the heads were fixed in Carnoy's solution. After paraffin embedding 5 micrometer sections were cut and incubated with an appropriate dilution of mouse monoclonal anti-BrdU. Primary antibody binding sites were identified using the avidin-biotin immunoperoxidase method. Diaminobenzidine was used as the chromogen substrate for peroxidase. With this technique a dark brown color is imparted to cell nuclei that incorporate BrdU and labelled nuclei can be clearly distinguished from unlabelled nuclei. In fetuses exposed to BrdU at E13 some labelling was seen in the facial motor nucleus. This correlates with autoradiographic studies (Altman, J. and S.A. Bayer, J. Comp. Neurol., 194:37-56, 1980) which reported that the majority of facial motor nucleus neurons are born on E13. Because 80% of BrdU is cleared from the blood of an adult rat within one hour, this technique can be used to pinpoint precisely the times of birth of dividing neurons. In addition, this technique is less time consuming and less tedious than autoradiography.

305.5 EXPRESSION OF HOMEO BOX-CONTAINING GENES IN THE LEECH *HIRUDO MEDICINALIS*. M. Baumgarten* and E.R. Macagno. (SPON: E. Holtzman). Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027.

We are interested in finding the mechanisms that underlie segmental differentiation in the central nervous system of the leech, *Hirudo medicinalis*. Although a great deal is known about leech development at the cellular level, the molecular level has received little attention due in large measure to our lack of knowledge of the genetics of this group of animals. The homeo box is a marker for genes which have important developmental functions in the fruitfly, *Drosophila melanogaster*, in particular genes that affect segmentation and segmental differentiation. A number of recent studies have demonstrated the expression of several homeo box-containing genes in neuronal tissue of the developing fruitfly. Several studies have also shown that many other animals, including the leech *Erpobdella octoculata*, contain homeo box-like sequences in their genomes (e.g., McGinnis, W. et al., C.S.H. Symp. Quant. Biol. L:263-270, 1985). Since insects have evolved from the annelids, we might expect that leech genes carrying the homeo box may also play important developmental roles. We are, therefore, determining (a) whether the *Hirudo* genome contains homeo box sequences, (b) whether genes so marked in the leech are part of a family, and (c) whether such genes are also involved in leech development, specifically in segmentation and segmental differentiation in the leech nervous system.

By probing Southern blots of total genomic DNA from *Hirudo* with radiolabelled homeo box sequences from *Drosophila* genes, we have detected a number of regions in leech DNA which contain homology to the homeo box, suggesting the existence of a homeo box-containing gene family in the leech. We have constructed a phage library of *Hirudo* genomic DNA and screened it with the same probes used for Southern analysis. One clone containing a 20kb insert has been isolated and subcloned. A 180bp region within a 500 bp fragment has been sequenced and its putative translation product is 83% homologous to that of the fly *Scr* homeo box. Using this 500bp clone, we have constructed a single stranded probe complementary to the hypothetical mRNA which would give rise to such a translation product. Northern analysis indicates that such a transcript, about 2.5kb long, is expressed in the adult leech.

Developmentally staged and tissue-specific Northern blots are in progress, and in situ hybridization experiments are planned in order to determine the spatial expression of this gene.

305.6 AN ANTIGENIC MARKER FOR NEUROEPITHELIAL PRECURSOR CELLS IN THE CNS OF THE RAT. K.Frederiksen*, R.McKay, Departments of Biology and Brain Sciences, E25-435, Whitaker College, MIT, Cambridge, MA 02139.

We have shown that the relative number of cells in the embryonic CNS of the rat that express an antigen recognized by monoclonal antibody Rat-401 (Hockfield and McKay, 1985 J.Neurosci.5 3310) increases from 0 % at embryonic day 9 (E9) to more than 98 % at late E11. The antigen is not expressed in the CNS of the adult rat. The decrease in the percentage of cells expressing the antigen correlates with the time when neurons are known to be born in the spinal cord and cerebrum. By using a combination of Fluorescence Activated Cell Sorting (FACS),3H-thymidine labeling and immunohistochemical staining techniques we showed that the Rat-401 pos. cells are a major class of proliferating cells at developmental stages when neurons become postmitotic. We have used two independant methods to measure the total number of cells in the CNS on different days of embryonic development. Knowing the total number of cells in the CNS allows us to calculate 1. the number of Rat 401 pos. cells and 2. the number of neurons born on each day of development in the spinal cord. This data shows that there are sufficient Rat 401 pos. cells present to account for the number of neurons born each day of embryonic development in the spinal cord. These results suggest that the Rat 401 antigen is expressed in a proliferating neuronal precursor cell.

Postnatally, at the time of the peak of gliogenesis very few cells remain Rat-401 positive, suggesting that glial cells are not immediately derived from a Rat 401 positive precursor in vivo. In contrast it is common to find cells double labelled with Rat 401 and the astrocytic marker GFAP in cultures of fetal brain cells.

This data suggests that the Rat-401 antibody recognizes both glial and neuronal precursor cells at E11 and early E12 (as 98% of the cells are stained). During neurogenesis neurons are born from Rat-401 positive cells, whereas the main proportion of glial cells are derived from precursor cells which have become Rat 401 negative.

Using Rat 401 as a marker we have shown that very few cells which are actively proliferating in vivo continue to do so when the cells are dissociated and plated under standard conditions. To overcome this problem we are now using retroviruses carrying immortalising oncogenes to establish cell lines from the early vertebrate nervous system. Some of these cell lines have properties which suggest they will provide simple models for a molecular analysis of early events in the development of the vertebrate CNS.

305.7 BLOCKING NEURONAL MIGRATION IN MOUSE RETINAE WITH A CELL SURFACE ANTIBODY. V.H. Perry, R.D. Lund and C.F. Lagenaur, Univ. of Oxford, United Kingdom, Univ. of Pittsburgh, School of Med. and Center for Neuroscience, Pittsburgh, PA 15261

A monoclonal antibody to a neuronal cell surface glycoprotein blocks neurite outgrowth in vitro (Lagenaur et al., Soc. Neurosci. Abstr., 10:759, 1984). We have examined the effect of the antibody on the developing retina by injecting 3µg of the antibody or 2µg Fab fragment into one eye of neonatal mice. Animals were fixed by perfusion with paraformaldehyde after 3 days to 6 weeks survival. Cryostat sections or flat mounts of the retinae were prepared for staining with cresyl violet or with a secondary antibody to demonstrate the presence of injected M6 antibody. The injected antibody penetrated the retinal layers and was detectable in the retina for one week after injection.

Nissl stained material showed many small cell bodies arrested in the inner plexiform layer, which were identifiable at all survival times examined. Counts made at 6 weeks postnatal showed, on average, a 5-fold increase in the number of cells in the inner plexiform layer as compared with saline injected controls. Cell numbers and sizes in the ganglion cell layer were normal, indicating that the antibody is not cytotoxic. Furthermore, since it is thought that the arrested cells are displaced amacrine cells destined for the ganglion cell layer, it suggests that these cells are not susceptible to the usual cell death occuring in the ganglion cell layer at early postnatal times.

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305.8 MONOCLONAL ANTIBODY CROSSREACTING WITH TRANSIENTLY EXPRESSED NEURAL NUCLEAR, AXONAL AND GROWTH CONE ANTIGEN. E. Pawlak-Byczkowska*., (SPON: L. Amini-Sereshki) Dept. Anatomy, Medical College of PA, Philadelphia, PA 19129.

Nuclear proteins are thought to be regulators of mammalian cell differentiation in several organ systems, including CNS. To support this hypothesis monoclonal antibodies were raised to the embryonic day 17 (E 17) neuronal cell nuclei (E. Pawlak-Byczkowska & P. Levitt, Soc. for Neuroscience,85). The transient appearance of several of these antibodies was chronicled previously and it was found that some of them were immunoreactive with radial glial fibers and mitotic nuclei. To obtain nuclear markers for earlier events in neuronal differentiation new antibodies to embryonic day 14 neuronal nuclei were developed. This report is on the monoclonal antibody crossreactivity with antigen(s) present in axons, their growth cones and mitotically active nuclei as revealed by light-microscopic studies on frozen sections and fixed cell cultures. A discrete, speckled staining pattern was observed in the nucleoplasm of the neural tube in E8 and E11 rat embryos; the cell nucleoli and nuclear envelope remained unstained. Later in development, no antibody-positive nuclei were noted. Antibody staining of axons was present throughout the course of nervous system development. Expression and cellular distribution of antigenic determinants was also monitored on primary monolayer cultures of dissociated neural cells from E14 embryos using double immunofluorescence and Evans blue counter-stain technique; anti-GFAP and anti-NF antibodies were used to evaluate cell types and neurofilaments. Nuclear staining was observed on neurons only throughout the first week of cultivation; staining on growth cones and distal parts of axons remained prominent and constant during the 17 days of culture. On immunoblots of SDS-PAGE the antibody recognizes bands not co-migrating with the major NF-species.Further studies are underway to explore the possibility that (1) proliferative neuronal precursors are committed to their specific lineage prior to final mitosis or (2)proteins recognized by the reported antibody may arise from the same gene by differential processing as was recently suggested for other nuclear and NF proteins.

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305.9 IDENTIFICATION OF A NEURON-SPECIFIC BETA-TUBULIN. A. Frankfurter*, L.I. BINDER* and L.R. Rebhun* (Spon: J.B. Tuttle), Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901 and Dept. of Cell Biology and Anatomy, Univ. of Alabama at Birmingham, Birmingham, AL 35233.

Microtubules are filamentous protein polymers found in almost all eucaryotic cells. These linear organelles have been implicated in a variety of cellular activities, such as chromosome movement, cell migration, maintenance and modification of cell shape, intracellular translocation of macromolecules and organelles, and the lateral movement of proteins in the plasma membrane. The basic constitutent of microtubules is tubulin, a dimeric protein (Mr 110,000) composed of two similar but non-identical globular subunits designated alpha- and beta-tubulin. High-resolution isoelectric focusing methods have revealed that both the alpha- and beta-tubulin subunits can be resolved into multiple electrophoretic species. The most extensive subunit heterogeneity has been documented in adult mammalian brain. During neural development, this complex tubulin pool undergoes significant modulation. The apparent functional and biochemical heterogeneity of microtubules has raised the question of whether cells construct biochemically different microtubules to perform specific functions. To establish highly specific probes with which to address this question, we are developing a library of anti-tubulin monoclonal antibodies, using microtubule protein isolated from developing rat brain as the source for the antigen. Recently, we identified a beta-tubulin specific antibody (TuJi) which recognizes an antigenic determinant unique to neuronal tubulin. Biochemical and immunocytochemical analyses indicate that the beta-tubulin variant recognized by TuJi is not a component either of glial tubulin or tubulins present in a wide variety of non-neural tissues. An extensive characterization of this anti-beta-tubulin monocloned antibody is currently in progress.

305.10 PRODUCTION OF MONOCLONAL ANTIBODIES TO CYTOSKELETAL PROTEINS FROM THE NERVOUS SYSTEM OF ADULT XENOPUS LAEVIS FROGS. B. G. Szaro, S. House*, and H. Gainer. Laboratory of Neurochemistry and Neuroimmunology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

Xenopus embryos provide a unique system for studying early molecular events in the differentiation of axons and dendrites in vivo, because of their rapid initial development and suitability for cell marking and perturbation. The distinctive shapes of adult neurons suggest unique regulation of cytoskeletal associated proteins during development, and therefore, we have begun to study the expression of specific cytoskeletal proteins to structural differentiation in the developing central nervous system (CNS). Monoclonal antibodies (MABs) to SDS/urea homogenates of triton insoluble cytoskeletal preparations from adult frog CNS were made. Hybridoma supernatants were screened first by a modified dot blot assay against CNS cytoskeletal homogenates, then by western blots and immunocytochemistry of CNS and other tissues. Most antibodies gave complex staining on western blots, and relatively little tissue or cell-type specificity in immunocytochemical staining. However, 13 MABs produced relatively simple and distinctive patterns. The gel and immunocytochemical data obtained with these MABs were compared to results obtained with other antibodies against known mammalian intermediate filament and cytoskeletal proteins which cross-reacted in Xenopus. These included antibodies to various forms of 200 kD neurofilaments (L. Sternberger), a general intermediate filament antibody (R. Pruss), vimentin (R. Goldman), cytokeratins (R. Goldman and I. Huitfeldt), MAP-1, and MAP-2 (R. Klausner). Of the 13 Xenopus-derived MABs, two react with low molecular weight neuron-specific intermediate filaments proteins, one of which is relatively specific for nerve tracts in the brain; three react with antigens specific to neurons and axons, similar in molecular weight to 200 kD neurofilaments; one is antivimentin-like, and stains some neurons and axons in the adult; two are antikeratin-like, but stain distinct CNS structures; and 2 more stain regions of the brain typically stained by keratin antibodies (e.g. internal CNS capillaries), but cross-react with high molecular weight proteins. Three antibodies do not appear to react with intermediate filament proteins: one is nervous system specific and stains neuropil, neuronal somas, and some but not all axons; the second predominantly stains neuronal cell bodies and dendrites; and the third only stains peripheral nerves. With the exception of the last MAB, all antibodies stained larval swimming tadpoles by 3 days of development (stage 41), and showed distinctive tissue and cell type specific distributions. These MABs will be used to study the expression of specific proteins and the differentiation of structure in the early embryonic CNS.

305.11 SPATIAL AND TEMPORAL RELATIONSHIPS BETWEEN THE PHOSPHO- AND NON-PHOSPHO-DERIVATIVES OF THE 200 KILODALTON NEUROFILAMENT PROTEIN DURING DEVELOPMENT IN CULTURE. G.A.Foster*, D.Dahl and V.M.-Y.Lee. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20205, West Roxbury VA Medical Center, MA 02132, Div. of Neuropathology, Univ. of Medical School, Philadelphia, PA 19104.

Neurofilaments comprise at least three proteins, of molecular weights 70, 150 and 200 kilodaltons. The last of these (NF200) exists in various states of phosphorylation, and some of these phosphoproteins (NF200P) may be discriminated with monoclonal antibodies. The absence of NF200P, but not other NF proteins, from immature axons of rat optic nerve prompted the suggestion that a post-translational modification of nascent NF200, eg. phosphorylation, renders the subunit recognizable to the NF200P antibodies. However, no evidence exists indicating that the phosphorylation step does indeed follow NF200 expression. In order to determine which phenomenon occurs first, we have used antibodies against the phospho- and non-phospho-derivatives (NF200D) of NF, and compared their appearance in dissociated cultures of mouse spinal cord and dorsal root ganglion.

NF-like immunoreactivity (NF-LI) occurred in all neurones from day 1 in culture, and was maximal in intensity by days 16-20. The first labelling of neurones by NF200D antibodies occurred at day 3, in dorsal root ganglion cells, but only in their perikarya and proximal processes. At later times, the immunoreactive region extended to more distal parts of these neurones. Spinal cord neurones first exhibited NF200D-LI at day 9, but even in mature cultures many NF200D-negative neurones remained. NF200P-LI was first visualized, in axons but not cell bodies, at day 17 in culture; these fibres were predominantly NF200D-negative. However, in some fibres the proximal segment was NF200P-positive only, whereas the distal part exhibited solely NF200D-LI.

Compared to NF70 and NF150, the 200 kilodalton neurofilament protein appears much later in most neurones in culture. The non-phosphorylated epitope is seen earlier than the phospho-derivative. Post-translational phosphorylation of NF200 therefore seems to be at a later stage of development than the induction of NF200 itself, while a wide variation of rates of its phosphorylation occurs during its passage down different axons.

305.12 IMMUNOCYTOCHEMICAL STUDIES OF NEURONS CONTAINING VASOTOCIN, MESOTOCIN, AND NEUROPHYSINS IN XENOPUS HYPOTHALAMUS. K.M. Conway and H. Gainer. Lab. of Neurochemistry and Neuroimmunology, NICHD, National Institutes of Health, Bethesda, MD 20892

Hypothalamic neurons containing the peptide hormones, arginine vasotocin (AVT) and mesotocin (MT), and their binding proteins, the neurophysins (NPs), were studied by immunocytochemistry (ICC). Affinity purified rabbit antisera were used to detect AVT and MT, and two monoclonal antibodies (MABs) were used to probe for NP-containing neurons. One MAB (PS-45) was specific for arginine vasopressin (AVP) associated neurophysin, and another (PS-36) was specific for oxytocin (OT) associated neurophysin in the rat (Ben-Barak, Y., et al., J. Neurosci. 5:81, 1985). These MABs have several advantages as phenotypic markers in development: high titer, low background, ample supply, and affinity for the pro-hormone forms as well as for the fully processed NP polypeptides (Whitnall, M.H., et al., J. Neurosci. 5:98, 1985). Both MABs cross-reacted with Xenopus antigens in ICC (Ben-Barak Y., et al. Cell. Mol. Neurobiol. 5:339, 1984). Co-localization studies were performed in Xenopus in order to compare fluorescent ICC staining by these MABs versus that of the antisera specific for AVT and MT. The PS-45 antigen was found in each MT-containing cell, and the PS-36 antigen in each AVT-containing cell. Hence, the relationship of the NPs to the peptide hormones was reversed in Xenopus as compared to the mammal. (The PS-45 antigen is NP-AVP in the rat, but was found in MT-, not AVT-positive cells in the frog, and conversely for PS-36). Since these NP MABs recognize epitopes which in mammalian genes are coded on a different exon from the peptide hormones, these results suggest that the amphibian genes may have 'switched' exons relative to the mammal. Using these MABs we mapped the locations of immunoreactive (IR) cells in the adult and developing Xenopus hypothalamus. In adults, PS-36 and PS-45 IR cells were intermingled and scattered throughout the magnocellular preoptic area, as are AVT- and MT-containing neurons in other amphibian species (Vandesande, F. and Dierickx, K., Cell Tissue Res. 175:289, 1976). In Xenopus, small and medium size, as well as large cells contain these antigens. Many IR cells were found in a large contiguous region extending caudally, dorsally, and laterally from the preoptic area. Intense NP and peptide hormone IR fibers were seen in the preoptic area, the neurohypophysial tract, and the neural lobe of the pituitary. PS-45 IR fibers first became detectable by our methods in the pituitary of young tadpoles between stage 41 and stage 45 and in neuronal somata of tadpoles between stage 45 and stage 49. NP IR neurons were found in marker strains (albino, Xenopus borealis), affirming the feasibility of a grafting analysis of the lineage of these two closely related cell types, and of environmental influences during development on the expression of their particular peptidergic phenotype.

305.13 IMMUNOCYTOCHEMISTRY OF GABA IN THE THORACIC GANGLIA OF ADULT AND EMBRYONIC LOCUSTS. D.A. Kuterbach and A.H.D.Watson*. Dept. of Zoology, Cambridge Univ., Cambridge CB2 3EJ, England.

The distribution of gamma-aminobutyric acid (GABA) immunoreactive cell bodies and axonal tracts was investigated in the thoracic ganglia of adult and embryonic locusts (Schistocerca gregaria) using an anti-GABA antibody. Embryos were staged according to Bentley et al. J. Exp. Embryol and Morphol. 54:47 (1979). Thoracic ganglia were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.6, for 2-4 hours, and embedded. Ten µm serial sections were processed for immunocytochemistry using a modification of the peroxidase anti-peroxidase method (Sternberger et al. J.Histochem Cytochem. 18:315 1979). Anti-GABA antibodies (SFRI, Bordeaux, France) were diluted 1:800 or 1:1600 (see Segula et al. PNAS 12:3888 1984, for specificity). Controls were made by preadsorbing the antibody with a GABA/BSA conjugate. Embryonic sections were intensified with 0.1% cobalt chloride before reacting with diamminobenzidine.

The pattern of staining in each of the adult thoracic ganglia is similar (Watson, Cell Tiss. Res. in press). The three pairs of inhibitory motor neurones stain with the antibody. There is biochemical (Emson et al. J.Neurobiol. 5:33 1974) and physiological (Usherwood and Grunfest, J.Neurophys. 28:497 1965) evidence that these are GABAergic. Four clusters of medial somata and two clusters of dorsal somata are also immunoreactive. One contains somata of spiking local interneurones that integrate sensory information from the leg (Siegler and Burrows, J.Neurophys. 50:1281 1983). Dorsal commissures IV and V are composed of stained fibres. The supramedian, posterior ventral and second ventral commissures and longitudinal tracts are mixed. The other commissures are unstained. There is an even distribution of stained processes through most of the neuropile though this is sparser in the anterior ventral association centre.

Neurones in embryonic ganglia consistantly stained with the antibody at 60% development and less consistantly as early as 45% development. Groups of immunoreactive cell bodies, similar to those in adult ganglia, were identified by 70% development and were seen to arise from larger groups consisting of a mixture of staining and non-staining somata. In stages earlier than 70% development, the immunoreactive and non-reactive somata are mixed and distributed across the entire surface of the ganglia. The neuropilar tracts that are immunoreactive were the same as in the adult ganglia.

The results of this work will be discussed in reference to the formation of groups of neurones with similar physiological function and the cell lineage of spiking interneurones. This work is supported by NIH grant NS07896 to DAK and a Beit Memorial Fellowship to AHDW.

305.14 PROPERTIES OF CATECHOLAMINE-POSITIVE CELLS WHICH DIFFERENTIATE IN NEURAL CREST CULTURES G. D. Maxwell and D. S. Christie, Dept. of Anatomy, Univ. of Connecticut Health Ctr., Farmington, CT 06032

We have been studying the ontogeny of catecholamine-positive (CA+) cells in quail neural crest cultures. Some of these CA+ cells also develop somatostatin-like immunoreactivity (SLI). One of our goals is to analyze the structural and functional properties of the CA+ cells which differentiate in neural crest cultures.

Greater than 85% of the CA+ cells which developed in our neural crest cultures were positive when stained with the monoclonal antibodies HNK-1 or A2B5. In contrast, fewer than 10% of the CA+ cells bound the monoclonal antibody R24. The uptake of 3H-norepinephrine by the cultures was inhibited by desmethylimipramine and by incubation at 0°C, but was unaffected by normetanephrine, consistent with the presence of an uptake I system. Overnight treatment with reserpine eliminated the histochemically detectable CA fluorescence. Chronic reserpine treatment from days 2-7 in vitro prevented the appearance of histochemically detectable CA+ cells, while normal numbers of tyrosine hydroxylase-positive and SLI+ cells developed. Exogenously added Nerve Growth Factor did not dramatically alter either the number or light microscopic morphology of the CA+ cells under our culture conditions. The CA+ cells in these cultures survived when treated with a regimen of 6-hydroxydopamine which killed older embryonic quail sympathetic neurons.

Using the method of Grillo et al. 1974 (J. Comp. Neurol. 153: 1-14) we have demonstrated that the CA+ cells in the light microscope correspond to cells containing cytoplasmic granular vesicles (GV) characteristic of catecholamine storage granules observed in other systems. By morphometric analysis we have determined that the GV diameters were quite similar in cells examined after 5, 7, 14 and 21 days in vitro. Most GV diameters were 50-200 nm with mean diameters of 145, 137, 160, and 190 nm after 5, 7, 14, and 21 days in vitro respectively. The number of GV per µm^2 of cytoplasm also remained quite constant at all ages examined. Nerve terminal profiles containing 50 nm agranular vesicles and a few 100 nm GV were observed. Some of these terminals contacted GV-containing cell bodies in cultures after 14 days in vitro. At present it is unclear whether these terminals emanated from GV-containing CA+ cells or from another class of neuronal cells.

Our data suggest that the CA+ cells which develop in our neural crest cultures may be an intermediate stage of adrenergic differentiation which is normally observed only transiently during avian embryonic development, but which can persist under our tissue culture conditions.

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305.15 EXPRESSION OF TYROSINE HYDROXYLASE, NEUROTENSIN AND NEUROPHYSIN BY HYPOTHALAMIC NEURONS IN SERUM-FREE CULTURE. J. P. Grierson*, S. McElligott, R. Ventimiglia, C. Coent and H. M. Geller. Dept. of Pharmacology, U.M.N.D.J., R.W. Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854 and †Dept. of Anatomy, King's College, London.

The hypothalamus contains heterogeneous populations of neurons involved in a variety of reproductive and somatic functions. One aspect of this heterogeneity is the diversity of neurotransmitters synthesized by hypothalamic neurons. We have used immunocytochemical methods to examine the expression of the enzyme, tyrosine hydroxylase (TH), and the neuropeptides, neurotensin (NT) and neurophysin (NP), by hypothalamic neurons in dissociated culture. Populations of neurons expressing each of these substances were identified in serum-free (SF) as well as serum-supplemented (SS) cultures.

Dissociated cultures of rat embryonic hypothalamus were prepared as previously described (Neurosci. Abstr. 11: 1065, 1985). Cultures were fixed in 5% acrolein in PBS and permeabilized with 0.25% Triton prior to incubation in antisera. Specific binding of antisera directed against TH (A. W. Tank, S.U.N.Y) and NT and NP (Immunonuclear Corp.), were visualized using the avidin-biotin-peroxidase technique. The majority of neurons were unlabeled; immunoreactive neurons varied in their intensity of staining. Staining of non-neuronal cells was not observed.

TH immunoreactivity was detected after 5 days in vitro in both SF and SS cultures. The percent of TH immunoreactive neurons and the average intensity of labeling increased with time in culture. In 12 day old cultures, approximately 2% of neurons were heavily stained. Immunoreactivity was observed in the neuronal perikarya, processes and growth cones. TH positive neurons were typically bipolar with a 15 µm cell body diameter and processes of up to 300 µm in length with multiple varicosities.

Peptide immunoreactivity (NT & NP) was investigated in 12 day old cultures. NT positive neurons were typically multipolar with perikarya 12µm in diameter. Soma and processes were uniformly labeled. NP immunoreactivity was also observed in cultures of comparable age. A striking observation was that neurons associated in clusters expressed similar peptide immunoreactivity.

Our results indicate that hypothalamic neurons cultured in serum-free medium are heterogeneous in expression of neurotransmitter phenotypes. The increase in TH immunoreactivity with time may indicate a selective enrichment for this phenotype in dissociated culture. The control of expression of these and other neurotransmitters normally found in the hypothalamus is under current investigation. (Supported by grants from N.I.H. and N.S.F. (H.G) an N.I.H. Training Grant (S.McE.) and a Merck Fellowship (R.V.).

305.16 CHANGES WITH TIME IN VITRO IN THE NUMBER AND DISTRIBUTION OF ACETYLCHOLINE RECEPTORS ON DISSOCIATED CHICK MONONUCLEATED MYOGENIC CELLS. M. Morgan* and G.D. Fischbach. Dept. of Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

When nerves invade the chick limb buds, few multinucleated myotubes are present, therefore we have begun to investigate the appearance and role of acetylcholine receptors (AChRs) in myogenic precursor cells. In our first experiments, AChRs were visualized with rhodaminated α-bungarotoxin (RH-BTX). Chick pectoral muscle was minced and triturated without exposure to proteolytic enzymes. The dissociated cells were either labeled with Rh-BTX in suspension and examined immediately after they settled on collagen coated glass coverslips (in MEM with 5% chick embryo extract and 10% horse serum), or they were labeled at various periods up to 48 hours after plating. Between 50 and 100 cells were examined at each time point. The label was visualized by epifluroscence microscopy with a 63x objective (N.A.=1.4) and images were collected with a SIT (Dage MTI66) video camera.

In cultures examined immediately after plating, 70% of the cells were labeled. This must be considered an underestimate because at this stage, myoblasts could not be distinguished from fibroblasts. Within a few hours of plating, the two cell types were distinguishable by morphologic criteria. The percentage of labeled myoblasts decreased gradually to below 50% during the first 24 hours in culture, and then it increased to a maximum of approximately 70% after 48 hours. Coincident with this fall and rise in the proportion of labeled myoblasts, there was a change in the distribution of receptors on individual cells. Newly plated myoblasts exhibited dense receptor aggregations, or "hot spots," that were usually located near the nucleus. As the number of labeled cells declined, the receptor aggregates disappeared and the label became diffusely distributed over the entire surface. After 30 hours in vitro, small, punctute clusters reappeared while the diffuse label persisted.

Results obtained with enzymatically-dissociated cells differed from those described above. Very few (5%) of the cells dissociated with 0.01% trypsin were labeled with Rh-BTX immediately after plating. The percentage increased to 20% by 12 hours in culture. After 16 hours, the percentage labeled was comparable to that observed in cultures seeded with mechanically-dissociated cells (45%). In addition, the large, intense clusters present in cells examined immediately after mechanical dissociation were never seen in the trypsinized cells. The appearance of diffuse label and punctate aggregates occurred about 8 hours earlier in trypsin treated cells.

Cell-cell contact apparently plays a role in the regulation of AChR expression, as myoblasts in contact with other myogenic cells were more intensely fluorescent than were isolated myoblasts. Intracellular injections of carboxyfluorescein showed that cytoplasmic fusion was not required for this phenomenon.

305.17 SOME ELECTROPHYSIOLOGICAL PROPERTIES OF LOCUS COERULEUS NEURONS IN FETAL RATS. S.Nakamura and T.Sakaguchi*. Dept. of Neurophysiology, Inst. of Higher Nervous Activity, Osaka University Medical School, Kita-ku, Osaka, 530 Japan.

The locus coeruleus (LC), composed principally of noradrenergic (NA) neurons, is known to appear early in the course of brain development. In the rat, NA neurons of the LC can be seen between embryonic days (E) 12 and 14, and the NA terminals in the cerebral cortex become detectable by E16-E17. A considerable number of morphological and biochemical studies on the development of the LC are now available, whereas the electrophysiological properties of LC neurons in early stages of development have not fully been known. In a series of experiments, we have previously elucidated that LC neurons are functionally active at birth (Kimura and Nakamura, Dev. Brain Res., 23: 301 1985), and now report on the electrical activity of LC neurons in the fetal rats which were kept in contact with the dams by the intact umbilical cord. Pregnant rats at gestation days 18 to 22 were anesthetized with urethane (1.3 g/kg, i.p.) which was supplemented as necessary during experiments. The anesthetic was injected directly to the fetuses if required. The head of the fetus was fixed in a conventional stereotaxic apparatus using a simple device for head fixation. Stimulating electrodes were implanted into the dorsal NA bundle (DNB) and frontal cortex (FC), and the single unit activity of LC neurons was recorded extracellularly by means of a glass micropipette filled with 0.5 M sodium acetate containing 2% pontamine sky blue. The location of the LC was determined by the appearance of field responses to DNB stimulation. Antidromic responses of single LC neurons were evoked superimposed upon the field responses. Although most of LC neurons recorded were not spontaneously active, a small number of them revealed epochs of sporadic firing, which appeared to occur synchronously in many or all LC neurons. From the measurement of distance between the DNB and the LC, the mean conduction velocity of LC axons was calculated to be 0.28 m/sec. Some LC units were activated antidromically from the FC with latencies ranging from 25 to 60 msec, that were similar to values obtained in adult rats. In addition, sensory stimulation (e.g., air puffs to the skin) was effective in activating LC units. These results indicate that LC neurons are already active in prenatal periods.

305.18 EFFECT OF SKELETAL MUSCLE ON THE EXPRESSION OF A CALCIUM-BINDING PROTEIN (CALBINDIN) IN DORSAL ROOT GANGLION OF THE CHICK EMBRYO. E. Philippe* and B. Droz* (SPON: European Neuroscience Association). Inst. d'Histologie et d'Embryologie -Faculté de Médecine -1005 Lausanne - Switzerland.

Immunoreactive calbindin (28 KD vitamin D-dependent Ca-binding protein) was revealed in two subpopulations of chick dorsal root ganglion (DRG) neurons: the so-called large A1 neurons and the small B1 neurons. All the other ganglion cell bodies, satellite cells and Schwann cells were devoid of immunostaining. Large myelinated axons, originating from the A1 cell bodies, and small unmyelinated axons, originating from the B1 cell bodies, were also immunostained in peripheral roots and nerves. Among tested peripheral target tissues, nerve endings of the neuromuscular spindles were found to display immunoreactivity.

Since calbindin immunoreactive nerve fibers are closely associated with skeletal muscles, the question could be raised whether this tissue could influence the expression of calbindin in subpopulations of DRG cells.

To answer this question, immunostaining of DRG was performed in chick embryo under various conditions:

- In the chick embryo, the immunoreactive material was not detected at E7, that is at a time preceding peripheral sensory projections, but appeared by E10, after innervation of the hindlimb.
- When DRG were transplanted at E7 on the chorioallantoic membrane of a host embryo, no immunoreactivity was observed at 3 or 8 days after grafting but when DRG were co-transplanted at E7 with skeletal muscle, the immunostaining was detected in several neuronal cell bodies.
- However, in DRG transplanted at E12 in the absence of muscle, immunoreactive subpopulations were present after 3 or 8 days of grafting.

It is concluded that the initiation of the calbindin expression in sensory neurons seems to be dependent of the interaction with skeletal muscle. In contrast, the maintenance of the calbindin expression would be independent of the target tissues.
Thus the phenotypic expression of primary sensory neurons could be modulated by the epigenetic influence of peripheral projections.
(S.N.S.F. 3.447-0.83/Dr M. Celio is gratefully acknowledged for his gift of anti-calbindin anti-serum).

CLINICAL CNS NEUROPHYSIOLOGY: EPILEPSY

306.1 ELECTROPHYSIOLOGICAL FEATURES OF EPILEPSIA PARTIALIS CONTINUA. O.W.Witte*, H.Hefter*, V. Hömberg*, E. Niedermeyer* and H.-J. Freund. Neurologische Klinik, Univ., Moorenstr. 5, D-4000 Düsseldorf, F.R.Germany

Epilepsia partialis continua (EPC) is regarded as a form of rhythmical cortical myoclonus usually characterized by giant somatosensory evoked responses (SSEP) and by jerk-related EEG potentials (Marsden et al., in Movement Disorders, Butterworth, London, 1982, p. 196). We analyzed the epileptic activity in a case of EPC without jerk-related EEG potentials revealed by back-averaging and without giant SSEP.

Neurological examination gave normal results and brain imaging did not reveal a structural brain lesion. The EEG showed left-sided epileptic potentials not related to muscle jerks. Positron emission tomography revealed a generalized left-sided hypometabolism but no focal hypermetabolism.

The muscle jerks involved the finger flexors and extensors of the right hand. They lasted 30 ms and had a regular repetition rate of 7/s. This frequency was constant during maintenance of posture and slow movements. The muscle bursts could not be triggered or reset by external stimuli. Rapid finger extensions led to a continuous increase of burst rate up to 25/s.

Comparison with experimental focal epilepsy in animals reveals that the described features of EPC could be explained by endogeneous neuronal bursting as observed in the chronic cobalt foci in the interictal state. In contrast to paroxysmal depolarization shifts as recorded in penicillin foci such endogeneous neuronal bursting increases in direct relation to the amplitude of excitatory postsynaptic potentials impinging upon these cells up to comparable repetition rates. Such interictal neuronal bursting is not expected to cause focal hypermetabolism and is not accompanied by significant EEG potentials.

It is suggested that this form of EPC without giant SSEP, jerk-related EEG events and focal hypermetabolism may arise from endogeneous membrane potential oscillations of motor cortical neurons.

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306.2 ALPHA-BAND ACTIVITY AT OCCIPITAL AND CENTRAL DERIVATIONS -- A SELECTED FREQUENCY ANALYSIS. L.S. Leung and R.E. Steenhuis, (SPON: M.A. Goodale) Depts. Psychology, Clinical Neurological Sciences and Physiology, Univ. Western Ontario, London, Canada N6A 5C2.

Subjects were seated in a dark, sound-attenuated room and alpha-band activity was recorded from the central (C3, C4 and Cz) and occipital (O1 and O2) derivations with reference to linked ear electrodes. Signals were digitized at 200 Hz and blocked into 1.28 sec segments. After discarding segments with artifacts, pairs of signals of 1.28 sec duration were subjected to Fourier analysis to yield autopower, coherence and phase spectra (each with df=6). For each subject, segments yielding an O2 autopower peak of one particular alpha-band frequency were selected. The autopower spectra at O1, O2 and C4, and the coherence and phase spectra of the O1-O2 and O2-C4 signals were all averaged for segments yielding the selected alpha peak frequency at O2. For example, if the 1st, 10th, 14th and 21st segments were found to have a peak frequency of 7.8 Hz at O2, the spectra corresponding to these segments were then averaged to give an average spectrum with O2 peak frequency at 7.8 Hz. The use of 1.28 sec segments limits the resolution of the frequencies to 0.78 Hz. (see Leung, Electroenceph. Clin. Neurophysiol. 58: 553, 1984 for a similar application to the hippocampal theta rhythm). This selective averaging technique allows investigation of the dependence of various parameters on the O2 alpha peak frequency (independent variable).

Detailed analysis from 4 subjects in the dark show that the maximal alpha power lay at 9.4 or 10.2 Hz. However, significant periods of occipital alpha had lower (7-8.6 Hz) or high (10.9 Hz) peak freqencies, but usually of lower power than the 9.4-10.2 Hz alpha. Alpha activity at all frequencies (7-10.9 Hz) was highly coherent (linear coherence >0.76) between O1 and O2. Coherence in the alpha-range frequency was lower between O2 and C4 (range of linear coherence 0.3-0.7). The O1-O2 phase at the alpha peak frequency was always indistinguishable from zero, while O2-C4 phase ranged 0°-80°, often significantly different from zero. This is no consistent dependence of phase on frequency. Occasionally, alpha peaks at O2 and C4 were at different frequencies. The selected alpha frequency analysis emphasizes the difference between central and occipital alpha activities. It extends the finding of a high coherence between occipital alpha rhythms to a range of frequency of 7-11 Hz for each subject.

306.3 CARDIAC AND RESPIRATORY CHANGES ASSOCIATED WITH TEMPORAL LOBE EPILEPTIFORM ACTIVITY. R.C.Frysinger and R.M. Harper. Dept. of Anatomy and Brain Research Inst., UCLA, Los Angeles, CA 90024.

Electrical stimulation of mesial temporal lobe structures in animals elevates arterial pressure, accelerates heart rate, and disrupts respiration, whereas experimentally induced seizures produce similar, but exaggerated effects on the cardiovascular system, including a variety of cardiac arrhythmias. Mesial temporal lobe structures may thus play an important role in the control of cardiac and respiratory function. In patients suffering from partial seizures, epileptiform discharge in mesial temporal structures could disrupt cardiac and respiratory function, possibly playing a role in sudden death associated with epilepsy. These dysfunctions may be particularly prominent during sleep because of the heightened potential for ictal discharge, and, since different sleep states elicit a variety of cardiorespiratory patterns, seizure-initiated cardiac or respiratory disturbances may be unmasked.

We have recorded ECG together with chest movement and airflow measures of respiration in nine patients being evaluated as candidates for surgical treatment of medically refractory complex partial seizures. These individuals were undergoing long-term telemetry monitoring, with depth electrodes implanted into the amygdala and hippocampus. We were thus able to correlate spontaneous ictal or interictal depth EEG abnormalities with cardiac rate or respiratory changes.

Both simple and complex partial seizures typically produced cardiac acceleration. Heart rate was often labile in the postictal period, with R-R intervals varying by as much as 600 msec between successive beats. Differentiating state effects on the cardiac consequences of seizure activity was difficult, because of transient wakenings associated with ictal episodes.

Simple and complex partial seizures also produced apneustic episodes up to 45 sec in duration. In addition, three of the nine patients recorded to date suffered from varying degrees of sleep apnea, with two showing severe mixed or central apneas over most of the night. These apneas were not reliably associated with EEG abnormalities, but they may be a consequence of an underlying brain lesion, antiepileptic medication, or CNS reorganization associated with the seizure disorder.

These findings suggest that epileptiform discharge in the mesial temporal lobe is associated with powerful descending influences on cardiac and respiratory systems. Moreover, they demonstrate the importance of obtaining measures of state-related cardiorespiratory patterning, since sleep deprivation resulting from severe apnea could increase seizure frequency and exacerbate psychiatric deficits commonly found in this patient population. Supported by NS 02808-25.

306.4 Long-lasting Alteration of Evoked Activity in Piriform Cortex Induced by Kindling of Olfactory Bulb. T.P. Sutula & L. B. Haberly. Depts. of Neurology and Anatomy, University of Wisconsin, Madison, WI 53706

Chronic recording in rats (Russell and Stripling, Brain Res. 361:61) has demonstrated alterations of evoked activity in the piriform cortex (largest subdivision of olfactory cortex) in response to activation of the olfactory bulb (OB). We are investigating the neuronal events underlying these changes in urethane anesthetized rats in which stimulating and recording locations and depths can be accurately controlled.

Rats were stimulated in the OB with daily trains of 60 Hz bipolar pulses until kindled class 5 seizures were evoked. Two to 9 days after the last seizure, potentials were evoked in piriform cortex by stimulation of afferent fibers in the lateral olfactory tract. The most striking change was the development of a high amplitude complex field potential that occurred in all-or-none fashion both spontaneously and at variable latency in response to stimulation of afferent or association fibers. In deep layers the spontaneously occurring potential consisted of a biphasic positive-negative slow wave (60-80 msec duration) with a high amplitude negative "spike" component near the onset of the initial positive slow wave. When evoked by afferent or association fiber stimulation the waveform was dependent on latency of occurrence, although it usually resembled the spontaneously occurring form when overlying potentials were subtracted. Frequency of occurrence of the all-or-none potential is increased by paired pulse afferent fiber stimulation with a 30 msec intershock interval and is higher with a stimulus repetition rate of 1 Hz than 0.1 Hz. This potential occasionally occurs twice or more in succession giving rise to an afterdischarge-like response. Extracellular recording revealed a synchronous discharge of single action potentials in the pyramidal cell layers during the fast spike component of the all-or-none field potential. Intracellular recordings revealed that the initial deep positive slow wave component is associated with a high amplitude EPSP and the second deep negative slow wave associated with an IPSP. Long latency, long duration depolarizing and hyperpolarizing potentials also occur.

An intriguing feature is that interictal epileptiform events occur in the olfactory cortex of kindled rats in absence of obvious alteration in afferent fiber evoked excitation and in the presence of both fast and slow inhibitory processes. We are testing the hypothesis that kindling induces a change in the balance of intrinsically mediated excitatory and inhibitory processes. Supported by NIH grant NS19865 (LBH), the Epilepsy Foundation of America (TS), & TIDA K07-NS808 (TS).

306.5 DEEP PREPIRIFORM CORTEX AND AMYGDALA KINDLING. D.Zhao* and S.L.Moshé Dept. of Neurology, Albert Einstein College of Medicine, Bronx, N.Y.10461.

The deep prepiriform cortex (DPC) has been recently identified as a crucial epileptogenic site in the rat brain (Piredda, S. and Gale, K., Nature, 317:623, 1985). In this report we studied the susceptibility of the DPC to the development of electrical kindling as compared to that of the superficial prepiriform cortex (SPC) and amygdala. Adult rats had electrodes implanted in the DPC, SPC or amygdala and were kindled using standard techniques.

There were no differences in the afterdischarge thresholds as a function of site. The behavioral seizures induced by DPC and SPC kindling were identical to those induced by amygdala kindling. Both DPC and SPC were as sensitive to the development of kindled seizures as the amygdala. The number of stimulations required for the development of the first stage 5 seizure was $\bar{x}\pm$ SE = 12.6 $\pm$ 1.2 for the DPC, 13.2 $\pm$ 1.7 for the SPC and 12.9 $\pm$ 1.3 for the amygdala, $F(2,17) = 0.04$, ns. There were no statistically significant differences in the afterdischarge durations during the first generalized seizures among the three groups $F(2,17) = 2.8$. However, EEG recordings demonstrated that the amygdala developed long lasting, self-sustained afterdischarges independent from the DPC or SPC afterdischarges even when the DPC or SPC were the site of stimulation.

These results indicate that the prepiriform cortex (DPC and SPC) is highly susceptible to the development of kindling although the amygdala appear to be more important in the kindling circuitry.

306.6 FOUR-AMINOPYRIDINE INDUCED AFTERDISCHARGE ACTIVITY IN THE HIPPOCAMPAL SLICE OF IMMATURE RATS. T.J. Chesnut and J.W. Swann. Wadsworth Center for Laboratories and Research, N.Y.S. Department of Health, Albany, NY 12201.

Most models used for the study of epileptogenesis in the *in vitro* brain slice preparation produce epileptiform activity by blockade of synaptic inhibition. We report here that the convulsant 4-aminopyridine (4-AP) can elicit prolonged seizure-like afterdischarges in the developing hippocampus even though synaptic inhibition is present.

Transverse slices 500 µm thick were cut from hippocampi of immature rats (10-15 days of age) and transferred to the surface of a nylon net in the experimental chamber. All slices were incubated at 32-33°C in oxygenated artificial cerebrospinal fluid (ACSF) constantly perfused under the net at 0.5-1.0 ml/min. The upper surfaces of the slices were exposed to humified 95% O_2/5% CO_2. The slices were incubated for at least 60 minutes before exposure to the 4-AP (20-60 µM) which was added directly to the ACSF. As with other convulsants, slices from the immature animals displayed more prolonged epileptiform activity in response to the 4-AP than did slices from mature animals. Extracellular field recordings obtained from the pyramidal cell layer of area CA3 of slices from the immature animal indicated regularly occurring ictal-like episodes consisting of prolonged afterdischarges (ADs) in response to the 4-AP. Often, interictal burst discharges were also present.

Intracellular recordings from presumed pyramidal cells indicated action potential activity concurrent with the extracellularly recorded population spikes during the ADs and the interictal bursts. The slices displayed a number of patterns of epileptiform activity in response to the drug. Although there were variations between slices, the activity changed very little within a slice once a given pattern was established. The slices did have a tendency to suddenly stop displaying epileptiform activity, as if the activity had been so intense that the transmitter supply had been exhausted. Simultaneous extracellular recordings from both CA1 and CA3 indicated that AD activity spread from CA3 to CA1. Population spike activity during the AD in CA1 followed that in CA3 in a one-to-one fashion with a latency of 20-60 msec. The delay was constant in any one AD for a given position of each electrode pair.

Intracellular recordings also indicated that inhibitory synaptic potentials were present during the 4-AP induced epileptiform activity. These inhibitory potentials often occurred during the interictal bursts and were capable of inhibiting action potential generation. Current injection indicated that they had a reversal potential similar to those of gamma-aminobutyric acid mediated IPSPs activated during recurrent inhibition.

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306.7 SECONDARY BILATERAL SYNCHRONY FROM STIMULATION OF SUBSTANTIA INNOMINATA IN RATS WITH FOCAL PENICILLIN SPIKES. F. Bihari* and R.S. McLachlan (SPON: T. Feasby). Epilepsy Unit Research Lab., Departments of Clinical Neurological Sciences and Physiology, University of Western Ontario, London, Ontario N6A 5C1.

Secondary bilateral synchrony is the development of generalized synchronous electrographic seizure activity from a cortical focus. Proposed mechanisms include triggering of generalized discharges from subcortical structures by the cortical focus and reduced inhibition in the cortex from multiple foci allowing synchronization of seizure activity. The substantia innominata (SI) is a subpallidal region of the basal forebrain with widespread cholinergic efferents to the neocortex. Since cholinergic pathways have long been implicated in the pathophysiology of seizures, the possible role of SI in the mediation of generalized seizure activity was assessed.

A bipolar stimulating electrode was placed in the SI (histologically confirmed) of urethane anesthetized Wistar rats and the electrocorticogram recorded from both hemispheres. Unilateral stimulation (0.2 - 0.8 mA, 0.2 - 1.0 ms, 10 - 50 hz) was carried out both before and after a cortical spike focus was established over the convexity of the ipsilateral hemisphere using a 1 mm^2 pledget of filter paper soaked with Penicillin G 100,000 IU/ml. Stimulation before Penicillin either had no effect or induced low voltage generalized 3-4 hz synchronized waves on the electrocorticogram. Penicillin induced focal spikes which frequently spread to homologous cortex of the opposite hemisphere. Penicillin spike frequency was inhibited during SI stimulation and at times increased after stimulation. In addition, generalized bisynchronous polyspikes and polyspike waves appeared simultaneously over both hemispheres with minimum latency of one second after SI stimulation onset persisting for up to 70 seconds and outlasting the stimulation offset for up to 60 seconds. These seizures could be blocked by topical application of atropine to the cortex. Secondary bilateral synchrony from a cortical focus may be related to enhanced activity of diffuse cholinergic afferents from the basal forebrain to the cortex, or to an alteration in cortical responsiveness to such inputs.

(Supported by PSI Foundation)

306.8 D(-)APV AND KYNURENIC ACID SUPPRESS ICTAL-LIKE ACTIVITY IN PENICILLIN-TREATED HIPPOCAMPAL SLICES. R.J. Brady and J.W. Swann. Section for Dev. Neurophysiol., Wadsworth Center for Laboratories and Research, NYS Department of Health, Albany, NY 12201.

Our laboratory has previously demonstrated that hippocampal slices taken from immature rats 9-19 days of age, when exposed to penicillin have a pronounced capacity to undergo ictal-like activity. Paired intracellular and extracellular recordings in the CA3 region show that these discharges consist of a depolarization shift (DS) and associated field epileptiform burst followed by a self-sustaining series of afterdischarges of up to 30 sec in duration. It is thought that N-methyl-D-aspartate (NMDA) preferring excitatory amino acid receptors are involved in CNS hyperexcitability, including epileptiform phenomena. We have previously observed that ketamine, and other NMDA receptor antagonists thought to act at the PCP/sigma opiod recognition site, effectively suppress afterdischarges and shorten the depolarization shift. These effects occur at concentrations that selectively antagonize the NMDA response of CA3 pyramidal cells.

The involvement of NMDA-receptor mediated processes in afterdischarge generation is further supported by the results of experiments presented here. Bath application of D(-)2-amino-5-phosphonovalerate (D(-)APV), thought to be a highly specific NMDA-receptor antagonist, completely and reversibly suppresses afterdischarges in penicillin-treated immature hippocampal slices. However, a concentration of 400 µM is necessary for this blockade. At this concentration the duration of the DS is also decreased. In contrast, bath application of only 100 µM of kynurenic acid (KYN) produces essentially identical effects. In perfusion media containing tetrodotoxin, no calcium and 2 mM manganese, 400 µM D(-)APV or 100 µM KYN reversibly suppress the response of CA3 pyramidal cells to the iontophoretic applications of NMDA while leaving the response elicited by the application of quisqualate relatively unaffected.

These and other observations led to the conclusion that NMDA-receptor dependent depolarizations are a necessary part of the mechanism of afterdischarge generation. The observation in the immature hippocampal slice that KYN seems four times more potent in suppressing afterdischarges and NMDA responses than D(-)APV is unexpected. It is possible that differences in receptor selectivity make KYN more potent. It is also an interesting possibility that in the CA3 area of the immature hippocampal slice, kynurenine preferring NMDA receptors predominate.

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306.9 INHIBITION OF FOCAL PENICILLIN SPIKES BY ELECTRICAL STIMULATION OF THE SUBSTANTIA NIGRA IN RATS. R.S. McLachlan and D. Whitney*. Epilepsy Unit Research Lab., Departments of Clinical Neurological Sciences and Physiology, University of Western Ontario, London, Ontario N6A 5C1.

Lesions of substantia nigra (SN) and GABA agonists injected into SN both inhibit behavioural and EEG generalized seizure activity suggesting that SN may help regulate widespread neuronal excitability.

We examined the effect of electrical stimulation of the SN on Penicillin induced focal cortical spike activity in urethane anesthetized Wistar rats. Penicillin G 100,000 IU/ml was topically applied to the cortex using a 1mm square pledget of filter paper to induce focal spiking which could be maintained for several hours. Some homologous spread of the discharges to the opposite hemisphere also occurred. Stimulation of SN on the side of the focus using bipolar electrodes (0.2 - 0.8 mA, 0.2 - 5 ms, 10 - 50 hz) most commonly inhibited spike frequency during and for a brief period after stimulation, although a slight increase in spike frequency during stimulation or no change also occurred. Inhibition was greatest when focal spike frequency was high. Seizures were not activated by SN stimulation and in some animals which developed generalized EEG seizures, this activity could be terminated by stimulating SN. This was in contrast to stimulation of the reticular formation dorsal to SN which regularly increased spike frequency and induced seizures. Inhibition of interictal and ictal activity by local release of GABA within SN by electrical stimulation would be consistent with previous data indicating a proconvulsant action of SN. However, a direct activation of SN efferents could also be responsible for the effects of electrical stimulation.

(Supported by PSI Foundation)

306.10 CARBAMAZEPINE ABOLISHES ICTAL BUT NOT INTERICTAL EPILEPTIFORM DISCHARGES IN IMMATURE HIPPOCAMPUS IN VITRO. K.L. Smith* and J.W. Swann. (SPON: V. Madelian). Section for Dev. Neurophysiol., NYS Dept. of Health, Wadsworth Ctr. for Labs & Res. Albany NY, 12201.

Carbamazepine (CBZ) is a widely used anticonvulsant and is particularly effective in controlling focal seizures in children. Since this form of epilepsy often originates from hippocampal formation, we have studied CBZ's effects on epileptiform discharges produced by penicillin in the CA3 subfield of slices from immature rats. As previously reported, bath application of penicillin to slices from rats 1-2 weeks of age produces interictal discharges similar to those recorded in mature hippocampus. In immature tissue, however, the interictal discharge is followed by repetitive and rhythmic synchronized discharges of CA3 pyramidal cells. These discharges are often 30 sec in duration.

Hippocampal slices were taken from rats 9-14 days of age. Once exposed to penicillin (1.7 mM), epileptiform discharges were established. Intracellular and extracellular recordings were obtained from the CA3 pyramidal cell body layer. CBZ was bath applied at concentrations of 5-100 µM. In some instances, effects on spontaneous epileptiform discharges were studied. However, in most experiments, epileptiform events were evoked by orthodromic stimulation of fiber tracts in stratum radiatum. In experiments in which dose response relations were studied, a low frequency (0.1-0.2 Hz) repetitive stimulation paradigm was employed. This paradigm resulted in interictal spikes followed by brief afterdischarges of relatively constant duration. Bath application of therapeutic concentrations of CBZ to slices suppressed synchronized afterdischarge generation. Concentrations of 30 µM completely abolished ictal-like discharges. These effects were highly dose dependent and the IC_{50} was 8.5 $\pm$ 1.4 µM (SEM N = 7). Interestingly the duration and amplitude of the interictal spike was unaffected by CBZ at these concentrations. In addition, the frequency of spontaneous interictal discharges was unaltered and, in some instances, actually increased. At supratherapeutic concentrations (100 µM), the duration of the interictal spike decreased minimally (only by 24.4% $\pm$ 1.2). Threshold stimulation strength remained unaltered; the interictal spike maintained its characteristic all-or-none property.

Intracellular recordings from CA3 pyramidal cells revealed that CBZ had no effect on resting membrane potential or resistance. In keeping with extracellular recordings, at concentrations of 30 µM, intracellularly-recorded afterdischarges were eliminated by CBZ but the amplitude and duration of the depolarization shift was unaltered. At 100 µM the drug decreased the duration but had no effect on depolarization shift (DS) amplitude. Taken together these results suggest that a CBZ-sensitive physiological process, distinct from those underlying DS generation, plays a key role in afterdischarge generation. (Supported by NIH Grant NS 18309).

306.11 DIFFERENCES IN RELATIVE ANTIEPILEPTIC DRUG EFFICACY BETWEEN CORTICAL FOCI IN THE RAT. M.W. Kalichman, K.A. Moss* and V.J. Iragui*. Depts. of Anesthesiology and Neurosciences, VA Medical Center and University of California, San Diego, San Diego, CA 92161

The relative efficacies of phenobarbital (PB), phenytoin (PHT), carbamazepine (CBZ), and valproic acid (VPA) were tested for the suppression of focal seizures produced by electrical stimulation of cortical areas 3 and 10 in the rat. Twenty-six (26) male, Sprague-Dawley rats (250-300 g) were prepared for the study by stereotaxic placement of bipolar electrodes either in cortical area 3 (mm from bregma: AP=0.0, Lat=3.0) or 10 (AP=4.0, Lat=2.0). Following recovery from surgery, individual afterdischarge thresholds were estimated and their stability was determined. Stimulation consisted of a 1-s train of 60 Hz, biphasic 1-ms square waves. Afterdischarge thresholds (mean ± SEM) were not signifcantly different between cortical areas 3 (5,680 ± 1,325 uA) and 10 (5,710 ± 1,540). Using a randomized, repeated measures design, subjects were each pre-treated with a coded solution prior to threshold electrical stimulation by an investigator blinded to the identity of the drug and dose being tested. The incidence and duration of subsequent afterdischarge activity were recorded. Each subject was tested once at each of 3 doses of each drug and with a drug vehicle as control and on each test day each drug or control dose was tested in one subject in each of the cortical groups. At the conclusion of the study the results were unblinded and the resulting dose-response data were analyzed for possible differences in slope or elevation. The two cortical sites were distinguished by signicantly different dose-response curve slopes for the suppression of afterdischarge duration by PHT, CBZ and VPA (P 0.05), which suggests more than one mechanism of action for these drugs. The dose-response curve slopes for PB, on the contrary, were not significantly different, although its potency was signicantly greater in area 10 (P 0.002). It is concluded that focal seizures elicited by the stimulation of different cortical sites are differentially refractory to antiepileptic drugs. The significance of these results to the clinical use of antiepileptic drugs is the implication that the choice of antiepileptic treatment might depend in part on the cortical focus for partial or partial-generalized seizures.

306.12 EFFECTS OF ANTICONVULSANT DRUGS ON AFTERDISCHARGE THRESHOLD IN KINDLED RATS: A MODEL OF TEMPORAL LOBE EPILEPSY. M.G. Vartanian*, P.J. Mickevicius*, B.Z. Stieber*, and C.P. Taylor, Dept. of Pharamcology, Warner-Lambert/Parke-Davis Research, Ann Arbor, MI 48105

The kindling phenomenon as first described by Goddard et al, (Exp. Neurol. 25: 295, 1969) is a progressive intensification of seizures after repeated subconvulsive electrical stimulation of the brain, that eventually results in generalized motor convulsions. These changes in neuronal excitability are apparently permanent, and may be similar to clinical epilepsy.

This study investigates changes in the electrical threshold for focal seizures (afterdischarges) in hippocampal-implanted kindled rats. We have developed a standardized pharmacological procedure as an animal model of temporal lobe epilepsy. A separate group of unimplanted rats were used to determine behavioral side-effects of each drug, allowing therapeutically relevant doses of each drug to be determined.

Effects of several drugs were quantified; ED50 (mg/kg IP) follows drug name (carbamazepine 7.4, phenytoin 23; zonisamide 13; sodium valproate 150; phenobarbital 41; diazepam >10; ethosuximide >200; baclofen >10; acetazolamide 15; imipramine 19). Carbamazepine, phenytoin, zonisamide, and acetazolamide were most effective in elevating the afterdischarge thresholds without causing behavioral side-effects, while sodium valproate, phenobarbital, and imipramine were somewhat less effective. Diazepam, ethosuximide, and baclofen were relatively ineffective at doses below those causing side effects. These results correlate with clinical anticonvulsant actions against partial seizures, suggesting that this procedure is an appropriate and sensitive animal model.

306.13 COCAINE'S EFFECTS ON EVOKED POTENTIALS IN THE PYRIFORM CORTEX: LOCAL ANESTHETIC AND MONOAMINERGIC MECHANISMS. D. K. Patneau and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Previous investigations in our laboratory have examined cocaine's pro- and anti-convulsant effects on kindling and kindled seizure expression in the olfactory bulb (OB) and pyriform cortex (PC). Although evidence indicates that these effects are due primarily to cocaine's local anesthetic action, the contribution of cocaine's monoaminergic action needs further evaluation. This study examined the pharmacological basis of cocaine's effects on the olfactory forebrain by comparison with lidocaine, another local anesthetic, and amphetamine, a behavioral stimulant which also potentiates monoaminergic transmission.

Male Long-Evans rats were chronically implanted with a stimulating electrode in the OB, a recording electrode in the PC, and an intravenous catheter, and divided into 3 groups. Evoked potentials (EPs) were recorded every 5 sec from 2 min before to 5 min after infusion of each of 3 doses of either cocaine, lidocaine, or amphetamine. Drug effects were analyzed on the initial two waves of the potential evoked by OB stimulation: period 1, reflecting both mono- and di-synaptic excitation in the PC; and period 2, believed to reflect inhibitory processes. A1, the monosynaptic component of period 1, reflects activation of PC pyramidal cells via the lateral olfactory tract (LOT), while B1, the disynaptic component, is produced by association fibers within the PC.

The results indicated that cocaine has both local anesthetic and monoaminergic effects on PC evoked potentials which are distinguishable. Cocaine and lidocaine depressed the amplitude of A1, which is consistent with local-anesthetic blockade of Na+ channels in LOT axons. Local anesthetic effects on B1 and period 2 were more complex, and their evaluation required consideration of differential effects of local anesthetics on myelinated and unmyelinated fibers and/or various intrinsic conductances. The monoaminergic effects of cocaine and amphetamine appeared to be restricted to the polysynaptic response within the PC to OB stimulation, and included an increase in B1 and/or a decrease in period 2. These changes suggest that PC excitability is modulated by central monoaminergic afferents, and may explain why cocaine has a substantially lower convulsive dose than lidocaine.

Implications of these findings for models of seizure development and expression will be discussed, with emphasis on the olfactory forebrain as a seizure-prone cortical system, and on the mechanisms which may underlie the convulsant and anticonvulsant effects of local anesthetics in the CNS.

306.14 NALOXONE AND CORTICAL SPREADING DEPRESSION. R.C.A. Guedes*, T.P. Hicks and F.A.M. de Azeredo* (SPON: H.B. Sarnat). Dept. of Nutrition, Fed. Univ. of Pernambuco, Recife, Brazil; Dept. of Med. Physiol., Univ. of Calgary, Calgary, Alta., T2N 4N1; and Dept. of Neurobiol., Fed. Fluminense Univ., Niteroi, RJ, Brazil.

Leão first described the phenomenon of cortical spreading depression (SD) over forty years ago (J. Neurophysiol. 7:359, 1944), but it still remains a puzzle as to the exact mechanism underlying the associated process of the slow potential change (SPC) accompanying the phenomenon. To test our hypothesis that GABA-mediated processes are involved in the SPC and its conductance along the cortical surface, we have used topical application and microiontophoretic administration of pharmacological compounds (GABA, glutamate, bicuculline (BMI) and naloxone) to cells of the cerebral cortices of rats and gerbils in several experimental paradigms. Iontophoretic experiments were performed together with SPC recording and SD elicitation using surface-applied K^+ in 10 Sprague-Dawley rats anaesthetized with a mixture of urethane and chloralose. Topical application of naloxone in conjunction with SPC recording and SD elicitation with K^+ was conducted in 8 gerbils and one rat under the same anaesthetic conditions. In 6 experiments with unanaesthetized gerbils prepared under ether, maintained with local anaesthesia on wound margins, electroshock-induced SD was recorded and the SPC tested with i.p. naloxone (25-50 mg/kg).

Fourteen cortical cells were recorded from rats, using multibarrel micropipettes; these were inhibited by GABA and excited by glutamate. Surface stimulation with K^+ (1 min) brought about a propagating wave of SD and accompanying SPC. The SPC was blocked by iontophoretic naloxone (8 of 9 cells) but not by BMI (5 cells). Additionally, the transient phase of neuronal inexcitability, which occurs subsequent to a brief period of excitation preceeding the SPC, was prevented by iontophoretic naloxone but was little affected by BMI. Topically applied naloxone blocked the SPC completely on 8 gerbils and one rat tested in this paradigm. Electroshock treatment to 6 gerbils evoked seizure patterns with tonic and sometimes clonic seizure-like activity, after which a short period of paralysis was observed - this latter phase invariably was accompanied by surface DC recording of spike-epileptiform activity and subsequent SD elicitation. Following i.p. naloxone, we blocked the appearance of SD, and therefore could dissociate the seizures from the occurence of SD.

These experiments provide evidence to support the view that the phasic neuronal inexcitability following the SPC of SD is not due to synaptically released GABA or to conversion of glutamate to GABA, and further that a naloxone-sensitive process is required for the local expression of the SPC and perhaps also for the integrity of the process responsible for the propagation of SD.

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306.15 EARLY CHANGES IN FELINE ALUMINUM HYDROXIDE FOCAL CORTICAL EPILEPSY D.L. Knox, M.D.* J.C. Godersky, M.D., and T. Yamada, M.D.*Division of Neurosurgery, University of Iowa Hospitals, Iowa City, IA 52242

Few studies have been designed to elucidate the early electroencephalographic changes occurring in the aluminum hydroxide model of epilepsy. Most studies have assumed that the primary site must undergo a maturation period and develop epileptiform activity prior to the development of abnormalities in other areas.

In order to characterize the early changes, 13 adult cats were studied. Utilizing aseptic conditions and general anesthesia, commercially manufactured concentric bipolar recording electrodes were placed bilaterally into the hippocampus, amygdala, putamen, thalamus, frontal and occipital cortex. Baseline electroencephalographic recordings were done prior to subpial placement of 0.04 cc aluminum hydroxide ($Al(OH)_3$) in the left sigmoid gyrus. EEG recordings were obtained daily for two weeks, then every three days for 11 weeks until sacrificed by intracardiac perfusion.

Clinical epilepsy was induced in 7 of 13 animals, two of which developed status epilepticus. All cats went through an evolution of EEG changes which were divided into acute, preictal, and ictal phases. The acute phase typically began the day following $Al(OH)_3$ injection and lasted two weeks. Bursts of sharp and theta transients and spike and wave activity predominated in the right frontal and occipital areas but also occurred in the left frontal and the left and right thalamus, amygdala and hippocampus.

The initial period of the preictal phase was characterized by a resolution of the acute phase changes. This generally lasted 5-7 days. The later portion of the preictal phase, lasting 3-6 days, revealed a resurgence of cortical sharp and theta transients with spike and wave formation with left frontal predominance. Deeper structures were also involved in a similar fashion with the formation of spike and wave activity. In the 6 cats that did not develop clinical seizures, the sharp theta transients persisted with multiple spike and wave formation diffusely throughout the cortex and deep nuclear structures. The 7 cats that developed seizures went on to develop the primary focus with frequent spike wave activity. Clinically the seizures were short lived and consisted of intermittent myoclonic head movement. During this ictal phase typically spike and waves and theta transients persisted throughout the left hemisphere and left cortex.

Extensive abnormalities become manifest throughout the cortex and in a number of subcortical areas prior to the onset of clinical or electroencephalographic seizures. Apparently the mechanism of a "single maturing primary focus" is too simplistic and the interaction of the primary cortical focus with other cortical and deep nuclear structures is critical for the later development of a primary focus and must be considered in the mechanism of the $Al(OH)_3$ model of feline epilepsy.

306.PO EFFECTS ON KINDLED AMYGDALA SEIZURES OF GAMMA VINYL GABA INFUSION AND FETAL CEREBELLAR GRAFTS IN ENDOPIRIFORM AREA OF RAT BRAIN. J.R. Stevens, I. Phillips, R. Beaurepaire and W. Freed. Neuropsychiatry Branch, NIMH, Washington, D.C. 20032

In 1985 Piredda et al. (Life Sci. 36:1295) reported finding a sharply circumscribed site in the deep prepiriform cortex of the rat where unilateral infusion of only 49 picomoles of the GABA receptor blocking agent bicuculline caused bilateral clonic seizures. We now report the suppression of amygdala kindled seizures following infusion of this area with gamma vinyl GABA, (GVG), an irreversible inhibitor of GABA transaminase. To study the effects of sustained increase in GABA release in this site, a second group of kindled animals has been implanted with tissue from fetal cerebellum in the endopiriform area (EPA) bilaterally.

Thirty-two Sprague-Dawley rats were used in these experiments. Under chloral hydrate anesthesia the animals were implanted stereotaxically with a bipolar stimulating-recording electrode of twisted teflon insulated stainless steel wire in left basolateral amygdala. In 18 animals stainless steel cannulas were implanted bilaterally just dorsal to the deep prepiriform cortex (A-4;L-3;V-7) and bilateral dural electrodes were placed over parietal regions for cortical EEG recording. Following identification of the critical site for inducing generalized seizures by infusion of bicuculline methiodide through the left and right cerebral cannulas, the animals were kindled by daily electrical stimulation. The lowest stimulus current required for inducing stage IV-V seizures was then determined on three consecutive stimulations. Twenty-four to 48 hours after determining threshold, GVG (2.5 ug/ul) was infused in each prepiriform cannula at a rate of .25 ul/min at the depth that had given maximum clinical or EEG response to bicuculline. Five animals had generalized seizures during or immediately following the GVG injection. Two to six hours following the GVG infusion and daily thereafter animals were again tested for kindled seizure threshold starting with stimulus strength 10-20 ua below previously established threshold and increasing current strength at 1 minute intervals to a level 110-800% of pre-GVG threshold. The GVG infusion raised seizure threshold or prevented seizures for 1-12 days in 12 of 14 animals.

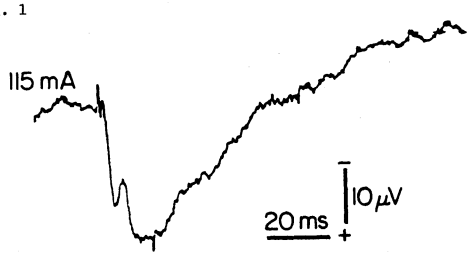
Fourteen additional rats were kindled in similar manner and threshold for Stage IV-V seizures established. Half of these animals were then implanted stereotaxically in the EPA with cerebellar tissue dissected from Sprague-Dawley embryonic rats of 15-16 days gestation. The remainder were implanted with cortex from the same embryonic brains. The effects of this procedure on kindled seizure threshold 2,4,6 and 8 weeks after grafting and results of histologic and immunocytochemical studies of grafted tissues will be reported.

CLINICAL CNS NEUROPHYSIOLOGY: EVOKED POTENTIALS

307.1 HUMAN CEREBRAL CORTICAL RESPONSES TO CONTRALATERAL TRANSCRANIAL STIMULATION. V.E. Amassian and R.Q. Cracco. Depts. of Physiology and Neurology, SUNY Health Sci. Ctr., Brooklyn, N.Y. 11203.

We wished to develop a non-invasive electrophysiological measure of conduction in and transsynaptic activation of human cerebral cortical neurons, e.g. subserving the transcallosal response. Merton and Morton showed that limb movements could be evoked by large transcranial stimuli (Nature, 1980, 285:227). Using ourselves as subjects, shock artefacts were reduced by: 1) stimulating percutaneously with a focal anode (5 cm^2) situated midway between F_4 and C_4 (i.e. right frontal region, standard 10-20 nomenclature) and a medially located, semicircular grounded cathode (26 cm^2) no closer than 3-4 cm away; 2) recording with the focal scalp electrode over homologous left frontal cortex versus two or more reference electrodes interconnected through variable resistors and at least 6 cm away. Pulses of 90-140 mA and lasting 100 μs elicited a brief diphasic deflection (latency 2-4 ms), followed by an initially positive, often bifid wave with a latency of 10-14 ms, lasting 25-44 ms and reaching 5-10 uV (Fig. 1 shows 10 summed responses). The initial diphasic deflection greatly increased when the stimulating anode was moved laterally over temporalis muscle, indicating an EMG origin. The EMG, but not the positive wave was elicited when the interpolar distance was reduced to one cm, which greatly reduced the fraction of current traversing the cortex; furthermore, occluding the circulation to the scalp for 30 min by rubber tubing above the zygomas and brows dulled sensation, reduced the EMG, but did not alter the latency or rising phase of the positive wave (J. Physiol., 1986, in press). Early somatosensory components were small compared with the positive wave. We conclude that the positive wave is mainly a corticocortical (CC) response. Stimulus intensities required for CC responses were not distressing; those required for movement of digits during voluntary contraction were much higher (>170 mA). The CC response may be valuable in the comatose patient in establishing cortical viability.

Fig. 1



307.2 INPUT-OUTPUT RELATIONSHIP OF SOMATOSENSORY EVOKED POTENTIALS IN HUMANS UNDER NORMAL AND PATHOLOGICAL CONDITIONS : G. Adler* and A.C.Nacimiento; Neurosurgical Research Laboratory, Saarland University School of Medicine, 6650 Homburg/Saar, F.R.G.

A study was made of the relationship between the amount of afferent activity (input) elicited by stimulation of the median nerve at the wrist at various strengths and frequencies, and the corresponding somatosensory evoked potentials (SEP) (output). SEP were recorded from cervical, parietal and frontal electrode locations in normal subjects, and in patients following either head trauma or various cerebral ischemic conditions. Stimulation frequency was varied between 1/s and 5/s. Input was measured as the size of the mass action potentials recorded with an electrode pair placed over the median nerve proximal to the stimulating one. Output was determined by measuring the amplitude of the evoked cortical N20-P27 complex. Input-output curves were obtained by plotting amplitude changes of the N20-P27 complex as a function of size of afferent activity triggered by increasing stimulation strengths or frequencies. In normal persons, N20-P27 amplitude rose steeply at only moderately increased stimulation strengths, thus displaying the low degree of spatial summation needed for effective synaptic transmission in the afferent pathways involved. In contrast, N20-P27 amplitude decreased approximately linearly with increasing stimulation frequency. Under the pathological conditions mentioned above, the most striking changes were a) a significant decrease in steepness of the input-output relationship based on stimulation strength; and b) a loss of gradualness in the frequency-dependent one. In the latter case, SEP amplitude decreased abruptly at stimulation rates much lower than its normal counterparts. A systematic study of the correlation of the pathophysiological basis of these changes with the corresponding clinical pictures is being made. Present results suggest that the determination of input-output relationships may constitute an useful parameter in the clinical assessment of SEP changes.

307.3 EFFECTS OF HYPOXIC-HYPOXIA ON THE CEREBRAL BLOOD FLOW AND THE SOMATOSENSORY EVOKED POTENTIAL IN THE CAT-OBSERVATIONS OF OXYGEN AVAILABILITY AND CORTICAL NEURONAL ACTIVITY. P.E. Coyer, J.J. Michele*, and F.A. Simeone*. Department of Neurosurgery, The Pennsylvania Hospital, Philadelphia, PA. 19107.

The purpose of these experiments was to measure the effects of hypoxic-hypoxia on the responses of cerebral blood flow (CBF) and the somatosensory evoked potenital (SEP). CBF was monitored in the gray matter of 7 cats utilizing the hydrogen clearance technique. The latency from the time of stimulus to the occurrence of the cortical component of the SEP was determined by signal averaging 256 successive sweeps of the electroencephalogram (EEG) during contralateral forepaw stimulation. Cortical neuronal activities and oxygen availabilities were monitored using standard electrophysiological techniques. Five other cats served as controls to ascertain the effects of experimental length on the CBF and SEP. All animals were premedicated with ketamine (35 mg/kg) and received subsequent doses at 1 hr intervals. The animals were paralyzed with a neuromuscular blocking agent (pancuronium; 0.05 mg/kg) and artificially ventilated under conditions of 25, 19, 14, 9, and 3% O_2. Arterial PO_2, PCO_2, and pH were determined for animals at each change in the nitrous oxide/oxygen mixture. The range of PCO_2 variability during these changes for all animals was from 27.9 ± 7.6 to 35.6 ± 6.3 torr. Arterial pH was invariant over the range of oxygen mixtures although slight acidosis occurred at the 3% level (pH=7.28 ± 0.01).

As the mean arterial PO_2 decreased from 140 ± 5.8 to 115.6 ± 9.8 torr during the step-wise change from 25 to 19% O_2, the mean cerebral blood flow decreased to 92.4 ± 5.3% of control corresponding to flow values of approximately 46 ± 3 ml/100gm/min. A subsequent decline to 91.4% of control flow was noted as the percent oxygen was reduced to 14. Absolute blood flow values were compared on an individual animal basis using paired t-tests. The results of these tests showed significant decreases in CBF at the 19 and 14% levels compared to the control value of 25%. Further reductions in oxygen resulted in more variability of CBF values. For the responses of the SEP recorded in these experimental animals, a significant extension ($p<0.05$) in the absolute latency of its cortical component occurred at 14 and 9% O_2 (paired t-tests). Control animals had SEP's with relatively stable cortical wave latencies over the duration of the experiment. Oxygen availability was significantly reduced at the lowest O_2 percentage (3%) as the cortical component of the SEP was no longer present. At this level of tissue oxygen availability, cortical neuronal activity was still persistent. The fact that cortical neurons remained active supports the premise that the cortex is able to support the cortical component of the SEP during graded hypoxia but that lengthening of the conduction time reflects alterations in blood flow in other areas of the somatosensory pathway such as the thalamus and radiating fibers.

307.4 MIDLATENCY AUDITORY EVOKED RESPONSES: DIFFERENTIAL EFFECTS OF AGE AND SEX. R. Erwin and J. Buchwald, Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

The middle latency auditory evoked responses (MLRs) which occur between 10-100 msec following stimulus onset, have received renewed attention in recent years. Two of the most prominent MLR components are Pa (25-35 msec latency) and P1 (50-75 msec latency). We have recently shown that P1, but not Pa, disappears during slow wave sleep and reappears during REM (Erwin & Buchwald, EEG, in press). Parallel data in the cat MLR model suggest that P1 is generated by projections of the ascending reticular activating system (RAS) to the intralaminar thalamus (Chen & Buchwald, EEG, in press). The present experiment evolved from data in the human sleep study which suggested that P1 latency differentially increased in older women.

Four groups of 10 subjects were tested: young males (18-32 years, X=22), young females (18-33 years, X=23), older males (39-57 years, X=47), and older females (42-56 years, X=47). Stimuli were binaurally presented rarefaction clicks (55 db HL, 1/sec). MLRs were recorded from a vertex (Cz) electrode referenced to linked mastoid electrodes. The EEG activity was amplified 200K with a filter bandpass of 10-300 Hz. Five hundred stimulus trials were used to generate each MLR average. The components selected for analyses were ABR V, Pa, and P1. Peak amplitude and peak latency data for each component were independently submitted to analyses of variance with levels for Age (2) and Sex (2).

Amplitude analyses revealed a significant Age main effect for Pa amplitudes ($p<.05$). The amplitude of Pa was larger in both older groups than in the younger groups, in agreement with previous reports (Clayworth & Woods, Neurosci. Abst. 10: 1140, 1984).

Not previously reported was a significant Age by Sex interaction obtained only for P1 ($P<.01$) latencies, with no significant effects obtained for either ABR V or Pa latencies. The P1 interaction effect was attributable to the P1 latencies in the older women (65-83 msec), which were significantly longer than those in the remaining groups (50-65 msec).

These data clearly differentiate the effects of aging on the MLR components Pa and P1. Pa shows increased amplitude, with no increase in latency, in both males and females. In contrast, P1 shows a marked increase in latency, but only in the older females. The age range (42-56 years) of this group can be a period of hormonal instability during which menopause often commences. Hormonal changes which occur during menopause may have some functional relationship to the P1 generator system (hypothetized to be a component of the ascending RAS) and, as a result, account for the observed P1 latency shifts. (Supported by USPHS HD-05958 and HD-04612).

307.5 MIDLATENCY AUDITORY EVOKED RESPONSES IN THE CAT: EFFECTS OF SCOPOLAMINE, A POSTSYNAPTIC ANTAGONIST OF MUSCARINIC CHOLINERGIC NEUROTRANSMISSION. L. Dickerson, J. Buchwald and B.M. Chen, Brain Research Institute, Departments of Physiology and Neuroscience, Ment. Ret. Res. Center, UCLA Med. Ctr., Los Angeles, CA 90024.

A sequence of auditory evoked potentials can be recorded from the vertex of the awake cat in response to repetitive click stimulation. The auditory brainstem responses (ABR) originate from the auditory brainstem relay nuclei within the first 10 msec following the click stimulus. Subsequent to these short-latency responses arise middle-latency and long-latency potentials. A positive potential with a 20-25 msec latency (wave A) follows the response generated by primary auditory cortex (10-15 msec latency). Previous work has shown that wave A arises from a generator system extending from the dorsomedial midbrain reticular formation to the centre median and centralis lateralis nuclei of the intralaminar thalamus. Furthermore, wave A vanishes under pentobarbital anesthesia whereas the preceding ABRs and primary cortical potentials are unaffected. In the unanesthetized cat, wave A and its subsequent negativity diminish or disappear completely during stage 3 and 4 sleep (as indicated by neck EMG, eye movement, and EEG measures). Wave A reappears during REM sleep, at or above its amplitude in the awake cat (Chen and Buchwald; EEG, in press).

These results, in combination with studies from other laboratories on midbrain cholinergic pathways led us to propose that wave A activity might be diminished or even abolished by administration of scopolamine, a postsynaptic antagonist of muscarinic cholinergic transmission. Our experiments indicate that this in fact is the case. A total of 6 cats (3 male, 3 female) were studied with a scopolamine dose response series (0.1, 0.2, 0.4, 0.8, and 1.2 mg I.M.). In all cases, the amplitude of wave A was diminished at the lowest doses of the drug, and wave A disappeared at moderate and high doses of the drug, with no discernible effect on the preceding ABRs or primary auditory cortical potential. In addition, the negative potential immediately following wave A tended to be reduced in amplitude with a similar time course and dose response behavior, suggesting that the two potentials are functionally linked in some way. These results, taken together with results from other laboratories, suggest that cholinergic activation of post-synaptic target cells is an essential feature of wave A production, and that the wave A generator system includes cholinergic components of the ascending reticular activating system which project to the intralaminar thalamus. (This research supported by USPHS grants HD-05958 and HD-04612).

307.6 COMPARISON OF SCALP-RECORDED EVENT-RELATED POTENTIALS IN PRE- AND POST-SURGICAL EPILEPSY PATIENTS. R. Johnson, Jr. and Paul Fedio*. National Institutes of Health, NINCDS/MNB, Building 10, Room 4N246, Bethesda, MD 20892.

In recent years, numerous attempts have been made to localize the neural generator of the P300 component of the event-related brain potential (ERP). Whereas recordings from indwelling, temporal lobe electrodes in epileptic patients have placed the P300 generator in the amygdala, pes, uncus, and anterior hippocampal areas, studies of post-surgical patients with unilateral removal of these structures suggest that these brain structures do not contribute to the scalp-recorded P300. To date, no comparisons have been made between the scalp-recorded P300 activity in pre- and post-surgical epileptic patients to determine whether the changes in the ERP waveforms observed in the operated patients are due to the surgery or to the disease process itself.

Seven patients with a left temporal lobectomy (LTL) (average age = 34; 2 males), seven with a right temporal lobectomy (RTL) (average =37; 5 males), four with a left temporal epileptic focus (LT), (average age = 31; 1 male), four with a right temporal focus (RT) (average age = 30; 2 males), and seven normal controls (average age = 41; 3 males) were tested in the Oddball paradigm. The task was to count mentally the number of infrequent (10%) 1000-Hz tones that were randomly presented in a series of frequent (90%) 1500-Hz tones. The stimuli were delivered binaurally through headphones at 50 dB SL for 50 ms. The EEG was recorded from Fz, Cz, Pz, F3, F4, C3, C4, P3, and P4, all referred to linked mastoid electrodes. The amplifiers were set to a high-frequency cut-off of 35 Hz and a time constant of 10 sec. The EEG was sampled at 200 Hz for an 1150 msec epoch beginning 150 msec prior to stimulus onset. Eye movements were recorded and contaminated trials were excluded from the averages.

Overall, ERP activity in both the pre- and post-surgical groups closely resembled that of the controls. The P300 component appeared to be little affected by the surgical removal of anterior temporal lobe structures: none of the patient groups showed any signs of a hemispheric asymmetry that was related to the location of either the surgical resection or epileptic focus. However, the hemispheric asymmetry in the amplitude of the N1 component that we observed previously (i.e., whereby the largest N1s are recorded over the side of the surgery) appears to be the result of the surgical pocedure since neither preoperative group showed this result. Finally, the long lasting frontal- central negative wave recorded in the LTLs was greatly diminished and restricted to the frontal electrodes in the LTs. We interpret these data as providing further support for our position that the limbic P300 generator does not contribute to the scalp-recorded P300.

307.7 EVOKED POTENTIALS IN SUBGROUPS OF SCHIZOPHRENIC PATIENTS WITH ABNORMAL CT SCANS. C. Kessler*, A. Kling, K. Tachiki, A. Steinberg*, N. Sachinvala*, and H. VonScotti*. Psychiatry Service, Sepulveda Veterans Administration Medical Center and Department of Psychiatry and Biobehavioral Science, UCLA School of Medicine, Los Angeles, CA 90024.

Auditory and visual evoked potentials were obtained from scalp electrodes in subgroups of schizophrenic patients and were examined for variation associated with abnormality in cerebral tomographic scan. Ten adult male inpatients meeting DSM-III criteria for schizophrenia and 5 adult male hospitalized controls (non-psychotic psychiatric inpatients with diverse diagnoses) participated in this study. The subjects were between 23 and 45 years of age and were free of neuroleptic medication for a least 10 days prior to testing. Four of the subjects were residual schizophrenics (RS), 6 were paranoid schizophrenics (PS), and 5 were hospitalized controls (HC). Auditory and visual evoked potentials (AEPs and VEPs, respectively) were obtained over 500 millisecond epochs for 500 presentations in each modality at frontal, temporal and occipital recording sites. Concurrent measurements were also made of ventricle-to-brain ratios (VBRs) and cortical atrophy via computerized tomographic scanning for each subject. When PS and HC groups were compared, significant differences were observed in the N100 and P300 components of the AEP. These differences were particularly pronounced in the left temporal area and were subject to habituation effects. Corresponding differences in these components were not found in VEP measures. When RS and HC groups were compared, significant decreases in amplitude were observed in the N100 and P200 components of the VEP and AEP. These differences were resistant to habituation and were widespread, occurring at occipital and frontal sites as well as temporal. The schizophrenic patients were divided into 2 groups on the basis of VBR. One group (HVBRS) consisted of individuals with VBRs of 9.0 or more; the other group (LVBRS) had VBRs of less than 9.0. When compared to LVBRS patients, the HVBRS patients tended to show amplitude differences, resistant to habituation, in early components of both AEPs and VEPs. The HVBRS group showed significant left-right differences in amplitude and latency of N100, P200, N200, and P300 components of both AEPs and VEPs at temporal, but not frontal nor occipital sites. These results appear to support the presence of diffuse brain pathology in residual schizophrenia. This research was supported by the Veterans Administration.

307.8 CAT 'P300': EFFECTS OF ASSOCIATION CORTEX ABLATION. J. Harrison and J. Buchwald. Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol. , UCLA Med. Ctr., Los Angeles, CA 90024.

The human P300 potential is a scalp-recorded positivity of approximately 300 msec latency which occurs to unexpected stimuli randomly presented within the context of expected stimuli of the same modality. It appears to be a correlate of short-term memory and sequential information processing and is abnormal in demented patients, e.g. Alzheimer's patients. We have been studying an animal model of the P300 in an attempt to elucidate the generator substrate. The 'P300' in the cat occurs with a typical P300 protocol, is task-related, and shows positivity in the 200-500 msec range which is significantly greater for rare than for frequent stimuli.

We previously showed that complete bilateral ablation of the primary auditory cortex did not affect the cat auditory 'P300' (J. Harrison and J. Buchwald, EEG and clin. Neurophysiol. 63: 180-187, 1986). In the present study we assessed the role of polysensory association cortex (anterior lateral gyrus ALA and medial suprasylvian gyrus MSA). We were interested in the role of the polysensory cortical areas ALA and MSA, as the P300 can be elicited by stimuli of several modalities.

Our P300 protocol involved daily recording of the EEG in awake cats from a skull screw previously implanted at the vertex under general anesthesia. Randomly ordered stimuli included frequent loud clicks (P=.80) and rare soft clicks (P=.15) in two 500-trial blocks, with stimulus probability counterbalanced in two alternate 500-trial blocks. A 4KHz tone also occurred rarely (P=.05), and an instrumental conditioning paradigm provided eyelid shock only when the EMG did not reach a specified level during the tone. Thus the cat's attention was focused on the auditory stimuli. After 12 preoperative recording sessions, MSA and ALA cortex was bilaterally aspirated under pentobarbital anesthesia. After recovery from surgery, responses were recorded for 12 more daily sessions. The cat was then terminated and the brain perfused for histology. Cats with MSA and ALA ablated continued to show a 'P300'. These data suggest that polysensory association cortex, MSA and ALA, may not be essential for generation of the auditory 'P300' in the cat model. (Supported by USPHS HD-05958, AG-04088, and HD-04612).

307.9 BRAINSTEM AUDITORY EVOKED RESPONSES ARE DIFFERENTIALLY AFFECTED BY HYPOTHERMIA AND ANESTHESIA IN RATS. Raelyn Janssen*, Bruce Hetzler[1] John P. Creason[2]* and Robert S. Dyer (SPON: Lester D. Grant). Neurotoxicology Division, and [2]Biometry Division, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711, and [1]Department of Psychology, Lawrence University, Appleton, Wisconsin.

Hypothermia and anesthesia are both common conditions under which the brainstem auditory evoked response (BAER) is recorded. Hypothermia in the presence of anesthesia increases BAER peak latencies and may decrease amplitudes. In humans, mild hypothermia increases BAER latencies without anesthesia. Others have reported that anesthetics have no appreciable effects on the BAER when temperature is maintained within normal limits. In the present study, body temperature and sodium pentobarbital dosage were varied independently.

Two experiments were performed in which Long-Evans rats with chronically implanted electrodes were cooled to selected rectal temperatures in a cold room. In Experiment I, rats were tested awake at 37.5, 37.0 and 36.5°C in order to observe effects of slight hypothermia on the BAER. In Experiment II, two groups of rats were maintained and repeatedly tested at 37.5, 36.0, 34.5 and 31.5°C. One group was tested while awake and the other was anesthetized with an ip injection of sodium pentobarbital (40 mg/kg body weight).

In both experiments, hypothermia significantly increased peak latencies and increased peak-to-peak amplitudes. Sodium pentobarbital produced additional latency increases at all temperatures. The drug's effects on amplitudes varied across peaks: it prevented the cold-induced amplitude increase of P4, but increased amplitudes of other peaks over and above the effect of hypothermia.

In contrast to prior reports that anesthesia does not affect the BAER, we found that sodium pentobarbital significantly alters both peak latencies and amplitudes in the rat, independently of the effects of hypothermia.

307.10 EVENT-RELATED CORTICAL FIELD POTENTIALS IN MONKEYS DISCRIMINATING VISUAL PATTERNS: EFFECTS OF MANIPULATION OF PROBABILITY. R.K. Nakamura, R. Coppola, J. Johannesen*, National Institute of Mental Health, Bethesda, MD 20892

We are attempting to map information processing across the cerebral cortex of the monkey based on cortical evoked potentials from arrays of bipolar electrodes. Up to thirty electrodes are placed throughout the cortical convexity of one hemisphere, using sulcal impressions in the concavity of the skull as landmarks. The basic task is a go/no-go visual pattern discrimination with reversal based on categories that have no local feature distinctions. The visual stimuli are presented for 100 ms on a shutter controlled screen. Evoked responses are recorded from 16 electrodes simultaneously, are bandpassed at .1 to 100 Hz, and sampled at 200 Hz.

Manipulation of the probability of a rewarded simulus is known to affect a component in the ERP that has been called P300 or P3. This component is thought to reflect cognitive processing of surprise, reorientation, or end-of-sensory processing. The magnitude of the P3 component is larger for low probability, unexpected, or even missing events. We have run our monkeys on the go/no-go task with systematic variations of the probability of a go trial (from 80% to 20%) with asymmetrical reinforcement. The results indicate that a monopolar scalp lead demonstrates a positivity at approximately 400 ms in each of two monkeys. This component increases with reduced probability of a go trial. None of the bipolar electrodes in cortex appear to reflect this component. In all recorded locations late components either have different time course or a different response to behavioral manipulation. These results suggest that the P3 component is not generated at the cortex and thus supports earlier work suggesting a subcortical source.

307.11 POTENT IPSILATERAL CONTRIBUTION TO FLASH EVOKED POTENTIALS RECORDED FROM RAT CORTEX. Robert S. Dyer, Neurophysiology Branch, U.S.E.P.A., Research Triangle Park, NC 27711.

In the hooded rat, only about 10% of retinal ganglion cell axons project to the ipsilateral lateral geniculate nucleus (LGN). The uncrossed fibers originate from the temporal retina (Jeffrey, 1985) and project to a portion of the LGN which receives both crossed and uncrossed ganglion cell fibers. The LGN, in turn, projects to a strip of visual cortex which forms the lateral border of area 17, or OclB, and which also receives both homotopic and heterotopic fibers from the contralateral visual cortex by way of the corpus callosum (Olavarria and Van Sluyters, 1985).

While flash evoked potentials (FEPs) recorded from the visual cortex are commonly used to assess the functional integrity of the visual system in rats, little is known about the relative roles played by ipsilateral and contralateral fibers in these recordings. Since amplitudes of certain FEP peaks, particularly N1, vary systematically with flash intensity following full field stimulation, it might be assumed that amplitude of N1 reflects the number of stimulated axons. If this assumption is correct, then amplitudes of FEP peaks produced by stimulation of the ipsilaterally projecting fibers should be about 10% of those produced by stimulation of the contralaterally projecting fibers.

In the present study, FEPs were recorded from rats with skull screw electrodes implanted over the visual cortex. One eye was enucleated under general anesthesia, and 2-10 days later recordings were obtained either ipsilateral or contralateral to the intact atropine-dilated eye. In a series of 57 rats it was evident that the ipsilateral input produced a response considerably greater than would be predicted if amplitude was proportional to percentage of fibers. The FEP P1, N1, P2, N2, P3 and N3 peak amplitudes recorded from the ipsilateral input were 38, 40, 32, 66, 118 and 75 percent of the responses recorded from contralateral input. The ipsilateral response had a shorter latency N2 peak (62 $\pm$ 2 vs 73 $\pm$ 3.5 msec), and a clearly bifurcated N1 peak. In addition, preliminary data from rats with unilateral enucleation followed by section of the corpus callosum suggest that the large response to ipsilateral stimulation is not mediated via callosal input. These data indicate that while FEP peak amplitudes may reflect stimulus intensity, they do not reflect proportionately the number of fibers projecting to the visual cortex. Some FEP peaks evidently require very little input to achieve maximum amplitude.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

307.12 EVOKED MAGNETIC FIELD DETECTED FROM ISOLATED TURTLE CEREBELLUM IN VITRO DURING PURKINJE CELL ACTIVITY AND SPREADING DEPRESSION. Y. C. Okada, M. Lauritzen* and C. Nicholson. Depts. Psychology and Physiol. & Biophys., New York Univ., New York, NY 10003.

The evoked magnetic field (EMF) from turtle cerebellum in vitro was recorded to determine the neural generators of magnetic signals. The cellular elements of the turtle cerebellum conform to a Cartesian coordinate system, allowing for selective measurements of the magnetic field due to currents in orthogonal directions. The cerebellum was placed in a bath of oxygenated Ringer solution, with parallel fibers oriented normal and Purkinje cells tangential to bath surface. The normal component (Bz) of the magnetic field outside the bath was thus expected to be due to currents oriented along the longitudinal axis of Purkinje cells. Neural activity was elicited by stimulating the dorsal surface with a brief pulse. The resulting Bz was measured with a superconducting detector (an asymmetric second order gradiometer with a 1.0 cm diameter pickup coil and 4.0 cm baseline) coupled to a dc-SQUID (superconducting quantum interference device) with its axis normal to the bath 16 mm above the midline of the cerebellum. The extracellular potential (Ve) was monitored simultaneously with Bz using a glass micropipette. The averaged Bz showed a complex waveform, consisting of an initial biphasic spike ending within 2-3 ms and slower biphasic variation ending at about 20 ms after stimulus onset. The late magnetic component and the postsynaptic component of Ve were abolished when 3 mM Mn^{2+} was added to the Ringer solution. Tetrodotoxin abolished all components of Bz and of Ve. The results suggest that the fast component was due to direct activation of Purkinje cells, whereas the late component was postsynaptic in origin. In another study, the real time dc-coupled magnetic signal was recorded during spreading depression (SD). SD was elicited with tetanic stimulation of the dorsal cerebellar surface, after conditioning the brain with a modified Ringer solution. During SD, Ve went negative (20-40 mV) simultaneous with a monophasic large magnetic signal ranging from 2 to 10 pT. The electrical response was long (2-5 min) compared to the magnetic signal which only lasted 0.5-1 min. Measurements of Bz at several locations showed mirror-image signals along the rostro-caudal direction of cerebellum with polarity reversal. This indicated that the magnetic signal was due to currents perpendicular to the cerebellar surface, directed from the dorsal to the ventral side. The above results demonstrate that the in vitro turtle cerebellum is a valuable tool in the analysis of the magnetic fields associated with different forms of nerve cell activity. Supported by Grants NINCDS NS21149 and NS13742.

MONOAMINES AND BEHAVIOR I

308.1 EFFECTS OF HIGH CARBOHYDRATE DIET ON LEARNED HELPLESSNESS AND BRAIN MONOAMINE METABOLISM IN RATS. K. Arasteh, H.W. Sampson*, J.R. Nation*, P.J Wellman* and M.E. Trulson. Dept. Anatomy, Coll. Med. and Dept. Psychol., Texas A&M Univ., College Station, TX 77843.

Mood disorders have been associated with diets that are high in carbohydrates. Mineral metabolism in mood disorders has been investigated for many years. Calcium metabolism in mood disorders has been studied since the 1940's. Altered calcium homeostasis associated with depressive illness has been reported on numerous occasions. It has been suggested that there may be a correlation between carbohydrate consumption and levels of serum calcium. To more closely examine the role of dietary carbohydrate on brain monoamines, calcium and mood disorders, we used an animal model of depession to examine the effects of high carbohydrate diet on these measures. Rats were maintained on either a standard laboratory diet or a high carbohydrate diet for 6 weeks prior to testing. The high carbohydrate diet consisted of 50% sucrose with all other constituents being identical to the standard diet with the exception of modification in the total amount of fiber to make up the difference in carbohydrate. Learned helplessness, locomotor activity (open field), pain sensitivity (tail flick), food and water intake, calcium measurements (atomic absorption spectophotometry), and monoamine assays (high performance liquid chromatography) were all measured according to standard methods. Rats maintained on a high carbohydrate diet exhibited significantly shorter escape (3 lever presses) latencies (mean= 9.7 vs. 16.6 sec in control animals, maximum time = 20 sec) ($P<0.01$) when tested on a learned helplessness paradigm. These animals showed no significant changes from control values on locomotor activity or pain sensitivity. The rats maintained on a high carbohydrate diet showed significant increases in brain (+12.9%) and serum (+11.2%) glucose. The animals showed no significant changes in serum calcium. Neurochemical studies revealed no significant changes in brain serotonin, 5-hydroxyindoleacetic acid, dopamine, homovanillic acid or dihydroxyphenylacetic acid. However, norepinephrine was significantly decreased by 10.4% and its major metabolite MOPEG-SO_4 was significantly increased by 21.7%. The data suggest that prolonged exposure to a high carbohydrate diet makes the animals "manic" and that this response may be due to an increase in brain norepinephrine metabolism. These data may have implications for clinical conditions which have suggested that diets high in carbohydrates may produce behavioral and learning disorders in children. The case histories of these children demonstrate that behavioral abnormalities including irritability, hyperactivity, and short attention span are normalized following manipulations of dietary constituents which decrease the amount of carbohydrates.

308.2 POSSIBLE ROLE OF NOREPINEPHRINE IN THE IMINODIPROPIONITRILE(IDPN)-INDUCED DYSKINETIC SYNDROME. J.L. Cadet, T. Braun*, F Karoum, D.M. Chuang and W.J. Freed. Neuropsychiatry Branch, NIMH, Saint Elizabeth's Hospital, Washington, D.C. 20032.

A critical issue in clinical neurosciences is the elucidation of the mechanisms involved in the development of the neuropathology of degenerative disorders such as Huntington's disease(HD). While the intrastriatal injection of kainic acid mimics some of the biochemical changes seen in the brain of HD patients, this drug does not cause a dyskinetic syndrome. The neurotoxic substance IDPN causes a set of behavioral abnormalities consisting of excitement, circling behavior, choreoathetoid neck movements (ECC-syndrome), and increased startle response in mice and rats. These changes last throughout the lifespan of the animals. These behaviors are similar, but not identical, to those induced acutely by catecholaminergic and serotonergic (5HT) agonists. The syndrome has recently been proposed as a possible model for HD. Because the behavioral syndrome induced by 5HT agonists can be inhibited by norepinephrine(NE) antagonists and because rats that show increased acoustic startle reflex after ICV injection of 6-OHDA have increased α1-noradrenergic receptors (Astrachan, D. I. et al. Brain Res 26:81, 1983) we decided to evaluate the possible involvement of the NE system in the IDPN-induced syndrome.

Swiss-Webster mice (20-30gm) were used in behavioral studies. The animals were injected with IDPN (100mg/kg) until the behaviors developed (7 days). One month after the last injection, they were tested using a dyskinesia scale (Diamond, B. I. et al. Adv Neurol vol. 35 p 221, 1982). After a half hour baseline, the animals were given IP injection of prazosin (1-4mg/kg), clonidine (.050-1mg/kg), or yohimbine (1-4mg/kg). and were monitored for another half hour. Sprague-Dawley rats (150-175gm) were used in biochemical studies. Rats were injected with IDPN as described above. They were killed at various intervals during the period of injection and up to 6 weeks after the last injection. Various brain areas were dissected out for measurement of catecholamines and their metabolites. Striatal α1 - adrenergic receptors were assayed using 3H-prazosin. Studies of phosphatidyl inositol (PI) turnover were carried out using both striatal and cortical slices.

Both prazosin and clonidine inhibited the IDPN-induced dyskinesia, whereas yohimbine had no effect. There was no significant change in DA in either the striatum or the hypothalamus. NE was increased in the striatum one month after the last injection of IDPN. There was, in addition, a transient increase in MHPG levels in the hypothalamus. Receptor binding assays revealed a 70% increase in prazosin bindings sites in rat striata one month after the last injection of IDPN. This was accompanied by a significant increase in NE-stimulated, but no change in carbamylcholine-induced, PI turnover in striatal slices of treated animals as compared to controls. These results suggest that the NE system is involved in the ECC-syndrome. The increase in NE-stimulated PI turnover suggests that phospholipid plus calcium-stimulated mechanisms may be involved in the development of persistent dyskinesia.

308.3 SYNERGISTIC EFFECTS OF ALPHA-2 ADRENERGIC AND OPIATE RECEPTOR BLOCKADE ON VOCALIZATION IN ADULT SQUIRREL MONKEYS. J. C. Harris and J. D. Newman. Div. of Child Psychiatry, Johns Hopkins University, Baltimore, MD 21205 and Lab of Comparative Ethology, NICHD, NIH, Bethesda, MD 20892.

Synergistic effects of naloxone and yohimbine on anxiety and sexual function have been reported in human volunteers (Charney and Heninger, 1985). An opiatergic mechanism has been shown to be involved in the production of separation-induced vocalization in squirrel monkeys (Newman, Murphy and Harbaugh, 1982). We have also implicated an adrenergic mechanism in this vocalization by demonstrating that yohimbine reverses clonidine inhibition of separation calls in that species, as well as producing a small but significant increase in separation calls when given alone (Harris and Newman, 1985). Some subjects increased the production of another vocalization, the twitter, when administered yohimbine. The present experiment tested whether naloxone and yohimbine administered together would produce a synergistic enhancement in either of these vocalizations using the same testing procedures. Four adult squirrel monkeys used as subjects were given an IM injection of 0.2 mg/kg yohimbine, 0.4 mg/kg naloxone, yohimbine and naloxone concurrently in these same doses, or sterile water vehicle as control. Both yohimbine and naloxone produced increases in separation vocalization when given alone; the extent varied between subjects. Only yohimbine produced an increase in twitters, in agreement with our earlier findings. Yohimbine and naloxone together resulted in up to three-fold increases in twitters in 3 subjects; in the fourth subject (which never produced twitters in any test), there was a seven-fold increase in separation vocalizations. Other behavioral changes noted following the naloxone-yohimbine combination were increased somatic and autonomic disturbances (scratching, paw rubbing, excessive salivation). There was no consistent increase in penile tumescence. The consistent increase in vocalization with the combination of naloxone and yohimbine compared with the same doses of these drugs when administered alone provides the first evidence of an interaction of opiate and adrenergic systems in the expression of primate vocalization.

308.4 HPLC ANALYSIS OF SEVERAL MONOAMINE TRANSMITTERS AND METABOLITES IN EIGHT BRAIN REGIONS OF ISOLATED FIGHTING MICE M. Gary Hadfield Neurochemistry Research Laboratory, Division of Neuropathology, Medical College of Virginia/VCU, Richmond, Virginia 23298

Fighting behavior produces important neurochemical changes in the brains of aggressive animals. In particular, monoamine transmitter function is altered. Yet, it is difficult to know whether the changes are directly responsible for fighting behavior, if they simply modulate the process or if they are due to extraneous or secondary behaviors that accompany fighting.

To help analyze this problem, we have employed the following behavioral and biochemical methods. Adult male ICR mice were isolated for several weeks to incite "natural" aggression. Three groups of animals were utilized: (i) Resting, non-fighting (baseline) animals never exposed to a fighting partner (R), (ii) animals placed in the fighting arena with a combat partner for whom no fighting behavior was recorded for either animal (NF) and (iii) bona fide fighting animals (F). Fighting duration, defined as aggressive body contact (biting, sparring), was measured during a five minute combat period. The brains were rapidly removed and eight discrete regions of the brain, rich in monoamine transmitter nerve terminals were dissected [olfactory tubercles (OT), olfactory bulbs (OB), prefrontal cortex (PC), septum (SP), striatum (ST), hypothalamus (HT), thalamus (TH) and hippocampus (HC)]. These tissues were subjected to HPLC analysis using a new method presented here last year (Abs. #263.8 and Hadfield et al., J. Liquid Chromat. 8:2689-97, 1985). It permits the simultaneous analysis of several monoamines during a single run of less than 20-25 minutes without prior clean-up of samples.

We are presenting results for norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), normetanephrine (NM), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3MT), serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA). Significant increases and decreases in monoamine levels over R are tabulated below.

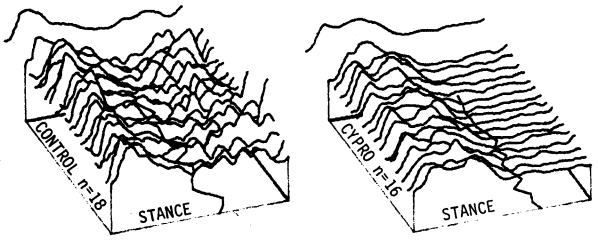
	NE		MHPG		NM		DA		DOPAC		HVA		3MT		5HT		5HIAA	
	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
OB						↑		↓					↑		↓			
OT				↑		↓				↑			↑	↑				
PC	↓	↓				↓		↑						↓	↓	↓	↑	↑
SP							↓									↓		↑
ST								↓					↑	↑		↓		↑
HT									↑	↑		↑			↓	↓	↑	↑
TH			↑	↑														
HC	↓	↓																

In general, decreases occurred in NE, DA and 5HT and increases occured in their metabolites, indicating utilization of neurotransmitter. The differences in pattern for NF and F are remarkable because totally different regions are often involved and sometimes metabolites. Where identical, F was usually greater than NF. The significance of these findings will be discussed.

308.5 MODULATORY ACTION OF CYPROHEPTADINE ON THE LOCOMOTOR PATTERN OF SPASTIC PARETIC PATIENTS. M. Wainberg* and H. Barbeau. School of Physical and Occupational Therapy, Faculty of Medicine, McGill University, Montreal, Quebec, Canada, H3G 1Y5.

Recent studies indicate an important role of the descending serotonergic pathways in the modulation of the locomotor pattern in adult chronic spinal cats (Barbeau H, Rossignol S. Soc Neurosci Abstr 8: 163; Rossignol S, Barbeau H, Julien, C. In Development and Plasticity of the Mammalian Spinal Cord, Spoleto, Italy (in press)). In addition, Barbeau et al. (J Neurol Neurosurg Psychiatr. 45: 413-426) and Wainberg et al. (J Neurol, in press) have recently demonstrated the antispastic action of the 5-HT antagonist cyproheptadine (CYPRO).

The intent of the present study was to determine the modulatory effect of CYPRO on the locomotor pattern of patients with partial spinal cord lesions transection and presenting spasticity. Four patients (age 24 to 56, 7 months to 7 years post injury) participated in the study. Electromyographic (EMG) and kinematic (not reported here) recordings were made of patients walking on a treadmill. During CYPRO the variability (standard deviation) of temporal distance factors (cycle, stance and double support duration) is reduced (range 13.2% - 41.3%). Greater decreases are noted in EMG duration variability (gluteus maximus-GM, vastus lateralis) (59.2% - 61.4%) and important changes are seen in the EMG pattern. In the control phase (below left), GM activity is characterized by discharge throughout the gait cycle and high variability (coefficient of variation-CV=157%). With CYPRO (below right), the GM main burst is prolonged but restricted to stance phase, and the pattern is less variable (CV=87%). In some cases, appearance of biphasic activity in medial hamstrings and tibialis anterior, and alternating activity of antagonists are observed. Functionally, patients report less "stiffness" and are capable of higher walking speeds (17%-34%).



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308.6 PUTATIVE PRECLINICAL ANTIPSYCHOTIC ACTIVITY OF PIPERAZINE SEROTONIN AGONISTS. L.K. Chambers, J.B. Harrell*, R.A. Mastroeni*, T.H. Andree, I. Lucki and J.A. Moyer, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, PA 19101 and the Dept. of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104

The piperazine serotonin agonists, TFMPP, m-CPP and MK 212 produced profiles similar to those of antipsychotics in a discrete trial/lever press conditioned avoidance procedure in rats. Therefore, these compounds were tested for their potential to inhibit apomorphine (APO)-induced behaviors in mice.

TFMPP, m-CPP and MK 212 were all effective in antagonizing APO-induced stereotypy and climbing done as a single test in mice. MK 212 and m-CPP resembled atypical antipsychotics in this test since doses required to antagonize climbing were much lower than those which antagonized stereotypy. TFMPP, on the other hand, resembled the typical antipsychotics, since climbing and stereotypy were antagonized at equivalent doses.

The blockade of avoidance behavior by the piperazine serotonin agonists was antagonized by metergoline but not ketanserin in the discrete trial/lever press conditioned avoidance procedure in rats. Therefore, metergoline and ketanserin were also tested for their ability to block the effects of the piperazine serotonin agonists on apomorphine-induced behaviors. Metergoline (2.0 mg/kg), a nonselective serotonin 5HT-1 and 5HT-2 antagonist, had no effect on MK 212's antagonism of APO-induced stereotyped sniffing. However, metergoline did block MK 212's inhibition of APO-induced climbing. Ketanserin (4.0 mg/kg), a selective 5HT-2 antagonist, blocked the MK 212-induced inhibition of both stereotyped sniffing and climbing. Metergoline and ketanserin are also being tested in combination with m-CPP and TFMPP. These data and the fact that the potent 5HT-1A agonist, 8-OH-DPAT was ineffective in both conditioned avoidance and apomorphine tests, suggest that the effects of the piperazine serotonin agonists on apomorphine-induced behaviors may be related to the stimulation of 5HT-2 receptors.

Receptor binding profiles of these serotonin agonists demonstrated differential selectivity at serotonin receptor subtypes and low affinity for D-2 and muscarinic receptors. These results suggest that 5HT-1A receptors do not mediate the behavioral effects observed in these tests, although 5HT-1B and 5HT-2 receptors may mediate these effects. These studies further support a preclinical antipsychotic profile for the piperazine serotonin agonists, TFMPP, m-CPP and MK 212.

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308.7 PHYSIOLOGICAL STRESS AND THE ACTIVITY OF DORSAL RAPHE SEROTONERGIC NEURONS IN FREELY MOVING CATS. C. Fornal, D.A. Morilak, and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Serotonergic neurons of the dorsal raphe nucleus (DRN) have been implicated in the global modulation of the CNS response to stress. These neurons project to many regions of the brain involved in the central regulation of sympathetic nerve activity and neuroendocrine function. We report here on the single-unit activity of serotonergic DRN neurons in awake freely moving cats in response to two physiological challenges: 1) hypoglycemia induced by insulin injection, and 2) hypotension induced by peripherally-acting vasodilators. Single-unit activity was recorded using movable electrode bundles consisting of 32 and 64 μm dia. insulated nichrome microwires implanted in the DRN. Serotonergic neurons were identified on line by their slow (< 5 spikes/sec) and regular discharge pattern, and complete cessation of activity during REM sleep and in response to the serotonin agonist 5-methoxy-N,N-dimethyltryptamine (250 μg/kg,i.m.). The dose of insulin used was 2 IU/kg, i.v. Sodium nitroprusside (5-15 μg/kg, i.v.) and hydralazine hydrochloride (1-3 mg/kg, i.v.) were used to induce brief and prolonged periods of hypotension, respectively. These drugs were administered via an indwelling jugular catheter which was also used for obtaining blood samples. Plasma samples were assayed for glucose, norepinephrine (NE), and epinephrine (EPI) concentrations. Serotonergic neurons displayed no significant changes in unit activity following insulin injection, although plasma glucose levels were greatly reduced (∿ 70%) and plasma catecholamines were elevated (EPI ∿ 400%; NE ∿25%). Similarly, there were no significant changes in serotonergic unit activity following bolus injections of sodium nitroprusside, although mean arterial pressure was reduced in some animals by as much as 50 mm Hg. Likewise, no significant changes in unit activity were observed following hydralazine administration, despite marked reflex activation of sympathetic outflow as indicated by increases in both heart rate (∿50%), and plasma catecholamine levels (NE ∿200%; EPI ∿ 25%). These studies demonstrate that the physiological stress of hypoglycemia and hypotension have no appreciable effect on serotonergic DRN unit activity. These results are consistent with our previous findings that thermoregulatory challenges had no effect on DRN serotonergic units (Fornal et al., Soc. Neurosci. Abstr. 11:769, 1985), and parallel the lack of effect of environmental stressors on these neurons (Wilkinson et al., this meeting). We conclude that any involvement of DRN serotonergic neurons in stress may be mediated by modulation of transmitter release at the axon terminal and not by a change in unit activity. Supported by NIMH grant MH23433 and postdoctoral fellowship MH 08869 to C.F.

308.8 ENVIRONMENTAL STRESS AND ACTIVITY OF DORSAL RAPHE SEROTONERGIC NEURONS IN THE FREELY MOVING CAT. L.O. Wilkinson, E.D. Abercrombie, and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Activity of serotonergic neurons in the DRN is positively correlated with behavioral arousal. Since these neurons are connected with areas of the brain involved in behavioral and physiological aspects of emotion, it is hypothesized that they may play a role in the CNS response to highly arousing and emotional stimuli. The present studies describe the effects of three environmental stimuli, loud white noise, restraint, and presentation of a dog, on 5-HT DRN unit activity in awake, freely moving cats. 5-HT neurons were identified on-line by electrophysiological and pharmacological criteria described previously (Fornal et al., this meeting). Cats were subjected to 15 mins. of white noise or restraint as described by Abercrombie et al. (this meeting), or to five mins. of exposure to a dog through a steel mesh screen. Heart rate was monitored continuously during stressors, and in some cats blood samples were withdrawn via a jugular catheter, before and immediately after the stressful stimulus. Plasma samples were analyzed for NE levels after extraction with acid-washed alumina, using HPLC-EC. All three stressors produced behavioral activation, increases in heart rate (∿ 25%), and plasma NE levels (∿ 120%). Dog presentation induced a strong feline defense reaction, including piloerection, broadside stance, and hissing. Despite these increases in measures of behavioral and physiological activation, the discharge of serotonergic neurons never significantly exceeded the normal baseline range. The maximal change in DRN unit activity during restraint, loud noise and dog presentation is seen during the first minute of the challenge, when firing rates are 35%, 42%, and 20% above quiet waking (QW) baseline, respectively. These increases are not significantly different from the increase in unit activity seen during spontaneous changes from QW to active waking (AW) behavioral state in unstressed animals. These results are consistent with Fornal et al., (this meeting), and suggest that the CNS response to environmental stress is not accompanied by a specific activation of 5-HT DRN unit activity. This contrasts with the dramatic increase in NE unit activity in the LC seen after these manipulations (Abercrombie et al., this meeting) and suggests a differential involvement of these two neurotransmitter systems in the coordination of the stress response. (Supported by NIMH grant MH23433 and an NSF predoctoral fellowship to L.W.)

308.9 PHYSIOLOGICAL STRESS AND THE ACTIVITY OF LOCUS COERULEUS NORADRENERGIC NEURONS IN FREELY MOVING CATS. D.A. Morilak, C. Fornal, and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Noradrenergic (NE) neurons of the locus coeruleus (LC) have been implicated in a wide variety of functions, all of which may be part of a more global process underlying the stress response. We tested this hypothesis by recording the single unit activity of LC-NE neurons in awake freely moving cats in response to several physiological stimuli of varying intensities (magnitude or duration), none of which increased behavioral arousal. These studies address two questions: 1) Do LC-NE neurons respond to physiological stimuli independent of behavioral arousal, or do they respond only to stimuli which possess a behaviorally activating component? and 2) Do LC-NE neurons respond to graded stimuli in a graded fashion (i.e. as a homeostatic regulatory system), or only to more extreme stimulus intensities (i.e. as a general stress-response system)? For recording methods, see Fornal et al, (this volume). NE units were identified by their a) slow discharge rate (< 3 Hz), b) long-duration action potential, c) complete suppression of activity during REM sleep, and d) complete suppression following systemic injection of the α_2 agonist clonidine (25 μg/kg). Blood pressure was decreased with hydralazine (1-3 mg/kg, i.v.). Hypoglycemia was induced by insulin (2 IU/kg, i.v.) and reversed by glucose (150 mg/kg, i.v.). Thermal stress was produced by increasing ambient temperature to 43°C for 60-90 min. In addition to unit activity, measures were taken of plasma epinephrine and NE, plasma glucose, heart rate, respiratory rate, and rectal temperature. Hypotension, hypoglycemia and heating all increased unit activity above quiet waking baseline (mean increase = 96%, 158% and 127%, respectively). However, the changes in unit discharge were not directly related to any of the variables studied, but were related more to the occurrence of the peak stimulus effect. Furthermore, unit activity was further elevated if animals became behaviorally active. Thus, we conclude that: 1) LC-NE neurons can respond to physiological stimuli independent of changes in behavioral arousal. These effects appear to be additive with those induced by behavioral arousal. 2) LC-NE neurons respond more like a general stress-response system than as a primary homeostatic regulatory system. Taken together with the data regarding LC-NE response to emotionally arousing environmental stressors (Abercrombie et al., this volume), the LC may function to integrate and coordinate the emotional and physiological responses to both internal and external stressors. Supported by Air Force grant AFOSR 85-0034 and NIMH grant MH23433.

308.10 ENVIRONMENTAL STRESS AND ACTIVITY OF LOCUS COERULEUS NORADRENERGIC NEURONS IN FREELY MOVING CATS. E.D. Abercrombie, L.O. Wilkinson and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Noradrenergic neurons of the locus coeruleus (LC) have been hypothesized to play a role in the CNS response to stress. This hypothesis derives from studies showing increased norepinephrine (NE) turnover during stress and studies showing that depletion of brain NE diminishes the effectiveness of the organism's response to stress. The present studies describe the effects of environmental challenges, namely 15 min of restraint or noise stress, upon NE neuronal activity in freely moving cats. LC neurons are identified as noradrenergic by physiological and neurochemical criteria published previously (see Morilak et al., this volume). Stress effects upon peripheral physiological systems as measured by heart rate and plasma catecholamine (CA) levels are also assessed. Restraint stress involves placing the animal in a canvas handling bag and wrapping elastic bandage about the torso thus precluding movement of the limbs. Noise stress is achieved by the presentation of 100 db white noise. In order to examine the unit response to a non-stressful environmental stimulus, the cats are also exposed to a pair of laboratory rats housed in a wire mesh cage. Both restraint stress (n=6) and noise stress (n=8) cause a significant increase in NE unit activity above the normal quiet waking/active waking range. During restraint there is a mean increase in firing rate of 162% by the first min. By 15 min post restraint onset, the mean firing rate remains elevated 105%. During noise stress, activity increases 234% in the first min and is sustained at 123% at 15 min. Following termination of both stressors, activity normalizes within 5 min. In contrast, during exposure to inaccessable rats (n=4) unit activity never deviates significantly from the baseline range despite overt signs of arousal in the cat. Changes in heart rate and unit activity are correlated: r=.83 for restraint, r=.73 for noise and r=.83 for inaccessable rats. The plasma CA data reveal significant sympathetic activation in response to both stressors (see Wilkinson et al., this volume). These results support the contention that activation of the LC system occurs in response to environmental stimuli which mobilize the stress response. Furthermore, this activation is significantly greater than the fluctuations in firing rate seen during normal behavior and is, indeed, the greatest we have observed. Both the magnitude and sustained nature of the LC response indicate that this NE system may be importantly involved in coordinating responses to stimuli perceived as stressful. (Supported by NIMH Grant MH09256/MH23433 and Air Force Grant AFOSR85-0034).

308.11 EFFECTS OF TRIFLUOROMETHYLPHENYLPIPERAZINE (TFMPP), A SEROTONIN AGONIST, ON REM AND NREM SLEEP IN RATS. R.H. Pastel and J.D. Fernstrom. Department of Psychiatry and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15213.

Serotonin (5HT) neurons may have a role in sleep. Previous experiments in our laboratory and others have shown that 5HT reuptake inhibitors have a suppressive effect on REM sleep in rats while having no effect on non-REM (NREM) sleep. To further examine the role of 5HT neurons in sleep, we tested the effect of TFMPP, a specific 5HT agonist. TFMPP is an unusual 5HT agonist in that it does not induce the 5HT motor syndrome [Lucki I, Frazer A. Soc Neurosci Abstr 8:101, 1982].

Administration of TFMPP (0.1-1.25 mg/kg, ip) at the onset of the daily light period suppressed REM sleep min/hr in a dose dependent manner. The time course of the effect was also dose dependent, with the 0.1 mg/kg dose having no effect, the 0.3 mg/kg dose suppressing REM for 4 hours, the 0.625 dose for 5 hours and the 1.25 dose for 6 hours. TFMPP decreased the number of REM episodes in the first six hours and increased REM latency at all but the 0.1 mg/kg dose.

TFMPP had no effect on NREM sleep min/hr at 0.1 and 0.3 mg/kg. At the 0.625 dose, NREM sleep was enhanced in the second hour after injection. The 1.25 mg/kg dose had a mixed effect, suppressing NREM in the first hour and increasing it during the second and third hour. Sleep latency was not changed except at the 1.25 mg/kg dose which increased latency. Although TFMPP had no consistent effects on sleep latency or min/hr of NREM, delta waves were increased in a dose-dependent manner, with the effect seen at the 0.1 mg/kg dose.

The dose-dependent suppression of REM by TFMPP, along with similar results with 5HT uptake inhibitors support the notion that enhancing 5HT transmission suppresses REM sleep. The dose-dependent increase in delta waves is very interesting. Our previous research with fluoxetine did not reveal a significant increase of delta waves. Very few compounds are known that increase delta waves. Other NREM sleep measures were not changed in a consistent or dose-dependent manner. The results suggest that 5HT neurons may play a role in the production of delta waves, but not in the induction of sleep. Since TFMPP is reported to be selective for 5HT-1B receptors, the data suggest that the suppression of REM sleep and the enhancement of delta waves may be due to the action of 5HT on 1B receptors.

308.12 ELECTROCONVULSIVE SHOCK DOWNREGULATES BETA-NORADRENERGIC RECEPTORS IN RAT BUT FAILS TO ENHANCE AGGRESSION. B. Eichelman and L.R. Hegstrand. Wm. S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705, and Dept. of Psychiatry, Univ. of Wisconsin.

Beta-noradrenergic receptor supersensitivity has been found correlated with aggressive behavior in the rat (Hegstrand, L.R. and Eichelman, B. Pharmac. Biochem. Behav., 19:313-320, 1983). Antidepressant drugs which downregulate beta-noradrenergic receptors also increase rodent aggression (Eichelman, B. and Barchas, J. Pharmac. Biochem. Behav., 3:601-604, 1975). Consequently, in two experiments rats were treated with electroconvulsive shock (ECS) chronically while tested for aggressive behavior. After treatment the rat cortices were measured for beta-noradrenergic receptor number using a ^{125}I-Pindolol ligand.

In the first experiment adult male Sprague-Dawley-derived rats were treated for baseline levels of shock-induced fighting (SIF) on five days (50 footshocks/test of 2 mA at 0.5 s duration recycled every 7.5 s). Experimental pairs were exposed to ECS daily for five days after anesthetized with ether. Current parameters were 150 v, biphasic alternating current of 0.5 s duration. Controls received ether anesthesia. Pairs were tested for SIF five hours after the ECS. The experimental group had no change in fighting from baseline levels after 5 days, while the controls showed an increase in fighting. This difference was statistically significant, $p<0.01$, 2-tailed Mann-Whitney Test. At the end of behavioral testing, the rats were killed; their brains removed, dissected on ice, frozen on dry ice; and the neocortex assayed with ^{125}I-Pindolol to determine the density of beta-noradrenergic receptors. For the ECS group the B_{max} was 33.0 fmol/mg which was significantly less than the B_{max} for the control group which was 40.3 fmol/mg ($p<0.05$, 2-tailed).

To assess the role of ECS on the same day as fighting a second experiment was carried out. The parameters were similar. However after baseline testing for SIF, experimental rats received five ECS exposures interspersed by five non-ECS days on which they were treated for SIF. In this paradigm experimental and control groups did not differ in change from baseline levels of fighting when compared at the end of five ECS treatments. The results of these behavioral experiments indicated that enhanced central noradrenergic function is not always correlated with increases in rodent SIF and may not be the primary causative mechanism in the enhancement of this behavior.

Supported by the Veterans Administration.

308.13 IS DOPAMINE INVOLVED IN THE HYPERACTIVITY PRODUCED BY INJECTIONS OF MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS? D. Wirtshafter, M. Klitenick, C. McWilliams and K.E. Asin. Dept. Psychol., Univ. of Illinois at Chicago, Box 4348, Chicago, IL 60680

Several authors have demonstrated that injections of the GABA agonist muscimol into the median raphe nucleus (MR) lead to a pronounced increase in locomotor activity, but little is known as to the neural basis of this effect. Since a number of workers have suggested that the raphe nuclei may tonically inhibit the activity of dopaminergic cells projecting to the basal forebrain, one possibility is that intra-MR muscimol injections produce hyperactivity through disinhibition of dopaminergic mechanisms. The current studies were designed to investigate this possibility.

We first examined the effects of intra-MR injections of muscimol on serotonin (5HT) and dopamine (DA) turnover. Rats received injections of 100ng of muscimol and were placed in activity boxes for one hour and then were sacrificed and the hippocampus, striatum and nucleus accumbens assayed by HPLC for levels of DA, 5HT and their metabolites. Muscimol injections were found to decrease levels of the 5HT metabolite 5HIAA only in the hippocampus, confirming that the injections inhibited the activity of serotonergic MR cells. Levels of the DA metabolites DOPAC and HVA were increased by 80% and 102% respectively in the nucleus accumbens whereas much smaller changes were seen in the striatum. These results are compatible with the notion that inhibition of MR cells by muscimol leads to an increase in DA turnover within the nucleus accumbens.

In our second experiment, we examined the effect of systemic treatment with various doses of the DA antagonist haloperidol (0, 0.2, 0.8, 3.2 mg/kg, s.c.) on muscimol-induced hyperactivity. Overall, haloperidol was without significant effect on the muscimol response, and even at the highest dose used, tended to produce only a small reduction in activity. In contrast, haloperidol is able to completely abolish amphetamine-induced activity at a dose of 0.4 mg/kg. These results demonstrate that muscimol-induced hyperactivity is not dependent on dopaminergic mechanisms.

In summary, the current results suggest that although intra-MR injections of muscimol result in increases in accumbens DA turnover, this change in DA release is unlikely to underly the locomotor response to the injection. It is possible that the observed changes in DA turnover may result from the hyperactivity produced by the muscimol injection, rather than the converse.

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308.14 ROLE OF SEROTONERGIC AND NON-SEROTONERGIC MECHANISMS IN THE ROTATIONAL BEHAVIOR PRODUCED BY ASYMMETRIC MEDIAN RAPHE LESIONS. W.E. Montana, D. Wirtshafter and K.E. Asin (SPON: W. Giardina) Dept. Psychol., Univ. Illinois at Chicago, Chicago, IL 60608

It has been well established that asymmetric electrolytic lesions of the median raphe nucleus (MR) result in pronounced, spontaneous contralateral rotation in rats. Since the MR is one of the major sources of forebrain serotonin, several authors have suggested that the rotation may be the result of asymmetrical depletion of serotonin. The current experiment was designed to test this possibility. We reasoned that if serotonergic mechanisms play an essential role in MR-induced rotation, prior depletion of serotonin should modify this effect.

Twenty rats were given two injections of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA) (300 mg/kg x 2 (i.p.)) or its vehicle. The injections were separated by 48 hrs. Subjects were then given asymmetric, electrolytic MR lesions 24 hrs. after the last injection. Twenty-four hours later, rats were placed in a circular rotometer and spontaneous turns were counted over a period of 10 minutes. It was found that PCPA was without effect on the vigorous contralateral rotation produced by the MR lesions. Since we have found in other animals that the PCPA regimen used here reduced forebrain serotonin levels by more than 90% 48 hrs. after the last injection, these results suggest that serotonergic mechanisms do not play a significant role in MR lesion-induced rotation.

If MR lesion-induced rotation does not depend on serotonin, it must reflect damage to non-serotonergic cells or fibers of passage. One possible substrate is the crossed component of the tectospinal tract which borders the MR. To investigate this possibility we severed this pathway by placing knife cuts (using a tungsten retractable knife) through the dorsal tegmental decussation two days prior to the production of asymmetric MR lesions. Preliminary results suggest that these knife cuts attenuate the lesion-induced rotational behavior.

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308.15 LOCOMOTOR ACTIVITY FOLLOWING LESIONS WITHIN, ROSTRAL, CAUDAL OR VENTRAL TO THE MEDIAN RAPHE NUCLEUS. T.R. Stratford and D. Wirtshafter. Dept. Psychology, Univ. Illinois at Chicago, P.O. Box 4348, Chicago, IL 60680.

It is well documented that electrolytic median raphe (MR) lesions produce a substantial increase in locomotor activity. Studies utilizing intra-MR injections of the excitotoxin ibotenate have suggested that this hyperactivity is the result of damage both to cells within the MR and to fibers of passage (Asin & Fibiger, Brain Research,268, 1983).

In the present study we evaluated the relative contributions of various pathways in the region of the MR to the hyperactivity by comparing locomotor activity levels in rats given electrolytic MR lesions with those of animals given lesions or knife cuts rostral, ventral or caudal to the MR. Locomotor activity levels were assessed in a 5-min open field test, a 1 h photocell activity cage test, a 2 h tilt box test, and a 10 min free-running test conducted in an eight-arm radial maze.

In the first experiment, coronal knife cuts were placed immediately rostral to the MR in order to minimize damage to adjacent nuclei, while severing most of the ascending fiber systems. These lesions produced massive depletions of striatal and hippocampal serotonin. They also significantly increased activity in the tilt cages and photocell cages, but decreased activity in the open field and radial maze. In the second experiment, rats were prepared with discrete bilateral lesions of the nuclei reticularis tegmenti pontis (NRTP); these lesions failed to affect any measure of activity, in contrast to the report of Brudzynski & Mogenson (Br.Res.Bull.,1984).

The previous studies suggest that the pathway through which MR lesions alter open field activity emerges from either the caudal or dorsal border of the MR. In pilot work we were able to demonstrate a small descending tract of degenerating axons following MR lesions. The tract ran caudally, along the midline, and we chose to disrupt it with a lesion at the level of the nucleus pontis caudalis. These lesions resulted in significant increases in activity during all of the tests. The increases in open field and tilt cage activity were equivalent in magnitude to those observed after MR lesions. Rats with combined MR and caudal lesions showed levels of open field activity equivalent to those of animals with either lesion alone.

These results support the conclusion that two or more neural systems are involved in the increases in locomotor activity levels observed following electrolytic MR lesions and suggest that the open field hyperactivity is the result of damage to a descending fiber tract passing through the MR.

Supported by NIH RO1 NS21350.

308.16 EFFECTS OF NORADRENERGIC LOCUS COERULEUS LESIONS ON SQUIRREL MONKEY EVENT-RELATED POTENTIALS. J. A. Pineda, S. L. Foote, and H. J. Neville. Departments of Neuroscience and Psychiatry, U.C., San Diego, Salk Institute and Scripps Clinic and Research Foundation, La Jolla, CA.

The P3a component of the human event-related potential (ERP) is elicited under similar conditions as the autonomic indices of the orienting response, and thus P3a may reflect the activation of orienting mechanisms by novel events in the environment. Since the noradrenergic (NA)-locus coeruleus (LC) system has been postulated to be involved in mediating orienting behavior, the present study investigated the effects of NA-LC lesions on monkey surface-recorded ERPs analogous to the human P3a.

ERPs were recorded from three squirrel monkeys (Saimiri sciureus) using small screws embedded in the skull. These electrodes were placed over midline and lateral sites, including one on the supra-orbital ridge to monitor the EOG. All sites were referenced to an inion electrode. Behaviorally-passive monkeys were exposed to a repetitive acoustic stimulus (2 KHz or 6 KHz tones, 40 ms duration and 60 dB above human threshold) presented 90% of the time, randomly intermixed with an infrequent tone (2 KHz or 6 KHz) presented 10% of the time. Following four weeks of baseline recordings, animals were placed under halothane anesthesia; a metal electrode was introduced at the anterior pole of the LC and an electrolytic lesion created (0.5 mA/15 sec), followed by a medio-lateral knife cut (750 um in each direction). The same procedure was repeated in the other hemisphere. After one week of recovery, ERPs were recorded twice a week for four weeks. Then, tissue histology and immunohistochemistry, utilizing anti-dopamine-β-hydroxylase (DBH) and anti-tyrosine hydroxylase (TH), were used to verify the extent and specificity of the lesion.

Pre-lesion ERPs exhibited a P30, N103, and P150 to both tones, while a late positive complex (LPC) and a negative slow wave (SW) only appeared following the infrequent tones. The LPC exhibited a bi-peaked morphology (mean latencies of 248 and 369 ms, respectively) and was of maximal amplitude over parietal sites. LC lesions in the anterior pole of the LC, histologically verified for all three monkeys, had a marked effect on these surface-recorded ERPs. In two of the monkeys, P30, P150, LPC, and SW recorded over frontal and parietal sites were attenuated 40-70% following the lesion, while N103 over parietal sites increased 40-100%. In the remaining monkey, while there was a 30-70% reduction in P30, N103, P150, and SW, there was a broadening of the LPC. Immunohistochemistry revealed that the two monkeys exhibiting an attenuation of P30, P150, LPC, and SW, showed a marked reduction in DBH immunoreactivity in most areas of the diencephalon and telencephalon. By contrast, in the third monkey, regions of the neocortex showed a reduction in DBH immunoreactivity, but hippocampus, septum, and entorhinal cortex did not. In addition, NA-LC lesions did not appear to affect the recovery periods of P30, N103, and P150 in two monkeys tested.

These findings suggest that NA-LC may play a role in the modulation of surface-recorded ERPs without affecting some of their characteristic properties, such as recovery periods. NA-LC lesions that cause extensive NA depletions in diecephalic and telencephalic regions appear to have marked effects on most components of the monkey ERP, including an LPC which appears similar to the human P3a. (Supported by PHS Grant NS21384 and MH40008).

308.17 **ENRICHED AND IMPOVERISHED ENVIRONMENTS: EFFECTS ON MONOAMINE NEUROTRANSMITTERS, PRECURSORS, AND METABOLITES.** M. J. Renner (Department of Psychology, University of Wisconsin, Oshkosh, WI 54901), C. L. Blank, K. Freeman*, and L. Lin* (Department of Chemistry, University of Oklahoma, Norman, OK 73019)

The effects of environmental enrichment and impoverishment on fine and gross neuroanatomy, numerous behaviors, and the chlinergic system have been studied extensively. Less, however, is known of the plasticity of other neurotransmitter systems in response to manipulation of the behavioral environment. Por, Bennett and Bondy (Behav. and Neur. Biol., 1982, 34, 132-140) reported finding no differences in receptor binding sites for several transmitters including spiroperidol, which putatively binds to dopamine (DA) and serotonin (5HT) receptor sites. No previous work has been done, however, on the monoamine transmitters themselves. In two replications, 25 weight-matched pairs of 70-day old Sprague-Dawley rats were randomly assigned, one to an enriched condition (group housing in a 75 x 75 x 40 cm cage, with a number of junk objects) and one to an impoverished condition (solitary housing in a small cage without cagemates), for 30 days. After 30 days in these conditions, subjects were sacrificed (under code numbers that did not reveal an individual's environmental history) by 800 msec of 10kW microwave irradiation to the head at 2.45 GHz (NJE-2603 Microwave Irradiator, New Japan Radio Company, Ltd.). Brains were then removed from the skull and dissected into 11 sections: four sections from the cortex (occipital, somesthetic, frontal pole, and remaining dorsal) and seven others (hippocampus and amygdala, corpus striatum, hypothalamus, cerebellum, medulla-pons, midbrain, thalamus). Samples are analyzed via liquid chromatography combined with electrochemical detection (LCEC) using a reversed phase column packed with 3 micron particles (P. Y. T. Lin., et al., J. Liquid. Chromatog., 1984, 7(3), 509-538), permitting determinition of 10 species of catecholamines, indoleamines, precursors and metabolites (Dopa, DA, Norepinephrine (NE), Epinephrine, Dopac, 3-Methoxytyramine, Homovanillic Acid, 5HT, 5-Hydroxytryptophol, and 5-Hydroxyindoleacetic Acid). Preliminary analyses of the first of two experiments indicate that DA and metabolites are not substantially affected in either occipital cortex or hippocampus - amygdala, but that NE levels may be elevated in the occipital cortex of impoverished animals, without being altered in hippocampus - amygdala. Complete analyses will be reported, and will apparently be the first direct evidence concerning the influence (or lack thereof) of enrichment or impoverishment of the behavioral environment on monoamine neurotransmitter systems.

309.1 MESOLIMBIC DOPAMINE LESIONS BLOCK THE UNCONDITIONED AND CONDITIONED MOTOR EFFECTS OF AMPHETAMINE. L.H.Gold, N.R.Swerdlow and G.F.Koob, Div. of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Unconditioned responses in rats can be conditioned to specific environmental stimuli following repeated drug administration in a distinctive environment. Demonstrating the neuropharmacological changes that dictate conditioned drug effects may reveal important factors in the maintenance of drug addiction and the psychopathology associated with chronic drug use.

The ability of various stimulants to engender a conditioned locomotor response was evaluated in photocell activity cages. Rats were injected daily with d-amphetamine sulfate 0.75mg/kg subcutaneously (SC) (amph test cage group) or saline (sal test cage group) for five days (pairing trials). The unconditioned response, locomotor activity, was measured for 120 minutes. In addition, a peanut butter smell (conditioned stimulus) was introduced into the photocell room to enhance the saliency of the environment. At the completion of each testing session, rats were returned to their home cages and injected with the alternate solution. On the sixth day (conditioning test day), all subjects received saline injections and were retested in the photocell cages in the presence of the conditined stimulus. Subjects in the amph test group showed a statistically significant increase in locomotion on the conditioning test day compared to the saline group on measures of total beam interruptions and crossovers.

In a second experiment the role of mesolimbic dopamine in this phenomenon was studied. Rats given either bilateral 6-OHDA lesions or sham lesions of the nucleus accumbens were injected with 0.75mg/kg d-amphetamine SC, or vehicle. Subjects with lesions did not demonstrate an unconditioned or conditioned locomotor activation when compared to animals with sham lesions, after repeated amphetamine injections. Thus, the removal of mesolimbic dopamine by a neurotoxin lesion, prior to pairing trials, eliminated the conditioned locomotor response. These results are consistent with the studies of others (Beninger,R.J. and Hahn,B. Science 220:1304, 1983) showing that intact dopamine function is required for the development of a conditioned locomotor response to d-amphetamine. Furthermore, this study suggests that the mesolimbic dopamine system may be responsible for both the unconditioned and conditioned locomotor responses to psychomotor stimulants.

309.2 SELECTIVE D1 AND D2 DOPAMINE RECEPTOR ANTAGONISTS: SIMILAR PROFILES IN CATALEPSY AND SUPERSENSITIVE LOCOMOTOR TESTING IN RATS. N.R. Swerdlow[1,2], I. Creese[2], G.F. Koob[1], and M. Amalric[1]. [1]Div Neuroscience and Endocrinol., Scripps Clinic and Res Fdn, La Jolla, CA 92037, [2]Dept Neurosci., UCSD, La Jolla, CA 92093

The dopamine receptor antagonists SCH 23390 and spiperone show highly selective in vitro affinity for D-1 and D-2 dopamine receptor subtypes, respectively. It is of great importance to establish whether such specificity in vitro is parallelled by similarly specific behavioral profiles in vivo. We studied the ability of SCH 23390 and spiperone to produce catalepsy and to antagonize the supersensitive locomotor response to apomorphine in rats following 6-hydroxydopamine (6OHDA) lesions of the nucleus accumbens (NAC).

In the first experiment, rats (n=56) were tested in a simple step-down catalepsy paradigm. Animals were injected with one of four doses of SCH 23390 (0, 0.005, 0.01, 0.05 mg/kg SC) or spiperone (0, 0.05, 0.1, 0.5 mg/kg SC). 30 min later, each animal was positioned with front paws resting on a bar 9 cm above the floor, and the latency to return both paws to the floor was recorded by an experimenter blind to drug condition. Both SCH 23390 and spiperone produced statistically significant dose-dependent increases in step-down latency. This effect was significant for SCH 23390 at a dose of 0.01 mg/kg, and for spiperone at a dose of 0.1 mg/kg.

In the second experiment, animals (n=15) received 6OHDA lesions of the NAC. Beginning one week later, animals were tested for their locomotor response to apomorphine (0.1 mg/kg SC) in photocell cages on four days, with each test day separated by three non-test days. 30 min prior to treatment with apomorphine, animals were injected with one of four doses of SCH 23390 (0, 0.005, 0.01, 0.05 mg/kg SC; n=9) or spiperone (0, 0.05, 0.1, 0.5 mg/kg SC; n=6) using a Latin Square design. Both SCH 23390 and spiperone produced statistically significant dose-dependent decreases in apomorphine-stimulated locomotor activity in NAC 6OHDA-injected rats. This effect was significant for SCH 23390 at a dose of 0.005 mg/kg (ED50 = 0.01 mg/kg), and for spiperone at 0.10 mg/kg (ED50 = 0.08 mg/kg). In separate unlesioned animals, neither of these threshold doses depressed baseline photocell activity.

These results indicate that drugs with distinct in vitro dopamine receptor binding affinities produce strikingly similar effects on simple motor behavior and supersensitive apomorphine-stimulated locomotion in the rat. Although it is possible that the specificity of these agents in vitro may not be maintained in an intact animal, it is more likely that these results reflect functional similarities of brain D1 and D2 dopamine receptors.

309.3 ATTENUATED ACQUISITION OF SCHEDULE INDUCED POLYDIPSIA FOLLOWING 6-HYDROXYDOPAMINE INJECTIONS INTO THE MEDIAL PREFRONTAL CORTEX OF THE RAT. C.N.Ryan* and S.B.Dunnett, Department of Experimental Psychology, University of Cambridge, UK.

The dopaminergic projections from the ventral tegmental area to the nucleus accumbens, as originally described by Ungerstedt (Acta Physiol. Scand. Suppl. 367, 1-48, 1971), have now been extended to include cortical areas. Alternatively, Bjorklund and Lindvall (Handbook of Chemical Neuroanatomy, Volume 2, Eds. Bjorklund and Hokfelt, 1984) have proposed that the dopaminergic projections to the prefrontal cortex and the nucleus accumbens form part of different systems (the mesocortical and mesostriatal systems, respectively). In the present study, the functional role of the dopaminergic innervation of the medial prefrontal cortex (PFC) was investigated in the development of schedule-induced polydipsia, a behavioural function which is sensitive to 6-OHDA induced lesions of the nucleus accumbens (Robbins and Koob, Nature, 285, 409-412, 1980). Microinfusions of 6-hydroxydopamine (6-OHDA) into the PFC of rats, following peripheral administration of desmethylimipramine and pargyline, deplete PFC dopamine by over 80%, and noradrenaline by 40%, without influencing levels in subcortical areas, either in the caudate or the nucleus accumbens. These were compared to prefrontal electrolytic lesions, in the same sites as those used for 6-OHDA infusion. Whereas rats with 6-OHDA induced lesions showed attenuated adjunctive drinking, rats with prefrontal electrolytic lesions exhibited significantly increased drinking. However, in both lesion groups drinking induced by water deprivation was unaffected. These results indicate that the dopaminergic innervation of the medial prefrontal cortex is essential for schedule-induced polydipsia, contrary to previous suggestions, (Christie et al, Behavioural Brain Research, 19, 183-186, 1986), supporting the functional relatedness of areas innervated by the mesencephalic dopamine-containing cell bodies in the ventral tegmental area.

309.4 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF NOREPINEPHRINE, DOPAMINE, AND SEROTONIN DEPLETION WITHIN THE FRONTAL NEOCORTEX OF THE RAT. G.L. Wenk, D.J. Hughey, B. Cribbs*, T. U'Prichard* and D.S. Olton. Dept. of Psychology, The Johns Hopkins Univ. Baltimore, MD 21218

Dopamine (DA), norepinephrine (NE), and serotonin (5HT) are all found in the frontal neocortex. However, these three neurotransmitters differ in their origin and patterns of distribution, suggesting functional divisions. The present study investigated the involvement of these systems in memory by making selective lesions with neurotoxins applied to the neocortex, and testing rats in a spatial delayed alternation task. Each neurotoxin was injected into five sites, 1 mm below the pia, in the frontolateral neocortex of each hemisphere. DA was depleted by 6-hydroxydopamine, 10 ug/ul, following pretreatment with desipramine HCl, 25 mg/kg, i.p. 5HT was depleted by 5,7-dihydroxytryptamine, 5 ug/ul. NE was depleted by DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine HCl), 10 ug/ul. Control rats were given equal volumes of the vehicle, 0.9% saline containing 2% ascorbic acid.

Each rat was food deprived and trained in a spatial alternation task in a T-maze. Accurate performance in this task requires trial-dependent, working memory for the arm of the maze visited during the immediately preceding trial. For the first trial of each day, one arm was blocked and the rat was forced to enter the other arm to obtain food. For the next 10 trials, the block was removed so that the rat could enter either arm. However, food was located only in the arm not entered during the immediately preceding run. The intertrial interval was 15 sec. After 30 days of behavioral testing, the rats were given pharmacological challenges using atropine (2-5 mg/kg), propranolol (10-15 mg/kg), and other selected receptor antagonists, 30 min prior to behavioral testing.

At the start of behavioral testing, only rats with DSP-4 injections showed impaired choice accuracy. The accuracy of these rats gradually improved until it returned to normal levels by Day 15 of testing. The biochemical and pharmacological data suggest that there is a complex interaction between the cholinergic, serotonergic, dopaminergic, and adrenergic afferents to the frontal neocortex of the rat.

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309.5 FROG-LIKE JUMP IN HALOPERIDOL TREATED RATS
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Lewis rats treated with haloperidol (2 mg/kg) stayed in akinesia and catalepsia. However, if they were braced forward on the surface of or at the edge of a table, they could display an energetic jump as far as 70 cm and as high as 40 cm. After the jump they returned to akinesia and catalepsia state immediately. Time course study revealed that akinesia and catalepsia appeared earlier and ended later than frog-like jump did. Haloperidol induced akinesia and catalepsia in five minutes and this effect lasted for more than one hour. On the other hand,the latency of haloperidol induced jump was about 15 minutes and the frog-like jump could not be induced 50 minutes after haloperidol injection. Dose response study revealed that akinesia and catalepsy could be induced by 0.5 mg/kg of haloperidol. At this dose, frog-like jump could not be induced. It appears that there are two subsystems mediating haloperidol effects.

309.6 AUTOMATED ANIMAL ACTIVITY MONITOR: ANALYSIS OF THE EFFECT OF HALOPERIDOL AND CYCLO(LEUCYL-GLYCYL) ON APOMORPHINE-INDUCED STEREOTYPY. J.Z. Fields, L.P. Gonzalez, L.R. Meyerson, J.M. Lee, K.A. Steece, K.M. Federowicz, F.A. DeLeon-Jones* and R.F. Ritzmann. Research Services, Hines V.A. Hospital, Hines, IL 60141, Westside V.A. Hospital, Chicago, IL 60680 and Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL 60680

Our previous studies indicated that the peptide cyclo(leucyl-glycyl) (cLG) prevents the development of supersensitivity to dopamine in several animal models at both biochemical and behavioral levels. We therefore tested cLG in a paradigm more commonly used to model tardive dyskinesia, namely, chronic haloperidol administration to rats.

We found that cLG administered subcutaneously at a dose of 8 mg/kg blocked about 50% of the supersensitizing effects of haloperidol on apomorphine-induced stereotypic behaviors including sniffing and licking. The peptide by itself increased the stereotypic behaviors compared to controls.

Further, we used a novel method, radio-frequency analysis, that quantifies sniffing and other stereotypic movements. Unlike methods that rely on visual observation of stereotypy and utilize an ordinal scale, these measurements are rated by an automatic motility monitor and utilize a ratio scale. Unlike other automated motility monitors, this device can distinguish between various forms of stereotypic behaviors. Since parametric statistics can be used, there is a significant improvement in the efficiency of the task of rating and comparing stereotypy scores. There is an identical rank order and a good correlation ($p<.01$) between the two behavioral rating methods indicating the reliability of the automated method.

309.7 2-DEOXYGLUCOSE IMPAIRS STRIATAL DOPAMINE RELEASE IN RATS: ENHANCED EFFECT AFTER 6-HYDROXYDOPAMINE PRETREATMENT. M.J. Zigmond, H.J. Morris*, R.W. Keller, Jr. and E.M. Stricker. Department of Biological Sciences, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

2-Deoxyglucose (2DG), an inhibitor of glucose metabolism, normally increases food intake in rats. However, after subtotal damage to the dopamine (DA)-containing nigrostriatal bundle (NSB), 2DG elicits acute akinesia and sensory neglect. Because these symptoms are similar to the effects of DA antagonists or complete NSB injury, we sought to determine whether 2DG impaired NSB function. One to three months after rats were given of 6-HDA (200 ug), coronal slices (350 u) of striatum were superfused (100 ul/min) with Krebs bicarbonate buffer containing tyrosine (75 uM), nomifensine (10 uM), glucose (11.5 mM) and 2DG (0-46 mM), with total osmolarity maintained constant by reducing the concentration of NaCl. After one hour of preincubation, slices were exposed to electrical field stimulation (4.5 Hz, 20 mA, bipolar) for 10 min and DA efflux was measured and compared with that which occurred during the 10 min before stimulation. In addition, we measured the DA content of incubated and nonincubated slices. Values were expressed as ng DA per mg tissue protein.

Stimulation of intact slices increased DA efflux 7-fold (1.1 ± .2 to 8.0 ± .5 ng/mg), with no depletion of tissue DA (82 ± 6 ng/mg). Comparable results were obtained after stimulation of slices from 6-HDA pretreated rats, despite a 69% loss of DA terminals. As a result net DA overflow due to stimulation expressed as a fraction of tissue DA was considerably greater from lesioned slices than from intact slices (.30 ± .04 vs .08 ± .01).

Incubation of intact slices in the presence of 23 mM 2DG had no effect on either spontaneous or stimulated DA efflux, although tissue DA content at the end of stimulation was reduced by 51 ± .09%. In contrast, whereas 2DG did not affect spontaneous efflux from 6-HDA-treated tissue, it reduced overflow by 78 ± .06% and depleted tissue content by 67 ± .08%, results comparable to those obtained with intact tissue only when the concentration of 2DG was increased 2-fold.

These results suggest that 2DG may inhibit DA synthesis and thereby cause the loss of DA stores during depolarization. They further indicate that transmitter release from striatal DA terminals is greatly enhanced after 6-HDA-induced brain lesions and is particularly sensitive to the inhibitory effects of 2DG, possibly due to the greater dependence of release from these terminals on new DA synthesis. The debilitating neurological impact of 2DG on NSB-lesioned animals may reflect this increased sensitivity.

309.8 THE HIPPOCAMPUS AS A DOPAMINE MODULATOR: A MOTOR SEQUENCING INTERPRETATION OF HIPPOCAMPAL FUNCTION. J. Kelley* and B. Osborne. Dept. of Psychology, Middlebury College, Middlebury, VT 05753.

The present experiment examined the possible role of the hippocampus in maintaining a balance of dopamine in neural areas. The dopamine antagonist haloperidol was injected interperitoneally into fornix transected and operated control rats. The experiment consisted of two phases. In phase I the rats were observed in a novel open field environment; in phase II the rats were observed during one hour of limited access to food. Behavioral changes in locomotion, exploration, rearing, and frequency of eating and drinking were consistent with those found previously after fornix transection. In general, haloperidol produced greater behavioral changes in rats with fornix transections than in control animals in the first phase of the study. The drug induced changes in the fornix transected animals made their behavior more like that of control animals. Injections of haloperidol were found to have no effect on either control or fornix transected animals in the second phase of the experiment. The results of the experiment support the role of the hippocampus as a neural neuromodulator in the open field environment. Further investigation is necessary before a conclusion can be drawn as to the neuromodulatory role of the hippocampus in the context of feeding.

309.9 DIFFERENT STIMULI CONTROL AMPHETAMINE- AND APOMORPHINE-INDUCED ROTATIONS. M.G. Ziegler* and H. Szechtman. Dept. Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

Administration of amphetamine (AMP) or apomorphine (APO) yields circling (rotations) in rats with unilateral 6-OHDA lesions of the substantia nigra (SN). In studies which make use of these drug-induced rotations, it is often assumed that the behavioral responses to AMP and APO are equivalent, except for the direction of rotation. However, if this assumption is valid, one would expect that under AMP or APO both the motor form of circling and the stimuli controlling these rotations, would be similar. Yet, our studies suggest that differences exist. In previous work, we showed that the stepping patterns observed during AMP- and APO-induced rotations, differ (Ziegler & Szechtman, CCNP, 1986), as does the topography of rotations (see poster by Szechtman & Ziegler). In this poster, we also show that different stimuli control these drug-induced rotations. Specifically, we found the following in rats with unilateral 6-OHDA lesions of the SN: (1) edges attract the animals under APO but very little under AMP; (2) edges maintain a directional bias under APO but eliminate the bias under AMP; and (3) edges reverse the typical APO-induced directional bias observed in a rotometer, so that almost no contraversive circling is seen. Rats (n=7) were given 1-2 minute swimming trials in a circular pool (7' dia). Following two swimming trials without drugs, they were given either APO (.25 mg/kg s.c.) or AMP (2 mg/kg i.p.) and tested again 10, 25 and 50 min later. Their behavior was videotaped for subsequent analysis of time spent swimming ipsiversively or contraversively at or away from the edge of the pool. The chosen drug doses yield similar numbers of circles in 1 hr rotometer tests. Results indicate: (1) Under AMP, rats spent 24.6% of their total swimming time aligned with the edge ($p<.05$); in contrast, under APO or without drugs, they spent about half of their swimming time at edge (time at edge vs away from edge, n.s.). (2) When away from the edge, rats swam in the direction consistent with that observed in the rotometer. (3) In the undrugged and AMP conditions, they showed no directional bias when swimming aligned with the edge (ipsiversive vs contraversive swimming at edge; n.s.) but under APO they spent 97% of their time at edge swimming in one direction ($p<.025$). (4) Under APO, the directional bias at the edge was opposite to that found in the rotometer (ipsiversive vs contraversive swimming at edge: 132 ± 43 vs 4 ± 2 sec; $p<.025$). Thus, AMP- and APO-induced rotations may be controlled by different stimuli. Since the topography of those rotations differs as well, it is possible that they reflect the activity of different neural processes. (Supported by NSERC. HS is a Research Associate of the Ontario Mental Health Foundation.)

309.10 DIFFERENCES IN THE MOTOR FORM OF CIRCLING INDUCED BY AMPHETAMINE AND APOMORPHINE. H. Szechtman and M.G. Ziegler* (SPON: M.D. Coughlin). Dept. Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

In rats with unilateral lesions of the substantia nigra (produced by 6-OHDA), the administration of amphetamine (AMP) induces circling (rotations) towards the side of the lesion and the injection of apomorphine (APO) produces circling in the opposite direction. In studies which use this phenomenon to investigate striatal function and mechanisms of drug action, it is generally assumed that the two behavioural responses are equivalent, reflecting the side of greater striatal activation. Yet, our analyses indicate that different stimuli may control APO- and AMP-induced circling (see poster by Ziegler & Szechtman). Moreover, rats use different stepping patterns during circling under APO and AMP (Ziegler & Szechtman, CCNP, 1986), and, as we show in this poster, there are additional differences in the topography of these rotations. Thus, at least at some level, APO- and AMP-induced rotations are not equivalent (symmetrical) phenomena. Rats were tested for circling in clear Plexiglas hemispheres; their behaviour was videotaped or filmed for subsequent analysis using the Eshkol-Wachman Movement Notation. They were injected with AMP (2 mg/kg i.p.) and APO (0.25 mg/kg s.c.) which yield similar number of circles in a 1 hr test. Results indicate that rotational behaviour under APO and AMP differed in: the degree of lateral bending of the head and middle torso (maximum under APO but variable under AMP); amount of turning in the non-preferred direction (minimal under APO, large under AMP); presence of snout contact (continuous under APO, minimal under AMP); extent of dorsal arching of the torso (maximum under APO, minimal under AMP); distribution of weight (predominantly over the hindlegs under APO, evenly balanced over the 4 limbs under AMP); and, amplitude of rotations (large and almost continuous under APO, variable and punctuated by frequent arrests under AMP). These differences may indicate that APO and AMP activate different neural circuits to induce circling. Alternatively, differences in behaviour may reflect that: (1) the same neural circuits are activated by both AMP and APO but in the first case the activated system interacts with an intact hemisphere while in the second case it interacts with a hemisphere in which there is a lesion; and, (2) both AMP and APO activate turning via the same mechanism but each drug has additional, different behavioural effects, that are superimposed on the turning. (Supported by NSERC. HS is a Research Associate of the Ontario Mental Health Foundation.)

309.11 LESIONS OF NORADRENERGIC PATHWAYS SUPPRESS THE BEHAVIORAL DEFICITS INDUCED BY ELECTROLYTIC LESIONS WITHIN THE VENTRAL TEGMENTAL AREA. K. Taghzouti*, H. Simon*, J.M. Deminière*, D. Hervé*, J.M. Studler*, G. Blanc*, J. Glowinski*, J.P. Tassin* and M. Le Moal. (SPON: A. Calas) Laboratoire de Psychobiologie des Comportements Adaptatifs, INSERM U.259, Université Bordeaux II, Domaine de Carreire, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex and Laboratoire de Neurobiologie, INSERM U.114, Collège de France, 11 place Marcelin Berthelot, 75231 Paris Cedex 05. France.

Biochemical results indicate that the development of dopaminergic (DA) D1-receptor supersensitivity in the prefrontal cortex following electrolytic lesion of the ventral tegmental area (VTA), the region where the dopamine cell bodies are located, depends of the presence of an intact noradrenergic (NA) innervation originating from the locus coeruleus (LC). Infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) into the lateral pedunculus cerebellaris superior (PCS), before or after the electrolytic lesions of the VTA abolished the supersensitivity of D1-receptors (Tassin et al., J.Neurochem., 46:243,1986) and suggested the existence of co-regulations between the two neurotransmistters. These co-regulations have been investigated at the behavioral level: We report that bilateral 6-OHDA lesions of noradrenergic fibers within the PCS reduced some behavioral deficits induced by the VTA electrolytic lesions. Locomotor hyperactivity induced by VTA-lesions was abolished after 6-OHDA infusions when the two lesions were performed either simultaneously or successively. Deficits in spontaneous alternation in a Y-maze in rats bearing VTA-lesions disappeared after the noradrenergic lesions. Moreover, enhanced vulnerability to d-amphetamine self-administration induced by VTA-lesions was not observed when 6-OHDA-PCS infusion was added to the electrolytic lesions of the VTA. Biochemical controls indicate that combined treatments produces a large decrease of cortical DA (-65%) and NA concentrations (-100%). It is important to note that the noradrenergic lesions do not induce behavioral deficits in the three tests. It is concluded that cooperative neuronal modulations may exist between both DA and NA transmission at both the molecular and the behavioral levels.

309.12 CUES SIGNALING MEAL ONSET PRODUCE INCREASES IN DOPAMINE TURNOVER. J.R. Blackburn, A.G. Phillips, A. Jakubovic and H.C. Fibiger. Departments of Psychology and Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6T 1W5.

A conditioned feeding paradigm was used to study the role of brain dopamine (DA) systems in feeding behaviour. Rats were individually housed in sound attenuating chambers for eight days. During this time, the only food they received was delivered as six signaled meals of a liquid diet each day. Within a few days, the animals would respond to the conditional stimulus (CS+) signaling meal delivery by engaging in various anticipatory behaviours. For example, they would enter into the niche where the food was about to be delivered. Previous studies have demonstrated that these anticipatory behaviours are severely attenuated by doses of the DA antagonist pimozide that do not disrupt consummatory feeding behaviours. The present study was conducted to determine if these behaviours were accompanied by increases in dopaminergic activity.

DA turnover was investigated using high pressure liquid chromatography coupled with electrochemical detection. Rats in the experimental group (n=7) were sacrificed 4 min after CS+ onset, while controls (n=7) were sacrificed immediately prior to CS+ onset. DA turnover, as measured by the ratio of the DOPAC to DA, was increased in the n. accumbens of the rats of the experimental group. No significant differences in striatal DA turnover between the two groups. In contrast, a separate experiment failed to show any differences in DA activity between a group of rats (n=8) that consumed an unsignaled meal of liquid diet in the same apparatus and a control group (n=8). These data are consistent with a role for DA systems in behaviours related to the anticipation of food delivery, as opposed to consummatory feeding behaviour.

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309.13 EFFECTS OF DOPAMINE BLOCKADE IN THE NUCLEUS ACCUMBENS AND LATERAL CENTRAL STRIATUM ON MOTIVATED BEHAVIOR. J.B. Richards* and D. Neill* (SPON: G. Audesirk).Dept. of Psychology, Emory Univ., Atlanta, GA 30322.

There is a large amount of data which suggests that anatomically separate dopamine (DA) terminal areas underlie different kinds or classes of behavior. We present data which supports a "classes of behavior approach" to understanding the behavioral function of DA. Specifically, we suggest that lateral central striatal (LCS) DA modulates the occurance of stationary behaviors while Nucleus Accumbens (NAS) DA modulates the occurance of non stationary locomotor behaviors. The classes of behavior approach can be contrasted to the anhedonia hypothesis (Wise, R., Behav. Brain Sci.,5:39, 1982) which suggests that NAS DA is primarly involved in the appreciation of reward.

Rats were trained on a task in a runway involving two distinctly different behaviors: snout poking and locomoting. In one componet of the task the rats remained stationary at one end of the runway, snout poking for liquid diet. With each drop of liquid diet the time to the next drop increased by .25 sec. At any time, the rat had the option of locomoting down the runway and interrupting a photo cell which reset the apparatus and returned the time between drops to a minimum value.

Twelve rats were trained on the travel box task described above. Six of the rats were bilaterally implanted with cannulae aimed at the NAS (AP9.2;L1.8;V0.0) The other six rats were bilaterally implanted with cannulae aimed at the LCS (AP6.8;l4.0;V1.0). Local DA blockade was induced by injecting 0, 1, 2.5, amd 5 ug of haloperidol (in 1 ul vehicle) into the NAS and LCS. In addition, the concentration of liquid diet was diluted inorder to determine how changes in its rewarding value effected the behavior of rats in this task.

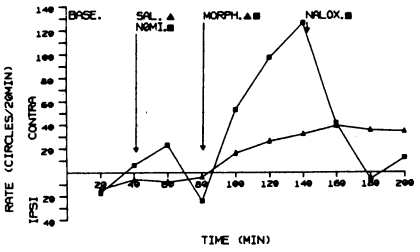
In reponse to decreasing the rewarding value of the liquid diet the rats spent less time nose poking and more time locomoting (resetting). In contrast, local DA blockade in NAS increased the amount of time the rats spent nose poking for liquid diet and decreased the locomotor componet of the task. Local DA blockade in the LCS had effects similar to those obtained by diluting the liquid diet and were opposite to the effects of local DA blockade in the NAS. That is, the LCS rats decrease the amount of time spent nose poking and increased locomotion.

These results are consistent with the classes of behavior approach and not the anhedonia hypothesis of the mesolimbic DA system.

309.14 POTENTIATION OF VENTRAL TEGMENTAL MORPHINE-ELICITED CIRCLING BY NOMIFENSINE. A.Gratton[1], G.Gerhardt[2], R.A.Wise[3] and B.J.Hoffer[1]. Departments of Pharmacology[1] and Psychiatry[2], University of Colorado Health Sciences Center, Denver, CO, 80262 and Department of Psychology[3], Concordia University, Montreal, Canada, H3G 1M8.

Unilateral infusions of morphine into the rat ventral tegmental area (VTA) elicit contraversive circling. There are indications in the literature that such circling is dopamine-dependent. VTA sites positive for morphine-elicited contraversive circling are coextensive with the medial-lateral and dorsal-ventral distributions of the A9 and A10 dopaminergic cell groups. Furthermore, morphine-elicited circling is blocked by both dopamine receptor (pimozide) and opiate receptor (naloxone) antagonists. In the present study, the interaction of morphine with VTA dopaminergic cells was further studied by examining the effects of the catecholamine reuptake blocker, nomifensine, on the rate of morphine-elicited circling. We reasoned that if morphine produces contraversive circling by causing a unilateral increase in dopaminergic activity, then the increased concentration of dopamine at postsynaptic sites following nomifensine pretreatment should potentiate the rate of morphine-elicited circling.

Animals exhibiting contraversive circling following a 0.5 ul infusion of 7.5 nanomoles morphine sulfate (prepared in physiological saline) into the VTA, via a chronic indwelling cannula, were pretreated with nomifensine (2.5 mg/kg s.c.). As shown below, nomifensine pretreatment resulted in a 4-5 fold increase in the rate of morphine-elicited circling. Administration of naloxone (1.5 mg/kg s.c.) produced a complete cessation of nomifensine-potentiated circling. The present data thus are consistent with the idea that the behaviorally activating properties of intra-VTA applications of morphine are dependent upon an opiate receptor population which interacts with dopaminergic mechanisms in A10. (Supported by USPHS (DA-02702) and NSERC (Canada) to A.G.)



309.15 STRIATAL DOPAMINE D_2 RECEPTOR CONCENTRATIONS DECREASE DURING HIBERNATION. T.S. Kilduff[1], S.S. Bowersox[1], R.P. Hammer[2], C. M. Radeke[3], R.D. Ciaranello[1], H.C. Heller[3], and W.C. Dement[1]. [1]Depts. of Psychiatry and [3]Biological Sci., Stanford Univ., Stanford, CA 94305 and [2]Dept. of Anat. and Reprod. Biol., Univ. of Hawaii, Honolulu, HI 96822.

Physical or functional deafferentation of neural structures commonly results in supersensitivity of post-synaptic receptors. During mammalian hibernation, electrical activity of virtually all brain regions is markedly reduced. Based on analysis of perfusates obtained from the caudate nucleus of hibernating and non-hibernating ground squirrels, Salzman et al. (1985) have proposed that, during hibernation, pools of conjugated and free extracellular dopamine (DA) prevent the development of receptor supersensitivity. Using radioligand binding, we have directly assessed whether alterations in dopamine D_2 receptor characteristics occur in the striatum during the hibernation cycle.

Golden-mantled ground squirrels (Citellus lateralis) were implanted with abdominal telemeters to monitor body temperature (T_b) continuously. Animals were placed in a constant temperature environment at 5°C under an LD 12:12 photoperiod. Four animals were sacrificed between 1200-1600 P.S.T. during each of five phases of the hibernation cycle: euthermia (T_b=37°C); entrance to hibernation (T_b=20°C); day 1 of deep hibernation (T_b<8°C); days 4 or 5 of deep hibernation (T_b<8°C); and arousal (T_b=20°C). The brains were removed, dissected into subregions and frozen on dry ice. Dopamine D_2 receptors were measured in unwashed striatal homogenates using 15-600pM [^{3}H]spiperone in the presence of 40nM cinanserin to block striatal $5HT_2$ receptors. Non-specific binding was measured in the presence of 300nM butaclamol. Scatchard analyses revealed that the B_{max} values of both deep hibernation groups were significantly decreased relative to euthermic animals (day 1 vs. euthermia: 44.5% decrease, $p<0.01$; day 4/5 vs. euthermia: 32.0% decrease, $p < 0.01$; 2-tailed t-tests corrected for multiple contrasts). B_{max} values of both entrance and arousal groups were intermediate between the euthermic and hibernation values. Binding affinities (K_d) did not significantly change.

In another 3 hibernating and 3 euthermic squirrels, D_2 receptors were localized autoradiographically using 0.6nM [^{3}H]spiperone in the presence of 50nM ketanserin to block $5HT_2$ receptors. Preliminary analyses confirm the findings described above.

These results are consistent with a down-regulation of striatal D_2 receptor concentrations as a consequence of the increased free and conjugated DA during hibernation documented by Salzman et al. Our results argue against the proposal that the DA system is protected against supersensitivity during hibernation, since a down-regulation of D_2 receptors is indicated during deep hibernation.

Salzman et al. (1985). Brain Res. 343:95-103.

309.16 EFFECTS OF 6-OHDA LESION OF DOMINANT OR NONDOMINANT NIGROSTRIATAL PATHWAY: SEX DIFFERENCES? B.A. Pappas and B. McBride*. Unit for Behavioral Medicine and Pharmacology, Department of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

Female but not male rats show asymmetry in neostriatal dopamine (DA) content. Furthermore, amphetamine elicits rotations contralateral to the neostriatum (NS) with the higher DA content (dominant NS) in females (Robinson et al., in Glick, S.D., Cerebral Lateralization in Nonhuman Species, 1985). This relationship between NS DA and rotation has not, however, been observed for males. Since these results imply sex-related functional asymmetry of NS DA, are the sequelae of unilateral 6-OHDA lesion related to the side lesioned (i.e., dominant vs. nondominant) and/or to gender?

Male and female Wistar rats were twice tested for rotation after amphetamine (females during estrus). While females rotated more than males, males were more lateralized in terms of percent of total rotations which were in one direction. The most highly lateralized 20 rats of each sex underwent unilateral 6-OHDA lesion (8 µg/4 µl, DMI pretreatment) of the dominant or nondominant substantia nigra. Ten control rats of each sex received vehicle. When retested 30 days postsurgery, dominant lesioned rats had reversed their rotation direction only if their NS DA depletion exceeded 90%. Sex did not influence this outcome. Of the control unlesioned rats, females but not males were found to rotate contralateral to the NS with the highest DA content.

There was a significant weight reduction due to the lesion but this was not related to whether the dominant or nondominant side was lesioned. Sex had no effect except that males showed a more persistent weight reduction. Daily somatosensory testing with a Von Frey Hair showed lower sensitivity contralateral to the lesioned side when this was compared to sensitivity on the ipsilateral side. This was not related to dominance or sex. When compared to unlesioned controls, however, the lesioned rats did not show hyposensitivity on the side contralateral to lesion but rather, hypersensitivity on the ipsilateral side. This underscores the necessity of unlesioned controls for the interpretation of unilateral lesion effects. Finally, lesioned rats showed impaired acquisition of 2-way avoidance. This was unrelated to sex or to dominance.

Thus, neither gender nor dominance influenced the outcome of unilateral NS DA lesion for body weight regulation, somatosensation or avoidance acquisition. Gender was relevant to rotation, however, as females but not males rotated contralateral to the NS with the higher DA content. Furthermore, 6-OHDA lesion of the dominant pathway reversed rotation direction only if the depletion exceeded 90%. Our results generally agree with those recently reported by Robinson et al. (op. cit.).

309.17 REDUCTION IN FOOD-INDUCED ACTIVITY IN RATS WITH ADMINISTRATION OF HALOPERIDOL J.D. Salamone* (SPON: E.Wong) MSD Neuroscience Research Centre, Eastwick Rd., Terlings Park, Harlow, Essex, U.K.

A food-induced activity procedure was developed in which 45mg food pellets were periodically delivered to food-deprived rats. The rats were tested in a rectangular activity chamber (52x33x33cm) with three wire mesh floor panels balanced on a rod which could trigger microswitches outside the box. Presentation of food pellets led to increases in activity which were directly related to the rate of food presentation. For drug studies, all rats received pellets on a fixed time 30 second schedule in 30 minute sessions. Haloperidol (0.1, 0.2, 0.4 mg/kg IP) reduced total activity in a dose-related manner ($F_{3,15}=20.32$, $p < .01$). Following 0.4 mg/kg haloperidol, proportionately more activity counts were registered in the first 5-seconds after pellet presentation than after saline (sign test $p < .05$). Administration of 0.2 and 0.4 mg/kg haloperidol resulted in declines in activity across the test session, while activity stayed relatively constant across the session after saline treatment. Since the food-induced activity paradigm is seen as related to the incentive properties of food, the present results are consistent with a dopaminergic involvement in incentive-related motor activity. The pattern of deficits produced by haloperidol suggests that the activation produced by food delivery was not maintained in time across the session or within the inter-pellet interval.

309.18 NO LATERALIZATION OF STRIATAL DOPAMINE METABOLISM DURING PREFERRED OR FORCED USE OF ONE PAW R. Schwarting and J.P. Huston. Institute of Psychology III, University of Düsseldorf, D-4000 Düsseldorf, Federal Republic of Germany

A number of studies have shown that unilateral manipulations in the mesostriatal system can lead to asymmetrical changes in behavior. Thus, it has been repeatedly demonstrated that unilateral destruction of mesostriatal dopamine (DA) neurons impairs or abolishes use of the contralateral forepaw in specific handedness tasks like reaching for food or bar pressing. However, information is lacking as to whether striatal DA metabolism is asymmetrically affected in intact animals performing such tasks. Therefore, the present study was designed to analyze DA metabolism (DA, DOPAC, HVA) in animals as a function of handedness.

In the first experiment, water-deprived male Wistar rats were trained to press a bar for water only with the left or right forepaw as determined by the experimental apparatus (forced handedness). About 20 days of training (25min/day) were employed to establish response rates of 700-1000 bar presses/20min under an FR10 schedule. Rats were killed during a training session and ventral and dorsal striata of both hemispheres were analyzed by means of HPLC-EC. Forced handedness was not accompanied by asymmetrical changes in DA metabolism (DA, DOPAC, HVA). Levels of DA, DOPAC and HVA in the dorsal striata were similar to those of untrained controls or to those of rats killed 2hrs after a training session. Levels of DOPAC and HVA in the ventral striata were higher in animals killed during the task than in those killed 2hrs later.

In the second experiment, the paw used was not determined by the experimental situation (preferred handedness). All animals showed clear paw preference; i.e., they made 80-95% of all bar presses with the same paw. Neurochemical analysis yielded the same results as with forced handedness; i.e., no asymmetrical levels of DA, DOPAC or HVA.

The present results provide evidence, for one, that forced handedness and paw preference are not necessarily accompanied by asymmetrical changes in striatal DA metabolism, and secondly, that paw preference (as measured in this task) is not correlated with endogenous DA asymmetry.

Supported by grant Hu 306/4-1 from the DFG.

309.19 DEPOLARIZATION-INDUCED STRIATAL DOPAMINE RELEASE IN RATS SENSITIZED TO AMPHETAMINE. E. Castaneda, J.B. Becker, and T.E. Robinson. Department of Psychology and Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, MI 48104-1687.

The repeated intermittent administration of amphetamine (AMP) produces an enduring hypersensitivity ("sensitization") to its motor stimulant effects and an enduring enhancement in AMP-stimulated endogenous dopamine (DA) release from striatal tissue in vitro. Therefore, it has been hypothesized that increased striatal DA release may be partly responsible for sensitization to AMP. Following sensitization to AMP animals also exhibit an enhanced response to environmental stimuli (e.g. stressors), and if an enhancement in DA release mediates this phenomenon one would also expect depolarization-induced release of DA to be enhanced. However, it is not known if the calcium-dependent DA release produced by synaptic depolarization is enhanced in rats sensitized to AMP. (AMP-stimulated release of DA is not calcium-dependent.) To address this question we have studied the effects of prior AMP treatment on endogenous striatal DA release in vitro induced by either high potassium (K+) or electrical stimulation.

In the first experiment ovariectomized (OVX) female rats received either one weekly injection of 3 mg/kg d-AMP (i.p.) for 5 weeks, or saline injections (1 ml/kg, i.p.). One week after the last injection the rats were decapitated, and striatal tissue placed into superfusion chambers. Effluent samples were collected at 5 min intervals and DA release stimulated by the addition of 25, 35, or 45 mM K+ to the infusion medium for 5 min. The effluent samples were later assayed for DA with HPLC-EC. The addition of K+ to the medium stimulated endogenous DA release in all cases. However, when DA release was stimulated with 45 mM K+ striatal tissue from sensitized rats showed a significantly greater enhancement in DA release than tissue from control animals. In contrast, lower concentrations of K+ stimulated the same amount of DA release from both groups.

A second experiment concerns the long-term effects of AMP on striatal DA release induced by electrical stimulation, and is currently in progress. OVX rats will receive either: (1) a single daily injection of 3 mg/kg of AMP for 6 days; (2) a series of saline injections; or (3) no treatment (i.e. non-handled controls). Non-handled control rats are included because we have found that the stress of saline injections and associated handling can also enhance AMP-stimulated striatal DA release. DA release from striatal tissue fragments obtained from these rats will be studied in a superfusion system similar to that described above, except DA release will be induced by biphasic rectangular pulses (20 mA, 2 msec duration). The frequency of the stimulus will be varied from approximately 10-40 Hz. Pilot studies have established that DA release in this situation is calcium and temperature-dependent. The results will be presented at the meeting.

309.20 EFFECTS OF DOAPAMINE INJECTIONS IN GENETICALLY INBRED HYPERACTIVE RATS. R.E.Musty, L.H. Conti*, S. Valent* Neuropsychology Laboratory, Department of Psychology, University of Vermont, Burlington, VT 05405-0132.

The Vermont Hyperactive Rat (HA) is hyperactive but normotensive. and is inbred from the Spontaneously Hypertensive Rat (SHR) and the parent strain, the Wistar-Kyoto rat (WKY) [Hendley,E.D., et al. Hypertension,5:211, 1983] The HA rat is hyperactive, aggressive, and has learning impairments (Musty, R.E.,Neurosci. Abstr.,11,558, 1985). The dopamine system of the nucleus accumbens plays a major role in the control of spontaneous locomotor activity (Cools, A., et al. Neurosci. Letters, 3:335, 1976); administration of dopamine in the nucleus accumbens leads to increased activity in randombred rats. The present study was designed to determine how dopamine administration in the nucleus accumbens of HA rats would affect spontaneous locomotor activity of HA rats.

Eleven female HA rats of the F(7) generation and 5 female randombred Wistar rats (Charles River) were bilaterally implanted with 24 gauge stainless steel cannulae in nucleus accubens. Each animal was tested in an automated activity cage (67 cm square) equipped with infrared light sensors on the x and y axis of the floor such that each each broken light beam constituted one unit of activity. Each animal was tested immediately after bilateral injection of dopamine (HCl, 1 ug-10ug/ul) or the vehicle (buffered ringers). Half of the rats were injected with dopamine on day 1 and vehicle 72 hrs. later and vice-versa. Cannulae placement was histologically verified.

Change in activity was expressed as log (100 x activity units after dopamine/activity units after vehicle) and plotted as a function of log (activity units after vehicle). These plots show the effect of the dopamine on activity as a function of activity after control treatment. All HA rats showed decreases in locomotor activity while Wistar rats showed increases or no change in activity in response to intra-accumbens dopamine. The effect of dopamine in HA rats was significantly rate dependent, that is highly active HA rats showed the greatest decrease in activity while less active HA rats showed smallest decreases in activity. These data strongly suggest that the hyperactivity of HA rats is mediated by a dopamine system in the nucleus accumbens which functions differently that that of the normoactive, randombred rat and is similar to results obtained in hyperactive children who are administered L-Dopa systemically.

310.1 ACCUMBENS INFUSION OF AMPHETAMINE INCREASES AND PICROTOXIN DECREASES REWARD FROM HYPOTHALAMIC STIMULATION. D. Spencer* and J.R. Stellar, Department of Psychology, Harvard University, Cambridge, MA 02138.

Dopamine blockade of the nucleus accumbens (NAC) decreases lateral hypothalamic (LH) self-stimulation (SS) reward, as indicated by the reward summation function (RSF) method, which discriminates between reward and motor/performance effects (Stellar et al. Neurosci.Abst. 11:1985). Amphetamine infusions into NAC have been shown to increase the lever-pressing rates of SS behavior (Stephens and Herberg. Psychopharm. 54:81-85, 1977), but this increase could be independent of reward and simply due to the drug's demonstrated ability to elevate general behavioral activity (Anden. Adv. Bio. Pharm. 16:385-390, 1977). The current study uses the RSF method to examine the reward effects of d-amphetamine infused into the NAC. In addition, the LH SS effects of NAC picrotoxin, a behavioral activator (Jones et al. Neuropharm. 20:29-37, 1980) were examined with the RSF paradigm. Finally, behavioral activity was monitored following infusions of both drugs into the NAC.

In LH SS testing, a stable RSF baseline was first established. Just prior to behavioral testing, amphetamine doses of 0, 5, 10, 20 ug/ul and picrotoxin doses of 0, .05, .1, .2 ug/ul were infused bilaterally into the NAC of freely moving animals through chronically implanted cannula. In two separate groups of rats, behavioral activity was measured using a Stoelting Co. monitor. All infusion procedures were the same as for RSF testing.

NAC infusion of amphetamine increased LH reward, but NAC picrotoxin decreased LH reward. For example, under amphetamine the stimulation frequency required to sustain half of the maximal responding of the RSF curve decreased an average of 0.21 $\pm$ 0.09 log units at the highest dose; whereas, under picrotoxin the average required frequency increased 0.16 $\pm$ 0.13 log units at the highest dose. Operant motor/performance capacity increased under amphetamine, and under picrotoxin it showed variable effects. NAC infusions of amphetamine produced a dose-dependent increase in general behavioral activity, and picrotoxin did so less reliably.

Most importantly, this study demonstrates with psychophysical behavioral measures that NAC amphetamine increases LH SS reward. In addition, the opposite actions of these agents on LH SS reward, with common effects on activation, suggests that NAC dopamine and GABA have different ways of producing behavioral activation.

310.2 NEUROLEPTIC-INDUCED CATALEPSY: D-1 RECEPTOR BLOCKADE PHENOMENON? W. R. Klemm and Helmuth Block* Brain Res. Lab., Dept. Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

Haloperidol and related neuroleptics cause catalepsy, a state of immobility in which animals maintain awkward postures. This action is generally attributed to action of the D-2 type of dopamine receptor, because these drugs have a preferential, though not exclusive, binding which blocks the D-2 receptor. Recently, this concept was strongly supported by demonstration that molindone, a relatively specific D-2 receptor blocker, could cause catalepsy in mice (Pharmacol. Biochem. Behav., 1985, 23:911-915).

Here, we evaluated the D-1 receptor, and interactions with the D-2 receptor, with selective D-1 drugs, an antagonist, SCH 23390, and an agonist, SKF 38393. Catalepsy was measured as the time that mice remained immobile in awkward postures imposed by placing them head down on a wire grid that was inclined at 45°.

The D-1 blocker caused distinct catalepsy at 1 and 10 mg/kg, i.p., but not at lower doses. Thus, catalepsy can be caused by blockade of either D-1 or D-2 receptors, suggesting that joint blockade would be additive. This was confirmed in that catalepsy was greatest after combined injection of the D-1 blocker (1 mg/kg) with the D-2 blocker, molindone (10 mg/kg).

The role of these receptors in catalepsy may not be as straightforward as these data suggest. Paradoxical effects have been reported (Psychopharm., 1985, 87:12-15); injection of haloperidol-treated mice with the mixed agonist, apomorphine (4 or 8 mg/kg) caused a marked potentiation over that caused by haloperidol alone. This effect was tested in the present study with the selective D-1 and D-2 antagonists. Apomorphine (4 mg/kg) potentiated the catalepsy caused by the D-1 blocker, but had little effect with the D-2 blocker. The selective D-1 agonist actually seemed to interfere with the catalepsy induced by D-2 blocker.

We conclude that catalepsy is not a simple D-2 blockade phenomenon and that selective blockade of either receptor type can cause catalepsy. Catalepsy is most profound when both receptor types are blocked. Catalepsy is augmented when the mixed agonist, apomorphine, is given to a D-1 blocked animal; but the selective D-1 agonist, 38393, does not augment catalepsy in the D-2 blocked preparation.

310.3 ABNORMAL GAIT SEQUENCE IN THE LOCOMOTION RELEASED BY ATROPINE IN CA-DEFICIENT AKINETIC RATS. S. M. Pellis*, V. C. Pellis* and P. Teitelbaum. Department of Psychology, University of Florida, Gainesville, FL 32611.

Excessive, abnormal locomotion can be released with a high dose (25-50 mg/Kg) of atropine sulfate in rats made otherwise akinetic by catecholamine deficiency, produced by intraventricular administration of 6-OHDA. Atropine, however, does not induce an abnormal form of locomotion in intact rats, but only in animal models of Parkinsonism. Using Eshkol-Wachman Movement Notation (EWMN) we found that this abnormal locomotion involves an abnormal gait sequence (RH, LH, LH, RF instead of the normal gait sequence RH, RF, LH, LF). In such animals, atropine progressively (a) decreases hindleg step size, (b) decreases arching of the trunk, and (c) increases foreleg step size. These independent factors combine to change the ratio of front/hind body support. If the body becomes too stretched out, and the hindleg step becomes too small, then a given hindleg step does not support sufficient weight behind it to unweight the foreleg on the same side, so the opposite foreleg must step next, producing the abnormal gait sequence. Thus atropine affects gait sequence indirectly. It acts on at least three variables that affect how the body weight is distributed and shifted during locomotion. To maintain stability during such locomotion the gait sequence is appropriately altered.

310.4 **DOPAMINE AND HUMAN COGNITION: rCBF STUDIES IN PARKINSON'S DISEASE.** D.R. Weinberger*, K.F. Berman and T.N. Chase (SPON: A.P. Oliver). Intramural Research Program, NIMH, NINCDS, Bethesda, Maryland 20892.

Dopaminergic projections from midbrain to prefrontal cortex have been implicated in the monkey in certain cognitive functions mediated by prefrontal cortex, e.g. delayed response tasks (Brozowski et al. 1979). While the role of these projections in human cognitive functon is less clear, diminished mesoprefrontocortical dopaminergic activity is found in Parkinson's Disease, as are cognitive deficits suggestive of prefrontal cortical dysfunction, e.g., difficulty with problem solving (Scatton et al. 1982). The purpose of the present study was to investigate prefrontal physiology (i.e., rCBF) and cognitive function simultaneously in patients with Parkinson's Disease before and after dopamimetic treatment.

Ten medication free patients (mean age $\pm$ S.D.; 63.9 $\pm$ 2.8) with idiopathic Parkinson's Disease (stage I=3, II=4, III=2, IV=1) who had never received dopamimetic agents and eleven similarly aged normal volunteers underwent in counterbalanced sequence two Xenon-133 inhalation rCBF procedures: once during performance of an automated Wisconsin Card Sort Task (WCS), a test of prefrontal cortex (PFC) cognitive function that in normals selectively increases PFC rCBF (Weinberger et al. 1986), and once during a simple number matching test (NM) which served as a baseline. These procedures were repeated in the patients after three months of optimum doses of L-dopa/carbidopa.

Compared with normals, patients had a slight (~15%) but nonsignificant reduction in mean brain blood flow during both conditions; L-dopa treatment did not significantly increase rCBF. It did, however, affect the pattern of rCBF during the cognitive tests. Prior to treatment, patients tended to show generalized cortical activation during the WCS, which became more selectively prefrontal (i.e., more like normal) after treatment. Furthermore, the degree of PFC activation during the WCS (i.e., PFC rCBF during WCS divided by rCBF during NM) correlated with performance on the WCS ($r=-.73$, $p<.01$), and also with stage of illness ($r=-.80$, $p<.01$), degree of rigidity ($r=-.66$, $p=.05$), and bradykinesia ($r=-.65$, $p<.06$), but not with tremor, age, or duration of illness.

These data strongly suggest that dysfunction of prefrontal cortex underlies some of the cognitive symptomatology of Parkinson's Disease and that reduced dopamine afferentation may be the pathophysiological mechanism. This supports the notion that mesoprefrontocortical dopaminergic projections are involved in PFC physiological activation and cognitive behavior.

310.5 HYPOTHALAMIC KNIFE-CUTS AND BIOGENIC AMINES: THEIR ROLE IN PRECOCIOUS TESTES DEVELOPMENT. B. A. Davison and W. J. Kuenzel. Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742.

Two experiments were performed in which parasagittal knife-cuts isolating the hypothalamus from lateral connections were made in two-week-old broiler chicks. In both experiments, by five weeks of age operated chicks had significantly reduced body weights and elevated testes weights compared to sham-operated controls. Chicks were sampled at five weeks of age as it had previously been determined that plasma luteining hormone peaks three weeks following lateral hypothalamic deafferentation (Kuenzel, W. J. and P. J. Sharp, Br. Poultry Sci., 26:199, 1985). Brains were removed, and the hypothalami dissected into three regions: anterior hypothalamus, posterior hypothalamus, and median eminence. The tissues were then homogenized in .05 N hydrochloric acid, and assayed for biogenic amine content using reverse phase high performance liquid chromatography with electrochemical detection.

The first experiment was designed to determine if static hypothalamic levels of the biogenic amines were altered in chicks showing precocious puberty as a result of surgery. "Responders" were chosen from the operated chicks on the basis of comb appearance, and were compared to sham-operated controls. Responders had significantly ($P<.05$) reduced norepinephrine (NE) and epinephrine (E) levels in anterior hypothalamus, and significantly ($P<.05$) elevated NE and dopamine (DA) in median eminence. Serotonin was significantly ($P<.001$) increased in all hypothalamic regions of responders.

The second study was conducted to determine the surgery's effect on biogenic amine turnover. Pargyline (100 mg/kg), was injected into half of the operated and sham-operated chicks, and saline into the remainder of the chicks. The difference between the biogenic amine level in the saline-treated and in the pargyline-treated chicks was used as a measure of turnover. No significant differences ($P>.05$) in turnover were found between operated and sham-operated chicks, probably due to the high variability in this study. However, within posterior hypothalamus NE turnover was found to be positively correlated with testes weight in operated chicks ($P<.002$), and to be negatively correlated with testes weight in sham-operated chicks ($P<.02$).

310.6 LEVELS OF MONOAMINES AND METABOLITES IN THE NUCLEUS ACCUMBENS AND FRONTAL CORTEX OF SELECTIVELY BRED ALCOHOL PREFERRING AND NONPREFERRING LINES OF RATS. W.J. McBride, J.M. Murphy, L. Lumeng* and T.-K. Li*. Depts. Psych., Med. and Biochem., Inst. Psychiatric Res. and Regenstrief Institute, Indiana Univ. Sch. Med. and VA Med. Ctr., Indianapolis, IN 46223

The objectives of this study were to determine the levels of serotonin (5-HT), dopamine (DA), norepinephrine (NE) and the metabolites of 5-HT and DA in the nucleus accumbens (ACC) and frontal cortex (FCTX), which are regions reportedly involved in the brain reward system, of two lines of rats selectively bred for their preference or aversion to drinking ethanol. The alcohol preferring (P line) rats will drink, in a free choice situation with water, 10% ethanol and food freely available, greater than 5g ethanol/kg body wt/day while the nonpreferring (NP line) group will drink less than 1.5g ethanol/kg/d. Adult male rats used for this study were alcohol free for at least one month before being killed by the near-freezing technique. The two regions were dissected at -20°C in a cold box and stored at -70°C until assayed by HPLC with EC detection for 5-HT, DA, NE, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA).

The content of 5-HT was approximately 20% lower ($P<0.001$) in the ACC (7.94 ± 0.15 vs. 9.95 ± 0.38 nmol/g wet wt; N=8,10) and FCTX (5.02 ± 0.19 vs. 6.06 ± 0.10 nmol/g wet wt) of the P line relative to the NP line. There was also a slightly lower level (12%) of 5-HIAA in the FCTX of the P relative to the NP rats (2.24 ± 0.07 vs. 2.54 ± 0.09 nmol/g wet wt; $P<0.05$). The contents of DA, DOPAC and HVA were approximately 25% lower ($P<0.001$) in the ACC of the P rats than in the NP line (51 ± 2 vs. 67 ± 2 for DA, 4.7 ± 0.2 vs. 6.2 ± 0.2 for DOPAC, 1.5 ± 0.1 vs. 2.2 ± 0.1 nmol/g wet wt for HVA, N=9,10) whereas no differences were observed in the levels of these compounds in the FCTX. The content of NE in the ACC and FCTX was not different between the two lines.

The lower content of 5-HT in the ACC and FCTX of the P rats may indicate a smaller number of terminals and/or lower metabolism of the serotonergic neurons in the dorsal raphe nucleus which project to these regions. Likewise, the lower contents of DA, DOPAC and HVA in the ACC of the P group may indicate a smaller number of terminals and/or lower metabolism of the dopaminergic neurons in the ventral tegmental area which project to the ACC. These differences in 5-HT and DA could indicate an imbalance in the brain reward system of the P line of rats which might be related to its excessive alcohol drinking behavior. (Supported in part by AA 03243).

310.7 PARADOXICAL EFFECTS OF DEXTROAMPHETAMINE ON INTRASPINAL TRANSMISSION TO SYMPATHETIC PREGANGLIONIC NEURONS. Chaichan Sangdee*, Scott C. Steffensen*, and Donald N. Franz (SPON: P.R. Burgess). Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah 84132.

The paradoxical effects of d-amphetamine in Attention Deficit Disorder are well-known but are poorly understood. The present study on sympathetic preganglionic neurons (SPGNs) in the spinal cord may provide some understanding of these effects in Attention Deficit Disorder. These neurons appear to be innervated by excitatory norepinephrine (NE) pathways and by inhibitory epinephrine pathways (Neurosci. Lett. 37:167, 1983).

Sympathetic discharges, recorded from upper thoracic preganglionic white rami, were evoked at 0.1 Hz by stimulation of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized, spinal cats. Intravenous injection of 0.5 mg/kg of d-amphetamine gradually increased intraspinal transmission to more than 300% of control which was sustained for several hours. Enhancements by 1 mg/kg and 2 mg/kg were progressively more rapid, smaller, and briefer. Enhancement by 4 mg/kg was only 15%. However, this dose produced only marked and prolonged enhancement (over 200%) after yohimbine (0.5-1 mg/kg) and only marked and prolonged depression (80%) after chlorpromazine (1-3 mg/kg), which also rapidly blocked enhancement of transmission by the smaller doses. Desipramine (1 mg/kg) completely prevented enhancement by amphetamine.

These results support the hypothesis that NE pathways are excitatory to SPGNs but do not directly innervate the inhibitory alpha-2 receptors. However, activation of remote alpha-2 receptors by progressively increasing the release of excessive NE into the SPGN neuropil would account for the diminishing effects of progressively increasing doses of d-amphetamine on intraspinal transmission. Similar events occuring at the inhibitory alpha-2 receptors on locus ceruleus and other central neurons could account in part for the calming effects of large doses of d-amphetamine in patients with Attention Deficit Disorder. Recent observations that clonidine, which depresses both locus ceruleus and preganglionic neurons by activating alpha-2 receptors, is also effective in these patients adds clinical support for this mechanism of action of d-amphetamine (J. Am. Acad. Child Psychiatry 24:617, 1985).

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310.8 **BEHAVIORAL INDEPENDENCE OF AMPHETAMINE REWARD AND LOCOMOTOR STIMULATION DEMONSTRATED BY CONDITIONED PLACE PREFERENCE.** C.D. Carr, A.G. Phillips & H.C. Fibiger. Departments of Psychology and Psychiatry, University of British Columbia, Vancouver, BC, Canada, V6T 1W5

The relationship between the rewarding and locomotor stimulant properties of psychomotor stimulants such as amphetamine remains to be resolved. Both effects are mediated by the drug's stimulation of dopaminergic activity in the nucleus accumbens. Given this close link between these behavioral effects at the neural level, a question is can they be dissociated in behavioral tests. A critical question is whether behavioral tests for reward may be confounded by increased locomotor activity. Swerdlow and Koob (Psychopharmacology, 1984, 84, 163) addressed this question using the conditioned place preference (CPP) paradigm, a frequently used measure of drug reward. They reported that the preference for a test compartment paired with amphetamine treatment was not observed when drug-induced locomotor activity was eliminated by placing subjects into small restraining boxes. Consequently, they attributed the previously reported CPPs produced by amphetamine to the locomotor stimulant property of the drug rather than a conditioned association with its rewarding effects. The present study examined this hypothesis in detail. The first experiment demonstrated that drug-naive rats showed a strong preference for the compartment in which they had been restrained for 6 trials over one in which they had been permitted to move freely. It was hypothesized that this preference was due to the novelty of the compartment that had inadvertently been maintained by the restraint. That stimulus novelty alone could produce a CPP was verified by demonstrating that limited exposure to one of the pairing compartments produced a strong preference for it on the test day. The previous report that restraint can eliminate amphetamine-induced CPP was replicated in the final experiment. However when the novelty of both compartments was reduced by 3 half-hour pre-exposures prior to conditioning, restrained subjects developed a strong preference for the compartment paired with amphetamine. The present experiments suggest that the absence of amphetamine-induced CPP in restrained rats can be attributed to the maintenance of stimulus novelty in both test compartments that occurred inadvertently as a consequence of restraint. The high incentive value of the novel stimuli in both compartments overshadowed the relatively weaker incentive produced by amphetamine pairings. The study also demonstrates that amphetamine reward is not dependent on locomotor stimulation, indicating that these two effects can be dissociated at a behavioral level.

310.9 EXPLORATORY BEHAVIOR OF RATS WITH VISUAL LESIONS AND APOMORPHINE TREATMENT. G. C. Midgley* (SPON: P. Wainwright). Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario, N2L 3C5.

The effect of visual cortex (VC) or Superior Colliculus (SC) lesions and .25 mg/kg of apomorphine (AP) or vehicle treatment on behavior in an open-field was assessed for 3 consecutive 2 min blocks on each of 5 consecutive days. Orienting responses to visual stimuli placed outside the open field, rearing responses, sniffing, grooming, and 2 measures of locomotion (number of squares crossed and distance travelled) were assessed from video-taped recordings. Habituation of these behaviors was also assessed.

Locomotor activity and orienting responses were enhanced by AP treatment for intact rats and rats with small VC lesions (area 17) on the first day of testing. Rats with SC lesions or large VC lesions (areas 17, 18 and 7) were hyperactive in the open-field on both measures of locomotion. Visual orienting, rearing, sniffing, and grooming were suppressed for these animals compared to intact controls.

AP treatment reduced the hyperactivity of rats with SC lesions with large VC lesions to that of intact controls. Visual orienting, rearing, and grooming were further suppressed by APO treatment but sniffing was enhanced. These results were compared to the effect of d-amphetamine on the activity and orienting behavior of rats with SC lesions. Amphetamine further exaggerated the hyperactivity of SC rats (1) and reinstated orienting behavior (2).

These results are discussed with regard to the processing of sensory information and the direction of attention during exploration by the visual and dopaminergic systems.

(1) Pope et al., Psychopharm., 1980, 31, 445-457.
(2) Midgley & Tees, Beh. Neurosci., 1986, 100, 246-251.

310.10 REGIONAL RESPONSES TO AMPHETAMINE IN THE NEOSTRIATUM AND NUCLEUS ACCUMBENS OF AWAKE, BEHAVING RATS. T.W. Gardiner, D.A. Iverson* and G.V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Both the neostriatum and nucleus accumbens have been implicated in the behavioral response to amphetamine. Although several laboratories have used electrophysiological recording techniques to elucidate the neural mechanisms underlying this response, different methodologies have led to different results. Single-unit recordings from the neostriatum of immobilized rats, for example, indicate that amphetamine elicits either excitatory or inhibitory effects depending on the dose and the location of the recording site (Rebec & Curtis, J. Neurosci., 3:2240, 1983). In contrast, multiple-unit recordings from awake, behaving rats indicate that the neostriatal response to amphetamine is uniformly excitatory (Warenycia & McKenzie, Pharm. Biochem. Behav., 21:53, 1984). Single-unit recordings from such animals have revealed either excitatory or inhibitory responses to amphetamine, but the pattern of these responses does not resemble that of immobilized rats (Rebec & Gardiner, Soc. Neurosci. Abstr., 11:550, 1985).

In the present series of experiments, we have attempted to relate the actions of amphetamine to the functional responsiveness of individual neurons in the neostriatum to tactile stimulation, movement, and other manipulations. Our results to date indicate that neurons in lateral neostriatum (n = 10) alter their activity in close association with specific movements and that during amphetamine treatment the neuronal response is linked tightly to behavioral changes. Neurons in medial neostriatum (n = 11), on the other hand, generally do not alter their firing rates in close temporal association with movement, nor does the neuronal response to amphetamine bear a close relationship to behavior or to the pre-drug neuronal response. Data also will be presented that describes these relationships for nucleus accumbens neurons.

310.11 BEHAVIORAL CORRELATES OF CONTINUOUS ADMINSTRATION OF AMPHETAMINE E. M. Hollingsworth* and K. J. Mueller. Texas Christian University, Fort Worth, Texas 76120.

Continuous amphetamine administration in animals has been employed as a model of schizophrenia. Acute and chronic regimens of amphetamine administration have been examined and have established behavioral effects of a wide range of doses. However, dose related differences in behavioral correlates of amphetamine administration have not been thoroughly examined with the continuous regime. The present study examined dose related differences in behavior patterns during continuous administration of amphetamine. Amphetamine (AMPH) was infused subcutnaeously in male albino rats for 96 hours. Three doses of AMPH were examined (0.2mg/kg/h, 0.55mg/kg/h, 0.9mg/kg/h) as well a saline (n=12). Locomotor behavior, self-grooming, gnawing and licking, sniffing and head-bobbing were observed for each animal for 1 hour in the light cycle and 1 hour in the dark cyle over the four days of administration. The low dose AMPH group (0.2mg/kg/h) exhibited increased locomotor activity over days. The medium (0.55mg/kg/h) and high (0.9mg/kg/h) dose groups displayed increased stereotypic sniffing and self-grooming over days of administration. Alterations in circadian rhythmicity of activity were observed in the first days of administration for the medium and high dose groups. Although the behaviors exhibited were similar to previous reports using continuous administration, the patterns of the behaviors are not. This finding may have important implications in that the mode of continuous administration of AMPH may be critical in the modeling of schizophrenia.

310.12 SEPARATION VOCALIZATIONS: ONTOGENY OF ADRENERGIC EFFECTS IN RAT PUPS. P.Kehoe, J.C.Harris. Trinity College, Department of Psychology and The Johns Hopkins University, Division of Child Psychiatry. Hartford, CT and Baltimore, MD.

Ultrasonic vocalizations (UV) emitted by rat pups following separation from their dam offer a model for the study of separation distress. Morphine produces a naloxone reversible decrease in separation vocalization (SV) in rats and in adult squirrel monkeys (Kehoe & Blass, 1986; Newman and McLean, 1984). Clonidine decreases SVs in chicks (Panksepp, 1979) and adult squirrel monkeys (Harris & Newman, 1985); an effect that is reversed with yohimbine, an α_2-adrenergic antagonist. We examined the ontogeny of the adrenergic effects on separation-induced UVs in rat pups' on days 10, 15, 16, 17 and 18 using two doses of clonidine and one dose of yohimbine IP.

Day 10 pups given clonidine, 0.05 mg/kg IP, prior to maternal separation showed a sustained high level of calling (200 per 5 min) for an entire 25 min isolation test period, whereas saline-control pups emitted 200 UVs for the first 5 min then became silent. Yohimbine, 0.1 mg/kg, given after the clonidine reversed the sustained high level of calling producing a vocalization profile similar to the saline-control siblings. Clonidine in a higher dose, 0.5 mg/kg, doubled the number of sustained vocalizations during the 25 min. test period.

Fifteen day old pups given saline, or clonidine followed by yohimbine showed identical profiles to those of the 10-day-olds but had lower baseline calling rates. On days 16 and 17, pups exhibited a dose-dependent dissociation in calling rate compared to the younger pups. Clonidine in low dose, 0.05 mg/kg, produced results similar to day 10 and 15, however, the higher dose, 0.5 mg/kg, caused a remarkable decrease in isolation-induced vocalizations. Yohimbine did not reverse the clonidine-induced reduction but caused a further decrease in calling. By day 18, clonidine in either dose caused a reduction in the baseline calling.

These results indicate that rat pups' isolation-induced UVs are affected by both the adrenergic and opioid systems. Morphine produces decreased UVs in day 10 rat pups while clonidine initially increases calling at this age followed by a decrease by day 18. The noradrenergic system's involvement in separation distress then undergoes ontological changes from day 10-18 paralleling changes in activity levels, i.e., ambulation and wall climbing (Reinstein & Isaacson, 1977). It is concluded that there may be an interaction between opioid and adrenergic systems in mediating SVs in rat pups. However, since the adrenergic mechanism undergoes maturational changes, the role of the specific adrenergic receptors involved requires further investigation.

310.13 **STRAIN DEPENDENT ALTERATIONS OF INTRACRANIAL SELF-STIMULATION FOLLOWING ACUTE STRESSOR APPLICATION.** R.M. Zacharko, G. Lalonde* and H. Anisman, Psychology Dept., Carleton University, Ottawa, Ontario, K1S 5B6, Canada

Exposure to inescapable footshock produces deficits in responding for brain stimulation (ICSS) from the dopamine (DA) containing mesolimbic system (e.g., nucleus accumbens, ventral tegmental area). These stressor induced alterations in ICSS are not evident from nigrostriatal structures (e.g., substantia nigra) where uncontrollable stressors do not appreciably alter DA activity. It was thus suggested that the response alterations induced by an acute stressor may be attributable to the brain region specific effects of uncontrollable stressors on DA lability. While previous ICSS investigations in this laboratory focused on the CD-1 mouse strain, the present experiments evaluated stressor effects in three strains (i.e., BALB/c, C57 BL/6, DBA/2J) which differ in neurochemical responsivity to uncontrollable stressors. Mice were stereotaxically implanted with a stimulating electrode in the nucleus accumbens septi and trained to respond for ICSS in a head dipping task. Following baseline training animals of each strain were exposed to either a single session of inescapable footshock (360 x 2 sec, 150 uA) or no shock, and tested for ICSS immediately, 24- and 168-hr afterwards. Mice of the DBA/2 strain demonstrated a stressor induced reduction of responding for ICSS which was evident over the three test intervals examined. In contrast, the stressor did not reduce responding in either BALB/c or C57BL/6 mice. In fact, the stressor enhanced responding in BALB/c mice. Furthermore, unlike the effects of repeated treatment with desmethylimipramine (DMI) on responding for self stimulation in the CD-1 strain, repeated drug administration was without effect on performance in DBA/2 mice. It appears that the effects of stressors on performance in an ICSS task vary considerably across strains, and might be a reflection of the stressor provoked neurochemical alterations. Furthermore, it appears that the therapeutic effects of DMI on stressor-induced response deficits may be strain dependent. The mechanisms responsible for the differential effects of the drug treatment have not yet been elucidated.

310.14 NEUROCHEMICAL CONSEQUENCES OF CONDITIONED CIRCLING: INFLUENCE OF INNATE DIRECTIONAL BIASES. C. Szostak*, A. Jakubovic, A.G. Phillips and H.C. Fibiger. Div. Neurol. Sci., Dept. Psychiat., Univ. British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Bilateral increases in dopamine (DA) utilization within the striatum (STR) and nucleus accumbens (NAS) have been reported in rats trained to circle for intermittent reinforcement. It is possible that the increases were not specific to the contralateral structures because innate directional biases, which have been correlated with striatal DA asymmetries, were not taken into account. The extent to which the neurochemical effects of conditioned circling are dependent upon innate positional biases was assessed.

Directional biases of rats were determined using two screening paradigms. The order of testing was counterbalanced and test sessions were separated by one week. According to the protocol described by Glick and associates, rats were placed in a rotometer for 15 min whereafter they were injected with 1.25 mg/kg d-amphetamine and observed for 60 min. The number of rotations to the left and right were recorded. The second paradigm involved water-depriving the rats for 24 h. Rats were then placed in a cylindrical apparatus for 20 min. Each rotation emitted was recorded and reinforced with water (i.e. non-differential reinforcement). Directional biases were found to be consistent across paradigms.

In a second experiment, rats were screened for directional biases using the non-differential reinforcement paradigm. This paradigm was employed as it does not involve the use of any drugs that could alter the neurochemical results. Half of the rats were then trained to circle in their preferred direction using intermittent reinforcement. The remaining subjects served as water-deprived controls. Neurochemical consequences of conditioned circling were determined upon attainment of stable levels of responding. Homovanillic acid (HVA) was increased in the STR contralateral to the direction of circling. This effect was also evident when HVA:DA ratios were analyzed. NAS HVA:DA ratios indicated that DA turnover was enhanced bilaterally in the NAS.

The effects of conditioned circling on striatal DA activity are dependent upon inherent directional biases. In contrast, mesolimbic DA activity is enhanced bilaterally regardless of such innate preferences.

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310.15 NEUROPHYSIOLOGICAL STUDIES OF SENSORY GATING IN RATS: EFFECTS OF AMPHETAMINE, HALOPERIDOL AND DSP-4. [1,3]L.E. Adler, [2,3]G.M. Rose, [2]K. Pang and [1-3]R. Freedman. Depts. of [1]Psychiatry and [2]Pharmacology, UCHSC, and [3]Medical Research, VAMC, Denver, CO 80262.

Central mechanisms of sensory gating were assessed in Sprague-Dawley rats using an evoked potential technique similar to one that we have previously employed to show diminished sensory gating in psychotic patients. Middle latency (15-50 msec) auditory evoked potential responses were recorded from the skull of unanesthetized, freely-moving rats. The recording electrode was a stainless steel screw implanted 4 mm posterior to bregma on the midline. Signals were referenced to a pair of stainless steel wires placed in prefrontal cortex which were connected to ground. Gating mechanisms were examined using a conditioning-testing paradigm in which pairs of 74 dB clicks were delivered; the interval between the conditioning and test stimuli was 0.5 seconds. Click pairs were given 20 seconds-2 minutes apart. Stimulus presentations were controlled by a computer, which also digitized, stored, averaged and analysed the evoked waveforms.

Two middle-latency components of the auditory evoked potential were examined: N25 and N50. Of these, only N50 demonstrated significant suppression to the test click. This effect was state dependent, as the greatest conditioning-testing inhibition was seen if the animal was awake but motionless at the moment of click delivery; less suppression was seen if the rat was moving or asleep. For experiments involving drug administration, only trials recorded during still, alert behavior were collected for averaging.

Systemic administration of amphetamine (1 mg/kg, i.p.) significantly reduced the amount of suppression observed in response to the test stimulus; haloperidol (1 mg/kg), injected after the amphetamine, returned the conditioning-testing suppression ratio toward normal values. To aid in determining the underlying mechanism of these effects, the animals were then treated with two doses, given at a one week interval, of N-(2-chloroethyl-N-ethyl-2-bromobenzylamine (DSP-4; 50 mg/kg, i.p.), an agent which selectively depletes central norepinephrine. The extent and selectivity of the depletions were confirmed at the conclusion of the experiment using HPLC-EC. Pretreatment with DSP-4 significantly attenuated the reduction in conditioning-testing suppression observed following the administration of amphetamine. Thus, this work suggests a role for norepinephrine in the modulation of sensory gating.

This work was supported by USPHS grants MH-38321 and AA-03527, and the Veterans Administration Medical Research Service.

310.16 THE ROLE OF SEPTO-HIPPOCAMPAL SEROTONIN PROJECTION IN NUTRITIONAL MODULATION OF RUNNING ACTIVITY IN HAMSTERS. R.Bonna* and K.T. Borer, (SPON: J.Swann).Dept. Kinesiology, Univ. Michigan, Ann Arbor, MI 48109-2214.

Serotonin inhibits, while endogenous opiates facilitate spontaneous running activity in rodents. Running is affected by the body energy content in rodents: overweight leads to hypoactivity and weight loss to hyperactivity.

Experimental aim was to determine whether septo-hippocampal serotonin fibers controlled obesity-induced hypoactivity. To test this hypothesis, three groups of mature hamsters of similar starting weight were exposed to three nutritional conditions: ad libitum Purina powder (AL), rationed (60% of ad libitum) Purina powder (REST), and high-fat supplementation (40% Purina powder, 60% shortening) of regular diet (HFD).

Within five weeks, dietary manipulation led to about 20% separation of body weights between the individual groups. All hamsters were then given a 1-2 day access to a horizontal activity disc under each of the following circumstances: injection of the opiate-receptor blocker naloxone (20 mg/kg) or saline ip 1 h prior of the onset of darkness, infusion of saline (2 µl) or of serotonin neurotoxin, 5,7 diOHtryptamine HCl (3 µg free base/2 µl of 0.1% ascorbic acid) three days beforehand. Dietary manipulation induced hyperactivity in DEP hamsters (29657 ± 2182 RPDs) and hypoactivity in HFD hamsters (18616 ± 1759 RPDs, $p < 0.01$). Naloxone reduced total activity in AL (14826 ± 3668 RPDs) and HFD (14394 ± 2411 RPDs) but not in DEP hamsters (25809 ± 2532 RPDs). Intraseptal saline did not affect running activity, while serotonin neurotoxin made all three groups hyperactive and abolished all dietary influences over running activity. The results suggest that septohippocampal serotonin mediates nutritional hypoactivity. EOPs, however do not mediate nutritional hyperactivity.

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310.17 ATTENUATION OF HALOPERIDOL-INDUCED CATALEPSY IN RATS FOLLOWING BILATERAL INFUSION OF HEMICHOLINIUM-3. L.L. Wing, D. Wirtshafter and K.E. Asin. Psychology Dept., Univ. of Illinois at Chicago, P.O. Box 4348, Chicago, IL 60680.

Many studies support the existence of a dynamic interaction between dopamine (DA) and acetylcholine (ACh) of an inverse or opponent nature. For example, the use of anticholinergic drugs concurrently with DA-blocking neuroleptics serves to decrease the intensity of extrapyramidal side-effects such as Parkinson-like tremor and rigidity. Similar results have been reported using animal models. For example, anticholinergic drugs markedly inhibit the cataleptic effects of neuroleptic agents. In the present study we sought to investigate the brain sites at which interference with cholinergic mechanisms may antagonize haloperidol-induced catalepsy.

Sprague-Dawley rats were operated on under ether anesthesia, and stereotactic injections of the high-affinity choline uptake blocker hemicholinium-3 (HC-3) were made into the caudate nucleus, the midbrain or pontine reticular formation, substantia nigra, thalamus or frontal cortex. Bilateral infusions of various doses of HC-3 (0.2ug - 10ug) dissolved in 1 ul of distilled water, or the vehicle alone, were made through a 27 gauge stainless steel cannula.

Animals were given a subcutaneous injection of haloperidol (0.15 mg/kg) three hours post-operatively, and tested for catalepsy at thirty and sixty minutes after administration of the neuroleptic. Catalepsy scores were based on the ability of the animal to maintain each of ten positions for 15 seconds after placement. Animals receiving bilateral injections of the vehicle alone attained high catalepsy scores when tested at 30 minutes and near-maximum scores at 60 minutes, irrespective of injection site.

Injections of HC-3 in the midbrain reticular formation were most effective in antagonizing haloperidol-induced catalepsy, producing a significant decrease of catalepsy at a dose of 0.2ug. No significant effects were produced by HC-3 at this dose at any of the other injection sites including the thalamic, nigral or caudal reticular formation locations. For example, rats receiving bilateral injections of HC-3 at caudate coordinates required a 10ug dose before catalepsy was significantly attenuated, while frontal cortex sites did not show any reduction in catalepsy in response to a 5ug dose of HC-3. These results suggest that the midbrain reticular formation, rather than the striatum, may be a major site at which interference with cholinergic mechanisms is able to antagonize neuroleptic induced catalepsy.

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310.18 INHIBITION OF SPONTANEOUS AND RITALIN-INDUCED ACTIVITY BY INJECTION OF KAINIC ACID INTO THE MEDIAN RAPHE NUCLEUS. C. McWilliams*, D. Wirtshafter and K.E. Asin (Sponsor: D. Brief). Dept.of Psychol., Univ. of Illinois at Chicago, Chicago, IL 60680 and *Dept. of Pharmacol., Loyola Univ. of Chicago.

Various manipulations of the median raphe nucleus (MRN) are able to profoundly influence locomotor activity. For example, electrolytic lesions of the MRN, and microinjections of the inhibitory compound muscimol, a GABA-A agonist, into the MRN produce locomotor hyperactivity which can exceed that produced by peripherally administered amphetamine. In contrast, it has been reported that electrical stimulation of the MRN can inhibit ongoing motor behavior. We now report that microinjection of excitatory amino acid agonists also inhibit locomotion.

In the first experiment, rats which had been previously habituated to the procedure were injected with kainic acid (KA) (0, 1.25, 5 or 20ng) and were then placed in the center square of a 3 X 5 ft. open field at 4 post-injection intervals (15, 30, 45 and 60 min.), and covered with a bucket for 15 seconds. The latency to leave the center square upon removal of the bucket, the number of squares entered, and the number of rears were counted for the following two minutes. Doses of 5 and 20 ng produced highly significant increases in the latency score, and highly significant decreases in the numbers of squares and rears, with 20 ng being of both greater effect and longer duration of action.

In the second experiment, rats which had been habituated to the procedure were placed in a photocell activity cage where locomotor activity was measured for one hour in 4 minute bins. They were then injected systemically with the indirect dopamine agonist methylphenidate (RIT) (6mg/kg, i.p.) and intracranially with KA (0, 5, or 20ng/0.5ul saline) into the MRN, and returned to the apparatus for one hour. KA (5 and 20 ng) was found to markedly attenuate RIT-induced hyperactivity in a dose-dependent manner. The effect was of immediate onset and lasted for about one hour.

We conclude that inhibitory and excitatory compounds can produce opposite behavioral effects in the MRN. Further work is being done with other excitatory amino acid agonists.

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310.19 THE EFFECTS OF SOCIAL STRESS (DEFEAT) ON HYPOTHALAMIC AND OLFACTORY BULB MONOAMINES IN C57BL/6 AND DBA/2 MICE. K. Ornstein (1), H. Milon (1)*, H.R. Frischknecht (2)* and B. Siegfried (2)*. (1) Nestlé Res. Dept., Nestec Ltd., 1800 Vevey, Switzerland and (2) Institute of Pharmacology, University Zürich, 8006 Zürich, Switzerland.

The effects of social stress (defeat of 50 bites) on central monoamines in the olfactory bulb (OB) and hypothalamus (HYP) of two strains of mice were investigated using HPLC. In C57 mice, defeat significantly enhanced ($p<.02$) dopamine content in the OB ($\bar{x}\pm SD$: $0.40\pm.05$ ng/mg tissue, n=11) when compared to control mice exposed to a nonaggressive confrontation ($0.33\pm.03$ n=9). In the HYP a similar but less pronounced increase of dopamine was found ($0.51\pm.08$, n=11 vs $0.43\pm.1$, n=10, $p<.1$). The dopamine metabolites DOPAC (Dihydroxy phenylacetic Acid) and HVA (Homovanillic Acid) were also significantly increased in the OB (DOPAC: $0.20\pm.05$, n=11 vs $0.12\pm.04$, n=9; $p<.001$; HVA: $0.28\pm.04$, n=11 vs $0.21\pm.05$, n=9; $p<.01$). In DBA mice, defeat enhanced the serotonin levels in the OB ($0.49\pm.07$, n=10 vs $0.42\pm.06$, n=10; $p<.02$) and in the HYP ($1.3\pm.2$, n=9 vs $1.0\pm.1$, n=10; $p<.02$). Dopamine level was increased in the HYP only ($0.65\pm.11$, n=9 vs $0.46\pm.16$, n=10; $p<.02$). It is concluded that defeat differentially affected the dopamine system of both strains, only enhancing dopamine turnover in the OB of C57 mice. On the other hand defeat only influenced the serotonin system in the DBA mice.

310.20 PLASTICITY OF STEREOTYPIC BEHAVIOR AFTER AN ACUTE INJECTION OF AMPHETAMINE: EVIDENCE FOR A TEMPORAL GRADIENT. C. H. M. Beck[1], H. L. Chow[2]* and S. J. Cooper[3].
[1]Department of Psychology, [2]Department of Educational Psychology, University of Alberta, Edmonton, Alberta, Canada T6G 2E1, [3]Department of Psychology, University of Birmingham, Birmingham, United Kingdom B15 2TT.

Saline-treated and amphetamine-treated (7 mg/kg, ip, immediate) male rats from a Sprague Dawley substrain, were observed in two test environments designed to elicit different investigative responses in normal rats. Snout contact with the substrate was generated by placing the rat in a small enclosed cage. Nonsnout contact was induced by placement of the rat on a square elevated platform. Detailed ethological records were kept of locomotion, rearing, sitting, grooming, gnawing and sleeping throughout the 90 min session.

Amphetamine-treated rats incorporated environmentally-contingent body postures into their forms of stereotyped behavior. The postures were characteristic of those envinced initially by the saline-treated rats in the same test environment. The control rats showed appropriate changes in their investigative behavior when the apparatus was changed at 10 min and at 30 min postinjection. The amphetamine-treated rats, however, were completely unresponsive to such changes at 30 min and only partially so at 10 min postinjection.

It was concluded that there is a temporal gradient of decreasing readiness to acquire new forms of adaptive behavior which occurs after a single, large dose of amphetamine.

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311.1 CLASSIFYING DENDRITIC BRANCH PATTERNS USING TERMINAL ORDERING OF SEGMENTS. D. E. Hillman. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016

The arrangement of dendritic segments forming arbors can be expressed globally as branch patterns irrespective of size and orientation. Since much of neuronal shape is revealed in the pattern of branching, a means of classifying arbors in numerical terms is essential for making comparisons and specifying types of branch patterns.

A method of incremental ordering of segments from terminals to the soma, provides values representing the number of segments lying distal to each segment. This method is called, "Terminal Ordering". The approach has been found useful in generating a numerical classification system of branch patterns that ranks arbors according to the degree of asymmetry.

In the procedure, the path that has the largest number of bifurcations from the soma to the terminal is ordered according to the terminal ordering rule. The terminal order values of each segment along this path are summed. A Symmetry Index (SI) value is generated by adding 1 to this sum and dividing by the total number of terminals on the arbor. A table of SI values designating 8 classes of branch patterns from Ia-IVb is used to classify the arbor. The rule for defining the 4 major ranges of SI values is to divide the range between 2 and the SI value for an arbor having maximum asymmetry into 4 ranges (8 for subclasses). The maximum asymmetry is approximately one-half of the total number of terminals on the arbor. The SI value of completely symmetrical arbors is always 2. This numerical approach allows computerized classification of neuronal arbors.

The results suggest a general rule for determining the SI ranges for the 4 major classes of branch patterns. The SI value range for Class I is between 2 and approximately one-eighth of the total number of terminals with the addition of 1 while the Class II range is from the latter to approximately one-fourth of the total number of terminals with the addition of 1. The range for Class III is from the Class II to three-eighths of the total terminal number with the addition of 1. The Class IV range extends up to about one-half of the number of terminals. Supported by USPHS Grant NS-13742 from NINCDS.

311.2 OPTIMUM COMPARTMENTAL NEURON MODELS. K Flach*[1], NT Carnevale* and SE Sussman-Fort*[1], Dept. of Electrical Engineering[1], and Depts. of Neurology and Neurobiology and Behavior*, State Univ. of New York, Stony Brook, N.Y. 11794.

Increasingly complex hypotheses of neuronal function, stimulated by a wealth of anatomical and biophysical data, in turn require more efficient methods for neuronal modeling. Therefore we have examined the problem of constructing multicompartmental models for optimum accuracy in simulations of linear electrotonus with minimum computational overhead.

Ladder network simulations of models with identical compartments led to the suggestion that neurites more than half a space constant long may be represented with satisfactory accuracy by models that have only "two or three regions [compartments] per space constant." [1] We expected that greater efficiency might result from designing each compartment individually so that the charging characteristics of the model would be experimentally indistinguishable from the neuronal structure that it represents. This approach would be particularly applicable to portions of a neuron that act only as a passive load, i.e. that are not the site of any synaptic input or active current.

We have tested this idea by systematically comparing theoretical predictions for equivalent cylinders of typical electrotonic lengths with the charging characteristics of optimized models that consist of one, two, or three compartments. Design constraints for these models include DC input conductance, characteristic frequencies of the impulse response, and relative error of the impulse response when loaded by a simulated soma or voltage clamp. We have addressed the following questions:

1. How well can models of different complexity approximate the theoretical predictions?
2. What is the optimum model design for a required degree of accuracy?
3. How should the values of the elements in the model (i.e. the series resistances and the parallel resistances and capacitances in each compartment) be chosen?
4. How sensitive are the simulations to variations in the parameters used to construct the models?

This study has resulted in a set of design rules that we can apply to improve the accuracy and speed of large-scale neuronal simulations.

Reference
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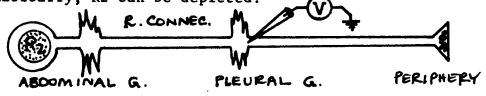
311.3 GIANT APLYSIA NEURON R2 HAS DISTAL DENDRITES: EVIDENCE FOR A SECOND SPIKE INITATION SITE AND PROTEIN SORTING. R. T. Ambron and S. G. Rayport. Departments of Anatomy & Cell Biology and Psychiatry, Center for Neurobiology & Behavior, Columbia P&S, New York City 10032.

The polarization of neurons into transmissive and receptive regions is reflected in the characteristic features of pre- and post-synaptic membranes. Such differences must arise from distinctive patterns of macromolecular sorting. Since neuronal somata are the site of macromolecular synthesis, mechanisms are required to sort and distribute components to their appropriate sites within the cell. We have been studying sorting using neuron R2 and have found postsynaptic structures resembling dendrites in the pleural ganglion some 4 cm distal to the cell body and 4 cm proximal to the R2 terminals in the body wall. Schematically, R2 can be depicted:



Stimulation of peripheral nerves elicits EPSPs in the R2 axon in the pleural ganglion. This input may modulate action potentials arising in the abdominal as well as evoke spikes in the pleural ganglion. A second spike initiation site in the pleural ganglion may explain the bi-directional spike conduction in the R2 axon in the right connective [Parsons et al., (1983) Science 221, 1203].

HRP injected into the cell body labels a series of evaginations from the main axon. These processes arise from within the axon, apparently suspended by glial infoldings. This orientation may selectively direct vesicles into the processes. In serial sections, numerous presynaptic elements are seen to contact the R2 processes. Under the EM the dendrites are seen to lack ribosomes, but contain the vesicle types commonly found in the right connective axon.

Given this cytoarchitecture, both pre- and post-synaptic components synthesized by the cell soma will be exported into the right connective axon for transport to the periphery. In the pleural ganglion, far from the cell body, these proteins must be sorted. Using a pulse-chase protocol with ^{35}S-Methionine, we have identified by SDS-PAGE 30 radiolabeled, rapidly transported proteins. Among these is a 70 kD protein band, that is transported to the pleural ganglion, but does not appear in the peripheral nerves terminating in the body wall. Possibly, this protein is deposited in the dendrites. Finding distal R2 dendrites adds a spatial dimension which should facilitate the elucidation of mechanisms involved in intracellular sorting.

311.4 ELECTRIC PROPERTIES OF ISOLATED SPINOTHALAMIC TRACT CELLS. L.-Y.M. Huang. Marine Biomedical Institute and Department of Anatomy & Neurosciciences, University of Texas Medical Branch, Galveston, TX 77550

The spinothalamic tract (STT) is thought to be one of the main pathways that transmit nociceptive information from the periphery to the brain. The conductance mechanisms of these cells and transmitter modulation of the ionic currents in these cells are central to an understanding of how pain is mediated by the nervous system. We have developed a method for in vitro recordings of spinothalamic cells.

Diamidino yellow dihydrochloride or fluorescent latex microspheres was injected into both the lateral and medial part of thalamus of rats. These cellular markers were transported retrogradely in the axons of the spinothalamic tract cells and subsequently were visible in the somata of STT neurons (Huang et al., J. Neuroscience Method, 14: 91-96, 1985). After 2-4 day survival time, the cervical or lumbar region of the spinal cord was dissected out. The tissue was then incubated in buffer solution containing 0.1-0.3 mg/ml papain for 45-60 min at 36°C. The solution was continuously stirred and bubbled with O_2 during this period. The tissue was then removed from the enzyme solution, washed with the fresh buffer solution and then placed in Dulbecco's modified eagle medium for storage. Immediate before use, a small piece of tissue was taken from the medium and triturated with a pasteur pipett. The dissociated cells were allowed to settle down in the recording dish. STT neurons displayed a diffuse fluorescence of the cytoplasm in their somata and proximal dendrites.

Electrophysiologic experiments were performed only on the fluorescent cells. The whole cell currents of these cells were measured under voltage clamp conditions using the patch clamp method. The neurons had a resting potential of -50 - -70 mV. When the clamped potential was stepped from a holding potential of -100 mV to -50 mV, a rapid inward current started to activate and then inactivated. When the test potential was more positive than -20 mV, the inward current was followed by a maintained outward current. Tetrodotoxin could block most the inward current, and tratraethylammonium effectively reduced the outward current. The voltage dependent properties of these currents will be discussed. (Supported by NIH grants NS 23061 and NS 19352.)

311.5 MORPHOLOGICAL INVESTIGATIONS ON THE INNERVATION OF CILIA IN LYMNAEA. W. Winlow, J.D. McKenzie, N.I. Syed and J. Tripp. Department of Physiology, University of Leeds, Leeds LS2 9NQ. England. U.K.

Ciliary beating is the primary mode of locomotion in pond snails such as Lymnaea stagnalis (L.). The paired pedal ganglia innervate the foot and are thought to be involved in the control of locomotion (1). The "A-cluster" neurones of the pedal ganglia contain serotonin (2) and preliminary results suggest that they are involved in ciliary locomotion. Most of the neurons within these clusters are weakly electrically connected to their near neighbours and have ipsilateral projections to the foot. Three pairs of giant neurones are electrically connected to their contralateral homologues and to the A-cluster cells that surround them. The giant neurones have both ipsilateral and contralateral projections to the foot.

Using the glyoxylic acid technique the presence of serotonin within the foot has been demonstrated using fluorescent microscopy. Immediately under the ciliated epithelium is a layer of serotonin-containing varicosities. Within the deeper tissues, tracts containing serotonin can be found. These originate from the ganglia. Ultrastructural investigation of the foot reveals numerous axon profiles at the base of the epithelium. Many of these contain dense cored vesicles. Apparent synapses, containing these vesicles, are made with ciliated cells, other epithelial cells and the sub-epidermal musculature. It is hypothesised that the A-cluster neurons innervate the ciliated cells and modulate locomotion via serotonin-containing "neuro-ciliated cell junctions". (Supported by an SERC grant to WW).

(1) Winlow, W. & Haydon, P.G. (1986). Comp. Biochem. Physiol. 83A, 13-21

(2) Casey, C. & Winlow, W. (1985). J. Physiol. 369, 169P.

311.6 ELEMENTAL COMPOSITION OF NEURONS: AN ELECTRON PROBE X-RAY MICROANALYSIS STUDY. D.A. Spero* and F.J. Roisen (SPON: R. Duvoisin). Department of Anatomy, School of Medicine, University of Louisville, Louisville, KY 40292.

Information on the distribution of elements in neurons is of considerable importance to understanding neuronal function. In the present study, we compared the intracellular distributions of elements in neurons prepared by the following methods: 1) rapid-freezing, freeze-drying at -80°C and 10^{-4} torr, 2) rapid-freezing, freeze-substitution at -80°C and 3) conventional fixation at 20° C. Neuro-2a neuroblastoma and 9-day embryonic chick dorsal root ganglia (DRG) were grown as described previously (Spero and Roisen, Dev. Brain Res., 13:37-48, 1984; Roisen et al., J. Neurobiol., 4:347-368, 1972). Cells were plated on formvar-carbon-coated gold grids treated with poly-L-lysine and maintained for 48 hr. For freeze-drying, cells were quick frozen in propane (-180°C) and dried at -80°C and 10^{-4} torr. For freeze-substitution, cells were frozen in propane, freeze-substituted in a mixture containing 1% glutaraldehyde and 0.02 M cacodylate buffer (pH 7.3) in absolute ethanol at -80°C and critical-point-dried. For conventional fixation, cells were fixed in 2% glutaraldehyde in cacodylate buffer (pH 7.3), dehydrated and critical-point-dried. Cells were examined with a Philips EM420T equipped with an EDAX 9100 x-ray microanalysis system. Multiple x-ray probes of 500 nm diameter were made in representative regions which included the soma, neurite shaft and growth cone to determine the concentration of elements in these regions. Background counts from extracellular regions were stored in the microprocessor and substracted from those obtained from intracellular regions. Freeze-dried cells had consistently higher levels of the diffusible elements K, Ca, Mg, and Na than cells prepared by either freeze-substitution or conventional methods, suggesting that freeze-drying provides a more accurate retention of elements than freeze-substitution or conventional procedures. Studies utilizing the rapid-freeze, freeze-drying procedure demonstrated higher Ca levels in the cytoplasm of nonneuronal cells and higher Mg levels in DRG and Neuro-2a neurons. An examination of the intracellular distribution of elements within these two neuronal types indicated high levels of P and S in the soma and high levels of Zn in neuritic processes and their growth cones. Within the neurites, high concentrations of Ca were associated with mitochondria and smooth endoplasmic reticulum. The rapid-freeze, freeze-drying method combined with x-ray microanalysis provides a useful technique for determining the intracellular distribution of elements and their associations with specific compartments. Supported by NIH grant NS11299.

311.7 EXPRESSION OF KERATIN IN THE CENTRAL NERVOUS SYSTEM. M.C. Franko*, C.J. Gibbs Jr.* and D.C. Gajdusek (SPON: I. Klatzo). Laboratory of Central Nervous System Studies, NINCDS, National Institutes of Health, Bethesda, MD 20892.

A monoclonal antibody derived from a mouse immunized with a homogenate of mouse brain infected with Creutzfeldt-Jakob disease virus recognized an epitope present in non-neural cytokeratins. By indirect immunofluorescence on frozen sections of adult hamster brain, the antibody reacted with intracytoplasmic filaments in a sub-class of astrocytes, loop structures within the cytoplasm of Purkinje cells and with ependymal cells. The immunofluorescence pattern that was seen in cultured astrocytes of mouse brains with this antibody consisted of an accumulation of antigen around the nucleus and arrays of cytoplasmic fibers extending to the cell borders characteristic of intermediate filaments. Using double-label indirect immunofluorescence, the staining of cultured astrocytes with anti-glial fibrillary acidic protein antibody revealed the coordinate expression of both antigens.

Immunoblot analysis of purified human epidermis keratin polypeptides and fetal mouse brain culture homogenate confirmed the reactivity of this antibody to keratin polypeptides. The antibody recognizes an epitope which is present in all of the epidermis keratin polypeptides and which is shared with a 58-60 kDa polypeptide found in cultured astrocytes.

This study shows that a sub-class of astrocytes in hamster brain contains a distinct intermediate filament of the keratin type and suggests that the loops within Purkinje cells represent a keratin intermediate filament organizing center. Additionally, this study shows that epidermal keratins share an epitope among themselves and with a "neural keratin".

311.8 VIP NEURONS IN THE THORACIC AND SACRAL CORD OF CAT AND MONKEY. C.C. LaMotte. Section of Neurological Surgery and Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

VIP immunoreactive neurons have been identified in horizontal sections of monkey and cat spinal cord (without colchicine pretreatment), using the PAP method and a fixative of 5% acrolein in phosphate buffer. In the thoracic cord cells were located in several areas; most were found from C8-T6 and the lower thoracic cord had few VIP neurons. One group of multipolar VIP neurons was in lamina X, lateral to the central canal; a few isolated VIP cells were also found in the intermediolateral nucleus and in the intercalatus nucleus. Another prominent group was located immediately dorsal and lateral to the central canal, often in close contact with ependymal cells. Each neuron had a single large apical dendrite which was oriented radially to the canal, appearing to span the ependymal layer and to contact the canal surface; smaller basal dendrites and a varicose axon were also present.

In the sacral cord two cell types were observed. One type was either subependymal or in the ependyma and varied in shape and size. These cells had long dendrites which stretched longitudinally along the canal surface. Branches of these long dendrites commonly penetrated between ependymal cells and ended as rounded terminals in the canal surface. Long thin varicose axons also arose from these cells. A second type of VIP neuron consisted of small cells with short, highly branched and varicose processes; these were located among ependymal cells. The processes from these 2 cell types were striking, particularly in the cat; they formed a massive intricate network extending dorsally, laterally and ventrally along the central canal. Electron microscopy of the central canal region confirmed that many VIP processes extended along the canal surface and these gave rise to large varicosities filled with round clear vesicles and dense core vesicles.

The results indicate both an expanded role of VIP in the autonomic nervous system by its presence in spinal neurons of the IML, IC and lamina X, and an additional function by its presence in CSF contacting neurons. (Supported by NS13335)

311.9 MYELIN-ASSOCIATED GLYCOPROTEIN AND Po GLYCOPROTEIN IMMUNOREACTIVE MATERIAL IN SMALL SENSORY NEURONS OF CHICKEN DRG.
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Myelin-associated glycoprotein (MAG) is an integral membrane protein, which is synthesized by oligodendrocytes and Schwann cells. The Po glycoprotein is a structural protein, which is specifically synthesized by myelinating Schwann cells and located in peripheral myelin; Po is absent from oligodendrocytes and CNS myelin. These two glycoproteins were thought to be specific for myelin forming glia and myelin of mammals.

We have reported that small B-sensory neurons of chicken dorsal root ganglia (DRG) express MAG or a closely related molecule (Omlin et al., Science 227, 1359, 1985). Using the PAP immunocytochemical method of Sternberger, specific reaction products were found in the Golgi apparatus and the rough endoplasmic reticulum of these neurons. A patchy distribution of immunoprecipitates was observed at the cell surface of the perikaryon. In contrast, large neurons of type A remained free of any reaction products.

To test whether other myelin-glycoproteins are also expressed in small B-neurons of chicken DRGs, adjacent sections were immunostained with polyclonal antibodies against Po and MAG, respectively. To our surprise the same MAG positive neurons were also immunostained for Po. Furthermore, the distribution of immunoprecipitates associated to these neurons was identical for both MAG and Po glycoprotein. All large neurons of type A were negative for the Po glyocoprotein as well as for MAG.

It is suggested, that these glycoproteins or closely related molecules, which were considered as specific constituents of myelinating cells in mammals, could be expressed by certain neurons derivating from the neural crest and play a role in cell adhesion and/or recognition. (S.N.S.F. 3.447-0.83/The anti-sera of anti-MAG and anti-Po are a generous gift from Drs R.H. Quarles and B.D. Trapp).

311.10 **Immunoelectron microscopic localization of a developmentally-regulated neuron-specific protein S54 in dendrites and cloning of its cDNA.**
T. Shirao*[1], H. K. Inoue*[2], Y. Kano*[3], N. Kojima*[1], Y. Kato*[4] and K. Obata[1]. Departments of [1]Pharmacology, [2]Neurosurgery and [3]Anatomy, Gunma University School of Medicine, [4]Institute of Endocrinology, Gunma University, Gunma 371, Japan

Recently we demonstrated three novel proteins S5, S6 and S54 (molecular weights 95,000, 100,000 and 110,000, respectively) in the chicken brain (Shirao and Obata, J. Neurochem. 44:1210-1216, 1985). They are developmentally regulated and immunologically cross-reacted with polyclonal and monoclonal antibodies. S54 protein is neuron-specific and concentrated in certain types of neuropil (Shirao and Obata, Dev. Brain Res. in press). In the cerebellar cortex immunostaining of S54 protein was observed only in the cerebellar glomeruli. It is proposed that S54 protein has a specific function in certain types of synapses; however, it had not been decided whether S54 protein is in presynaptic and/or postsynaptic sites.

The present experiments were undertaken to elucidate electron microscopic localization of S54 protein. Electron microscopy showed that in the cerebellar glomeruli immunostaining was observed diffusely in postsynaptic elements facing large mossy fiber terminals. In the optic tectum, S54 protein immunoreactivity was also observed in the postsynaptic components of axo-dendritic synapses. In addition, weak immunostaining was observed in the shaft of dendrites. These results suggest that S54 protein is synthesized in certain types of neuronal somata, transported along their dendrites, and concentrated in the postsynaptic sites of dendrites.

Then, we performed cDNA cloning using poly(A) enriched RNA from embryonic chick brain in order to elucidate the molecular relationship of S5, S6 and S54 proteins and the regulatory mechanism of their expression. A modification of the RNase H method of Okayama and Berg (1982) was utilized for generation of the second strand, followed by methylation, addition of Eco R1 linkers, and ligation into the Eco R1 site of the expression vector λgt11. Cloning efficiency was about 10^8 recombinants per microgram of poly(A)$^+$ RNA. Fusion proteins were screened immunologically using specific antibodies. Screening of 2 x 10^5 recombinants resulted in the isolation of positive clones.

311.11 PROTEIN PHOSPHORYLATION IN HIPPOCAMPAL TISSUE SLICES.
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Phosphorylation of synaptic junction (SJ) proteins by Ca^{2+}/calmodulin-dependent kinase II(CaM-KII) may mediate events which underlie synaptic transmission. CaM-KII contains a major 50K and a minor 60K subunit; each binds calmodulin and is autophosphorylated. The present studies examined if proteins phosphorylated in situ in hippocampal slices were similar to SJ proteins phosphorylated in vitro by endogenous CaM-KII. Conditions which influenced the in situ phosphorylation of CaM-KII and other endogenous substrates were examined. Adult rat hippocampal slices were pre-labeled with $^{32}P_i$ (1 mCi/ml) in Krebs Ringer buffer and depolarized using high K^+(50 mM). Slices were homogenized under conditions that inhibited virtually all detectable endogenous phosphatase and protease activities. Cytosolic and plasma membrane fractions were obtained; the latter were extracted with Triton X-100 to obtain SJ-enriched fractions. Proteins in each fraction were analyzed by (a) 2-D IEF/SDS-PAGE, (b) proteolytic phosphopeptide mapping, (c) immunoblotting, and (d) autoradiography. Our data indicated that the presence of Ca^{2+}(1mM) in pre-labeling and labeling media resulted in maximal in situ protein phosphorylation. This Ca^{2+} effect was diminished by 1mM EGTA, and abolished by Ca^{2+}-channel blockers. High K^+ depolarization resulted in small but variable increases in ^{32}P incorporation into slice proteins. 2-D autoradiographs showed that the 60K subunit of CaM-KII was more highly phosphorylated than the 50K. Enhanced ^{32}P incorporation into the 60K subunit was found mainly in Triton-soluble (TX_s) extracts of synaptic membrane fractions. Increased apparent 60K autophosphorylation was also observed in cytosolic (S1) fractions but to lesser degrees. Although SJ-enriched fractions contained 10-fold greater amounts of CaM-KII compared to S1 and TX_s fractions, phosphorylation of 50K and 60K subunits in the SJ-enriched fractions was considerably less and their 2-D autoradiographic spots were of similar intensity. Identification of 50K and 60K subunits in hippocampal fractions was verified by (a) co-migration in 2-D gels with purified CaM-KII, (b) immunoblotting using affinity-purified antibodies against CaM-KII, and (c) purification of kinase subunits by calmodulin-Sepharose chromatography. Peptide mapping of CaM-KII subunits phosphorylated in situ produced similar patterns when compared to 50K and 60K subunits autophosphorylated in vitro. Synapsin I, tubulin, and proteins of 138K, 87K, and 48K were also phosphorylated in hippocampal slices. These results indicate that the 60K subunit of CaM-KII is more highly autophosphorylated in situ relative to the 50K subunit and that the 60K subunit is enriched in TX_s extracts of synaptic membranes. A majority of hippocampal CaM-KII is recovered in SJ-enriched fractions, however, its apparent autophosphorylation is largely refractory to in situ labelling conditions. (Supported by NIH Grant #NS22452 & #NS01052.)

311.12 NEURONAL NADPH-DIAPHORASE: BIOCHEMICAL AND HISTOCHEMICAL CHARACTERIZATION.
B.T. Hope*, B.E. Boyes* and S.R. Vincent (SPON: D. Schwarz). Div. of Neurological Sciences, Dept. of Psychiatry, The Univ. of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Histochemistry based on the enzymatic reduction of a tetrazolium dye in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) selectively stains discrete neuronal populations. The endogenous enzyme responsible for this reaction has been termed NADPH-diaphorase. Striatal neurons containing the neuropeptides somatostatin and neuropeptide Y, and the caudal cholinergic column of the mesencephalic and pontine tegmentum are two of the cell groups known to contain this enzyme. We have begun to characterize and purify this enzyme from rat and human brains, and to compare the biochemical properties of this enzyme with those observed in histochemical studies.

NADPH-diaphorase activity was quantitated using a method similar to that employed for histochemistry. The incubation medium contained 1 mM EDTA, 20 μM DTT, 1% Triton X-100, 1 mM NADPH and 0.5 mM nitro blue tetrazolium, in 50 mM Tris-Cl (pH = 8.4 at 37°C). The production of formazan was assayed using the absorbance at the isobestic wavelength (585 nm). The enzyme activity was completely inhibited in the presence of 2 mM CDTA or after trypsin pretreatment.

Subcellular fractionation of the rat striatum indicated that greater than 75% of the total NADPH-diaphorase activity is cytosolic. Activity was high in the pituitary and adrenal glands and varied greatly between brain regions. Lesions of the striatum with kainic acid reduced NADPH-diaphorase when detected biochemically, confirming the neuronal localization of this enzyme observed histochemically.

Partial purification of the NADPH-diaphorase from human neocortex was accomplished using ammonium sulfate precipitation, ion exchange chromatography and size exclusion chromatography. In all of these chromatographic modes only a single peak of activity was observed. HPLC size exclusion chromatography yielded an apparent Stokes radius of 22 Å, corresponding to a globular protein with an apparent molecular weight of 15-20 kilodaltons. Affinity chromatography suggested the presence of a 'dinucleotide fold' domain.

The present results indicate that NADPH-diaphorase is a small oxido-reductase present in the cytoplasm of particular neurons. Further examination of the structure of this enzyme may yield clues to its function in these cells.
(Supported by grants from the Medical Research Council and the British Columbia Health Care Research Foundation)

311.13 INOSITOL TRIPHOSPHATE INDUCED CALCIUM RELEASE FROM BRAIN MICROSOMES. J. Shah* and H.C. Pant (SPON ˜: F.F. Weight), Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, Maryland 20852.

Calcium ion (Ca^{++}) accumulation and release from intracellular organelles may play an important role in regulating many cell functions. Recent studies show that several cell types which mobilize intracellular Ca^{++} in response to hormones or neurotransmitters also hydrolize phosphatidylinositol 4,5 biphosphate to produce diacylglycerol and a water soluble molecule inositol triphosphate ($InsP_3$) which may mediate mobilization of intracellular Ca^{++}. In the present study, we investigated in rat brain microsomes the possible involvement of $InsP_3$ in Ca^{2+} mobilization. The microsomes were prepared by differential ultracentrifugation by the method of Edelman et al (J. Neuroscience, 5: 2609-2617, 1985) and purity was checked by electron microscopy. Calcium uptake and release were determined by measuring the radioactive calcium trapped in the microsomes.

ATP dependent accumulation of calcium was observed in the brain microsomes and $InsP_3$ caused a rapid release of calcium which was followed by a slow re-uptake. This release was found to be $InsP_3$ concentration dependent and was maximal at a concentration of 0.2 μM. The $InsP_3$ induced Ca^{++} release required the presence of potassium chloride (100 mM) in the medium, as no significant release was observed with $InsP_3$ in sucrose (250 mM) or sodium chloride (100 mM) media under the identical experimental conditions. A23187 also showed a sudden large release of the sequestered calcium. The two organelles thought to be responsible for calcium storage are endoplasmic reticulum and mitochondria. We found that $InsP_3$-induced Ca^{++} release was not observed with mitochondria isolated by the method of Edelman et al. (J. Neuroscience, 5 : 2609-2617, 1985). Moreover, the presence of mitochondrial inhibitors, oligomycin (0.7 μg/ml) and sodium azide (500 μM), during calcium uptake did not affect the ability of $InsP_3$ to release Ca^{++} from the microsomes. In addition, electron microscopy did not reveal mitochondria in the microsomal fraction. These observations suggest that the organelle responsible for the $InsP_3$-releasable Ca^{++} store is endoplasmic reticulum. We conclude that microsomes from rat brain may provide a model for investigating the regulation of Ca^{++} mobilization in the central nervous system.

311.14 IMMUNOCYTOCHEMICAL LOCALIZATION OF BRAIN-SPECTRIN IN RAT CNS. L.S.Perlmutter, C.Gall, F.Avdin*, B.Passani and G.Lynch. Depts. of Psychobiology and Anatomy, University of California, Irvine, CA 92717.

Members of the spectrin family of proteins are found in most if not all cell types. These proteins cross-link actin and other elements of the cytoskeleton to the membrane, bind calmodulin, and may regulate cell shape and the position and mobility of cell surface proteins. Spectrin is also found in a cytoplasmic pool, possibly in a transport/assembly phase, and recent biochemical work (Siman et al., submitted) has shown that its concentration there varies markedly across brain regions. Immunocytochemical studies (Zagon et al., J. Neurosci., 4: 3089, 1984) using antibodies against erythrocyte spectrin also indicate that marked regional and cellular differences in spectrin concentration exist. The experiments to be reported employed antibodies against brain spectrin to provide further information on this issue.

Young adult rat brains were either perfusion or immersion fixed with 2-4% paraformaldehyde, 0.0-0.4% glutaraldehyde. Sections were cut on a freezing microtome or vibratome and spectrin was immunocytochemically localized in brain and cervical spinal cord using rabbit anti-rat brain-spectrin (Siman et al., PNAS, 81: 3572, 1984) and the biotin-avidin-peroxidase technique (Vectastain). Spectrin-like immunoreactivity (SI) was localized within neuronal perikarya, axons and dendrites in all areas studied although regional differences were observed in the intensity and compartmentalization of immunostaining. SI was particularly dense in the dendritic processes of cortical, hippocampal, and subicular pyramidal cells. The perikarya of these neurons were less densely immunoreactive. In contrast, the large multipolar neurons of the mediodorsal and ventrobasal thalamic nuclei were densely but evenly immunoreactive through perikarya and proximal dendrites. Neuronal perikarya and dendrites were only lightly immunoreactive in some other areas including the midline thalamus, granule cell layer of the cerebellum and globus pallidus. Interneurons in several regions were unlabelled. Dense staining was observed in axonal processes including the corpus callosum, internal capsule, cerebral peduncle, deep cerebellar white matter, spinal tract of V, white matter of spinal cord, as well as pericellular baskets in the cerebellum. No SI was observed in glial cell bodies, glial processes, or myelin in brain or spinal cord.

These data indicate that spectrin concentration does vary markedly between brain areas (e.g. striatum vs. globus pallidus; Purkinje vs. granule cells in cerebellum) and that the within-cell distribution differs greatly as well. The origins of these variations and their potential significance to cell functioning will be discussed.

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311.15 MICROTUBULES AND NEUROFILAMENTS: RELATIONSHIP TO AXON CALIBER IN CENTRAL AXONS. K.B. Logvinenko* and R.A. Nixon. Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 02178

The caliber of axons in peripheral nerves has been reported to correlate with neurofilament (NF) and/or microtubule (MT) number and to vary with NF delivery to axons, suggesting that one or both of these cytoskeletal systems may specify, or reflect, the radial size of axons. To identify determinants of axonal caliber in CNS neurons, we carried out morphometric analyses of NF and MT along axons of mouse retinal ganglion cell (RGC) neurons. We previously showed that the distribution of axon caliber sizes is the same at various levels along the optic nerve and tract, indicating that, on the average, RGC axons exhibit a uniform caliber along their lengths (J. Cell Biol. 102:647, 1986). In the present study, optic pathways from perfused mice were cut in transverse section at levels of 1, 4, and 7 mm from the eye, representing proximal, middle, and distal regions of RGC axons. At each level, 200-280 axons were selected randomly from the population in which most NF and MT appeared in exact cross-section. The NF and MT in these axons were counted visually from electron micrographs (mag. 75,000 x). NF number correlated strongly with axon cross-sectional area at each level ($r = 0.92-0.94$, $p < 0.001$); however, the slopes of the lines relating these parameters were significantly larger ($p < 0.001$) at progressively distal axonal levels, indicating that NF density (number of NF per μm^2 of axoplasm) was 75% higher at the 7 mm level than at 1 mm. MT number also correlated highly with axon caliber at the three levels ($r = 0.81-0.90$, $p < 0.001$); however, the slopes of lines relating MT and axonal area decreased ($p < 0.001$) at more distal axonal levels, indicating a decrease in MT density of 75% between the 1 mm and 7 mm levels. The sum of NF and MT (MT number was multiplied by 2.5 to reflect their larger size relative to NF) exhibited the highest correlations with axonal area at each level ($r = 0.94-0.96$, $p < 0.001$). In addition, the slopes of the lines relating NF + 2.5 MT to axonal area were identical at each axonal level, indicating that this parameter remains constant along the lengths of RGC axons. We conclude that the distributions of NF and MT are non-uniform along RGC axons and are reciprocally related. The robust correlation between NF + 2.5 MT and axonal area, which is independent of axonal level, suggests that both of these major constituents of the group V phase of slow axoplasmic transport are important in determining the radial dimension of central axons. The seriate variation in the NF:MT ratio along RGC axons supports the view that the NF-MT cytoskeleton is assembled in final form within axons while unassociated or weakly associated NF and MT are being conveyed by axoplasmic transport. The decreasing density of MT at distal axonal levels also raises the possibility of regional differences in transport rate or capacity along RGC axons. Support: NIA, NINCDS, Anna and Seymour Gitenstein Foundation.

311.16 A MONOCLONAL ANTIBODY TO TAU PROTEINS LOCALIZES WITH A SUBSET OF MICROTUBULES IN CONTROL AND β,β'-IMINODIPROPIONITRILE (IDPN)-TREATED LARGE MYELINATED AXONS OF SPINAL NERVE ROOTS. S. Ch. Papasozomenos. Dept. of Path. and Lab. Med., Univ. of Texas Med. Sch., Houston, TX 77225.

I used a monoclonal antibody (Tau-1, provided by Dr. L. I. Binder) which binds to all of the tau polypeptides (Binder, L. I. et al., J. Cell Biol., 101:1371, 1985), and the peroxidase-antiperoxidase staining technique at the light and electron microscopic levels to study the distribution of tau proteins in control and IDPN-treated rats. Epon-embedded and Vibratome sections were taken from the spinal cord, ventral and dorsal roots, optic nerve, brainstem, and brain.

Tau immunoreactivity localized exclusively with axonal microtubules but it was absent from axonal terminals, dendrites, neuronal cell bodies and glial cells. In spinal ventral and dorsal roots, while in unmyelinated and small myelinated fibers (<10 μm^2 axonal cross sectional area) all microtubules were intensely stained, in large myelinated fibers only a subpopulation (∿20-30%) of microtubules were labeled. In white matter tracts all axonal microtubules were labeled, but, at the light microscopic level, a gradient of staining intensity was present, with tracts containing the smallest axons staining most intensely. In general, the staining intensity was inversely proportional to the axonal cross sectional area.

IDPN produced segregation of axonal microtubules and membranous organelles from neurofilaments in distal axons of ventral and dorsal roots and accumulation of neurofilaments in proximal axonal swellings (Papasozomenos, S. Ch. et al., J. Cell Biol., 81:866, 1981; ibid, 100:74, 1985). In segregated microtubules there were groups of stained among unstained microtubules, but in proximal axonal swellings all microtubules were intensely labeled. Since all microtubules became segregated in the central region of distal IDPN axons, these findings suggest that tau proteins do not play a role in at least some, if not in all, microtubule-neurofilament interactions deranged by IDPN. (Supported by NIH grant NS22453).

311.17 MECHANISMS FOR LONG TERM SURVIVAL OF ENUCLEATED AXONS I: INTERCELLULAR PROTEIN TRANSFER. J.W. Moehlenbruck*, J.W. Zeagler*, K.R. Seshan, R.A. Sheller and G.D. Bittner. Department of Zoology, The University of Texas at Austin, Austin, TX 78712.

Severed distal stumps of many invertebrate axons survive for weeks, months, or years. There have been scattered reports for decades of long term survival (LTS) in severed distal stumps of vertebrate axons (see Bittner. Amer. Zool. 13:379, 1973; Comp. Biochem. Physiol. 68A: 299, 1981). Perhaps the most convincing example of LTS in vertebrates is that recently reported for giant Mauthner (M-) axons in goldfish kept at 13.5°-15.0°C (Zottoli, et al, Neurosci. Abs., 1985). Such LTS must result from some combination of (1) axoplasmic protein synthesis, (2) low rate of axonal protein turnover and/or (3) intercellular transfer of trophic substances, including proteins. Vertebrates and invertebrates may have different mechanisms for LTS, as may different neurons in the same organism.

We now report that the proximal stumps of goldfish M-axons kept at 20-25°C usually degenerate at least several mm rostrally within days. This degeneration is evidenced by axonal collapse and disorientation of the myelin sheath (The myelin sheath around control, intact, M-axons appears to lack well defined nodes and to be less regular compared to sheaths around mammalian or amphibian myelinated axons). In contrast, the severed distal stumps of M-axons and other non-giant axons remain relatively intact as evidenced by the maintenance of axonal diameter and cytoplasmic organelles. The myelin sheaths around severed distal stumps appear to be more irregular than those around control axons.

We have demonstrated glial-axonal and axonal-axonal transfer of ^{3}H-labelled proteins and horseradish peroxidase (HRP) in crayfish medial (MGA) and segmental lateral (SLGA) giant axons, both of which exhibit LTS (Bittner, ibid; Sheller, et al, Neurosci. Abs., 1985). We find that HRP, a 40kd protein, transfers non-artifactually between adjacent SLGA's and that this transfer can be blocked by reduced Ca^{++} or by the addition of Ca^{++} antagonists such as Mn^{++}. We also find that HRP perfused into MGA's transfers to adaxonal glia. HRP placed extracellularly to MGA's and SLGA's is found within the axoplasm, often associated with vesicles.

These and other data are consistent with the hypothesis that SLGA's have a major trophic dependence on adjacent SLGA's whereas MGA's depend upon adjacent glia. Since severed stumps of crayfish MGA's, SLGA's, and goldfish M-axons all have LTS, we are currently examining the ability of HRP to transfer between severed M-axons and glia or adjacent neurons. If the myelin sheath surrounding M-axons has properties similar to that in mammals and amphibians, one might a priori expect that this M-axon sheath would reduce glial-axonal exchange compared to crayfish MGA's. Whatever the mechanism of LTS, in invertebrates it is associated with rapid and specific regeneration of those axons that can regenerate. It is yet to be determined whether LTS is associated with rapid and specific regeneration of M-axons in goldfish.

311.18 MECHANISMS FOR LONG TERM SURVIVAL OF ENUCLEATED AXONS II: AXONAL PROTEIN SYNTHESIS. R.A. Sheller, S.A. Halls, G.D. Bittner, and K.R. Seshan. Department of Zoology, The University of Texas at Austin, Austin, TX 78712.

One possible mechanism for long term survival (LTS) of vertebrate and invertebrate enucleated axons is local axonal protein synthesis (see above abstract). Although ribosomal material in the form of rough endoplasmic reticulum or polyribosomes has not been identified in any axonal process by electron-microscopy, there have been reports that RNA in axons can be identified biochemically. Koenig (Brain Research 174:95, 1979) has reported 4S, 5S, 15S, 18S, and 28S RNA in myelin-free goldfish Mauthner (M-) axons. Analyses of extruded axoplasm from squid giant axons show much 4S RNA, but little (Giuditta, A., et al., J. Neurochem. 34:1757, 1980) or no (Lasek, R.J., et al., Nature 244:162, 1973) 18S and 28S RNA. None of these studies have conclusively identified the RNA species nor have they established that the RNA present is actually utilized for axoplasmic protein synthesis. M-axons in goldfish exhibit LTS (see above abstract), but squid giant axons have not been tested for LTS because even non-operated animals usually survive only a short time in captivity. Medial giant axons (MGA's) of crayfish exhibit LTS, but the RNA found in these axons has not been identified as to function or molecular weight (Anderson, E., et al., Acta Phys. Scand. 78:491, 1970).

We have identified RNA in the axoplasm of the crayfish ventral nerve cord by electrophoretic techniques. We are currently investigating the chemical identity of this RNA in MGA's and goldfish M-axons. A quantitative analysis of axoplasmic RNA is being performed by RNA extraction procedures which control for rRNA and cytoskeletal protein associations. RNA is extracted and separated on agarose microgels with rRNA and tRNA standards. Centrifugation is used to remove mitochondrial RNA from the axoplasm. Isolated axoplasm is being tested for the ability to incorporate tritiated amino acids into TCA precipitable protein with and without the addition of cycloheximide, chloramphenicol, and other inhibitors of mitochondrial versus cytoplasmic protein synthesis. Axoplasmic or mitochondrial protein synthesis may be an important mechanism for LTS in crayfish MGA's and goldfish M-axons.

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312 TITLE:
PHOSPHOINOSITIDE HYDROLYSIS, PROTEIN KINASES AND SYMAPTIC TRANMISSION.
B.W. Agranoff, F.T. Crews, E. Costa, L. Kaczmarek and J.L. Barker.

Recent discoveries have indicated that the hydrolysis of phosphoinositides (PI) leads to the formation of at least two second messengers, diacylglycerol and inositol 1,4,5-triphosphate (IP3), which can activate protein kinases and release intracellular calcium stores. This symposium will deal with the chemistry, receptor coupling, the plasticity of PI coupled receptors, and the changes in membrane conductance subsequent to stimulation of phosphoinositide hydrolysis. A large variety of neurotransmitters and neuromodulators have been shown to be coupled to phosphoinositide hydrolysis. These receptors include cholinergic, adrenergic, serotonergic, glutaminergic, and peptidergic receptors. Stimulation of these receptors initiates a cascade which can activate at least two protein kinases, eg. phosphatidylserine-calcium dependent protein kinase (protein kinase C) and calcium-calmodulin dependent protein kinases. Protein kinases can alter membrane conductance. In addition, release of intracellular calcium by IP3 can directly activate calcium dependent changes in membrane conductance. The various protein kinases, their substrates and functional changes subsequent to activation of this cascade in brain will be discussed.

313 SYMPOSIUM. MECHANISMS OF NEUROPEPTIDE ACTIONS ON BEHAVIOR.
J. L. Martinez, Jr., University of California, Berkeley (Chair); A. Ettenberg, University of California, Santa Barbara; S. Levine, Stanford University; G.F. Koob, Scripps Clinic and Research Foundation; C.B. Nemeroff, Duke University Medical Center.

Neuropeptides dramatically affect many behaviors, and their actions may be either endocrine or directly in the CNS as neurotransmitters or neuromodulators. This symposium is focused on five peptides for which there is intense current interest: [leu]enkephalin, ACTH, CRF, neurotensin, and vasopressin. For each, recent rapid progress has been made in understanding their behavioral functions and neurobiological mechanisms of action.

Recent research shows that [leu]enkephalin affects learning in diverse conditioning situations. These effects are mediated outside the blood brain barrier. Further there appears to be an enzyme system that regulates the concentrations of [leu]enkephalin observed in plasma and the activity of this enzyme system is sensitive to the environmental experience of the animal.

Both ACTH and $ACTH_{4-10}$ retard extinction of a learned taste aversion. In the squirrel monkey the vocalization response elicited following separation is modulated by $ACTH_{4-10}$. The relationship between these seemingly disparate systems is discussed.

CRF is proposed to have a role in regulating endocrine and behavioral responses to stress. CRF injected into the ventricular system activates the sympathetic nervous system, promotes locomotor activation, facilitates acquisition of a two-way shuttle avoidance response, and produces an anxiogenic-like effect in a conflict test that is reversed by chlordiazepoxide.

Neurotensin may function to maintain behavioral homeostasis. Intraventricular administration of neurotensin produces actions that are similar to antipsychotic drugs. This peptide reduces locomotor activity, potentiates actions of ethanol and barbiturates, produces hypothermia, decreases food consumption, relaxes muscle, decreases conditioned avoidance responding, and blocks effects of amphetamine, methylphenidate and cocaine on activity.

Recent controversy has surrounded our understanding of the mechanism of action of vasopressin as regards memory. Evidence is presented to help clarify whether its actions are central or peripheral, or whether it acts directly on the neurobiological substrate of memory formation, or affects memory through indirect actions on hormonal systems.

314.1 NOREPINEPHRINE (NE) AND DOPAMINE (DA) INNERVATION OF MEDIAL PREOPTIC AREA (MPO) LHRH AND GABA NEURONS IN THE RAT F.Naftolin,* C.Leranth* and N.MacLusky* (SPON: L. Masukawa) Yale University, School of Medicine, Dept. of Obstetrics and Gynecology and Section of Neuroanatomy, New Haven, CT. 06510

Turnover rates for NE and DA increase during proestrus in the rat. It has been suggested that NE directly stimulates LHRH neurons, and that GABAergic control of LHRH release is exerted indirectly through presynaptic inhibition of NE axons (Lamberts et al, Exp. Brain Res. 52:356, 1983). However, our previous studies have failed to identify degenerated NE axons in direct connection with LHRH neurons following transection of the ascending NE pathways, while direct GABA innervation of LHRH neurons was observed. Based on these findings we hypothesised that NE may innervate GABA interneurons, which in turn influence the activity of the LHRH cells.

Two experiments were performed to test this hypothesis: a) Evidence for direct connections between the GABAergic, catecholaminergic and LHRH systems in the preoptic area was sought by means of EM double immunostaining, using avidin ferritin and immunoperoxidase as contrasting electron dense immunolabels to stain for either tyrosine hydroxylase (TH) and LHRH, or TH and glutamic acid decarboxylase (GAD); b) Immunostaining for GAD or TH was performed two days following surgical transection of the ventral and dorsal NE bundles, in order to characterize the postsynaptic elements of synapses formed by ascending NE fibres in the MPO. The results of these experiments demonstrate that: 1) LHRH and GAD neurons of the MPO both form synapses with TH immunoreactive boutons; 2) Degenerated NE axons immunoreactive for TH synapse on GAD immunoreactive profiles in the MPO. No contacts were, however, identified between degenerating NE axons and LHRH immunoreactive neurons.

We conclude that local dopamine interneurons innervate LHRH as well as GABAergic interneurons of the MPO. Ascending NE axons do not appear to make synaptic contact with LHRH neurons in the MPO. These findings suggest that the control of LHRH release by NE is not mediated through direct synaptic input to LHRH neurons within the preoptic area. The NE system may exert its effects indirectly, through GABAergic or other interneurons in the MPO, or through connections in the hypothalamus and median eminence. (Supported by NIH Grant HD13587)

314.2 NORADRENERGIC PROJECTIONS FROM THE LOCUS COERULEUS AND THE A7 GROUP TO THE SPINAL CORD. R. Grzanna, W.E. Lyons*, C.D. O'Bryan*, B.E. Kosofsky and M.E. Molliver. Department of Neuroscience, Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

Noradrenergic (NA) cells of the locus coeruleus (LC), subcoeruleus, A5 and A7 cell groups innervate all levels of the spinal cord. To determine whether different subgroups of NA cells project to separate regions of the spinal cord in the rat, we have traced descending projections from the LC and the A7 group using anterograde transport. The lectin PHA-L was iontophoresed in the ventral portion of the locus coeruleus and the region of the A7 group. After a 3-week survival sections of cervical, thoracic and lumbar spinal cord were processed for PHA-L immunohistochemistry. A double immunofluoresence staining method was used to verify uptake of PHA-L by NA cells of the A7 group.

Following injections of the tracer into the LC anterogradely labeled fibers were present at all levels of the spinal cord. Within a spinal cord segment the highest density of fibers was observed in the substantia gelatinosa and around the central canal. A small number of labeled fibers was seen in the ventral horn and in the region of the intermediolateral cell column thoracic levels. In contrast, transport from the A7 cell group labeled numerous axons in the ventral horn but only few fibers in the dorsal horn and the intermediolateral column.

In the substantia gelatinosa PHA-L labeled fibers formed short, thick and darkly stained short axon segments with closely spaced varicosities. Anterogradely labeled fibers in the intermediate zone and the ventral horn were of fine caliber and less darkly stained. These fibers could often be followed for several hundred microns in the plane of section. A unique morphologic feature of labeled NA fibers is the presence of numerous short axon collaterals which terminate as bulbous swellings.

The finding that NA cells of the LC and A7 group project to separate regions of the spinal cord provides the first direct evidence for topographic organization of the descending NA projections to spinal cord. NA cells projecting to the spinal cord do not constitute a homogeneous population but can be divided into subgroups based upon differences in their efferent connections: the LC projects preferentially to afferent nuclei of the dorsal horn while cells of the A7 group innervate predominantly motor nuclei of the spinal cord. (Support NIH NS 16654, NS 15199, NS 21011).

314.3 ACETYLCHOLINE STIMULATES THE PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN SITU ON DIFFERENT PEPTIDE SITES THAN ARE PHOSPHORYLATED IN VITRO BY CAM-KINASE, C-KINASE OR A-KINASE. J. Waymire, J. Johnston, K. Lickteig*. Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77025.

Tyrosine hydroxylase (THase), the rate limiting enzyme in catecholamine biosynthesis, is activated in response to altered neural activity through a mechanism believed to involve protein phosphorylation. Although CaM-kinase, C-kinase and A-kinase are able to phosphorylate THase in vitro, the protein kinase(s) responsible for the regulation of THase in situ is/are presently unknown. One strategy to investigate this issue is to compare the sites on THase phosphorylated in response to acetylcholine in situ with sites phosphorylated in vitro by known kinases. In the present studies reverse phase HPLC of limit tryptic peptides of THase phosphorylated in situ in response to acetylcholine were compared with THase phosphorylated in vitro in response to CaM-kinase, C-kinase or A-kinase. This approach shows that five distinct tryptic phosphopeptides are generated from THase phosphorylated in situ in bovine adrenal chromaffin cells prelabeled for one hour with ^{32}Pi. The distribution of ^{32}P CPM's in the five peaks separated on a 0-20% gradient of acetonitrile and 0.1% TFA over 30 min. is as follows: 1) 11 min., 1286±275; 2) 16 min., 810±59; 3) 18 min., 695±58; 4) 19 min., 810±59; and 5) 22 min., 585±173. Acetylcholine significantly(*) enhances the phosphorylation of three of these peaks such that the distribution of CPM's is: 1) 11 min., 1538±426; 2) 16 min., 1220±58*; 3) 18 min., 1064±56*; 4) 19 min., 838±160; and 5) 22 min., 1251±138*. When this pattern of stimulation is compared with in vitro phosphorylation carried out in the presence of each of the protein kinases known to phosphorylate THase, two unexpected findings become evident. First, two of the peptides phosphorylated in situ are not phosphorylated in vitro by any combination of the three kinases. Second, whereas all three of the kinases phosphorylate the peptide eluting at 19 min., this peptide is unaffected by acetylcholine stimulation in situ. These results suggest two possibilities; (a) either protein kinase(s), other than the three thus far known to phosphorylate THase in vitro, participate(s) in the acetylcholine induced phosphorylation of the enzyme in situ, or (b) that some additional covalent modification of THase may occur in response to acetylcholine which alters the retention times of the phosphorylated peptides. In either case, acetylcholine induced regulation of THase through covalent modification of the enzyme does not appear to simply involve phosphorylation of THAse by CaM-kinase, C-kinase or A-kinase.

Supported by NS11061.

314.4 PURIFICATION AND CHARACTERIZATION OF THE SUBUNITS OF DOPAMINE β-HYDROXYLASE. D. L. Wong and R. D. Ciaranello. Dept. of Psychiatry and Behavioral Sciences, Stanford Univ. Sch. Med., Stanford, CA 94305.

Three monomeric forms of soluble bovine adrenal medullary dopamine β-hydroxylase (DBH) have been identified on 10 percent sodium dodecylsulfate (SDS)-polyacrylamide gels. These monomers have been designated α, β and γ with relative molecular weights of 71, 75 and 78 kdaltons. Treatment of denatured native enzyme with various endoglycosidases, which specifically remove high and complex mannans from glycoproteins, suggested that these subunits might be glycosylation variants since only a single species of molecular weight 66 kdaltons is generated. In addition, a polyclonal antibody directed against native DBH reacts with each of the subunits as well as the deglycosylated enzyme. This antibody is specific for protein epitopes since periodate destruction of the carbohydrate in DBH does not interfere with its immunoreactivity. Thus, the various subunit forms of the enzyme appear to share common polypeptide components.

Each of the subunits has been purified and is presently being characterized. Homogeneous preparations of each subunit are obtained from purified native enzyme using preparative 10 percent SDS-polyacrylamide gels. Protein is localized on the gels by Coomassie blue staining, bands are cut and subunit is extracted in 50 mM Tris-HCl, pH 7.4 containing 0.1 percent SDS.

When each subunit is endoglycosidase-treated and examined by SDS-polyacrylamide gel electrophoresis or Western analysis, a single 66 kdalton species is again observed. Moreover, each deglycosylated subunit has lost its ability to bind Concanavalin A although it remains immunoreactive. Densitometric analysis of equal amounts of silver-stained subunits analyzed on SDS-polyacrylamide gels shows equivalent signals for the monomers while densitometric analysis of identical Western blots show equivalent amounts of the β and α subunits and negligible amounts of the γ subunit. Immunoreactivity therefore reconfirms decreased amounts of carbohydrate in the lower molecular weight subunits and further suggests that sugar substituents may hide potential antigenic, immunoreactive sites.

One dimensional mapping of chymotryptic digests of each of the subunits show that distinct peptides are generated. Western analysis of the peptide digests shows that proteolytic cleavage destroys polypeptide epitopes of both small and large peptides. Relatively limited changes in protein structure may thus greatly affect immunoreactivity.

314.5 IN VIVO BIOSYNTHESIS OF TWO SUBUNIT FORMS OF DOPAMINE ß-HYDROXYLASE IN RAT BRAIN. E.L. Sabban[1], L.J. Kuhn[1]* and B.E. Levin[2]. [1]Dept. of Biochemistry, New York Medical College, Valhalla, N.Y. 10595 and [2]V.A. Met. Ctr., East Orange, N.Y. 07019.

Dopamine beta-hydroxylase (DBH), the enzyme which catalyzes the biosynthesis of norepinephrine, is synthesized in the PC12 pheochromocytoma cell line as 2 subunit forms in about equal amounts. The 77K subunit of the membrane form is a precursor of the 73K subunit of the soluble form of DBH (JBC, 1983, 258, 7812).

In vivo biosynthesis of dopamine ß-hydroxylase (DBH) was examined in the rat brain. [^{35}S]methionine was injected into the noradrenergic neurons of the locus coeruleus (LC) using a stereotactic device. Several hours later, newly synthesized [^{35}S]Met-labelled DBH was immunoprecipitated and quantitated. Both 77K and 73K subunit forms were present in near-equal proportions after 4 hrs of labelling and these were indistinguishable, by electrophoretic mobility, from those isolated from PC12 cells. Both forms sedimented with the vesicular subcellular fraction of LC homogenates, and a portion of the 73K form could be released by hypotonic lysis of these vesicles. The 77K form predominated in the first 30 min labelling period, while the 73K form appeared more slowly over the next several hours. By 16 hrs, the 73K form comprised about 2/3 of the total [^{35}S]Met-labelled DBH present. Inhibition of protein synthesis with puromycin treatment 30 min after [^{35}S]Met injection into the LC did not prevent the subsequent appearance of the 73K form suggesting that this subunit form was the product of post-translational modification of the 77K subunit form in a fashion similar to that seen in PC$_{12}$ cells.

Axonal transport of newly synthesized [^{35}S]Met-labelled DBH from the LC to the anterior hypothalamus showed that predominantly the 73K subunit form was transported.

Treatment with the catecholamine-depleting drug reserpine (10 mg/kg, ip) produced a 17-fold increase in the relative synthesis of DBH two days later without affecting the proportion of its two subunit forms. Thus, induction of DBH synthesis was regulated separately from the post-translational modification of its subunit forms. (Supported by NIH grant NS 20440.)

314.6 PLASMA LEVELS OF FREE AND SULFATED CATECHOLAMINES DURING HYPOTENSIVE HEMORRHAGE IN DOGS AND IN CATS. M.K. Dousa*, T.L. Yaksh*, D. Gaumann*, and G. M. Tyce. Departments of Neurosurgery, Anesthesiology, and Physiology, Mayo Clinic and Foundation, Rochester, MN 55905.

Hypotensive hemorrhage (HTH) causes a strong sympathoadrenal stimulation resulting in increased plasma levels of free norepinephrine (NE), epinephrine (E), and dopamine (DA). The aim of this study was to determine the changes of conjugated (namely sulfated) catecholamines (CA) as well as free CA during HTH. The experiments were carried out on halothane-anesthetized (0.8%) dogs (n=8) and cats (n=10) divided in 2 groups: a) animals with induced HTH (4 dogs, 5 cats) and b) control group (4 dogs, 5 cats). Samples were taken simultaneously from cannulated left adrenolumbar vein (ALV) and abdominal aorta at 1 hour after cannulations and immediately after induction of HTH. In the control group the same protocol was followed except for hemorrhage. Free and sulfated CA (S-CA) in plasma were determined by high performance liquid chromatography with electrochemical detection. Under basal conditions the highest plasma levels of free CA were found in ALV with the free E being highest (152.2±90.4 ng/ml in cats and 68.9±18.7 ng/ml in dogs) followed by free NE (27.4±14.8 ng/ml in cats and 6.9±1.9 ng/ml in dogs) with DA having the lowest levels (0.8±0.4 ng/ml in cats and 0.9±0.3 ng/ml in dogs). S-CA in baseline samples represented a significant fraction of total plasma CA in both cats and dogs. Sulfated DA (S-DA) was equal to or exceeded the concentration of free DA in both species (ALV levels were 0.8±0.3 ng/ml in cats and 2.3±0.7 ng/ml in dogs). In cats, baseline adrenal arteriovenous (A-V) differences were positive for: free NE (30-fold), free E (300-fold), free DA (10-fold), and sulfated E (S-E) (10-fold). In dogs, baseline A-V differences were positive for: free NE (50-fold), free E (100-fold), free DA (15-fold), and S-E (5-fold). There were no baseline A-V differences for S-NE and S-DA in either species. In cats, HTH resulted in increases in ALV for: free NE (130-fold), free E (30-fold), free DA (100-fold), S-NE (180-fold), S-E (38-fold), and only a small increase in S-DA. In dogs, HTH resulted in increases in ALV for: free NE (20-fold), free E (17-fold), free DA (40-fold), S-E (14-fold), but little change in S-NE and S-DA. After HTH there were positive A-V differences for free CA and S-E in both species and in cats also for S-NE. The data indicate that sulfated, as well as free, CA are released from adrenals under basal conditions and after HTH. Cats respond to HTH by a higher release of CA than do dogs. Supported by NIH Grants NS 16541 and NS 17858.

314.7 ACTIVATORS OF PROTEIN KINASE C OPEN VOLTAGE-SENSITIVE CALCIUM CHANNELS OF THE CHICK SYMPATHETIC NEURONS GROWN IN CULTURE. T.D. Wakade, and A.R. Wakade (Spon: R. Margolis). Department of Pharmacology, State University of New York, Health Science Center at Brooklyn, NY 11203.

Recently we have shown that protein kinase C activators, phorbol 12,13-dibutyrate (Pdbu) and 12-0-tetradecanolynphorbol 13-acetate (TPA) markedly enhanced the stimulation-evoked release of norepinephrine from sympathetic nerves of the rat salivary gland (Wakade et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 331:122, 1985) and the secretion of catecholamines from the rat adrenal medulla evoked by nicotine and excess K (Wakade et al., Nature in press). In none of these experiments phorbol esters had a measurable effects of their own on the secretion. In this communication we have investigated the effects Pdbu and TPA on the release of tritium from 3H-norepinephrine-loaded sympathetic neurons and simultaneously measured the uptake of Ca^{45}. Sympathetic neurons derived from peripheral ganglia of 10-day-old chick embryo were cultured in a serum free-medium in the presence of 20 ng/ml nerve growth factor. Exposure to 30 nM Pdbu for 2 min increased the outflux of tritium from a basal level of about 2.5 (fractional overflow) to 3.5 and the overflow increased gradually to almost 6.5 after 12 min treatment with Pdbu. After removal of Pdbu the secretory response was restored towards the control level. Essentially similar results were obtained with 10 nM TPA. However the secretory response did not return toward the basal level in 20 to 30 min. 10 µM verapamil or omission of Ca from the medium markedly (70 to 90%) reduced the effects of Pdbu on the tritium outflux. Inactivator of protein kinase C, polymixin B (50 µg/ml), also produced a significant reduction (48%) in Pdbu-mediated outflow of tritium. Five min exposure to Ca^{45} medium followed by 4 washes with krebs solution resulted in the accumulation of about $22x10^3$ cpm/dish. If the same protocol was repeated in the presence of 30 nM Pdbu the accumulation was about $46x10^3$ cpm/dish. It is concluded that activation of neuronal protein kinase C results in the opening of calcium channels to trigger the release of neurotransmitter. Whether this property is unique to embryonic sympathetic neurons growing in a serum-free culture condition remains to be determined.

(Supported by Grants from NSF, BNS8409685 and NIH, HL18601)

314.8 DIFFERENCES BETWEEN PHARMACOLOGIC AND CONDITIONING-INDUCED REDUCTION OF HERMISSENDA CURRENTS. C. Collin*, J. Harrigan*, and D.L. Alkon (SPON: W.J. Adelman, Jr.). Sect./Neural Systems, Lab. Biophysics, NINCDS-NIH, Marine Biol. Lab., Woods Hole, MA 02543.

Recent experiments demonstrated that 0.1-1 mM of the α_2-receptor agonist, clonidine, reduced two outward K^+ currents, I_A and $I_{Ca}2+\text{-}K^+$ (but not $I_{Ca}2+$), across the soma membrane of Hermissenda type B photoreceptors which had been isolated by axotomy from all synaptic interactions and impulse activity (Sakakibara et al., J. Neurochem., in press). These two currents remain reduced on days following classical conditioning (but not control procedures) of intact Hermissenda (Alkon et al., Science 215:639, 1982; Behav. Neural Biol. 44:278, 1985). Clonidine's effect on I_A and $I_{Ca}2+\text{-}K^+$ was specifically blocked by 5 µM yohimbine, as were the EPSPs received by type B photoreceptors from ipsilateral optic ganglion cells. Clonidine (0.1-1 mM) had no effect on $I_{Ca}2+$, the sustained voltage-dependent inward Ca^{2+} current (-3.45±1.67 before vs. -3.2± 1.74, N=6, after 1 mM clonidine). However, we report here that conditioning reduced $I_{Ca}2+$, measured 1-2 days after training (-1.755±0.6, N=9, for conditioned vs. -4.025±1.0, N=8, for control, $p<.001$). This is a clear difference between the clonidine and conditioning-induced effects on type B membrane currents.

Using two microelectrode voltage-clamp, $I_{Ca}2+$ was measured after leak correction as the sustained inward current elicited by a command depolarization to the new equilibrium potential for K^+ flux (~0 mV) (from holding potential at -60 mV) in 300 mM K^+, 3 mM 4-aminopyridine, 100 mM TEA-ASW. The exact K^+ equilibrium potential was determined for each cell by on-line data acquisition and processing with a PDP 11/34 computer.

Other workers reported (Wu and Farley, Soc. Neurosci. 11:620, 1984) that 0.1 mM serotonin reduces I_A and $I_{Ca}2+\text{-}K^+$, although there is no evidence of serotonergic cells in Hermissenda's visual system. Effects of serotonin were assessed here in the presence of 1 mM ascorbic acid, in order to prevent its oxidation. 1 mM ascorbic acid alone did not have any effect on I_A and $I_{Ca}2+\text{-}K^+$. There was no effect on the K^+ currents when .01-1.0 mM serotonin was added to the external solution. For example, I_A at 0 mV (from a holding potential at -60 mV) was 46.9 ± SD before vs. 46.3±8.2 (N=12) after 0.1 mM serotonin. $I_{Ca}2+\text{-}K^+$ at 0 mV was 21.12±3.9 before vs. 21.16±3.9 (N=12) after 0.1 mM serotonin.

These results suggest that a clonidine-like substance, but not serotonin exerts an inhibitory effect on both I_A and $I_{Ca}2+\text{-}K^+$, but not on $I_{Ca}2+$, and thereby increases the excitability of type B photoreceptors. This modulation of K^+ currents could amplify the conditioning-specific depolarization of the type B cell during acquisition of the classically conditioned behavioral response. The presynaptic source of such modulation may be a catecholamine-containing optic ganglion cell which has been identified by histofluorescent methods.

314.9 BLOOD PRESSURE PERTURBATIONS ELICIT ALTERATIONS IN NOREPINEPHRINE RELEASE FROM THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IN UNANESTHETIZED FREELY MOVING RATS. J.M. Qualy* and T.C. Westfall (SPON: J. Gibbons). Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

The paraventricular nucleus of the hypothalamus (PVN) has been shown to play a major role in the regulation of the cardiovascular system. Since the parvocellular division of the PVN receives noradrenergic projections from the A_1, A_2, and A_6 cell groups the neurotransmitter norepinephrine (NE) may be involved in the regulation of blood pressure by the PVN. The purpose of the present study was to explore the effects of alterations in systemic blood pressure on the release of NE from the PVN using push-pull cannula in unanesthetized freely moving Spontaneously Hypertensive (SHR), Wistar-Kyoto (WKY), and Sprague-Dawley (SD) rats.

Male rats (8-9 wks) were anesthetized with 0.45 mg/kg pentobarbital and the head of the rat fixed in a stereotaxic apparatus. Based on stereotactic coordinates for localization of the PVN, a 22 gauge guide cannula was lowered through a burr hole drilled in the skull and secured with epoxy cement; a dummy cannula was inserted into the guide cannula to maintain patency. Five days post-implantation, the rats were reanesthetized and catheters were placed into the abdominal aorta and jugular vein to measure blood pressure and introduce pressor/depressor drugs, respectively. Two days later, a 33 gauge push-pull cannula assembly was introduced into the guide cannula in order to perfuse the PVN with artificial cerebrospinal fluid (18 µl/min). Cerebrospinal fluid effluent was collected in 10 min fractions for analyzation of NE with high performance liquid chromatography coupled to electrochemical detection. Cannula placement was verified histologically after each experiment.

The basal release of NE from the PVN was greater in the SHR vs WKY and SD rats. Administration of 100 µl of phenylephrine (10 µg/kg/i.v.) attenuated the release of NE from the PVN in all three strains; conversely administration of 100 µl of sodium nitroprusside (150 µg/kg/i.v.) exacerbated the release of NE from the PVN in all three strains. These doses of phenylephrine and sodium nitroprusside elicited increases in blood pressure of 40-45 mmHg and decreases in blood pressure of 45-50 mmHg, respectively.

The results of this study show that alterations in blood pressure can elicit alterations in the release of NE from the PVN in the rat. In addition, the increased basal release of NE from the PVN of the SHR, as opposed to the WKY and SD strains, suggests NE's involvement in the maintenance of hypertension.

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314.10 MIDBRAIN DOPAMINE NEURONS: NICOTINIC CONTROL OF FIRING PATTERN. T.H. Svensson, J. Grenhoff* and G. Aston-Jones. Dept. of Pharmacology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden.

Several observations point to a stimulating action of nicotine on dopamine (DA) containing neuronal pathways in brain. Nicotine has been shown to cause release of DA in brain slice preparations, and to produce increased homovanillic acid concentrations and increased DA turnover in the striatum (Lichtensteiger et al., 1976, 1982). When systematically administered or directly applied by means of microiontophoresis on identified DA neurons, nicotine has a dose-dependent stimulatory effect on neuronal firing, as disclosed in recordings of single neurons in the zona compacta (ZC) of the substantia nigra (SN) (Lichtensteiger et al., 1976, 1982; Clarke et al., 1985). Thus, the SN-DA neurons may be equipped with nicotine receptors, mediating the action of the drug. Here we report the effects of systemic administration of nicotine or the nicotinic antagonist mecamylamine on the midbrain dopamine (DA) systems of the rat studied with single cell recording techniques.

DA cells of the zona compacta, substantia nigra (ZC-SN) and the ventral tegmental area (VTA) were identified by their characteristic action potentials, antidromic stimulation methodology and conventional histological procedures. Firing rates as well as firing patterns were determined from computer generated interspike interval histograms describing burst-firing in relation to single spike firing. A larger proportion of burst firing DA cells was found in the VTA when compared with the ZC-SN area. (-)-Nicotine bitartrate (0.5 mg/kg, i.p.) not only increased the firing rate of ZC-SN neurons but also the amount of burst firing of the ZC-SN neurons and VTA neurons, respectively. Mecamylamine HCl (4.0 mg/kg, i.p.) decreased the firing rate of VTA cells, which, in principle, indicates a tonic nicotinic input in this area. The increase in firing rate of central DA neurons following nicotine administration was found to be associated with increased bursting of the burst firing cells whereas, in contrast, the non-bursting neurons did not respond with burst-firing. Generally, the correlation between nicotine induced changes in firing rate and in burst firing activity, was found to be low for midbrain DA neurons.

In conclusion, these observations, in conjunction with the previous demonstration of nicotinic receptors in these areas, indicate the existence of a nicotinic input, specifically regulating the firing pattern of these central DA cells. This neuromodulatory effect of nicotine may be significant for its behavioral stimulant action. In addition, it could alter the ratio of release of coexisting neuronal messengers from midbrain DA neurons.

314.11 EFFECTS OF LOCALLY INFUSED PHARMACOLOGICAL AGENTS ON SPONTANEOUS AND SENSORY-EVOKED ACTIVITY OF LOCUS COERULEUS NEURONS. L.M. Adams and S.L. Foote. Department of Psychiatry, University of California, San Diego, La Jolla, CA 92093

Microinfusion of chemicals directly into brain tissue has been used increasingly in attempts to more precisely determine the anatomical locus for the behavioral effects of pharmacological agents. Inferences about the effects of infused substances on the electrophysiological activity of certain cell groups is usually based on microiontophoretic studies. However, it is difficult to make meaningful comparisons between these two types of data because of marked differences in volumes, rates and duration of drug application between these two methods. Using a recently developed method for recording from locus coeruleus (LC) neurons while simultaneously infusing pharmacological agents 400 µM lateral to the recording site (Soc. Neurosci. Abst. #312.14, 1985), we have begun to investigate the effects of peptides and α-adrenergic drugs on the spontaneous and sensory-evoked activity of LC neurons.

The α-adrenergic agonist clonidine (CLON), in concentrations ranging from 5-20 µM (67-270 pg/50 nl injection) reversibly suppressed activity with latencies to onset of 5-15 min. and durations of 20-120 min. During initial periods of suppressed firing, the responses to sensory stimuli (footshock) were relatively preserved, but at later time points, the reliability of responses to footshock were greatly reduced. The α-adrenergic antagonist piperoxane (PIP) rapidly reversed the inhibitory effects of CLON and also had activating effects alone. Whether administered alone or subsequent to CLON, the excitatory effects of PIP (250-500 pg/50 nl) were invariably followed by a prolonged period of suppressed firing during which sensory responses were difficult to evoke.

These preliminary results indicate that relatively low doses of CLON can have prolonged effects on the spontaneous and stimulus evoked activity of LC neurons and might be useful in behavioral studies for testing the effects of reversible LC "lesions". The ability of PIP to excite LC neurons and block the inhibitory effects of CLON are consistant with previous reports of the effects of iontophoretic and systemic application. However, the subsequent inhibitory effect has not been previously reported. Although iontophoretic applications of PIP using high ejection currents or long durations have been shown to produce local anesthetic effects, this dose not appear to be the case in this instance since the inhibition was not associated with a reduction in spike amplitude. Additional studies are underway to further characterize the effects of α-adrenergic drugs and to determine the effects of fragments of adrenocorticotropin hormone which have been shown to induce abnormal motor behavior when infused directly into the LC and to excite LC neurons when applied iontophoretically.

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314.12 THE EFFECT OF CHRONIC CORDOTOMY, GM_1 GANGLIOSIDE AND FETAL MONOAMINERGIC IMPLANTS ON THE CONTENT OF CATECHOLAMINES IN THE SPINAL CORD OF THE RAT. J. Commissiong and G. Toffano, Dept. of Physiol., McGill Univ., 3655 Drummond St., Montreal, P.Q., Canada H3G 1Y6 and Fidia Res. Lab., 35031 Abano Terme, Italy.

Fetal monoaminergic neurons survive for several months after transplantation into the adult spinal cord (Commissiong, 1984). The monosial ganglioside GM_1 has also been shown to induce a dense sprouting of monoaminergic axons in the rostral region of the transected spinal cord (Commissiong and Toffano, 1986). Also, after a mid-thoracic transection of the cord in early postnatal life (before PN.14), the adult animals recover substantially, and walk with good four point coordination. In these animals, dopamine (DA) is present at 20-50% of the control values in the caudal region of the cord. In this study, a systematic assessment was done of the extent to which catecholamines in the cord are replenished under the experimental conditions listed above. At PN 0(4 hrs after birth), 7, 14, 21 and 28, and at 6 wk animals were anesthetized with ether, and the cord cut at the mid-thoracic level. Analyses were done 6 months later. E.16 fetal locus coeruleus (LC) or mesencephalic dopaminergic neurons (MDN) were implanted into the spinal cavity created by transection (4 implants), or into a small cavity in the dorsolateral region of the cord at L_1 (one implant) in young adult rats. Those animals implanted with MDNs were treated with GM_1 (25 mg/kg i.p.) daily for the first 21 days, and then every other day until sacrifice at 4½ months after implantation. Dopamine (DA) and noradrenaline (NA) were analyzed by gas chromatography - mass spectrometry. The main findings are: 1) After cordotomy at different postnatal ages, there was a very striking increase in NE in the rostral region adjacent to the transection. The effect is age dependent, being most marked for PN 14 (7.08 ± 0.95) and lowest for PN 28 (3.27 ± 0.71). The control value is 2.16 ± 0.18 nmol/g ± S.D. Surprisingly, except in the sacral region of the PN 0 animals, DA was present at between 50-100 pmol/g in the caudal regions of the transected cords. GM_1 also caused a very marked increase in NE in the rostral region of the transected cord, 6.90 ± 1.84 vs. 1.82 ± 0.21 nmol/g ± S.D. in the control. The fetal LC and MDN implants also caused significant replenishment of NE and DA in caudal regions of the cord at up to 25 mm from the site of implantation. A novel feature of the results is the presence of DA in the caudal region of the chronically transected cord. It would be interesting to know whether DA is involved in the oft reported ability of the chronically spinalized adult animal to regain a degree of motor recovery.

Commissiong, J.W. Neuroscience 12, 839, 1984.
Commissiong, J.W. and Toffano, G. Brain Res. In press.

315.1 ROLE OF THE ARCUATE NUCLEUS (ARC) IN VASOPRESSIN (AVP) RELEASE: STUDIES EMPLOYING MONOSODIUM GLUTAMATE (MSG). R.L. Dundore* and M.J. Brody (SPON: D.D. Lund). Department of Pharmacology and Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Previous work from this laboratory indicated that electrical stimulation of the hypothalamic ARC of rats produced an increase in arterial pressure mediated primarily by the release of AVP. The purpose of the work reported here was to determine if a lesion including the ARC, produced by neonatal administration of MSG, alters the release of AVP in response to physiological stimuli.

Male Sprague-Dawley rat pups were injected sc with MSG (40%, 4mg/g) or isomolar NaCl (13%, 0.01ml/g) on days 2, 4,6,8 and 10 of life. At 8-12 weeks of age, rats were separated into three groups: untreated controls (CONT), NaCl- or MSG-treated, and allowed free access to water (normally hydrated, NH) or were water deprived for 48 hr (WD). Trunk blood was collected by decapitation. Plasma [AVP] was measured by RIA; plasma [Na] was measured by flame photometry. In other animals, plasma volume was measured by dye dilution. Urine was collected from NaCl and MSG rats 2 days prior to and 3 days following removal of drinking water. Urine osmolality was measured by freezing point depression.

Neonatal MSG administration destroyed most neurons in the ARC. Basal plasma [Na] (mEq/l) did not differ among the groups. Plasma [Na] was increased during dehydration, however, MSG WD rats exhibited a significantly greater hypernatremia when compared to CONT or NaCl littermates.

		[Na]	[AVP]	PV
CONT	NH	134.4±0.3	6.2±0.5	------
	WD	140.4±0.8	25.8±1.9	------
NaCl	NH	135.5±0.6	7.1±0.7	42.1±1.2
	WD	139.9±0.6	24.3±2.3	35.1±1.1
MSG	NH	135.9±0.7	8.6±2.6	33.5±1.6
	WD	143.3±0.8	24.6±2.9	29.3±1.8

Similarly, basal [AVP] (μU/ml) did not differ among the groups. Plasma [AVP] was increased by dehydration, however, there were no differences in plasma [AVP] among the WD groups. Urine osmolality was similar in NaCl and MSG rats and increased to the same extent by water deprivation (NaCl = 2876±52, MSG = 3038±183 mOsm/kg). Plasma volumes (PV, ml/kg) were decreased by MSG treatment and water deprivation. Both NH and WD rats treated with MSG were hypovolemic when compared to littermates treated with NaCl.

These data indicate that rats treated with MSG are chronically hypovolemic and exhibit a greater hypernatremia during water deprivation. We conclude that lesions including the ARC may blunt AVP release, although the ability to concentrate urine during water deprivation is not impaired. (Supported by HLB-14388 and HLB-07121.

315.2 AFFERENT AND EFFERENT CONNECTIONS OF SOLITARY TRACT NUCLEI WITH A MIDBRAIN PERIAQUEDUCTAL GRAY REGION MEDIATING THE DEFENSE REACTION IN THE CAT. R.Bandler and I.Törk*.Dept of Anatomy,Univ.of Sydney, N.S.W., Australia 2006 and Sch.of Anatomy,Univ.of New South Wales, Kensington,N.S.W.,Australia 2033.

Electrical or chemical stimulation of a distinct region of the midbrain periaqueductal gray matter (PAG) of the cat elicits a set of autonomic and somatic responses collectively described as the "defense reaction". In this study, the connections of the PAG defense region with solitary tract nuclei were investigated by the use of wheat germ agglutinin-HRP (WGA-HRP) as a tracer. The technique of microinjection of excitatory amino acids was first used to identify that region of the PAG containing neurons whose excitation elicited a defense reaction in the unanesthetised, freely moving cat. Such PAG sites were subsequently injected with 50-150 nl of a 1.5% WGA-HRP solution and the anterogradely and retrogradely transported lectin-enzyme complex was demonstrated with the tetramethylbenzidine reaction. Within the solitary complex the majority of retrogradely labelled neurons (73% of total number of labelled solitary neurons) were distributed in approximately equal numbers in the ipsilateral medial solitary nucleus and both the ipsilateral and contralateral ventrolateral solitary nuclei. The remaining labelled neurons were found in the contralateral medial solitary nucleus, and bilaterally in the intermediate, lateral and commissural solitary nuclei. The densest PAG-solitary complex efferent projection was to the ipsilateral medial solitary nucleus especially in the region extending approximately 1.0 mm rostral and caudal to the obex. Lighter labelling was present in the contralateral medial solitary nucleus and bilaterally in the commissural and ventrolateral solitary nuclei. This study has demonstrated, for the first time, the existence of major connections between the PAG and solitary tract nuclei. Further, since the PAG injection sites were the same sites from which a defense reaction could be elicited the results are placed in a specific functional context. It has been demonstrated previously, for example, that the elicitation of a defense reaction in the cat is accompanied by a suppression of the baroreceptor reflex. It has also been reported that selective activation of chemoreceptor or baroreceptor fibers respectively elicits or blocks the occurrence of a defense reaction in the "thalamic" cat. Although it has been suggested previously that such effects may involve the hypothalamus, the results of this study provide an anatomical basis for considering a central role for the PAG.

315.3 **THE SYMPATHOINHIBITION ELICITED FROM THE CEREBELLAR FASTIGIAL NUCLEUS IS MEDIATED THROUGH THE C1 AREA OF THE ROSTRAL VENTROLATERAL MEDULLA.** K. Chida*, C. Iadecola and D.J. Reis (SPON: D. Rottenberg). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We have recently observed that the hypertension elicited by electrical stimulation of the rostral fastigial nucleus (FN), i.e. the fastigial pressor response (FPR), is preserved after destruction of intrinsic neurons in FN by ibotenic acid, indicating that the FPR is not mediated by local neurons but by fibers ending in or passing through FN (Chida et al., Fed. Proc., 45: 875, 1986). In contrast, stimulation of local neurons in FN by excitatory amino acids results in a marked hypotension and bradycardia, the fastigial depressor response (FDR) (Chida et al., Brain Res., 370:378, 1986). We sought to determine: (a) the efferent mechanisms of the FDR, (b) if neurons in FN participate to maintain resting arterial pressure (AP), and (c) if the FDR, like the FPR (McAllen, J. Physiol., 368:423, 1985), depends upon the integrity of the C1 area of the rostral ventrolateral medulla (RVL) (Ross et al., J. Neurosci., 4:474, 1984), a region containing the adrenergic neurons of the C1 group.

Rats were anesthetized (chloralose: 40 mg/kg), paralyzed (tubocurarine) and artificially ventilated. AP and heart rate (HR) were continuously monitored and recorded. The FN was stimulated electrically (10-100 μA, 50 Hz), through microelectrodes, or chemically, by microinjection of dl-homocysteate (DLH) (injection volume ≤ 100 nl) through micropipettes (tip diameter: 50 μm). Sites of electrical and chemical stimulation were histologically verified in all animals.

As before, electrical stimulation of FN (n=10) elevated AP by 48 ± 2 mmHg and HR by 49 ± 5 bpm. However, DLH (100 nmol/100 nl) microinjected into FN of intact rats (n=11) decreased AP by 44±4 mmHg and HR by 40±6 bpm ($p<0.001$). In contrast, microinjection of the inhibitory amino acids GABA (10-100 nmol/100 nl, n=5) or glycine (200 nmol/100 nl, n=5) or tetrodotoxin (10 pmol/100 nl, n=4) did not change resting AP or HR ($p > 0.05$). The fall in AP and HR elicited by DLH was not affected by bilateral transection of the vagi (n=4) (Δ AP= 42±9, Δ HR= 32±4, $p>0.05$, from intact) nor systemic administration of atropine (1.0 mg/kg, i.v., n=5). In contrast, transection of the cervical spinal cord (n=7), with AP maintained by infusion of phenylephrine (PE), abolished the hypotension ($p > 0.05$, from resting) and reduced the bradycardia (residual Δ HR= 17±5 bpm; $p < 0.05$). Bilateral electrolytic lesions of the C1 area lowered resting AP by 60±3 mmHg and HR by 247±16 bpm (n=5) and AP was maintained by PE infusion. After lesions, microinjections of DLH in FN (n=5) failed to elicit the FDR (ΔAP:8±3 mmHg; ΔHR = 7±4 bpm; $p>0.05$ from resting). Similarly, lesions of the RVL also abolished the FPR (ΔAP: 3±1 mmHg; ΔHR: 9±5 bpm; $p > 0.05$).

We conclude that neurons in FN: (a) do not tonically contribute to maintain resting AP; (b) when excited, they inhibit resting sympathetic tone; and (c) the sympathoinhibition is mediated through the C1 area of he RVL, probably by inhibition of neurons of the C1 group.

315.4 MODULATION OF NUCLEUS MEDIANUS NEURONS BY BRAINSTEM NORADRENERGIC CELL GROUPS. D.O. Nelson, C.A. Graham* and A.K Johnson. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

A number of studies have demonstrated the importance of structures in the anterior ventral third ventricle (AV3V) region in controlling body fluid homeostasis and arterial pressure. Of the structures in this region, the nucleus medianus (NM) appears to function as a critical integrative structure relaying signals related to cardiovascular control to the paraventricular nucleus (PVN), supraoptic nucleus (SON) and other hypothalamic structures. Recent neuroanatomical and behavior studies have demonstrated the importance of ascending noradrenergic projections from the A1 and A2 cell groups to the NM. This study investigated using electrophysiological techniques the role of ascending inputs from these regions to NM neurons, particularly those projecting to the PVN and SON. Small bipolar concentric stimulating electrodes were placed in the A1 and A2 regions, the PVN, and SON in urethane anesthetized rats. Spontaneous NM neuronal activity was recorded through one barrel of a multibarrel micropipette. Additional barrels contained L-glutamate (10 mM), norepinephrine (1 mM), yohimbine (100 uM), prazosin (100 uM), or marking dye and were connected to a custom micropressure injection system providing 200 pl resolution. Of 63 NM neurons examined, 32% and 49% showed increased probability of discharge following single shocks (15-100 uA, 0.5 msec duration) applied to the A2 and A1 regions, respectively. Nearly 85% of the NM neurons receiving brainstem input were antidromically activated from the PVN or SON compared to only 25% of the unresponsive neurons. Application of femto- and picomole amounts of yohimbine reduced or eliminated the neuronal excitation to A1 or A2 activation of 75% of the neurons examined. Prazosin was effective on approximately 15% of the cells. Several neurons showed an altered spontaneous discharge rate during application of norepinephrine, yohimbine or prazosin. Pretreatment with 6-OHDA in 4 animals significantly reduced the percentage of NM neurons responding to A1 or A2 activation while not significantly altering the distribution of cells antidromically activated from the PVN or SON. Average spontaneous discharge rates were, however, significantly reduced following 6-OHDA treatment. In a separate series of experiments, a midline stimulation electrode was inserted into the NM and neuronal activity in the A2 region was recorded as described previously. Single shocks applied to the NM elicited antidromic activity from about 50% of the cells recorded in the A2 region. The spontaneous discharge of a high percentage of these cells was correlated negatively or positively with arterial pressure, presumably through baroreceptor afferents.

Our results indicate that ascending input from the A2 and A1 regions may provide tonic excitatory drive to a large precentage of NM neurons and that this input is mediated, in part, by noradrenergic receptors. Some of this ascending input is likely related to baroreceptor activity since neurons in the A2 region projecting to the NM are sensitive to alterations in arterial pressure. These data support results from our *in vitro* studies showing noradrenergic modulation of NM cells and subfornical organ input to the NM. (Supported by HL29033).

315.5 THE AXONS OF VENTROLATERAL MEDULLOSPINAL SYMPATHOEXCITATORY NEURONS BRANCH IN THE MEDULLA. G.L. Gebber and S.M. Barman. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

We recently described (J. Neurophysiol. 53:1551-1566, 1985) the spinal axonal projection patterns of cat rostral ventrolateral medullary (VLM) neurons with sympathetic nerve-related activity. These neurons were classified as sympathoexcitatory in function since they were inhibited by baroreceptor reflex activation. Antidromic mapping of the thoracic spinal cord revealed that the axons of these neurons coursed through the dorsolateral or ventrolateral funiculus to innervate the region of the intermediolateral nucleus (IML). Some VLM-spinal axons that projected to the eleventh thoracic (T11) segment branched at upper and mid-thoracic levels. The axonal projections of other VLM-spinal neurons were restricted to upper thoracic segments.

The present study demonstrated that the axons of VLM-spinal sympathoexcitatory neurons also branch in the medullary lateral tegmental field (LTF). This was indicated by the results obtained with two tests. First, the antidromic response latency of VLM-spinal neurons (n=8) was shortened (8.6±3.8 ms) when the stimulus current applied to the LTF was raised above threshold. The longest latency antidromic responses (20.6±5.2 ms) elicited with threshold stimulus current likely reflected slowed conduction velocity in branches of the main axon. Second, intramedullary axonal branching of five VLM-spinal neurons was demonstrated using time-controlled collision of the action potentials initiated by LTF and T2 IML stimulation. Activation of a medullary axonal branch was indicated when the maximum interval after a threshold LTF stimulus at which the stimulus applied to T2 IML failed to elicit an antidromic response at the recording microelectrode exceeded the difference between the onset latencies of the antidromic responses of the VLM neuron evoked by spinal and medullary stimulation plus the spinal axonal refractory period. Although we have not yet located the sites of termination of the medullary axonal branches of these neurons, the data suggest that individual VLM neurons influence sympathetic nerve discharge by actions mediated at both spinal and supraspinal levels. (Supported by NIH grants HL-13187 and HL-33266.)

315.6 LATERAL TEGMENTAL FIELD NEURONS OF CAT MEDULLA: A SOURCE OF BASAL ACTIVITY OF VENTROLATERAL MEDULLOSPINAL SYMPATHOEXCITATORY NEURONS. Susan M. Barman and Gerard L. Gebber. Dept. of Pharm./Toxicol., Michigan State Univ., East Lansing, MI 48824.

We (J. Neurophysiol. 53:1551-1566, 1985; J. Neurophysiol. 54:1498-1512, 1985) recently showed that both the rostral ventrolateral medulla (VLM) and the medullary lateral tegmental field (LTF) contain the cell bodies of sympathoexcitatory neurons. Their spontaneous activity is correlated to that in the inferior cardiac sympathetic nerve (as demonstrated with spike-triggered averaging), and their firing rate is decreased during baroreceptor reflex activation. The axons of VLM neurons project to the intermediolateral nucleus (IML) of the thoracic spinal cord. Those of LTF neurons, however, do not project to thoracic spinal levels. Thus, LTF neurons must exert their influences on sympathetic nerve discharge (SND) via neurons located in other brain regions. The current study was designed to test the hypothesis that LTF neurons influence SND by activating VLM-spinal sympathoexcitatory neurons. If this hypothesis is correct, the axons of LTF neurons with sympathetic nerve-related activity should project to and branch in the rostral VLM. Moreover, VLM-spinal sympathoexcitatory neurons should be synaptically activated by microstimulation of the LTF. Finally, conduction time in the pathway from the LTF to the VLM should compare favorably with the difference in the times of occurrence of the spontaneous action potentials of the two groups of neurons.

We antidromically activated 31 LTF neurons with sympathetic nerve-related activity by microstimulation of the rostral VLM (as indicated by time-controlled collision of spontaneous and stimulus-induced action potentials). The firing rate of these neurons decreased during baroreceptor reflex activation. The axons of 27 of these neurons branched in the region of the rostral VLM as indicated by the decrease (5.3±1.3 ms) in antidromic response latency produced by raising stimulus current above threshold. The longer latency response (14.2±1.8 ms) elicited with threshold stimulus current likely reflected slowed conduction velocity in axonal branches. Two observations indirectly suggest that the axonal branches of LTF neurons terminate in the rostral VLM. First, in all cases tested the site requiring the least stimulus current to elicit the longest latency antidromic response was located in the rostral VLM. Second, 25 VLM sympathoexcitatory neurons were synaptically activated by microstimulation of the LTF. The axons of many of these VLM neurons projected to the thoracic IML. Importantly, the modal onset latency of synaptic activation of VLM neurons (25.6±2.6 ms) compared favorably with the difference (31 ms on the average) in the firing times of LTF and VLM neurons relative to the peak of the cardiac-related sympathetic nerve slow wave. These data are consistent with the view that LTF neurons are an important source of the basal activity of VLM-spinal sympathoexcitatory neurons. (Supported by NIH grants HL-33266 and HL-13187.)

315.7 SPINAL PROJECTIONS TO BRAINSTEM AUTONOMIC CENTERS. D.A. Ruggiero, R.L. Stornetta, S.F. Morrison and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Somatic-autonomic reflexes involve spinal and supraspinal components and appear to be integrated along multiple levels of the neuraxis (Mitchell et al., Ann. Rev. Physiol. 45:1983). However, the neuroanatomical substrates mediating these reflexes have not been characterized. We therefore used axonal transport techniques to map the distribution and origins of spino-autonomic projection fields.

In anesthetized rats (halothane, 2% in 100 O_2), anterograde tracers, wheat germ agglutinin-horseradish peroxidase conjugates (WGA-HRP) or tritiated amino acids were microinjected into lumbosacral segments innervated by the sciatic nerve . In other animals, control injections of WGA-HRP were made into the thoracic or cervical spinal cord. Animals were reanesthetized and perfused; brains and spinal segments were sectioned (20-40 um) and processed using tetramethylbenzidine or autoradiography.

Anterograde transport was localized to individual ascending spinal tracts as well as diffusely throughout the white matter of the anterior and lateral funiculi. Fibers in passage were also labeled in the posterior columns. Labeled terminals were identified in several brainstem autonomic nuclei including medial, and to a lesser degree, lateral aspects of the rostral ventrolateral medulla. Labeled projections were also found in lateral, ventral and commissural subnuclei of nucleus tractus solitarii, periventricular grey, lateral tegmental field (in the region of nucleus retroambiguus), dorsolateral tegmental nucleus and the noradrenergic A5 area. In the parabrachial complex, clusters of profuse labeling outlined subdivisions of the lateral parabrachial nucleus and nucleus of Koelliker-Fuse. Portions of the "precerebellar" lateral reticular nucleus, accessory olivary and dorsal column nuclei, nuclei x and f of the vestibular complex, cerebellum, pontine reticular formation, midbrain and thalamus were also labelled.

Injections of WGA-HRP into RVL (a site mediating the sciatic pressor response; Stornetta et al. Neurosci. Abstr. 1986) retrogradely labeled cells primarily in laminae IV-VI of the dorsal horn, and to a lesser extent the zona intermedia (lamina VII) and lamina X in lumbosacral and other spinal segments.

We conclude: (a) Neurons in the dorsal horn and central grey of the spinal cord project to several autonomic sites in the lower brainstem, and (b) These projections may serve to mediate various components of somatic-autonomic reflexes, in particular, the pressor response to somatic afferent stimulation.

315.8 CENTRAL PATHWAYS MEDIATING THE ARTERIAL PRESSURE INCREASE EVOKED FROM SURAL NERVE STIMULATION. R.L. Stornetta, S.F. Morrison, D.A. Ruggiero and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

The reflex elevation of arterial pressure (AP) elicited from somatic afferents, the somatic pressor response (SPR), is integrated within the medulla oblongata, however the exact site of integration is not established. Both precerebellar lateral reticular nucleus (LRN) (Mitchell et al. Ann. Rev. Physiol. 45:229, 1983) and rostral ventrolateral medulla (RVL) (McAllen, J. Physiol. 368:423, 1985) have been proposed to mediate the SPR in cat. We sought to establish in rat if the area of the RVL containing adrenergic neurons, a region known to participate in the tonic and baroreflex control of AP, also integrates the SPR.

In the first series of experiments, WGA-HRP was iontophoretically injected into subregions within the RVL in 16 rats. Retrogradely labeled cells were observed in all segments of cord including laminae IV-VII and X of lumbar cord. Injections localized to more ventromedial areas of RVL resulted in greater numbers of retrogradely labeled cells, corresponding to the topographic distribution of spinal afferents to the RVL. In another series of animals (n=6), HRP was applied to the cut end of sural nerve (SN) or the main sciatic trunk. Labeled terminals were observed in laminae I-VI of the dorsal horn of lumbar cord. When lumbar cord was double-labeled by injection of HRP into RVL and HRP application to the cut end of SN, terminal fields of SN were seen to overlap cells retrogradely labeled from the RVL.

The SPR was elicited by electrical stimulation of SN in urethane anesthetized, paralyzed and artificially ventilated rats. A threshold AP increase of 10 mm Hg was elicited at an optimal frequency of 20 Hz and stimulus intensity of 140 ± 12 uA (n=25). This intensity was 7x twitch threshold and 45-50x threshold for the A-fiber volley recorded as a compound action potential from dorsal roots, thus confirming that activation of C-fibers initiates the SPR (Schmidt and Weller, Brain Res. 24:207, 1970). After eliciting the SPR with supramaximal stimulation (20 Hz, 600 uA, 3 sec), small electrolytic lesions were placed in various regions of RVL and LRN. Lesions of RVL or LRN contralateral but not ipsilateral to SN stimulation abolished the SPR. RVL lesions overlapped areas containing PNMT-labeled neurons. The response was also eliminated by injection of 1 nmol of 100 nl kainic acid into the contralateral but not ipsilateral RVL. In contrast, kainic acid injected into the contralateral LRN did not abolish the SPR. Contralateral or ipsilateral electrolytic lesions of other autonomic areas receiving spinal afferents, including the parabrachial complex, the nucleus tractus solitarii, A5 region or the inferior cerebellar peduncle (output pathway of LRN) did not affect the reflex. We conclude: the RVL including the C1 area is a critical region for the integration of somato-autonomic reflexes.

315.9 RETICULOSPINAL SYMPATHOEXCITATORY NEURONS IN THE C1 REGION OF THE ROSTRAL VENTROLATERAL MEDULLA (RVL) MAY MEDIATE THE SUPRASPINAL COMPONENT OF THE SOMATOSYMPATHETIC REFLEX. S.F. Morrison, T. Milner, V. Pickel and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Neurons projecting from the C1 region of the RVL to the spinal cord provide the sympathoexcitatory drive necessary to maintain basal sympathetic nerve activity (SNA) and arterial pressure. Inhibition of these cells contributes to the vasodepressor response to baroreceptor reflex activation. Neurons in this region are also required for the pressor response to sural nerve (SN) stimulation. We sought to determine (1) if the same RVL-spinal neurons integrate baroreceptor and somatic reflexes and (2) the relationship of these neurons to C1 adrenergic cells. Reticulospinal neurons were antidromically identified in the RVL of rats anesthetized with urethane. Their conduction velocities were distributed bimodally with means of 3.7 ± 0.2 m/s (n=56) and 0.9 ± 0.05 m/s (n=24). Such RVL-spinal neurons may be elements of the C1 cell group since (1) their recording sites were consistently located within 100 um of cell bodies immunoreactive for PNMT and (2) electron microscopy revealed that PNMT was contained in both myelinated (0.6-2.1 um diameter) and unmyelinated (0.1-0.8 um diameter) spinal axons. The spontaneous discharges of RVL-spinal neurons were synchronized to the bursts of activity in the splanchnic nerve in the presence and in the absence of baroreceptor input. On the average, these cells fired 66 ± 3 ms prior to the peak of SNA. This time corresponded to the time to peak of the early excitation in SNA evoked by stimulation of the RVL. The influence of the baroreceptor reflex on the discharge of RVL-spinal neurons was demonstrated by (1) the strong cardiac-related component in their discharge and (2) the potent inhibition of their firing during the pressor responses to norepinephrine infusion or aortic occlusion. RVL-spinal neurons of both conduction velocity groups responded orthodromically to electrical activation of somatic afferents in the SN at latencies from 20-40 ms and at threshold intensities of about 100 uA. SN stimulation elicited an excitatory potential in SNA with two peaks (90 ± 2 ms and 172 ± 3 ms, n=7). These values were comparable to the sum of the times to peak SNA following RVL stimulation plus the latency to activation of RVL-spinal neurons following SN stimulation. Both peaks in the SN-evoked potential in SNA were abolished by microinjection of kainic acid into the contralateral C1 region of the RVL. We conclude that (1) sympathoexcitatory neurons in the RVL are located within, and may be elements of the C1 adrenergic cell group, (2) the effects of these cells are transmitted over rapidly and slowly conducting spinal pathways that could correspond to the myelinated and unmyelinated spinal axons containing PNMT, and (3) spinal afferent excitation of RVL-spinal sympathoexcitatory neurons underlies the pressor response to somatosympathetic reflex activation.

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315.10 CLONIDINE MODULATES THE CAUDAL VENTROLATERAL MEDULLARY CATECHOLAMINERGIC HYPERACTIVITY INDUCED BY CONTROLLED/ HYPOVOLEMIC HYPOTENSION. L. Quintin, J.Y. Gillon*, M. Ghignone*, O. Calvillo and J.F.Pujol*. Neuropharmacology,Roussel Uclaf, Romainville,France and Anesthesiology, Texas Tech U. Health Sci. Ctre, Lubbock, TX.

Catechol (CA) metabolic activity, measured with in vivo electrochemistry in the caudal ventrolateral (CVL) medulla (Suaud-Chagny J Neurochem in press), is increased during hypotension (Gillon Soc Neurosci Abst 1985,11(I):491).Clonidine has been shown to suppress the CA metabolic hyperactivity of the perikarya of the LC induced by immobilization in behaving rats(Quintin Brain Res 1986,362:366-9). Hence clonidine was thought of value to modulate the hyperactivity of the ventral NA bundle secondary to hemodynamic disturbances. Rats(350g,OFA),were anesthetized (maintenance: alphachloralose 10-20 mg kg^{-1} h^{-1} iv),rewarmed(38°C),ventilated ($P_{et}CO_2$ = 30-35 mmHg,F_iO_2=0.4,arterial blood gases q15-60 min) and had their blood pressure(BP) maintained with a dextran-bicarbonate infusion. Stable recordings of hemodynamic parameters and of a CA oxydation current(CA.OC,+ 55 mV,treated carbon fibre electrode, Biopulse,Tacussel) in the CVL area (P:0.3,L:1.9 mm to calamus scriptorius;DV: -2,5 mm, 30°from verticality) were obtained for 4 hours or more with less than 10 % variation after saline injection (n=6). Controlled hypotension (CH,sodium nitroprusside) lowered BP from 101 mm Hg+6 (x + sem) to 67+2 and increased the CA.OC to 173 %+17 of baseline (100 %) values (n=5). Clonidine(200 ug kg^{-1} ip) lowered BP (90 mmHg+5 to 67+2) and the CA.OC to 42 %+8 (n=6). Clonidine, injected 30 min(n=3) or immediately before CH(n=6), blunted almost totally the CA hyperactivity secondary to CH. Major hemorrhage(HGE,30 % blood volume) lowered BP(110 mmHg+5 to 37+3) and increased CA.OC to 187 %+13 (n=5). Blood reinfusion led to complete hemodynamic stabilization while CA.OC partially reverted toward baseline values. Deafferentation before HGE suppressed this increased CVL activity (n=5). Clonidine(200 ug kg^{-1} ip) partly suppressed this increased phasic hyperactivity upon HGE(n=5). CA nature of the O.C recorded in the CVL area was ascertained with alphamethylparatyrosine(n=4),pargyline(n=16),FLA 63(n=3).Immunohistochemical stainings revealed the presence of only NA perikarya in this area. That clonidine suppresses the CA metabolic hyperactivity of the ventral NA bundle secondary to circulatory disturbances is in keeping with a previous demonstration of a modulatory effect of this drug after a behavioral stimulus.Clonidine may be of interest to treat sympathetic hyperactivity occuring after CH in the anesthetic setting, or after trauma in the intensive care unit.

315.11 INTRATHECAL ADMINISTRATION OF A SUBSTANCE P (SP) RECEPTOR ANTAGONIST: CARDIOVASCULAR ASSESSMENT OF 'NON-SPECIFIC' EFFECTS. C.J. Helke, E.T. Phillips*, and J.T. O'Neill*. Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

SP is contained in ventral medullary projections of the intermediolateral cell column (IML) of the spinal cord and appears to be involved in mediating sympathoexcitatory cardiovascular responses from the ventral medulla. Intrathecal (i.t.) administration of the SP antagonist, [D-Arg^1, D-Pro^2, D-$Trp^{7,9}$, Leu^{11}]-SP (D-Arg-SP) (3.3 nmol, i.t.) decreased mean arterial pressure (MAP) and the pressor responses elicited by ventral medullary administration of bicuculline (Keeler & Helke, 1985). Although considerable evidence suggests that D-Arg-SP interacts with SP receptors located on IML preganglionic neurons, other data suggest that at least in some systems certain of the SP antagonists have actions unrelated to SP receptor blockade i.e. 'local anesthetic' and/or local vasoactive effects. Therefore, we tested the ability of D-Arg-SP to antagonize a sympathoexcitatory cardiovascular response elicited by the intrathecal administration of a non-SP receptor agonist, sodium L-glutamate (glu). In addition, regional blood flow was evaluated to assess local vasoactive and generalized vasodilatory actions of D-Arg-SP.

I.t. injections were performed in anesthetized, ventilated rats. In control rats, administration of glu (i.t.) caused a dose-dependent pressor response which was not mimiced with the intravenous administration of similar doses. Administration of glu (200 µg i.t.) at the peak of the D-Arg-SP (3.3 nmol i.t.) response consistently elicited a pressor response which was greater than or equal to that elecited in the absence of D-Arg-SP. Regional blood flow was measured with the radioactive microsphere technique before and after administration of D-Arg-SP (3.3 nmol i.t.). These studies showed that whereas D-Arg-SP reduced MAP, D-Arg-SP-induced vasodilation appeared to be selective for certain sympathetically innervated tissues, none was apparent in kidneys and adrenals. No changes in brain blood flow were noted, however flows in thoracic and lumbar cords were significantly reduced.

Whereas, the importance of the D-Arg-SP induced reduction in spinal cord blood flow remains to be determined, the ability to elicit a sympathoexcitatory response to glu in the presence of D-Arg-SP and the lack of a generalized vasodilation of sympathetically innervated organs by D-Arg-SP suggest that 'non-specific' neuronal dysfunction due to local vasoactive properties, or local anesthetic effects are not solely responsible for the actions of this SP antagonist. These data therefore support the notion that an interaction of D-Arg-SP with SP receptors in the spinal cord is an important component of its actions. Supported by NIH grants NS19317 and NS20991.

315.12 EVIDENCE FOR GABA-LIKE IMMUNOREACTIVE INPUT ONTO AND SURROUNDING RETROGRADELY LABELED SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT AND PIGEON. N. Bogan*, A. Mennone* and J. Cabot, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Physiological and biochemical evidence suggest that spinal GABAergic mechanisms may be important for the central regulation of autonomic function. Previous observations by our laboratory and others demonstrated that iontophoretic application of GABA onto sympathetic preganglionic neurons (SPN's) results in functional inhibition, though the ionic mechanism and sites of action are unknown. This study provides light and electron microscopic evidence showing the presence of GABA-like immunoreactive (GABA-IR) processes within the SPN neuropil and specifically documents, for the first time, that one class of somatic inputs onto SPN's contains GABA-IR. These data suggest that inhibitory synaptic events affecting SPN discharge may, in part, be mediated by GABA.

Male Sprague-Dawley rats (n=6) and pigeons (Columba livia)(n=8) were sacrificed 3 days after HRP injections were made into the adrenal medulla or paravertebral ganglion 14, respectively. Following intracardial perfusion, thoracic spinal cord was vibratome sectioned and DAB reacted. Sections were then incubated in anti-GABA (generously provided by Dr. B. Maley) and subsequently stained using either PAP or ABC immunohistochemical protocols.

Light microscopically, the pigeon SPN neuropil contains a dense network of GABA-IR processes which appear to completely outline and give rise to punctate swellings which appose SPN somata. GABA-IR interneurons are found intermingled among HRP-labeled SPN's, in the intermediate spinal gray and in dorsal horn. In rat the density of GABA-IR processes within the intermediolateral cell column (IML) is qualitatively less than in the pigeon; however, GABA-IR profiles distinctively dot SPN somata. GABA-IR cells are not in the IML, but present in dorsal horn and lamina X.

The ultrastructural pattern of GABA-IR in the SPN neuropil is similar in both species. Immunolabeled boutons contain electron-lucent vesicles 50-60nm in diameter and form symmetrical contacts. Utilizing a serial section analysis, we observe GABA-IR terminals contacting perikarya of HRP-labeled SPN's. Although more frequently, GABA-IR boutons formed synapses on proximal and distal dendritic elements. Less commonly observed are axo-axonic configurations. In addition to GABA-IR terminals, small caliber myelinated and numerous unmyelinated GABA-IR axons course through the SPN neuropil. Occasionally, these unmyelinated axons contained vesicles in regions adjacent to SPN's with no apparent synaptic specializations. Immunolabeled dendritic processes are present within the SPN neuropil of both species and are contacted by unlabeled (predominantly RA-type terminals) as well as GABA-IR terminals. (Supported by HL24103; JBC is an Established Investigator of AHA.)

316.1 OVARIAN STEROIDS POTENTIATE THE NOREPINEPHRINE-MEDIATED CYCLIC AMP FORMATION IN RAT HIPPOCAMPUS.
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Catecholaminergic neurons have been shown to be involved in the control of ovulation and reproductive behavior. The present studies were conducted to gain insight into the possible action of steroid hormones on brain adrenergic-receptor mediated responses.

Groups of ovariectomized female rats (OVX) were treated with 5 µg of estradiol benzoate (EB) 48 hours before sacrifice alone or in combination with 1 mg progesterone (P_4) four hours before sacrifice. The cyclic AMP (cAMP) formation induced by adrenergic agonists was measured in hippocampal slices, by means of a prelabeling technique and isolation of the radiolabeled cAMP formed by ion-exchange and alumina chromatography.

The conversion of labeled adenine nucleotides to labeled cAMP was stimulated 3 fold over basal by 10 µM norepinephrine (NE) in OVX controls and in EB treated animals. However in EB + P_4 treated animals the same dose of NE induced a 6 fold increase in cAMP formation. Isoproterenol 25 µM consistently induced a lower stimulation of the cAMP formation when compared with NE: 15% lower in OVX controls and 50% in EB and EB + P_4 treated animals. EB treated animals also showed a lowered responsiveness to the β-adrenergic agonist (-37%) when compared to OVX controls and EB + P_4 treated groups. The addition of the α-agonist metoxamine (100 µM) during ISO stimulation rised the cAMP formation to a level similar to that obtained with NE in all groups studied. Moreover the addition of phentolamine but not yohimbine, previous to the NE stimulation induced a reduction of the cAMP formation to a level similar to the one observed with ISO alone.

These data indicates that progesterone can induce an increased responsiveness to NE-mediated cAMP formation in the rat hippocampus, through an effect not on the β-adrenergic component of the response, but on the recently demonstrated potentation of β-adrenergic response induced by α_1-adrenergic receptor stimulation.

316.2 THE ROLE OF THE MEDIAL REGION OF THE AMYGDALA IN ALDOSTERONE-INDUCED SALT APPETITE. J. Marini*, J. Schulkin* and A.N. Epstein, (SPON: A.R. Morrison), Univ. of Pennsylvania, Philadelphia, PA. 19104-6018.

Aldosterone generates an appetite for salt. Female rats typically ingest more salt than male rats. Because the cortico-medial region of the amygdala (CM Amyg) is a site of receptors for both aldosterone and the gonadal steroids, we tested the idea that this brain region is critically involved in aldosterone-induced salt appetite and in the normally enhanced appetite for salt of female rats.

Male and female adult rats received electrolytic lesions of the CM Amyg and vicinity, and were adapted to periodic access to 3% NaCl. They were then placed on a sodium deficient diet and given one of three doses of aldosterone or the oil vehicle (60, 120, 240 ng in oil SC) once weekly in counter-balanced order. The following day they were offered a 3% NaCl solution in addition to water and their 24 hour intakes were measured while they continued to be maintained on the sodium deficient diet. In addition, male rats, with and without CM Amyg lesions, were given a sodium depletion-induced salt appetite (the removal of ambient sodium and 2 SC injections of 5 mg furosemide treatment separated by 2 hrs) and tested for salt appetite as described.

CM Amyg damage resulted in marked impairments in aldosterone-induced salt appetite in both sexes. In addition, female lesioned rats did not show the normally enhanced need free intake of salt, as shown by the fact that they drank very little salt when not treated with the hormone (2 ml in lesioned rats vs 23 ml in the non-lesioned rats). Female lesioned rats (n=5) ingested between 2 to 3 ml of salt following 60, 120, and 240 ng of aldosterone respectively. Non-lesioned females (n=5) ingested 26 to 30 ml of salt. Male lesioned rats (n=4) ingested 2 to 3 ml following vehicle or 60, 120, and 240 ng of aldosterone respectively: while the non-lesioned males ingested 7 to 19 ml of salt. But, CM Amyg damage did not abolish depletion-induced salt appetite in male lesioned rats (n=3). They ingested 9 to 12 ml of salt: non-lesioned male rats (n=3) ingested 16 to 24 ml of salt.

These results reveal that the CM Amyg is a critical central site for aldosterone-induced salt appetite and may also be critical for the enhanced avidity for salt normally seen in female rats.

Lastly, depletion induced salt appetite, which activates aldosterone secretion, was reduced but not abolished. Presumably, the lesion prevented the natriorexigenic effect of aldosterone, but other mechanisms which initiate salt appetite (in the rat) such as angiotensin and atrial volume receptors remained competent because their effects are mediated by neural structures other than the cortico-medial amygdala.

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316.3 **EFFECTS OF TESTOSTERONE ON THE UPTAKE OF TRITIATED ESTRADIOL BY CELL NUCLEI IN THE BRAIN OF THE MALE RHESUS MONKEY.** **Robert W. Bonsall*, Howard D. Rees and Richard P. Michael, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and Georgia Mental Health Institute, Atlanta, Georgia 30306.**

Testosterone (T) restores the sexual behavior of castrated macaques and also regulates gonadotropin releasing hormone secretion by a hypothalamic mechanism. Neurons that concentrate [^{3}H]T or its metabolites have been localized by autoradiography in the hypothalamus, preoptic area and amygdala. The major form of radioactivity in cell nuclei from these areas is [^{3}H]estradiol ([^{3}H]E_2), an observation that supports the view that aromatization may be involved in some of the central effects of T. To determine the extent to which nuclear estrogen binding sites in the brain are occupied by estrogenic metabolites of T, we castrated eight adult rhesus monkeys and injected four of them daily for 3 days with 2 mg testosterone propionate (TP) s.c. (a dose that produces high physiological plasma levels of T and is behaviorally effective). One hour before death, all males were injected with 0.47 mCi/kg [^{3}H]E_2 as an intravenous bolus (this raised blood levels above the normal levels of E_2 in intact females). Brains were rapidly removed and placed on ice. The left half of each brain was frozen for autoradiography, and cell nuclei were isolated from 14 regions dissected from the right half by centrifuging homogenates through 2M sucrose. The [^{3}H]E_2 contents of ether extracts of the nuclei were estimated by subjecting them to high performance liquid chromatography and measuring the radioactivity in fractions. In the four males that were not treated with TP, concentrations of [^{3}H]E_2 in nuclei were significantly ($P<0.001$) higher in hypothalamus, preoptic area and amygdala than in other regions of the brain. Autoradiograms showed highest frequencies of labeled cells in the bed nucleus (n.) of stria terminalis (which was combined with the preoptic area for biochemical analysis), medial preoptic n., anterior hypothalamic area, arcuate n., ventromedial n. and in the cortical, medial and accessory basal nuclei of the amygdala. In the four males that received testosterone pretreatments, nuclear concentrations of [^{3}H]E_2 were significantly ($P<0.025$) reduced compared with males not receiving testosterone pretreatments: in hypothalamus, 12,700 ± 2,000 vs 51,200 ± 5,000 dpm/mg DNA (means ± s.e.m.); preoptic area, 3,300 ± 300 vs 28,600 ± 3,900 dpm/mg DNA; and amygdala, 7,000 ± 900 vs 18,300 ± 2,500 dpm/mg DNA. There were no differences between groups in other brain areas. Importantly, there were no differences in the levels of [^{3}H]E_2 in supernatants from testosterone pretreated (2,090 ± 220 dpm/mg protein) and non-pretreated (2,720 ± 700 dpm/mg protein) animals, which suggested that the reduction in nuclear concentrations could be attributed to prior occupation of nuclear estrogen binding sites by estrogenic metabolites of T rather than to any effects of TP treatments on the availability of [^{3}H]E_2. This is consistent with the view that some of the central actions of testosterone in the brain of the male primate are mediated by estrogen receptor systems.

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316.4 EVIDENCE FOR THE REESTABLISHMENT OF COPULATORY BEHAVIOR IN CASTRATED MALE RATS WITH A BRAIN-SPECIFIC ESTRADIOL REDOX DELIVERY SYSTEM. W.R. ANDERSON, J.W. SIMPKINS, M. BREWSTER* AND N. BODOR*, Departments of Pharmacodynamics and Medicinal Chemistry, University of Florida, Gainesville, FL 32610.

We have developed a redox system for brain-specific drug delivery based on an interconvertible dihydropyridine ⟺ pyridinium salt carrier. The drug, when combined with the lipoidal carrier, readily crosses the blood-brain barrier. The carrier, upon oxidation, reduces the rate of exit of the drug from the brain and subsequent hydrolysis of the drug-carrier complex results in delivery of the drug to the central nervous system. The delivery system utilized in this study is an estradiol-chemical delivery system (E2-CDS), formed by conversion of 17β-estradiol to a 3, 17β-dinicotinoyl derivative, then hydrolyzed to the 17-monoester. The aim of this study was to evaluate the effects of this E2-CDS on copulatory behavior in four-week orchidectomized Sprague-Dawley rats. Either the E2-CDS or estradiol valerate (E2-VAL; another C-17 substituted prodrug of estradiol) was administered as a single i.v. injection at an equimolar dose of 3 mg/kg B.W. of estradiol and dimethyl sulfoxide (DMSO) served as the control vehicle. Behavioral parameters which were assessed were mounting latency and frequency (ML, MF), intromission latency and frequency (IL, IF), ejaculation latency and total copulatory performance (CP; calculated as total number of copulations within 15 minutes after the initial mount). Animals treated with E2-CDS responded with a decrease in ML and IL by 3 days and this effect persisted for at least 28 days. The ML and IL of rats exposed to E2-VAL were diminished for 3 to 7 days and to a lesser extent than in E2-CDS rats. The intromission frequencies of E2-VAL treated rats were increased only at 1 week while animals treated with the E2-CDS increased the number of intromissions by 300% over castrate levels through 14 days and eight-fold over E2-VAL treated animals through 28 days. Intromission frequencies were restored to pre-castrate levels through 4 weeks in E2-CDS treated rats while those exposed to E2-VAL responded similarly for 7 days. CP in E2-CDS rats was increased at 7 days and continued through 4 weeks while E2-VAL rats improved their CP for only 1 week. Neither ejaculation latencies nor penile reflexes were restored by either form of estradiol in 4-week orchidectomized rats. These studies indicate that the E2-CDS can restore male copulatory behavior in orchidectomized male rats to a greater degree and for a longer period of time than E2-VAL. These data suggest that a single injection of E2-CDS can maintain male copulatory performance for up to 4 weeks presumably by a local action of released E2 in the brain. (Supported in part by grants AG02021, GM 27167 and a grant from Pharmatec, Inc.)

316.5 SEXUAL PREFERENCE VS. SEXUAL RECEPTIVITY: EFFECTS OF CNS LESIONS AND LUTEINIZING HORMONE RELEASING HORMONE (LHRH). C.A. Dudley and R.L. Moss. Dept. Physiology, UTHSCD, Dallas, TX 75235

Sexual receptivity; i.e., lordotic behavior, and sexual motivation; i.e., willingness to approach a sexually active male, are both hormonally mediated but can be dissociated. The present study evaluates the effect of lesions placed in various behaviorally-relevant CNS sites on sexual receptivity and sexual preference, and tests the ability of estrogen (E), progesterone (P), and LHRH to modulate the lesion effect.

Bilateral electrolytic lesions were placed in the ventromedial hypothalamus (VMH;n=14), the medial preoptic area (MPO; n=13), or the midbrain central gray (MCG;n=14) of OVX female rats. An additional 5 to 7 animals per group received sham lesions. Sexual preference tests were conducted in a choice box apparatus (Pharmacol. Biochem. Behav. 22: 967, 1985) while sexual receptivity tests were conducted by placing the test female in a mating chamber with two sexually active male rats for 15 min or 15 mounts. The incentive choice box animals were a sexually active male, a castrate male, an OVX female, and an EP-primed female. Two weeks after lesion, the animals were primed with E (5µg), and 48 hrs later, tested in the choice box and then in the mating arena. In subsequent weeks the animals were primed with E and P (2.5mg) and E and LHRH (1000ng). All injections were subcutaneous. Results are tabled below. Sexual receptivity is expressed in terms of the L/M ratio and sexual preference (PREF) is reported as the percent of time spent close to SM (25% time being the amount expected by chance for each of the four incentives).

	E		E+P		E+LHRH		
	L/M	PREF	L/M	PREF	L/M	PREF	
VMH	.11*	25.5**	.65*	37.3	.29	42.9	LESION
	.27*	36.5**	.96*	46.1	.64	41.3	SHAM
MPO	.14	31.9**	.82	39.4	.08*	34.3	LESION
	.08	23.6**	.53	36.3	.46*	29.3	SHAM
MCG	.05	35.7	.60	36.3	.90*	43.6	LESION
	.18	40.2	.50	51.9	.39*	56.0	SHAM

lesion vs. sham: *p<0.05;t test **p<0.05; Kruskal-Wallis test

VMH lesions were effective in reducing L/M and PREF in E and EP primed animals, whereas MPO lesions tended to enhance both behaviors. LHRH was not effective in facilitating L/M or PREF in animals bearing VMH or MPO lesions. LHRH was effective in restoring L/M in MCG-lesioned animals, but did not increase PREF. Results indicate that exogenously administered LHRH may act through the MPO or VMH but not through the MCG and that sexual receptivity and sexual motivation can be dissociated by LHRH treatment. Supported by HD 11814.

316.6 ESTRADIOL INDUCES HYPOTHALAMIC PROGESTIN RECEPTORS AND LORDOSIS IN 2 TO 12 DAY OLD MALE AND FEMALE RATS. C.L. WILLIAMS, D. LORANG* AND J.D. BLAUSTEIN. Dept. Psychol., Barnard College, NY, NY 10027, and Div. Neurosci. and Behav., Univ. Mass., Amherst, MA 01003.

Both male and female neonatal rats show lordosis in response to tactile stimulation of the flanks and rump if they are deprived of their dam for 3-4 hrs and tested in a warm (33 ± 2°C), moist incubator. Moreover, these responses are facilitated by estradiol benzoate (EB). If 4-day-old infants are treated with 100 ug/10 g b.wt. EB 44 hrs prior to behavioral testing, lordosis frequency, duration and intensity are increased in females and lordosis duration is increased in males. Interestingly, lordosis frequency and intensity in unprimed males tends to be higher than in unprimed females. By 12 days of age lordosis is rarely displayed unless pups have been primed with EB, and at this time a sex difference in responsivity to estradiol emerges.

In adult rats induction of lordosis by estradiol has been associated with induction of hypothalamic progestin receptors. Therefore, we examined the development of estradiol inducible hypothalamic progestin receptors using hormone doses, timing and protocols optimal for the display of lordosis in the neonate. Male and female infants (2-, 4-, and 10-day-olds) were primed with either 100 ug EB/10 g b.wt. or oil, and 44 hrs later tissues from the MBH-POA and cortex were dissected out, frozen and then assayed for the presence of progestin binding sites. Although the concentration of receptors is lower in infant than adult brain, EB caused a significant induction of cytosol progestin receptors in MBH-POA, but not cortex, at all ages. Sex differences in induction did not begin to emerge until 12 days of age. The concentration of progestin binding sites was significantly higher in the MBH-POA of unprimed males than unprimed females at 4 and 6 days of age, but not at 12 days of age.

These data suggest: 1. Activational actions of estrogens on brain and behavior can occur as early as 2 days of age, when estrogens are organizing brain tissues mediating sexually dimorphic behaviors. 2. In infant males endogenous steroids, perhaps of testicular origin, may facilitate sexual behavior and induce progestin receptors. 3. Sex differences in estrogen inducible progestin receptors and behavior emerge at around 12 days of age.

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316.7 DIFFERENCES IN HYPOTHALAMIC SITES OF PROGESTIN ACTION ON AGGRESSION AND LORDOSIS IN FEMALE HAMSTERS. R.L. Meisel, I.G. Fraile*, and D.W. Pfaff. Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

Studies on social and sexual behaviors in Syrian hamsters have demonstrated that there is an inverse relationship between levels of aggression and sexual receptivity over the duration of the female's estrous cycle. In laboratory studies, low doses of estradiol have no effect on aggression or receptivity (lordosis), while subsequent administration of progesterone both inhibits aggression and facilitates lordosis responding. Given the effects of systemic progesterone on these behaviors, we have begun to examine the role of neural progestin receptors in mediating these behavioral changes.

Adult female hamsters, singly-housed, were ovariectomized and implanted bilaterally with stainless steel cannulae (23 g) aimed at the ventromedial hypothalamus (VM) or the caudal anterior hypothalamus (AH) near the VM. Experimental females were tested with a group-housed, ovariectomized female hamster (aggression test), followed an hour later by a test with a group-housed, intact male hamster (lordosis test). This procedure, conducted within the experimental female's home cage, maximized the observation of a female's potential for both aggression and lordosis within a single test session.

One week after ovariectomy and cannulae implantation, females were tested for their baseline levels of aggression and lordosis. Following this test session, all females were injected sc with 10µg estradiol benzoate (EB). About 46 h after EB injection, females were tested for their responsivity to EB alone. Following this test session, females received 27 g applicator cannulae containing a 10% crystalline mixture of the synthetic progestin R5020 (diluted with cholesterol) in the VM (N=7), AH (N=8), or implants of cholesterol (N=7) in the VM or AH, and tested about 6 h later.

EB alone had no effect on aggression or lordosis. R5020 implanted in the VM, but not AH, significantly ($p<.01$) reduced the number of attacks against stimulus females, as compared with the prior test following EB. Cholesterol-implanted females showed no significant change across tests in the number of attacks.

In tests with males, intracranial R5020 stimulated lordosis in 4/8 AH-implanted females, albeit with a low mean lordosis duration (40 sec of a 180 sec test). VM implants of R5020 did not stimulate lordosis in any female (0/7). Similarly no cholesterol-treated female (0/7) showed lordosis.

These results demonstrate that intracranial implants of the synthetic progestin, R5020, can inhibit aggression and facilitate lordosis in estradiol-treated female hamsters. Furthermore, it appears that there are differences in the hypothalamic sites of progestin action on these behaviors.

316.8 CYSTEAMINE INJECTED SYSTEMICALLY INHIBITS LORDOSIS. C. V. Mobbs, J. M. Rothfeld, and D. W. Pfaff. (SPON: Z. M. Wenzel) Rockefeller University, New York, NY 10021.

The female sexual behavior, lordosis, can be stimulated by infusing prolactin (PRL) into the midbrain central grey (MCG), and can be inhibited by infusing antiserum against prolactin (Harlan et al., 1982). Furthermore, immunoreactive prolactin (irPRL) can be detected by immunocytochemistry in cell bodies of the medial basal hypothalamus, and nerve endings in the MCG (Harlan et al., submitted). However, it is not known if irPRL in brain is identical to PRL from pituitary; furthermore, the biochemical mechanism by which PRL influences lordosis is unknown. Cysteamine (2-mercaptoethylamine; 100 mg/kg injected s.c.) reduces immunoreactive and bioactive PRL from pituitary and serum by greater than 80%. Cysteamine also reduces somatostatin in brain, but does not affect levels of LHRH or several other peptides. If brain irPRL is identical to pituitary PRL, then cysteamine should reduce lordosis; furthermore, depletion of brain irPRL, followed by controlled infusion of PRL, might provide a useful model for studying the mechanism by which PRL influences behavior. We therefore examined if systemic or central administration of cysteamine can block lordosis. Female Sprague-Dawley rats were ovariectomized, and given silastic implants containing estradiol. After 2 weeks rats which exhibited lordosis (manual reflex score ≥ 2 on a 0-3 scale) were given a single s.c. injection of saline (n=4), or cysteamine (90 mg/kg,n=6; or 270 mg/kg, n=5). Saline had no effect on lordosis. Lordosis scores in rats given 90 mg/kg declined from 2.3 ± 0.1 (mean ± SEM) before injection to 0.6 ± 0.4 24 h after injection ($p=0.008$, Mann-Whitney). Rats given 270 mg/kg declined from 2.6 ± 0.2 to 1.6 ± 0.4 ($p=0.018$, Mann-Whitney). Cysteamine did not influence righting, foot withdrawal, or startle reflexes. In most rats lordosis was lowest 1-3 days after cysteamine injection, and recovered fully by 10 days after injection. In a second study, female Long-Evans rats were ovariectomized (n=5), given estradiol implants, and fitted with bilateral cranial cannulae placed into the dorsal MCG. Infusion of 50 ug cysteamine in 2 ul solution into the MCG elicited no behavioral change. Infusion of 500 ug cysteamine into the MCG induced intervals of vocalization, running and defensive behavior, but no effect on lordosis. One week after infusion, injection of cysteamine s.c. (90 mg/kg followed, 24 h later, by 270 mg/kg) reduced lordosis from 2.9 ± 0.05 to 0.6 ± 0.4 ($p=0.004$, Mann-Whitney). These studies suggest that although cysteamine can inhibit lordosis, the site of action does not appear to be the MCG. Whether cysteamine reduces lordosis by depleting brain irPRL, for example in cell bodies of the medial basal hypothalamus, remains to be determined.

316.9 PROLACTIN REGULATION OF MATERNAL BEHAVIOR IN FEMALE RATS: BROMOCRIPTINE TREATMENT DELAYS AND PROLACTIN TREATMENT REINSTATES THE RAPID ONSET OF BEHAVIOR. R. S. Bridges and P. M. Ronsheim*. Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, MA 02115.

Recent findings have indicated that prolactin (PRL) helps stimulate the onset of maternal behavior in inexperienced, hypophysectomized, steroid-treated female rats (Bridges et al., Science, 227:782, 1985). In a series of four experiments we have further examined the involvement of PRL in maternal behavior using nonhypophysectomized rats. In Expt. #1 we measured plasma PRL levels in ovariectomized (ovx) rats that were treated for two weeks with a combination of progesterone (P, 3 x 30 mm Silastic implants, days 1-11) and estradiol (E2, 2 mm implant, days 11-13) that effectively stimulates maternal behavior in nulliparious rats (Bridges, Endocrinology 114:930, 1984). PRL concentrations remained low through the duration of P exposure. In contrast, plasma PRL levels exhibited daily diurnal (1700 h) increases from days 11-13 following P implant removal and E2 insertion (1000 h on day 11). In Expt. #2, ovx, steroid-treated (P/E2) rats were administered bromocriptine, CB-154, at a dose of 2 mg/kg or 0.5 mg/kg or vehicle twice daily (0900 & 1700 h) from day 11 to 13 of steroid treatment. The diurnal rise in plasma PRL found in the vehicle-treated rats (300-400 ng/ml) was suppressed by both doses of CB-154; total suppression was evident in rats given 2.0 mg/kg CB-154. In Expt. #3, the effects of CB-154 (2.0 mg/kg) treatment on the behavioral responsiveness of steroid-treated, ovx, nulliparous rats towards foster young were measured. Testing for maternal behavior was conducted daily from treatment days 12-22. CB-154 treatment (twice daily, days 11-22) significantly delayed the onset of maternal behavior. The latency to display full maternal behavior was 5.5 days for CB-154 treated rats, while it was 2.0 and 3.0 for noninjected and vehicle injected controls, respectively. In the final study (Expt. #4), the behavioral effects of PRL administration were measured in CB-154 plus steroid (P/E2) treated, ovx, nulliparous rats. Prolactin injections (500 ug Ovine PRL x twice daily, days 11-22) reversed the disruptive behavioral actions of twice daily CB-154 (2.0 mg/kg) treatment. The median latencies of the CB-154, CB-154 & PRL, and vehicle control groups to exhibit full maternal behavior towards foster young were 5.5, 2.0, and 1.0 days, respectively. These data demonstrate an involvement of prolactin in the induction of maternal behavior in the steroid-treated, nonhypophysectomized, nulliparous rat, and lend further support for a role for prolactin in the induction of maternal behavior in the parturient female. Supported by March of Dimes Birth Defects Foundation Grant #1-895, NIH #HD19789 and NIMH RSDA #KO2 MH00536 awarded to RSB.

HUMAN BEHAVIORAL NEUROBIOLOGY

317.1 NEUROPSYCHOLOGICAL CORRELATES OF THE FRONTOCAUDATE "COMPLEX LOOP" IN NEUROLOGICAL PATIENTS.
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It is now recognized that the effects of basal ganglia dysfunction are best understood by tracing efferent connections through the pallidum, nigra, and thalamus to the cortex (Alexander et al, Ann Rev Neurosci, 9: 357, 1986).

In Parkinson's disease, motor symptoms have been related to the supplementary motor cortex (motor loop) however, the functional manifestations attributable to caudate dysfunction have not been elucidated. Neuroanatomical studies have indicated a prefrontal cortical focus for those circuits (complex loop).

We have studied 150 patients with idiopathic Parkinson's disease and 50 matched normal control subjects using an extensive battery of neuropsychological tests. After ruling-out the complicating factors of depression, concurrent dementia, drug toxicity, education, age, and gender, a small cluster of cognitive deficits was defined (Taylor et al., Brain 109: 279, 1986 and Brain, 109: in press).

Patients were selectively impaired on 1) the ability to form mental sets; 2) delayed recall of spatial position; 3) supraspan verbal learning; 4) simulteneous motor programming; and 5) serial order recall. All of these processes have been related to prefrontal cortical integrity. Care was taken to ensure that the patient's motor deficits did not interfere with their ability to perform the required tasks. Degree of cognitive impairment did not necessarily correlate with severity of motor impairment thus indicating a functional dissociation between the complex and motor loops. Patients who experienced secondary treatment failure were more severely impaired cognitively but only within the domain of frontal lobe-based functions. These findings, which suggest that the caudate nucleus may contribute to the recognition of task-specific stimulus meaning, are consistent with unit recording studies (Rolls et al Behav Br Res, 1983 7: 179).

Preliminary results using a test of procedural learning based on the Tower of Hanoi have indicated impaired performance in patients with Parkinson's disease, Huntington's disease, and Wilson's disease, as well as in patients with damage restricted to the frontal lobes. These data support the hypothesis (Mishkin et al, Neurobiology, Learning, and Memory, Guilford press, 1984: 65) that the integrity of the frontocaudate complex loop may be necessary for the establishment of cognitive habit patterns.

317.2 **CORTICAL PHYSIOLOGICAL ACTIVATION IN ALZHEIMER'S DISEASE: rCBF STUDIES DURING RESTING AND COGNITIVE STATES.** K.F. Berman and D.R. Weinberger*. Section on Clinical Neuropsychiatry, NIMH, Saint Elizabeth's Hospital, Washington, D.C. 20032.

New techniques for measuring regional brain physiology in vivo may be useful adjuncts for diagnosing neurodegenerative disorders such as Alzheimer's Disease (AD) and for understanding the pathophysiology underlying them. However, previous studies have not been consistently successful in distinguishing patients from elderly controls.

In the current study fifteen patients with AD (mean age 63 years, range 55-68) and 10 normal control subjects matched for age, sex, and education had regional cerebral blood flow (rCBF) measured by the xenon-133 inhalation method. rCBF was determined during three different testing conditions: first during an eyes-closed resting state and then during two cognitive activation tasks, one an automated version of Raven's Matrices (RM), a task linked to posterior association cortex, and the other a similarly automated Number Matching (NM) baseline task. The order of the two activation tasks was counterbalanced across subjects.

During all three testing conditions, gray matter rCBF for every cortical area averaged 20% lower in patients with AD than in normal subjects ($p<.03$). However, during RM, both groups increased posterior rCBF over that during the baseline NM task. During the RM and the NM, but not during resting, clinical status (as assessed by the Mini-Mental State exam) correlated with rCBF values for several cortical areas.

Discriminant function analysis was performed on the rCBF values derived during each task to create a mathematical model that best differentiated the patients from the normal group. Each model was then retrospectively applied to classify each subject into one of the two subject categories (patient vs. normal), and the relative success rates for each model were assessed.

In the retrospective discriminant function analysis, rCBF for each of the three testing conditions correctly classified the majority of the patients and normals into the appropriate diagnostic category. Compared to the resting state, cognitive activations improved the ability of the rCBF models to distinguish the two groups (during resting 89% of the normals and 79% of the patients were correctly classified; during RM 89% of the normals and 89% of the patients; and during NM 93% of the normals and 89% of the patients). Discriminant function models derived from rCBF during all three conditions retrospectively classified subjects with 100% accuracy.

These data are consistent with previous reports of decreased coritcal activity in patients with AD. These findings further suggest that measuring coritcal activity during several different conditions, including cognitive activation, improves the ability of physiological measures such as rCBF to differentiate patients with AD from controls.

317.3 POST-STROKE MOOD DISORDERS: DYNAMIC CHANGES IN ASSOCIATED VARIABLES OVER TWO YEARS OF FOLLOW-UP.
R. M. Parikh, T. R. Price and R. G. Robinson, (SPON: Paul R. McHugh), Johns Hopkins Univ Sch Med

As part of a prospective study of 103 stroke patients, we have analyzed the relationship between several variables which may be associated with the development of depression at regular follow-up intervals of 3, 6, 12 and 24 months post-stroke.

We have previously reported that although lesion location was the single most important variable in determing the severity of depression ($r \simeq .7$)1,2,3, between in-hospital and six months follow-up there was an increasing relationship between depression and other variables such as activities of daily living as measured by the Johns Hopkins Functioning Inventory (r increased from .32 in-hospital to .56 at 6 months), cognitive function as measured by the Mini-Mental State (r increased from .29 in-hospital to .45 at six months), social functioning as assessed by the Social Functioning Exam (r increased from .25 in-hospital to .50 at 6 months)3. At one and two years follow-up, we found that the strength of the relationship between depression and other variables had generally declined (the strength of the correlation between Hamilton Depression Scale scores and the JHFI scores declined from .64 at 6 months to .56 at 12 months and .31 at 24 months). Similarly, the strength of the correlation between cognitive function and depression had declined (from .44 between mini-mental scores and Hamilton Depression Scale scores at 6 months to .28 at 12 months and .12 at 24 months).

Since other follow-up analyses of this patient group had found that the natural (untreated) course of major depression in these patients was about one year in duration, these decreases in correlation between 6 months and 2 years follow-up parallel the natural course of improvement in patients who are acutely depressed after stroke.

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317.4 RELATIONSHIPS BETWEEN DEPRESSION AND COGNITION IN STROKE PATIENTS.
J. D. Boston*, Karen Bolla-Wilson, E. Kaplan, and R. G. Robinson. (SPON: Larry Tune). Dept. of Psychiatry, The Johns Hopkins Univ Sch Med, Balt., MD. 21205

Previous research in stroke patients has demonstrated a relationship between depression and cognition. Using the Mini-Mental Status Exam (MMSE) we found that patients with major depression (MD) had a greater degree of cognitive impairment than comparable patients with lesions of the left hemisphere even though the two groups had comparable sizes. The present research attempts to further clarify the nature of cognitive differences between major depression and non depressed stroke patients by examining their performance on a variety of neuropsychological measures to determine which functions are most affected by depression.

Subjects were 65 patients, with no previous history of brain trauma, stroke, or psychiatric illness. Patients were predominantly in their 50-60's, black, right handed, and of a lower socioeconomic class. Using DSM III criteria groups with depression or no depression were established.

On demographic variables no differences were found between groups. Neurological deficits were evenly distributed across groups. Using multiple t-tests with a Bonferroni correction between group comparisons were made on the individual tasks of a neuropsych battery. Among patients with left hemisphere (LH) (N=33) those with major depression (N=11) performed less well than non-depressed patients on all 35 cognitive tasks. The differences were statistically significant on 9/35 subtests. Using measures of depression and performance on neuropsychological tasks, the relationships between severity of depession and cognitive deficits were ascertained. In the left hemisphere group severity of depression correlated inversely and reached statistical significance on 15 neuropsych subtests. The right hemisphere (RH) group (N=32) with major depression (N=7) compared with the nondepressed group performed less well on 16/35 cognitive tasks but none of the differences reached significance.

These results suggest that major depression associated with left hemisphere stroke significantly impacts on all aspects of cognition including functions which are generally "right hemisphere tasks" as well as "left hemisphere tasks". The effect of depression on cognition associated with right hemisphere injury is less clear. These findings which demonstrate the significant impact of depression on all aspects of cognitive function underline the importance of recognition and treatment of depression following stroke.

317.5 FOCAL BRAIN ACTIVITY DURING VISUAL LANGUAGE TASKS AS MEASURED WITH AVERAGED PET IMAGES OF EVOKED CBF CHANGE
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We identified language-related brain areas using measurements of regional cerebral blood flow from 12 normal volunteers. Responses to visually presented words provided a simple, readily controlled, but potent means of activating brain areas involved in reading silently, reading aloud, and producing words with a semantic relation (a use) to a cue word (an object).

Four conditions were performed by all subjects: 1) eyes-closed resting state; 2) passive observation of visually presented words (names of objects); 3) vocal repetition of visually presented object names; 4) saying a use for a visually presented object name. Gaze was stabilized with a fixation point. Stimulus duration (150 msec) and inter-stimulus interval (850 msec) were invariant.

Each image underwent inter-slice interpolation to create a data array with uniform pixel size (2 mm) in all 3 axes. Images then were translated into a standardized, stereotactic coordinate space (J. Comput. Assist. Tomogr. 9:141-153, 1985) and combined pixel-for-pixel into a single, averaged image for each condition. Focal blood flow changes induced by specific aspects of the langugage tasks were identified by image-to-image subtraction of selected pairs of averaged images. Brain structures corresponding to these areas of CBF change were identified in a stereotactic atlas.

Passive words (minus eyes closed) activated striate and extrastriate visual cortex, without apparent lateralization. Repeating words (minus passive words) activated: 1) inferior rolandic cortex (sensorimotor mouth), L > R; 2) supplementary motor area (SMA); 3) anterior, superior premotor cortex, L >> R; 4) caudate head; 5) cerebellum, R > L. Generating uses (minus repeating words) activated: 1) anterior cingulate gyrus; 2) left anterior, inferior frontal cortex; 3) SMA.

SMA activity was greater while generating uses than during word repetition, suggesting that SMA activation was not solely motor. This was confirmed when silently monitoring for semantic relations (N = 5) also activated SMA. Anterior cingulate was not activated by this monitoring task.

317.6 Evidence for Abnormal Serotonergic Function in Obsessive Compulsive Disorder.
W.K.Goodman, L.H.Price, S.A. Rasmussen, D.S.Charney, S.W.Woods*, G.R.Heninger., Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT., 06508.

The hypothesis that an abnormality of serotonin (5-HT) function is related to the etiology of obsessive compulsive disorder (OCD) is based primarily on satisfactory treatment response to antidepressants with potent 5-HT uptake blocking effects. In order to further assess the role of 5-HT function in OCD, patients were treated with the potent and selective 5-HT uptake inhibitor fluvoxamine and the biochemical effects of 5-HT stimulation were measured in drug-free OCD patients. Prolactin response to the intravenous administration of the 5-HT Type I receptor agonist 1-3-chlorophenylpiperazine (MCPP) was compared in patients and age- and sex-matched healthy subjects. METHODS: TRYP and MCPP tests: Patients (ages 20 to 48) and healthy subjects received 7.0 gm of L-tryptophan (n=15, 3 males), and .1 mg/kg of MCPP (n=13, 3 males), and placebo MCPP on 3 separate test days. Subjects fasted overnight and had a catheter inserted at 8:30 AM. Repeated blood sampling was carried out before and after the infusion. Plasma prolactin (PRL) was assayed with standard RIA methods. Fluvoxamine Trial: Ten inpatients (ages 20 to 58, 4 males) initially received placebo for at least 2 weeks and then were treated with fluvoxamine (max. dose = 300 mg daily). Treatment response was determined by scores on the global improvement subscale of the clinician global impression (CGI) scale with a change at 4 weeks from placebo baseline of "much improved" or better defining a patient as a "responder". Nurse raters and patients were blind to treatment conditions. RESULTS: Baseline PRL levels were significantly lower in patients compared to healthy subjects on the TRYP (2.8 ± 1.9 v 6.8 ± 5.0 ng/ml, $p<.01$) and MCPP (2.8 ± 1.5 v 5.2 ± 3.7 ng/ml, $p<.04$) test days. Prolactin increased significantly less at 5 out of 7 sample times in the patients (peak increase = 5.3 ± 3.7 ng/ml) than the healthy subjects (peak increase = 10.9 ± 9.2 ng/ml, $p < .04$) after MCPP compared to placebo administration. In contrast, although there was an increase in PRL levels at all sample times following infusion of TRYP, the patient-control differences were not significant. Preliminary results with fluvoxamine were very promissing with 7 out of 10 patients rated as "responders". DISCUSSION: Clinical improvement on fluvoxamine and a blunted PRL response of patients to MCPP suggests that a primary abnormality of 5-HT function or a disturbance of other neuronal systems regulating 5-HT activity may be involved in the pathogenesis of OCD. Further studies are needed to confirm these findings and to define the specific site of 5-HT dysfunction.

317.7 ABNORMAL REGULATION OF NORADRENERGIC FUNCTION IN PANIC DISORDERS
D.S.Charney, S.W.Woods*, W.K.Goodman, G.R.Heninger, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT., 06508.

There is extensive preclinical and clinical evidence suggesting that the pathophysiology of some forms of panic anxiety disorders is related to abnormal regulation of brain noradrenergic function. In a series of investigations in healthy subjects and panic disorder patients, noradrenergic function was evaluated by determining the behavioral, biochemical, and cardiovascular effects of the alpha-2 adrenergic agonist, clonidine, and the alpha adrenergic antagonist, yohimbine.

METHODS: Each patient or healthy subject had two test days. Placebo was given on one test day and yohimbine (20 mg po or clonidine 0.15 mg i.v. over 10 minutes) on the other. Plasma was obtained from blood samples drawn through an indwelling intravenous catheter, both before and at several time points following drug administration. Plasma MHPG was determined by gas chromatography and mass spectrometry. Plasma growth hormone and cortisol was measured by RIA. Visual analogue scales and a panic attack symptom scale were used to evaluate changes in behavior and physical symptoms. Platelet ^{3}H-yohimbine binding to platelet membranes was also determined.

RESULTS: Yohimbine produced panic attacks meeting DSM-III criteria in 37 of 68 panic disorder patients and only 1 of 20 healthy subjects. The patients reporting yohimbine induced panic attacks had significantly larger increases in plasma MHPG, cortisol, systolic blood pressure, and heart rate than the healthy subjects. Panic disorder patients (N=9) had a significantly decreased maximal number of yohimbine binding sites (Bmax) but normal affinity (Kd) in comparison to matched healthy subjects.

In a separate group of 26 panic disorder patients, clonidine produced significantly greater decreases in plasma MHPG levels and sitting and standing diastolic blood pressure and smaller increases in growth hormone and self-rated drowsiness in the patients compared to 21 healthy subjects.

IMPLICATIONS: These findings indicate that the regulation of noradrenergic activity is aberrant in some panic disorder patients. Similar effects of clonidine and yohimbine have not been observed in patients with schizophrenia, depression, generalized anxiety, or obsessive compulsive disorder thereby supporting the specifity of the present findings. The increased dynamic range of noradrenergic activity observed as an increased sensitivity to both clonidine and yohimbine may reflect abnormalities in the regulatory inputs to noradrenergic neurons, or dysfunction in the alpha-2 adrenergic receptor effector coupling mechanism or the intracellular effector system.

317.8 STIMULATION OF LOCUS COERULEUS IN MAN, VIA CHRONICALLY IMPLANTED ELECTRODES. B. Libet, B. Feinstein*, and C.A. Gleason*. Mt.Zion Hospital, Univ.Calif.,San Francisco, CA. 94143, and Stanford Res.Inst.,Palo Alto, CA.

Bipolar electrodes were chronically implanted by B.F. in 1977, in the anterior end of locus coeruleus (LC) for possible therapeutic relief in conditions involving hyperexcitable neuronal states. Of the 3 patients implanted, 1 had cerebral palsy with spastic quadriplegia (18-yr male), 1 had "psychomotor epilepsy" (26-yr female) and 1 had grand mal epilepsy (10-yr female),all intractable. Therapy was based partly on pharmacological reports and on our demonstration that stimulation of LC in rats could suppress subconvulsive seizure activity (Libet et al.,Epilepsia 18:451,1977).Effectiveness of LC excitation was checked by MHPG in jugular and systemic blood after stimulation (measured by Angelos Halaris).

Spasticity was and still is distinctly lessened clinically in the palsy patient; it returns to stronger levels with discomfort when the stimulator is not in use or nonfunctional for a day. Stimulation for therapy consists of 100 μsec pulses, 50 to 60 pps at 0.1 ma, 0.5 sec on and 1 sec off, for 6 min; sequence is repeated each 100 min. (Stimuli are delivered to a subcutaneous RF receiver coil via an overlying external transmitter coil connected to a portable stimulator box.) In a separate sleep study on this patient (Kaitin et al.,Biol. Psychiat.21,in press,1986), such LC stimulation paradoxically markedly reduced both REM and non-REM sleep, with increased wakefulness at night. (Clinical results on the other 2 patients also seemed positive but were more difficult to assess.)

LC stimulation in these human subjects never produced any indication of anxiety or other mental discomfort or disturbance, in contrast to reports for monkeys (e.g., Redmond et al.,Brain Res. 116:502,1976). It is possible that some of the routine medications may have affected any anxiety responses in the patients. The psychomotor epileptic, the only verbally articulate patient, repeatedly reported her thinking became clearer and with better concentration.

The results in these few human subjects suggest that LC activity may inhibit some generalized hyperexcitable neuronal states, and also may produce some generalized arousal. Clinical results with the one case of intractable spasticity are at least encouraging. (Supported in part by Mt.Zion Hosp.Med.Center Res.Funds and USPHS NS-00884.)

317.9 PERCEPTUAL SKILL LEARNING AND REPETITION PRIMING FOR NOVEL MATERIALS IN AMNESIC PATIENTS, NORMAL SUBJECTS, AND NEURON-LIKE NETWORK MODELS. N.J. Cohen*, I. Abrams*, W.S. Harley*, L. Tabor*, B. Gordon*, & T.J. Sejnowski. Departments of Psychology, Biophysics, & Neurology, The Johns Hopkins University and School of Medicine, Baltimore, MD, 21218.

Recent memory research has distinguished between the incorporation of experience into skilled performance and explicitly stored memory for particular processing episodes. Investigations of skilled performance have indicated both the generalizability of skill to new materials within the trained domain and the further superiority of performance for repeating materials (repetition priming effects). This is particularly clear in amnesic patients who, despite profound disorders in recall and recognition, nonetheless demonstrate both intact skill learning and repetition priming. The same dissociation between classes of memory can also be observed in normal subjects with the appropriate tests. Such findings have important implications for understanding the organization of normal memory systems, and have played a prominent role in several different theoretical accounts of memory.

Some accounts, most particularly those invoking a distinction between episodic and semantic memory, have predicted that the memory system preserved in amnesia could support a facilitation of performance based on experience only for familiar materials, i.e., materials for which there are already-established representations. The present studies investigated the acquisition of skills and repetition priming effects for novel or unfamiliar materials in amnesic patients, normal subjects, and neuron-like network models. In a series of studies involving detection of the axis of symmetry in mirror symmetric checkerboard-based visual patterns, each of three amnesic patients showed acquisition and retention of perceptual skill within and across daily testing sessions; and, extended testing with two of these patients also showed repetition priming effects that were at least as large as those shown by normal subjects. Parallel studies with quasi-neural, massively-parallel network models, in which no prior knowledge was built in about symmetry detection, topography, or checkerboard-based patterns, thus requiring completely de novo learning, showed both skill learning and repetition priming. Indeed, repetition priming could be demonstrated with as few as 6 presentations of the primed stimuli spread out among 20,000 visual patterns.

These findings suggest that skill learning and repetition priming are aspects of the basic, procedural learning mechanisms that serve to configure and reorganize processing modules as a consequence of experience.

317.10 EFFECTS OF SELECTIVE ATTENTION ON AUDITORY AND VISUALLY EVOKED FIELDS. L.Kaufman, S.Curtis*, B. Luber* and S.J.Williamson. Neuromagnetism Laboratory, Departments of Physics and Psychology, New York University, New York, NY 10003.

In one experiment subjects were given a dichotic listening task, and neuromagnetic fields associated with the stimuli to which they attended were compared with fields recorded from the same places outside the scalp that were related to concurrent but ignored stimuli. As in event related potentials (ERPs), the stimuli to which attention was paid resulted in relative enhancement of the neuromagnetic counterpart of the N1 component of the ERP. It also resulted in relative enhancement of the counterpart to the P2 component. The field was measured at about 60 different positions on each side of the head during this task. The locations of the best fitting equivalent current dipoles for the sources of N1 and P2 deduced from these data were in or near the primary auditory cortex, as we have found in other studies where attention was not manipulated. The present experiment thus demonstrates that attention affects activity in the vicinity of the primary projection area. The positions and orientations of these sources suggest that they can account for a significant portion of the variance in the electrical N1 and P2 recorded near the vertex.

In a similar experiement subjects attended to one pattern of bars in one quadrant of the visual field while ignoring another pattern in another quadrant on the ipsilateral side. Again, responses to the attended pattern resulted in much enhanced N1 and P2 components. However, the equivalent current dipole sources of these visual components were located in or near the visual cortex, apparently deep within the longitudinal fissure. Also, the sources of these two components seem to be in separate locations. Thus, attention has effects in different regions of the brain, depending upon the modality stimulated. The implications for theories of selective attention will be described.

317.11 EARLY DECLINE OF LOGICAL REASONING SKILLS IN ALZHEIMER'S DISEASE. A. Cronin-Golomb, S. Corkin, and J.H. Growdon. Dept. of Brain and Cognitive Sciences & Clinical Research Center, MIT, Cambridge, MA 02139, and Dept. of Neurology, Mass. Gen. Hosp., Boston, MA 02114.

Logical reasoning skills are at the core of intellectual functioning, and a decline in these skills is evident clinically in certain disease states of the brain, including Alzheimer's disease (AD). For neurological populations with language disorders, e.g., AD, it is useful to assess reasoning ability nonverbally. The Hukok Logical Thinking Matrices Test was developed to measure reasoning nonverbally and apart from visual discrimination factors (Darin, 1977). The test uses squares, circles, and triangles of 3 colors and 3 sizes. Eight stimuli and one blank appear in a 3x3 matrix. A response array of 9 to 12 items appears below the matrix, and the subject verbally or manually indicates which item completes the pattern established by the 8 stimuli of the matrix.

The Hukok matrices were presented for a prolonged duration (up to 30 seconds) to each hemi-field of three complete commissurotomy subjects (LB, NG, and AA). Mean scores for the three subjects fell within the normal range. No significant hemisphere difference in mean scores was seen (left hemisphere 5, right 6). This result supports the premise that the Hukok test, unlike the widely used Raven's Colored Progressive Matrices, does not involve visuospatial factors that may favor right hemisphere performance. The Hukok test may therefore be a test of logical reasoning abilities that is not directly tied to language or visuospatial proficiency.

The Hukok test has now been used to examine logical reasoning abilities in a variety of neurological populations, including AD (N=17), Parkinson's disease without dementia (N=8), cingulotomy (N=4), global amnesia (N=5), left (N=5) or right (N=8) penetrating head injury, and left hemispherectomy (N=2). Age-matched control subjects were also given the test (N=23). For all subject groups except AD, scores were normal ($\bar{X}$=6), indicating that test performance was uninfluenced by relatively discrete disorders of language, memory, visuospatial, or motoric abilities. In contrast, the AD group performed very poorly on the test ($\bar{X}$=2.6). Performance correlated negatively with scores on the Blessed Dementia Scale (BDS). It is noteworthy that even those subjects who were mildly demented (BDS<10) attained Hukok scores that were well below the normal range. The findings suggest that logical reasoning skills, as measured by this test, are among the cognitive functions to be disrupted early in the course of AD.

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317.12 LONG INTERVALS BETWEEN STIMULATION OF MEDIAN NERVE EVOKES P300-LIKE PEAK IN HUMAN SOMATOSENSORY EVOKED POTENTIALS. D.M. Chandross* B.H. Pomerans, and A. Kalpin.* Ramsay Wright Zoological Laboratories Univ. of Toronto, Toronto, Ontario, M5S 1A1.

We studied some of the late potentials on the scalp elicited by somatosensory stimulation of the hand and median nerve in normal human subjects. We compared the effects of presenting stimuli at a long interstimulus interval (long ISI)(1/18 sec., 0.05 Hz.) with those with a short interstimulus interval (short ISI)(1/2 sec., 0.5 Hz) on a late potential with a peak latency of 286 msec.(P286). We compared these data to those obtained by the oddball paradigm to evoke P300. Our data showed that the late potential (P286) can be evoked by increasing the ISI to 18 seconds.This P286 is greatly attenuated or absent when the ISI is decreased to 2 seconds.There is no attenuation or loss of earlier components N1,P2 or N2 which accompanies this frequency-dependent response decrement of P286. When randomised stimuli are given with an ISI of 2 seconds in the oddball paradigm (OP), large P300's are evoked which are similar to P286.Counting of the unrandomised long ISI or short ISI stimuli fails to affect the amplitude of P286. This amplitude is merely a function of ISI if task demand and equivocation are not emphasised in the experimental design(unlike the OP). Reliable late positive potentials can be evoked with long ISI or with the use of the OP. The long ISI technique of obtaining P286 is a new one and it may allow the study of late potentials without the use of the OP. It is easier, more reliable and more objective to increase the ISI rather than to ask a subject to focus attention. This model may prove to be a useful one for studying the interaction of habituation, attention and human information processing in the somatosensory system.

INVERTEBRATE NEURODEVELOPMENT II

318.1 EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT OF THE SEROTONIN PHENOTYPE IN *HELISOMA*. J.I. Goldberg*, P. Natvig* and S.B. Kater. Dept. of Biology, University of Iowa, Iowa City, IA 52242 and Program in Neuronal Growth and Development, Colorado State Univ., Fort Collins, CO 80523.

Recent experiments utilizing *Helisoma* embryos have supported the hypothesis that serotonin regulates the morphology and synaptic connectivity of specific identified neurons during development (Neurosci. Abstr. 11: 158, 1985). A corollary to this hypothesis is that development of neuronal form and connectivity depends in part on the precise temporal and spatial expression of the serotonin phenotype. In the present study, the number and location of serotonergic cells throughout embryonic and postembryonic development of the *Helisoma* CNS were determined by wholemount immunohistofluorescence.

Embryonic stages are expressed as a percentage of the total duration of embryogenesis (9 days at 24 °C). Serotonin-like immunoreactivity (SLI) was first detected within the soma and axon of a bilateral pair of cells at stage 20%. These cells were identified as adult neuron C1 on the basis of their extremely large size, transmitter phenotype, projections and ultimate location within the paired cerebral ganglia. Between stages 35%-45%, additional SLI was observed bilaterally in 2 smaller cells adjacent to each neuron C1 and in 2-3 cells within each of the paired pedal ganglia. At stage 55%, by which time the larval embryo has metamorphosed into a juvenile form, each cerebral ganglion contained a single cluster of 4-5 stained cells, while each pedal ganglion contained 8-12 serotonergic cells oriented along an anterior-posterior axis. At stage 70%, 2 distinct clusters of serotonin-immunoreactive cells within each cerebral ganglion were observed. Also at this stage, SLI within the posteriorly-located parietal and visceral ganglia was first detected in 5-10 cells. **Therefore, the number of cells that express the serotonin phenotype increased throughout embryogenesis.** Furthermore, the embryonic stage at which SLI was initially detected was distinct for each ganglion type.

Three postembryonic stages were also examined to determine whether development of the serotonin phenotype progresses beyond embryogenesis. Neonatals (2-4 days post-hatching), juveniles (sexually immature; 3 mm verticle shell height) and sexually-mature snails (10 mm verticle shell height) were studied. From neonatal to juvenile stages, there was a significant increase in the number of pedal and cerebral cells expressing SLI. Moreover, from juvenile to mature stages, the number of serotonin-immunoreactive cells increased in the cerebral, buccal, pleural and parietal ganglia, but not in the pedal and visceral ganglia. **Therefore, development of the serotonin phenotype is not limited to embryogenesis, but continues beyond hatching for a period determined in a ganglion-specific manner.**

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318.2 DIFFERENTIATION OF AN IDENTIFIED PAIR OF PEPTIDERGIC NEURONS IN THE STOMATOGASTRIC NERVOUS SYSTEM OF THE MOTH, MANDUCA SEXTA. P.F. Copenhaver and P.H. Taghert, Dept. Anat. & Neurobiol. Washington U. School of Medicine, St. Louis, MO 63110

We are interested in examining neuronal differentiation in a system that will permit controlled manipulations of cellular interactions during development. The insect stomatogastric nervous system, which innervates the gut musculature and several adjacent targets, has been examined in this regard. In Manduca, the central component of this system is the frontal ganglion (FG), which despite its diminutive size possesses many of the morphological features seen in the more complex ganglia of the CNS. Its 50 neurons are arranged in a cortical dome encompassing a ventral neuropil, positioned above the dorsal surface of the stomodaeum (foregut). It is joined posteriorly to the more diffuse hypocerebral ganglion (HG) via the recurrent nerve and with the brain by paired connectives. We have identified individual neurons by their size, position, transmitter phenotype, and axonal projections. In particular, we have identified a pair of neurons (F1 and F2) that express a substance immunologically related to the molluscan small cardioactive peptides (SCP) and that project through the recurrent nerve to the posterior stomodaeum. We have also begun to characterize the differentiation of these cells during embryogenesis.

Previous histological studies have indicated that the stomatogastric ganglia arise from the dorsal stomodaeal surface, coincident with the formation of the CNS. We have confirmed this developmental sequence by observations of living embryos under Nomarski optics and by immunostaining with monoclonal antibodies to cell surface antigens. A single proliferative zone appears to produce the cells of both the FG and the HG, which initially are contiguous structures. By 35% of embryogenesis, the FG is clearly visible and has established its bilateral connections with the brain. Subsequently, the FG and HG become increasingly distinct; the FG rounds into its characteristic form and lifts off the gut surface, while the HG assumes a more variable configuration along the recurrent nerve. By 50%, dye fills of F1 and F2 reveal that they have extended axons into the recurrent nerve. Shortly thereafter, SCP-like immunoreactivity is seen within F1 and F2; first within their somata at 60% of development and later (at 65-70%) within their axons and growth cones. Significantly, this expression precedes the arrival of the F1 and F2 growth cones at their target sites. This system can now be manipulated in culture to examine the mechanisms underlying the establishment of cellular phenotype in a relatively simple ganglion.

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318.3 SEROTONIN MOSAICS IN LARVAL CNS OF *DROSOPHILA* A.M. Vallés and K. White*. Dept. of Biology, Brandeis University, Waltham, MA 02254

Genetic mosaics in *Drosophila melanogaster* offer a unique system to study the distribution and organization of biogenic amines in developing nervous systems. Mosaic analyses can potentially provide information on a) functional foci b) causal interactions between subsets of cells and c) insights on the rules that govern the internal compartmentalization of biogenic amines.

In *Drosophila*, the gene *Ddc* encodes the enzyme dopa decarboxylase (Wright *et al*, Genetics, 84:287, 1976) that catalyzes the conversion of 5HTP to serotonin (5HT) as well as L-dopa to dopamine (DA) (Livingstone and Tempel, Nature, 303:67, 1983). We have constructed genetic mosaics for the gene *Ddc* using the Ring-X chromosome system (Hall *et al*, 1976). Central nervous systems (CNSs) from mosaic larvae, part deficient and part wild-type for the *Ddc* enzyme activity were scored for the presence of 5HT immunoreactive cells.

Three classes of CNS staining patterns were observed: the wild-type serotonergic pattern, the *Ddc* deficiency pattern, (Vallés and White, J. Neurosci., in press) and a third mosaic group. Within the class of CNSs that yielded mosaic patterns, we observed 5HT immunoreactive segments adjacent to non-immunoreactive segments, and 5HT immunoreactive segments adjacent to weakly immunoreactive segments. All CNS mosaics were bilaterally symmetrical. Either a wild-type genotype or the ability of mutant cells to accumulate 5HT (Vallés and White, J. Neurosci., in press) could produce 5HT immunoreactive neurons in these mosaics. (Supported by NIH Grant GM31503).

318.4 PERTURBED PATTERN OF CATECHOLAMINE-CONTAINING NEURONS IN A DOPA DECARBOXYLASE *Drosophila* MUTANT. V.Budnik*, L.Martin-Morris* and K.White* (SPON: R.Calabrese). Dept. of Biophysics and Dept. of Biology, Brandeis University, Waltham MA 02254.

Genetically mutant animals unable to synthesize a neurotransmitter provide *in vivo* model systems to study the effect of absence of the neurotransmitter on the development and function of the nervous system. We are studying the biogenic amine systems in *Drosophila melanogaster* as it is possible to generate animals that are genetically deficient in dopa decarboxylase (DDC) enzyme activity and thus are deficient in serotonin and catecholamines (Livingstone and Tempel, Nature, 303:67, 1983). Previously, we described the development of catecholamine-containing neurons in wild type CNS using the glyoxylic acid (GA) technique (Budnik *et al*, Soc. Neurosci. Abstr., 11:944, 1985). We are now able to stain the same GA-induced fluorescent cells using antibodies directed against either dopamine or tyrosine hydroxylase, suggesting that these cells are dopaminergic.

We have also extended our investigation to mutant larval CNS deficient in DDC enzyme activity. We find that in mutant CNSs "novel" neuronal subsets exhibit GA-induced histofluorescence. To determine if these novel cells are present in non-mutant CNS, wild type larval CNSs were incubated in exogenous catecholamine. Incubations in L-dopa (10^{-5}M) or dopamine (10^{-3}M), but not norepinephrine (10^{-5} to 10^{-3}M), revealed normally non-fluorogenic neurons that are able to take up the amine and become fluorogenic. Included among these neurons are subsets that are similar to the "novel" neurons in the mutant CNS. These results suggest that in the absence of DDC activity, subsets of normally non-fluorogenic neurons able to sequester L-dopa or dopamine accumulate a fluorogenic catecholamine. Studies are in progress to determine the identity of the "novel" cells in the mutant CNS. Supported by NIH grant NS 23510.

318.5 **GENETIC MAPPING, ALLELES AND GENE DOSAGE EFFECTS OF PASSOVER, A DROSOPHILA MUTANT ALTERING SYNAPTIC CONNECTIONS.** D.H.Baird. Dept.of Biology, Yale University, New Haven, CT 06511.

The mutation Passover (Pas) causes dominant disruption of the visually elicited escape response. Pas alters the ability of the Giant Fiber (GF) interneuron to drive two mesothoracic targets, a tergo-trochanteral ("jump") muscle motor neuron and the interneuron that drives the wing depressor motor neurons (Thomas and Wyman, 1984, J. Neurosci. 4:530-538). Pas maps in X chromosome band 19E4 based on uncovering of its altered GF respose by Deficiency (Df) 16-3-35 (deficient for genes in 19D2-19E4) and Df(1)LB6 (19E4;20A1-2). These deficient chromosomes uncover only one lethal complementation group in common, l(1)R-9-29 (Lifshytz and Falk, 1969, Mutation Res. 8:147-155) and its alleles. When Pas is crossed to R-9-29 or five of its alleles, the resulting heterozygote has the same GF response as Pas homozygotes. This non-complementation also places Pas in 19E4.

Since there are two other alleles of R-9-29 that complement the neural phenotype of Pas, the R-9-29 complementation group may consist of at least two regions or genes, one necessary for neural function, the other for post-embryonic viability. All R-9-29 alleles involve the lethal region; the six alleles that do not complement Pas affect both regions. R-9-29/Df(1)A118 or Df(1)16-3-35 /Df(1)A118 (Df(1)A118 lacks 19E5-E7) heterozygotes are viable yet have a mutant GF response. These results also suggest the presence of a region encoding a purely neural function. Pas is allelic to a previously isolated behavioral mutation shakingB (shakB) (Homyk, Sidzonya and Suzuki, 1980, Molec. Gen. Genetics. 177:553-565). shakB has the same GF response as Pas homozygotes and Pas/shakB heterozygotes. The mutant GF response of shakB maps to 19E4 as well.

The Pas phenotype is not caused by an elimination of wild-type (Pas^+) gene function: Df/Pas^+ heterozygotes have a wild-type GF response while Pas/Pas^+ flies do not. The Pas phenotype is not due to over-production of Pas^+ gene product: adding duplications of the chromosome region bearing Pas^+ to a Pas fly restores the wild-type GF response. Adding a Pas chromosome to a wild-type fly causes a mutant GF response. Thus the Pas gene product interferes with Pas^+ function. Supported by NIH NS07314 to R.J.Wyman.

318.6 **THE DEVELOPMENT OF A POSTEMBRYONIC TOUCH NEURON IN THE NEMATODE CAENORHABDITIS ELEGANS.** **W. W. Walthall and Martin Chalfie. Dept. of Biol. Sci. Columbia University, New York, NY 10027.**

The nematode C. elegans responds to a light touch on the head by moving backward. At hatching larvae possess a pair of anteriorly located mechanosensory neurons, ALML and ALMR, which form gap junctions with a pair of interneurons, called AVD, in the major area of neuropil in the animal, the nerve ring. The AVD interneurons synapse onto a set of motorneurons that are necessary for backward movement. Later a postembryonic anterior touch neuron, AVM, is added to this circuit. A branch of AVM enters the nerve ring and forms gap junctions with the ALM touch cells and the AVD interneurons. The formation of the branch by AVM is position dependent: if AVM is displaced posteriorly, it will not form functional synapses (Chalfie et al., Science 221, 61-63, 1983). We now report that two interneurons BDUR and BDUL are required for AVM to form functional synapses.

When the embryonic touch neurons ALML and ALMR were killed with a laser microbeam the resulting animals were partially sensitive to head touch as adults (n=34); thus AVM alone mediates a partial touch response. However when both the ALM and the BDU cells were killed in young larvae, the resulting adults were completely insensitive to head touch (n=25). Thus the BDU cells are needed for the partial sensitivity produced by AVM. When BDU cells were ablated more than 20 hours after AVM had arisen it had no effect on AVM mediated touch sensitivity (n=5). Because the BDU cells are only needed early it suggests they play a developmental rather than a functional role in the establishment of AVM synapses. Additional support for a developmental role for BDU comes from an examination of AVM branches in electron micrographs of serial sections from animals in which BDU had been killed. In four of six animals no branch was found, in a fifth a small branch was observed and in the sixth a normal branch was present. These data suggest that the BDU neurons induce the formation of the synaptic branch and/or stabilize the process as it threads its way through the nerve ring. It is interesting to note that two other postembryonic neurons, PVN and HSN, that enter the nerve ring late in C. elegans development also synapse extensively on the BDU cells. Thus, the BDU cells may serve to guide the entry of late developing neurons into the already established neuropil of the nerve ring.

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318.7 EXTRA CLEAVAGES INDUCED BY INJECTION OF ADENYLIC ACID (POLY A), INTO IDENTIFIED CELLS RESULTS IN SUPERNUMERARY TELOBLAST LINEAGES IN LEECH EMBRYOS. R.K. Ho* and D.A. Weisblat, Univ. of Calif. Dept. of Zoology, Berkeley, CA 94720

In leech embryogenesis, highly determinant cleavage divisions generate 13 large, yolky cells: 3 macromeres, (A, B and C); a bilateral pair of mesodermal teloblasts, (M); and four pairs of ectodermal teloblasts, (N, O, P and Q). Teloblasts arise exclusively from one of the initial macromeres, (D), which undergoes a strict series of symmetrical divisions, unlike the A, B and C macromeres which cleave no furthur. Teloblasts are stem cells that generate bandlets of primary blast cells which differentiate to produce the segmental tissues of the leech. The microinjection of various tracer molecules into identified teloblasts has revealed the lineal origins of most of the cells in the leech.

We have found that the microinjection of poly-adenylic acid, (poly A), causes the injected cell to undergo extra cleavages. In the A, B and C macromeres, poly A injection induces cleavages which normally do not occur. In general, injection of poly A into any of the teloblast precursors leads to the overproduction of teloblasts. In the teloblast precursors NOPQ and OP, which normally generate four and two teloblasts respectively, poly A injections induce extra cleavages which generate 2 to 4 times the normal number of teloblasts. These supernumerary teloblasts are capable of producing viable, nucleate blast cells which undergo apparently normal first mitoses. Injection of poly A into a differentiated N teloblast also induces extra cleavages producing 2 to 4 teloblasts that generate blast cells. The n blast cells normally produce, (in addition to other cells), the 5-HT containing neurons called cells 21, 61 and Retzius. When embryos in which extra N teloblasts have been generated are allowed to hatch and stained with anti-5HT antibodies, extra immunoreactive cells are seen within the final CNS pattern. These results suggest that poly A injections induce extra cell cleavages and produce supernumerary teloblasts able to generate viable blast cells which give rise to neurons capable of expressing a differentiated phenotype.

The effect of poly A injections into embryonic leech cells is very specific. These types of results are not seen with injections of tracer molecules such as derivatized dextrans or HRP. Moreover, other nucleic acid homopolymers have different effects. The injection of poly-deoxyadenylic acid, (poly dA), causes the injected cell to continue cleaving into as many as 40 cells, which never produce blast cells. And injections of poly G or poly C immediately stop normal cell divisions in the injected cell while leaving the cell otherwise viable.

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318.8 EFFECTS OF HYDROXYUREA ON NEUROBLAST DUPLICATION AND THEIR IMPLICATIONS ON THE MECHANISMS OF SURVIVAL OF IMMATURE NERVE CELLS IN THE CNS OF MANDUCA SEXTA. J. W. Truman and R. Booker Dept. of Zoology, Univ. of Washington, Seattle WA 98195.

The segmental CNS of an adult moth is a mosaic, consisting of remodeled larval neurons and cells that are unique to the adult stage. The latter cells come from stereotyped arrays of identified stem cells (neuroblasts) located in each ganglion. The neuroblasts become active early in the 3rd larval instar and generate a nest of up to 100 imaginal nest (IN) cells by the end of the last (5th) larval stage. In certain nests metamorphosis results in the death of some of the IN cells while the survivors then differentiate to form adult-specific neurons (Booker & Truman, 1985, Soc. Neurosci. Abstr., 11:958).

The segmental neuroblasts can be killed at various times during larval life by injecting larvae with the DNA synthesis inhibitor, hydroxyurea (HU). Beginning on Day 1 of the 3rd instar, HU effectively killed 80 to 90% of the neuroblasts while leaving postmitotic cells unaffected. The treated larvae continued growth and eventually underwent metamorphosis to form morphologically normal moths which had severe deficits in their number of central neurons.

Treatment with HU before day 1 of the 3rd instar resulted in little NB death, but about 10% of the identified nests contained twice the expected number of IN cells when examined late in the 5th instar. For reasons that are unknown, the HU treatment at this early time results in the random duplication of a small percentage of the neuroblasts. Both of the resulting stem cells then appear to make the appropriate number of progeny, thereby adding a double complement of cells to certain regions of the CNS. In the case of nests in which some of the IN cells die at metamorphosis, those arising from the duplicated neuroblasts showed twice the number of surviving cells, all of which differentiated into neurons having features characteristic for their respective lineage. Thus, the CNS has the capacity to support many more neurons from a particular nest than normally survive. These results suggest that the hypothesis that competition between IN cells in a nest is the sole factor which determines which cells will survive is not valid.

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318.9 DEVELOPMENTAL DISAPPEARANCE OF A MONOSYNAPTIC SENSORY-TO-MOTOR REFLEX IN THE TOBACCO HORNWORM, MANDUCA SEXTA. G.A. Jacobs and J.C. Weeks. Dept. of Zoology, Dept. of Entomological Sciences & Graduate Group in Neurobiology, UC Berkeley, Berkeley, CA 94720.

A conspicuous aspect of insect metamorphosis is the stage-specific loss of particular behaviors, which may be associated with structural changes in motoneurons. The larval-pupal transformation of Manduca includes the loss of the larval locomotory appendages, the prolegs, and involves the degeneration of the proleg musculature, and the structural regression and death of one of the proleg motoneurons. This motoneuron, PPR, innervates the principal planta retractor muscle. PPR loses over 40% of its dendritic arbor prior to its death in the pupa, and previous work has shown that this structural regression is accompanied by a reduction in synaptic drive to the motoneuron and its progressive elimination from behaviors (J. Comp. Physiol. 155:423,1984).

To study the effects of dendritic regression on synaptic inputs to PPR we have begun to characterize the proleg withdrawal reflex in larvae. Touching the sensory bristles on the tip of the proleg elicits bilateral proleg withdrawal in the stimulated segment. These bristles are singly innervated by sensory neurons which arborize ventrally in the segmental abdominal ganglion, and the afferents overlap anatomically with a ventral dendritic branch of PPR. Stroking the hairs elicits powerful excitation of PPR, and thereby evokes reflex retraction of the proleg. Simultaneous recordings from PPR and individual afferents reveal short latency, constant amplitude EPSPs in the motoneuron, which follow one for one at high spike frequencies. The EPSP is gradually and reversibly abolished in high magnesium saline, and persists in high calcium/high magnesium saline. These experiments are consistent with a monosynaptic sensory-to-motor pathway. The rapid and long-lasting excitation of PPR by hair stroke is due to the synaptic convergence of many hairs onto each motoneuron, and to homosynaptic facilitation shown by each EPSP. The EPSP is reversibly abolished in curare, suggesting a nicotinic cholinergic pharmacology.

Since the prolegs degenerate during normal development, we have utilized a hormonal technique to preserve the prolegs and the sensory afferents into the pupal stage to study how the dendritic regression of PPR affects its synaptic inputs. Treatment of the prolegs with a juvenile hormone analog during the larval stage causes preservation of the prolegs in the pupa, and we are presently using these developmentally mosaic animals to investigate changes in synaptic strength between the sensory afferents and PPR during the larval-pupal transformation.

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318.10 DISSOCIATION OF THE ECDYSTEROID-MEDIATED DENDRITIC REGRESSION AND PROGRAMMED DEATH OF A MOTONEURON DURING INSECT METAMORPHOSIS. J.C. Weeks. Dept. of Entomological Sciences & Graduate Group in Neurobiology, UC Berkeley, Berkeley, CA 94720.

The larval-pupal transformation of the tobacco hornworm, Manduca sexta, is accompanied by the loss of the principal locomotory appendages of the caterpillar, the abdominal prolegs. The elimination of the prolegs includes the degeneration of the proleg muscles, and the structural regression of their motoneurons. Subsequently some, but not all, of the regressed motoneurons die (J. Comp. Physiol. 155:423,1984). Previous studies have concentrated on a motoneuron designated PPR, which innervates the principal planta retractor muscle of the larval proleg. Morphometric analysis indicates that during the 3 days preceding the pupal stage, PPR's dendritic arbor is reduced by over 40%. Two days after pupation, PPR dies. These events are triggered by the prepupal rise in blood ecdysteroids (the 72 hr duration 'prepupal peak') (J. Neuroscience 5:2290,1985).

The observation that not all motoneurons which regress subsequently die suggests that dendritic regression and death are independently determined developmental options. I have now verified this hypothesis by experimentally dissociating the regression and death of PPR. By altering the duration of the prepupal peak of ecdysteroids it has been possible to cause PPR to regress partially or fully, but then survive indefinitely beyond the expected time of death. For instance, when the 72 hr long prepupal peak is shortened to 10, 34, or 58 hrs in duration, PPR respectively regresses partially but survives, regresses fully but survives, or regresses fully but dies. In the first two cases, PPR was made to resemble other proleg motoneurons which during normal development regress but do not die. These hormonal manipulations indicate that the magnitude of PPR's regression is proportional to the duration of ecdysteroid exposure, and that neuronal death is triggered by further exposure beyond that which is sufficient to induce full regression. Thus, dendritic regression and programmed neuron death in this system are separate developmental options, with different hormonal requirements.

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318.11 AGE-DEPENDANT REORGANIZATION OF SYNAPTIC CONNECTIONS IN THE CRICKET CERCAL SENSORY SYSTEM.
D. Shepherd* and R.K.Murphey (SPON: S.B.Tieman). Neurobiology Research Center, SUNY Albany, Albany, NY 12222.

A group of 3 identified sensory neurons (X-neurons) on the cricket cercus have axons that terminate in both ipsilateral and contralateral halves of the terminal abdominal ganglion. Anatomical studies of these 3 neurons have shown that they are structurally identical. Despite this apparant anatomical similarity of the 3 X-neurons, their synaptic connections in the CNS are different.

Simultaneous recordings from one of these sensory neurons, named vpX, and 2 bilaterally homologous postsynaptic cells the medial giant interneurons (MGI's) demonstrated that activity in vpX produces short latency (1.4 - 2.4 mS) epsp's in both MGI's (0 - 1.0 mV) (Shepherd and Murphey, in press). Similar recordings made from the other two X-neurons, pX and X, revealed that pX made only a relatively weak connection to the MGI's (0 - 0.25 mV) and X never made a detectable connection to either MGI.

The strength of the synaptic connection made by each of the X-neurons with MGI is correlated with the age of the neuron: vpX is the youngest and it makes the strongest connection and X is the the oldest and it makes no detectable contact. This does not mean that X fails to make functional connections in the adult CNS. Simultaneous recordings from X and another interneuron (10-2), show that X makes a clear contact in adult animals.

To determine if the differences in the connections of the X-neuron with MGI are specified by the age of the neuron, experiments were performed on larval crickets. Results from these investigations show a strong connection between X and MGI in 5th instar crickets, this connection is absent in adults.

The results suggest that as the specimen ages, its synapses between X and MGI are lost. Whether these changes in connectivity represent a sliding of X's connections from MGI in larvae to other interneurons like 10-2 in the adult or simply a shedding of contacts by MGI isn't presently clear, but further experiments will clarify this point.

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318.12 SYNAPSE FORMATION BY SENSORY NEURONS IS CONTROLLED BY PERIPHERAL POSITIONAL INFORMATION
G. Kaemper* and R. K. Murphey Neurobiology Research Center SUNY Albany, N.Y. 12222

We have shown that specific receptors, within an ordered array of receptors of similar modality, can form synapses with different postsynaptic interneurons. These differences are correlated with the position of the receptors at the body surface. It has been suggested that "positional information" directs this type of neuronal specificity.

In order to test this hypothesis at the physiological level we transplanted pieces of epidermis from the cricket Gryllus bimaculatus to another species, Acheta domesticus. These two species have epidermis of different colors and thus donor tissue can be recognized after transplantation. We first demonstrated that these cross species transplants generated functional synapses, by recording simultaneously from transplanted sensory neurons and appropriate interneurons. These experiments revealed that Gryllus sensory neurons could make synaptic connections with the Acheta interneurons.

The crucial test of the positional information hypothesis occurs when tissues from different locations on the cercus are juxtaposed. Under these conditions a process of pattern regulation is initiated which creates new types of receptors in a manner consistent with the new position of the graft (intercalation). In this way two classes of sensory neurons are available for testing: sensory neurons born before the transplantation but now located at an ectopic position and those born after the transplantation.

The second class is important. If these new cells make connections in predictable ways according to their new position rather than to the original position of their epidermal precursors, then a role for position in synaptogenesis is proven. We found that new neurons, formed by the transplanted tissue, made connections appropriate to their new positions and not to the original location of the precursors. Thus position at the time of their terminal mitosis dictates both the anatomy and the synaptic connections of sensory neurons.

Supported by DFG grant Ka 662/1-1 to G.K. and NIH grant NS15571 to RKM.

318.13 DEVELOPMENT OF THE ECDYSIS MOTOR PATTERN IN MANDUCA SEXTA: HOW ADULT-SPECIFIC BEHAVIOR ARISES FROM THE CONSERVATION AND MODULATION OF A LARVAL MOTOR PROGRAM. K.A. Mesce and J.W. Truman. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

What is the developmental fate of pattern-generating circuitry, for a particular stage-specific behavior, when that behavior is no longer expressed or has changed in form? We addressed this question by studying the developing hawkmoth, Manduca sexta, and its periodic expression of ecdysis behavior (the shedding of the old cuticle) which occurs as it molts from one stage to the next. Although larval, pupal and adult ecdyses are all "triggered" by the same neuropeptide, eclosion hormone, the adult ecdysis pattern (termed eclosion) radically differs from larval and pupal ecdyses. Such a behavioral distinction reflects the necessity of the pharate adult not only to shed its old cuticle but also to dig itself out from its underground pupation site. To accomplish this, the adult performs a series of abdominal retractions and extensions, the latter coupled with wing muscle contractions. This pattern is quite distinct from the slowly progressing, anteriorly-directed, abdominal peristalses expressed by ecdysing larvae and pupae.

It was assumed that the pattern-generating circuitry for larval and pupal ecdysis behavior was dismantled during metamorphosis and replaced with adult-specific circuitry. To the contrary, we found, using behavioral and electrophysiological criteria, that the larval/pupal ecdysis pattern-generating circuitry is conserved through metamorphosis but modified to produce new adult-like behavior (K.A. Mesce and J.W. Truman, 1985, Soc. Neurosci. Abs. 11:919.). When descending neural inputs from the thorax are interrupted surgically or by a reversible cold-block, the adult abdomen rapidly lapses into the larval-like ecdysis routine. Removal of the brain, subesophageal or prothoracic ganglion does not significantly alter the adult-specific motor program. Alterations in the pterothoracic ganglion, however, renders the adult abdomen incapable of producing the adult pattern and permits expression of only the larval-like behavior. Our data suggests that the adult-specific behavior is generated through the coupling of two motor programs: the persisting larval program that is distributed through the segmental ganglia, and an adult-specific program located in and descending from the pterothoracic ganglion. Interestingly, activation of the two motor programs is not equally dependent on eclosion hormone so that they can be uncoupled and studied independently. We are currently studying thoracic neural elements which interact with the larval motor program to yield the adult-specific expression of the behavior.

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319.1 A DECREASE IN PUTRESCINE ENHANCES BRAIN PLASTICITY ELICITED BY COMPLEX ENVIRONMENT. Vesna A. Eterović and P.A.Ferchmin. Dept. of Biochemistry, School of Medicine, Universidad Central del Caribe, Cayey, Puerto Rico.

The role of polyamines in brain plasticity was studied by comparing rats injected with difluoromethylornithine (DFMO) or saline and exposed to either a complex or impoverished environment.

DFMO is an irreversible inhibitor of ornithine decarboxylase which specifically inhibits putrescine synthesis and consequently slows cellular proliferation. Recently several authors reported that in some cell lines DFMO induces differentiation.

Rats exposed to a complex environment for seven hours a day during four days had heavier brains and increased cortical RNA content than littermates kept in an impoverished environment. These differences were larger in DFMO injected rats than insaline controls. At the dose used, 400 mg/kg, DFMO decreased the levels of cortical putrescine by 50%. Saline injected animals exposed to the complex environment showed a 12% decrease in cortical putrescine vs. littermates kept in impoverished conditions.

It is known that stimulants, like amphetamines, enhance the effect of the complex environment by stimulating the animal - environment interaction. The action of DFMO differs from amphetamines; DFMO decreases the interaction of rats with the complex environment. The enhancing effect of DFMO cannot be explained by the same mechanism as amphetamines.

In conclusion, DFMO which decreases putrescine concentration enhances the effect of environmental complexity, and the exposure to environmental complexity per se causes a decrease in putrescine. Therefore, a decrease in putrescine either accompanies or is a signal needed for increased brain plasticity.

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319.2 EXOGENOUS OXYTOCIN IMPAIRS THE REGENERATIVE CAPACITY OF THE HYPOTHALAMIC NEUROSECRETORY SYSTEM. J.P. Herman, M. Matise*, F.F. Marciano and D.M. Gash. Dept. of Neurobiology and Anatomy and the Center for Brain Research, Univ. of Rochester, Rochester, NY 14642.

We have previously demonstrated that chronic administration of vasopressin (VP) following neurohypophysectomy (NPOX) inhibits regeneration of hypothalamic neurosecretory system (HNS) VP neurons and blocks recovery of antidiuretic function (Soc. Neurosci. Abs. 11, 613, 1985). Oxytocin (OT) neurons, by contrast, were unaffected by VP treatment. In these experiments, we sought to determine the effects of chronic OT administration on regeneration and recovery of function of the HNS following NPOX. Male Long-Evans rats were NPOXed and immediately implanted with mini-osmotic pumps delivering 0.0, 0.1, 0.5, or 2.5 ug/h OT. Two weeks after NPOX, the mini-osmotic pumps were removed. Animals were monitored for daily water consumption and urine osmolality throughout the experiment, which terminated 7 weeks following NPOX. At this time rats were perfused, brains sectioned and tissue processed for VP and OT immunocytochemistry.

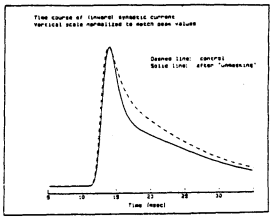
Administration of 0.5 or 2.5 ug/h OT significantly reduced the severity of the transient diabetes insipidus normally seen during the 2-week period following NPOX. After removal of the mini-osmotic pumps, a pronounced diabetes insipidus occurred in the 0.5 and 2.5 ug/h OT treatment groups which persisted for the duration of the experiment. Rats receiving 2.5 ug/h OT were the most adversely affected, excreting extremely dilute urine (224 mOsm/kg) and drinking copious quantities of water (87.1 gm/100 g body weight/day). In contrast, NPOX rats receiving saline showed an initial diabetes insipidus which significantly recovered over time. The 0.1 ug/h dose of OT did not elicit a pronounced antidiuretic response over the period of delivery, but did result in slight long-term impairment in antidiuretic function. Histological analysis revealed a dose-related decrease in number of HNS VP positive cells, with no substantial decreases in OT-positive cells.

The results of these experiments indicate that OT administration at high titers, like VP administration, impairs the ability of damaged VP neurons to regenerate following NPOX, resulting in decreases in the number of HNS cells expressing VP immunoreactivity and in long-term impairment of antidiuretic function. The deleterious effect of OT is directly related to its antidiuretic potency following NPOX. These data in addition provide further evidence that osmotic feedback signalling release of VP is important in determining capacity to survive following NPOX.

Supported by MH08883 and NS15109.

319.3 **RECEPTOR UNMASKING: EFFECT ON TIME COURSE OF POSTSYNAPTIC CURRENT** Donald H. Perkel, Theoretical Neurobiology Facility, Department of Psychobiology and Department of Physiology and Biophysics, University of California, Irvine, 92717.

It has been proposed that a chief cellular mechanism underlying long-term plasticity is the unmasking of previously nonfunctional postsynaptic receptors for neurotransmitter (G. Lynch and M. Baudry, Science 224: 1057-1063; 1984). For this mechanism, or related ones such as migration and immobilization of receptors, to be effective in increasing the amplitude of postsynaptic currents and potentials, it is essential that neurotransmitter be released in excess before long-term changes take place, and that afterwards, any excess be diminished or eliminated by the increased receptor binding capacity postulated. The dynamics of the bound transmitter and receptors are such that not only the amplitude of the postsynaptic current but also its overall time course can be expected to change following "learning".



We have calculated postsynaptic currents using a detailed model of Ca^{2+}-mediated transmitter release following an action potential in the nerve terminal, diffusion or uptake from the cleft, a cycle of state changes in the bound receptors, and return of transmitter to the cleft. Under a wide range of assumed parameter values, we found that postsynaptic currents (as well as corresponding conductances and potentials) not only increased in amplitude after receptor "unmasking" but also that the time course became noticeably narrower. A typical example is illustrated. The synaptic currents (inward plotted upwards) have been normalized at peak amplitude to facilitate shape comparison. Briefly, excess transmitter in the control case remains available for subsequent binding by receptors that become available after channel closing, while after "unmasking", the increased receptor population rapidly binds virtually all transmitter released into the cleft.

These theoretical findings suggest an experimental test of the proposal that long-term synaptic changes result from increased receptor capacity in the absence of proportional increases in presynaptic release of transmitter. Sufficiently localized field-potential measurements can furnish comparisons of synaptic currents before and after the changes. Failure to observe the predicted narrowing of synaptic currents could indicate a significant presynaptic contribution to the plasticity mechanisms.

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319.4 REACTIVE DENDRODENDRITIC SYNAPTOGENESIS IN THE ADULT OLFACTORY BULB: REAPPEARANCE OF CONJUGATE INTERNALIZATIONS OF APPOSED DENDRITIC MEMBRANES. Charles A. Greer, Sections of Neurosurgery and Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Endocytosis of extracellular material is a ubiquitous phenomenon in the CNS and is believed to mediate the internalization of extracellular molecules that cannot readily pass the lipid bilayer. (Anderson & Kaplan, 1983). Eckenhoff & Pysh (1979, 1983) reported a developmental endocytotic event in the cerebellum and olfactory bulb (OB), the conjugate internalization of apposed plasmalemma membranes. Although this occurred late in synaptogenesis one alternative they suggested was that this event may contribute to the remodelling of neuronal membranes.

The mutant mouse PCD, as an adult, loses a principal neuronal population in the OB, mitral cells (Greer & Shepherd, 1983). As a result, there is extensive reorganization of reciprocal dendrodendritic microcircuits and a period of reactive dendrodendritic synaptogenesis (Greer et al., 1986). The current report explores the possible involvement of conjugate internalization of apposed dendritic membranes (CIDMs) in synaptogenic events by examining their morphology and incidence in the adult PCD OB following the loss of mitral cells.

PCD mice, both homozygous recessive mutants and heterozygous littermate controls, and 6 - 12 day postnatal rats were perfused with aldehydes for conventional EM or prepared with the Golgi-EM procedure of Fairen et al. (1977). Micrographs of serial sections from the external plexiform layer of the OB were used to assess the morphological features and incidence of CIDMs.

The typical CIDM appeared as a coated pit composed of double plasmalemma membranes separated by 10 - 20nm. It was goblet shaped and extended up to 250nm into the apposed dendrite. CIDMs measured approximately 200nm in diameter at the widest point and narrowed to 30 - 60nm at the neck. It was not uncommon to recognize 1 or more vesicles contained within the CIDM. Furthermore, they were frequently seen in close association to the reciprocal dendrodendritic synapses characteristic of the OB. In the mutant mice the incidence of CIDMs increased significantly relative to controls ($2.26 \pm 0.37/100um^2$ versus $0.62 \pm 0.12/100um^2$). Moreover, the increase in CIDMs was greater in the deeper sublamina of the external plexiform layer which is more extensively affected by the loss of mitral cells.

These data demonstrate that normally quiescent developmental events can be reactivated in the adult and that membrane remodelling mediated by the internalization of apposed membranes may be central to the expression of dendrodendritic synaptic plasticity.

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319.5 METABOLIC PLASTICITY FOLLOWING CORTICAL INFARCTION: A 2-DEOXYGLUCOSE STUDY IN ADULT RATS. W. D. Dietrich, B. D. Watson, R. Busto and M. D. Ginsberg, Departments of Neurology and Anatomy and Cell Biology, University of Miami School of Medicine, Miami, FL 33101.

The clinical literature has shown for years that varied degrees of recovery may occur following stroke and other injuries to the central nervous system. Possible mechanisms underlying functional recovery following ischemic infarction include injury-induced structural alterations resulting in aberrant functional circuits. Additionally, recent studies have suggested that pre-existing circuits may also contribute to recovery processes. The documentation of these events following cerebral infarction, and their potential relationship to functional recovery necessitate experimental study. The present study was undertaken to determine whether experimentally induced cortical infarction in adult rats precipitates abnormal patterns of cortical field activation induced by unilateral whisker stimulation. Right barrel-field infarction of the first somatosensory cortex was produced photochemically in adult rats. Following 30 days, the pattern of increased cortical metabolic activity induced by unilateral whisker stimulation was assessed with ^{14}C-2-deoxyglucose autoradiography. Left whisker stimulation for 45 minutes activated contralateral cortical regions anterior and lateral to the infarcted zone. Lateral to the infarct, a single region of stimulation- induced increased glucose utilization was apparent, which was somewhat larger and extended more laterally than the normal SmII barrel-field region. Ipsilaterally activated regions (not apparent in control animals) included somatosensory cortical areas. Patch-like aggregates of metabolically activated ipsilateral and contralateral cortical regions were shown by three-dimensional reconstruction to vary from the activated patterns obtained in control rats following whisker stimulation. Glucose utilization was depressed within several thalamic nuclei underlying the infarcted zone. Additionally, a decreased capacity to activate the contralateral ventrobasal thalamus in response to whisker stimulation was also apparent in the experimental rats. The results demonstrate that as a consequence of chronic barrel-field infarction, abnormal patterns of stimulation-induced increased glucose utilization can be identified within cortical and subcortical brain structures. Altered field activation occurs in regions immediately surrounding the infarcted zone as well as in contralateral areas. These data provide metabolic evidence for functional plasticity occurring within the adult rat central nervous system. Mechanisms responsible for these plastic changes may include both functional changes in pre-existing connections as well as structural reorganization at sites adjacent to the destroyed cortical area. Supported in part by NIH grant NS 05820, WDD is an Established Investigator of the AHA.

319.6 HYPOTHESIS: PROTEIN KINASE C ACTIVATION ACTS SYNERGISTICALLY WITH A CALCIUM-MEDIATED EVENT TO INDUCE LONG-LASTING SYNAPTIC CHANGES IN THE HIPPOCAMPUS. P. Colley* and A. Routtenberg. (SPON: J.P. Rosenfeld) Cresap Neurosci. Lab., Northwestern University, Evanston, IL. 60201

Translocation of protein kinase C (PKC) to the plasma membrane and a change in the phosphorylation of a PKC substrate, protein F1, have been observed following the induction of long-term potentiation (LTP) in intact rat hippocampus [*Behav Neural Biol.* 44 (1985) 186]. Recently, the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA), a translocator and activator of PKC, when ejected into the hilar dentate gyrus prolonged potentiation induced by two brief trains (20 msec) of 400 Hz stimulation delivered to the perforant path [Routtenberg et al., *Br Res.* (1986) in press]. If TPA effects are mediated by PKC translocation and activation, then TPA alone should induce long-lasting changes in evoked field potentials similar to those seen in LTP. We now have evidence that this is not the case, suggesting a synergism between the effects of TPA and a Ca2+-mediated event.

Using ejection parameters similar to those previously described [Routtenberg et al., *Br Res.* (1986) in press] animals were used as their own controls to show that TPA alone had no effect on baseline synaptic activation, but prolonged potentiation when delivered just prior to the 400 Hz stimulation. Reports of effects of TPA alone in the hippocampus [*J Neurosci.* 6 (1986) 475] may be related to differences in preparation and dosage. TPA ejected 2 hrs prior to the 400 Hz stimulation did not have an effect on potentiation. Thus, this prolongation of potentiation depends upon the close temporal contiguity of the TPA and perforant path stimulation treatments.

These data support our recently proposed model [*Prog Br Res.* Vol.69 (1986)] in which TPA acts synergistically with elevated intracellular Ca2+ induced by 400 Hz stimulation to produce long-lasting synaptic potentiation [see also proposal by Nishizuka; *Nature* 308 (1984) 693]. Both TPA and Ca2+ could be activating PKC to produce this synergism: TPA via PKC translocation, and Ca2+ via an PLA_2/oleate pathway of PKC activation which has recently been described [*FEBS Letts.* 192 (1985) 189]. (This work was supported by MH 25281-12, and AFOSR83-0335 to A.R.)

319.7 PROTEIN KINASE C SUBSTRATE PHOSPHORYLATION IN PRIMATE CEREBRAL CORTEX (E.G. PROTEIN F1) IS INCREASED IN THOSE STAGES OF THE OCCIPITOTEMPORAL VISUAL PROCESSING PATHWAY IMPORTANT FOR INFORMATION STORAGE. R.B. Nelson*, D.P. Friedman, M. Mishkin, and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60201, and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

Translocation of protein kinase C (PKC) to membrane, and increases in the phosphorylation of one of its substrates, protein F1, have been previously related to the induction of long-term potentiation in the hippocampal formation [*Behav. Neur. Biol.* (1985) **43**:3; *Brain Res.* (1985) **343**:137; *Science* (1986) **231**:587]. Since LTP is a model for information storage, we predicted that a relation also exists between regional PKC substrate phosphorylation and brain regions involved in information storage. To test this prediction, we examined protein phosphorylation along the occipitotemporal visual processing pathway in rhesus monkey cerebral cortex since neurobehavioral evidence suggests that temporal lobe areas participate more than occipital areas in the storage of visual representations. We found that two acidic PKC substrates (of 50 and 81 kDa) showed a graded increase in mean endogenous phosphorylation moving rostrally along this pathway from occipital to temporal cortex then to entorhinal cortex ($F=22.9$, $df=5$, $p<0.001$). Based on kinase specificity, molecular weight, isoelectric point, and two-dimensional phosphopeptide maps, the 50 kDa protein appears to be homologous to protein F1 described in rat. No other phosphoproteins analyzed from one- or two-dimensional gels demonstrated systematic increases in phosphorylation along this pathway. Neither the phosphorylation of synapsin Ia and Ib, well characterized synaptic proteins, or that of the alpha subunit of pyruvate dehydrogenase, a key mitochondrial enzyme, increased along this pathway, suggesting that the increases in phosphorylation of 50 kDa and 81 kDa were not due to differences in the regional density of synapses or differences in energy utilization among the cortical regions assayed. These results suggest the hypothesis that regional participation in visual information storage in primate may be directly related to regional levels of PKC substrate phosphorylation. (Supported by MH25281-12 and AFOSR 83-0335 to A.R., and NRS fellowship DA05254 to R.B.N.)

319.8 PROTEIN F1, PREVIOUSLY RELATED TO SYNAPTIC PLASTICITY, EXHIBITS DECREASED PHOSPHORYLATION IN SENESCENT RAT HIPPOCAMPUS. D. Lovinger*. Cresap Neuroscience Laboratory (CNL), Northwestern Univ., Evanston, IL 60201, C.A. Barnes, S.J.Y. Mizumori. Dept. Psychol, Univ. Colorado, Boulder CO 80309, S.Y. Chan, D. Linden*, K. Murakami*, F-S. Sheu*, and A. Routtenberg. (CNL).

The in vitro phosphorylation of protein F1 (47kD, 4.5 pI), a synaptic membrane substrate of protein kinase C (PKC), is positively correlated with the persistence of long term synaptic enhancement (LTE) in the hippocampus [Br. Res., (1985) 343 137]. If protein F1 phosphorylation regulates the duration of LTE then one would predict that phosphorylation of this protein in hippocampus might be decreased in senescent rats which have been shown to exhibit faster decay of enhancement [J. Comp. Physiol. Psychol., (1979) 93 74]. Indeed we report here that phosphate incorporation from ^{32}P-ATP into protein F1 in vitro is reduced in dorsal hippocampus from 24 month old Fischer-344 rats ($\bar{x}=12.90 \pm 1.47$, n=11) relative to rats 10 months ($\bar{x}=22.00 \pm 1.53$, n=13), or 4 months ($\bar{x}=17.66 \pm 1.40$, n=12) of age. Protein F1 phosphorylation did not differ significantly between 4 and 10 month old animals, and no significant difference in protein F1 phosphorylation was detected between age groups in cerebellar cortex. No other protein in the 40-90 kD range exhibited an age-related difference in phosphorylation.

The decrease in protein F1 phosphorylation does not appear to result from a decrease in PKC activity. Total PKC activity in hippocampal tissue from the same animals used for in vitro phosphorylation analysis was not different among the three age groups. The distribution of hippocampal PKC between cytosolic and membrane fractions [measured as in Science (1986) 231 587] also did not differ with age. The decreased protein F1 phosphorylation may be related to a substrate decrease or an increase in phosphatase activity in aged animals. In a parallel report by de Graan et al. [Prog. Br. Res., (1986) Volume 69 in press] hippocampal protein F1 concentration as determined by radioimmunoassay was found to be lower in 28 month old- than in 2 month old rats. Protein F1 is likely the same as GAP43 [Snipes et al., this meeting], an axon terminal protein linked to neuronal growth, and may therefore play a role in synaptic growth during LTE. The loss of protein F1 in senescent rat hippocampus might reduce the capacity for synaptic growth leading to less persistent LTE. (Supported by MH25281-12, AFOSR-0335 to A.R., and AG-03376 to C.A.B.)

319.9 CHARACTERIZATION OF PURIFIED PROTEIN F1, A SPECIFIC PROTEIN KINASE C SUBSTRATE RELATED TO SYNAPTIC PLASTICITY. S.Y. CHAN, K. MURAKAMI* and A. ROUTTENBERG. Cresap Neuroscience Labortory, Northwestern University, Evanston, Ill. 60201.

The *in vitro* phosphorylation of protein F1 (Mr=47KDa, pI=4.5) was selectively increased and directly correlated with synaptic plasticity of long term potentiation (*Behav. Neural Biol.*, 1985,43, 3-11). Protein F1, a protein kinase C (PKC) substrate, was purified 800-fold from a synaptosomal membrane fraction using pH extraction, then hydroxylapatite and phenyl-sepharose column chromatography (Chan *et al.*, 1986, *J. Neurosci., in press*).

Maximal stimulation conditions for the purified F1 phosphorylation by PKC was 100 ug/ml PS, 200uM Ca^{2+}, 2.5 mM Mg^{2+} and 30 min incubation at 30^0C. DiC_8 and 1,2 diolein, in the presence of PKC and PS lowered the requirement of Ca^{2+} for maximal phosphorylation to 5 uM. Calmodulin inhibited F1 phosphorylation in a dose-dependent manner (maximal inhibition at 3.6 uM). S. Aureus V8 protease digest (200ug/ml V8 at 37^0C for 2 hrs.) gave 2 phosphofragments of 13K and 11K from a 20% SDS gel. Phosphoamino acid analysis showed 97% of the incorporated phosphate was associated with phosphoserine. Purified F1 phosphorylation was stimulated only by PKC but not by any other kinases so far tested including cAMP-dependent kinase, Ca-CaM-dependent kinases type I and II and phosphorylase b kinase.

Protein F1 is undoubtedly associated with synaptosomal membranes. Interestingly, based on the phenyl-sepharose column elution profile, protein F1 was observed to have a strong hydrophilic domain. Also, when F1 was applied to a P-100 gel filtration column, the protein was eluted at the void volume (i.e. >100K Da), raising the possibility that F1 may be a subunit of a larger molecule.

Protein F1 phosphorylation by PKC can also be stimulated by oleic acid (maximal stimulation: 50-200uM) in the absence of phospholipid and Ca^{2+}. However, the amount of phosphorylation (with 50uM oleate) in the presence of 200 uM Ca^{2+} was 5-10 fold higher than in the absence, indicating that oleate effect may be synergistic with Ca^{2+}. This synergism may contribute to the control of synaptic plasticity as discussed by Colley *et al.* and Linden *et al.* (this meeting). (This work was supported by MH25281-12 and AFOSR83-0335 to A.R.)

319.10 PROTEIN KINASE C (PKC) ACTIVATION BY CIS-FATTY ACID IS DISTINCT FROM PREVIOUSLY DESCRIBED PHOSPHOLIPID AND Ca2+ ACTIVATION K. Murakami*, S.Y. Chan, and A. Routtenberg, Cresap Neurosci. Lab., Northwestern Univ., Evanston, IL 60201. (SPON: Z. Iqbal)

Our laboratory has shown an increase in membrane associated PKC activity following long-term potentiation in rat hippocampus (Science (1986) 231:587). Moreover, a brain PKC substrate protein F1 (Brain Res. (1985) 334:147; J.Neurosci.(1986) in press) phosphorylation state is selectively changed by a brief high frequency stimulation which leads to LTP (Behav. Neural Biol. (1985) 43:3). Recently we have demonstrated that unsaturated fatty acids (oleate, arachidonate) can activate purified PKC independently of phospholipids and Ca2+ (FEBS Lett.(1985) 192:189). This suggests that another mechanism may exist for PKC activation, possibly mediated by cis-fatty acid liberation by PLA2, in addition to PLC/PI turnover signaling. Indeed, iontophoretic injection of oleic acid into hippocampus prolongs a short-lasting change in synaptic efficacy (see Linden et al. this meeting).

We have further characterized the new type of PKC activation by free fatty acid. Other cis-fatty acids such as linoleate or linolenate also activate PKC at concentrations similar to oleate in the absence of Ca2+. Saturated fatty acids (C4-C18), the trans-fatty acid elaidate, or detergents (Triton X-100 and SDS) weakly activated PKC. PKC activity induced by Ca2+/phosphatidylserine (PS) and diacylglycerol was strongly inhibited by these detergents, but not when induced by oleate. Lowering the critical micelle concentration of oleic acid by increasing ionic strength also strongly inhibited oleate activation of PKC. Dioleylphosphatidylserine activated PKC effectively (Ka=7.2uM), whereas dimirystoylphosphatidylserine containing saturated fatty acid in both acyl moieties, failed to activate PKC even in the presence of Ca2+. These observations suggest that: 1) PKC activation by free fatty acid is specific to the cis-form and is not due to its detergent-like action, 2) cis-fatty acid activation is due to the direct activation of PKC with the monomeric form of cis-fatty acids and 3) cis-fatty acids at acyl positions in PS are also important for Ca2+/PS activation of PKC.
(Supported by MH 25281-12 and AFOSR 83-0335 to A.R.)

319.11 OLEIC ACID, A PROTEIN KINASE C ACTIVATOR, ENHANCES HIPPOCAMPAL LONG-TERM POTENTIATION D.J. Linden*, K. Murakami*, and A. Routtenberg. (SPON:T. Bleck) Cresap Lab., Northwestern Univ., Evanston, IL, 60201.

Oleate, an unsaturated fatty acid, has been shown in this laboratory to activate protein kinase C (PKC) *in vitro* (*FEBS Letters*, 192:189, 1985). Since PKC is activated following hippocampal long-term potentiation (LTP) (*Science*, 231:587, 1986), and phorbol myristate acetate, a PKC activator, prolongs the duration of the potentiated response when applied to the hippocampus, (*Brain Research*, in press) we wished to determine whether a distinctly different PKC activator, oleic acid, would have the same prolongation effect on the potentiated response.

A micropipette was placed in the hilar dentate gyrus of urethane anesthetized rats for iontophoretic ejection of drugs and the recording of field potentials evoked by stimulation of the perforant path. After application of 1.4 nmoles of oleate, two 8 pulse trains of high frequency stimulation (400 Hz) were delivered.

Oleate ejection prior to the two 400 Hz trains caused the response to persist and grow beyond the initial potentiation, whereas a tris control or elaidate (trans stereoisomer of oleate, a very weak PKC activator) ejection had no effect on the response produced by the 400 Hz trains; the response decayed to baseline within 2 hr as monitored by 0.1 Hz test pulses. Ejection of arachidonate, which stimulates PKC to a lesser degree than oleate, caused the response to persist but not to grow. The mean (n=6/group) magnitude of the response measured two hours after drug application was positively correlated with PKC activation by the drug *in vitro* ($r=+.991$, $df=2$, $p<.02$).

Ejection of oleate without 400 Hz trains produced no alteration of the baseline response, and ejection of oleate two hours prior to the 400 Hz trains produced a response identical to the tris and elaidate controls. Oleate ejection into the molecular layer, where perforant path terminals synapse on granule cell dendrites was also seen to enhance the potentiated response, possibly at a lower dosage. Since neither oleate alone nor two 400 Hz trains alone produced a persistent enhancement of the LTP response, it suggests that oleate may act synergistically with the consequences of two 400 Hz trains to produce enhancement of LTP through PKC activation. (This work was supported by MH25281-12 and AFOSR83-0335 to A.R.)

320.1 OPIOID RECEPTORS AND ENDOGENOUS OPIOIDS IN DIVERSE HUMAN AND ANIMAL TUMORS. P.J. McLaughlin and I.S. Zagon, Dept. of Anatomy, The M.S. Hershey Med. Ctr., Penn State Univ., Hershey, PA 17033

Following our laboratory's discovery that endogenous opioid systems (i.e., opioid receptors and endogenous opioids) play an important role in regulating neurotumor development and growth (Science 221:671, 1983; Life Sci. 35:409, 1984), we have been especially interested in examining whether the components of the endogenous opioid systems are found in other forms of cancer. In the present study, we have evaluated a number of varied human and animal tumors for the presence of endogenous opioid immunoreactivity. In addition, we have performed opioid receptor binding assays to establish the presence of receptors, and pursued the characterization of these receptors as to type, affinity, and number.

Animal tumors harvested from tumor transplantation experiments included: S20Y murine neuroblastoma, N115 murine neuroblastoma, B16 mouse melanoma, R3230AC rat mammary, and Morris hepatoma (rat); in some cases, tumors were also grown in nude mice and these tissues evaluated. Human tumors grown in nude mice included: SK-N-MC neuroblastoma and HTB-14 glioblastoma. Human tumors obtained from biopsy included: adenocarcinoma of the breast, mixed Mullerian tumor of the uterus, squamous cell carcinoma of the tongue, prostatic rhabdomyosarcoma, squamous cell carcinoma of the skin, endometrial adenocarcinoma, B-cell lymphoma, adenocarcinoma of the cecum, and ovarian fibroma.

Radioimmunoassays of β-endorphin and/or met-enkephalin revealed measurable levels of immunoreactivity (at least 40 pg/mg protein) in those human and animal tumors tested. In nearly all cases, there was no saturable binding for a prototypic mu ligand (DAGO). Binding assays for delta receptors using DADLE (and appropriate blocking agents revealed saturable binding in most tumors; binding affinities (K_d's) ranged from 0.22 to 25.9 nM and binding capacities (B_{max}'s) ranged from 4 to 77 fmol/mg protein. Binding assays for kappa receptors using EKC (and appropriate blocking agents) also revealed saturable binding in most tumors; binding affinities ranged from 0.12 to 25.5 nM and binding capacities ranged from 10 to 606 fmol binding/mg protein.

Our data show that endogenous opioids and opioid receptors are characteristic of a broad spectrum of human and animal tumors, representative of diverse embryological backgrounds. The function of endogenous opioid systems in neural and non-neural tumors is unknown. Given information derived from explorations in murine neuroblastoma, the most intensely studied system thus far, endogenous opioids and opioid receptors may be involved in aspects of development and growth of neoplasia.

Supported by NIH grants NS-20623 and NS-20500, and grants from the Pennsylvania Research Corporation.

320.2 OPIOID ANTAGONIST MODULATION OF MURINE NEUROBLASTOMA IN NUDE MICE: STUDIES ON THE ROLE OF IMMUNITY IN ENDOGENOUS OPIOID CONTROL OF CANCER. L. Nagy*, P.J. McLaughlin and I.S. Zagon (SPON: T. Lloyd). Dept. Anatomy, The M.S. Hershey Med. Ctr., Penn State Univ., Hershey, PA 17033.

Opioid agonists have been shown to modulate the growth of neuroblastoma, in vitro and in vivo, in a stereospecific and naloxone-reversible fashion. Opioid antagonists also influence tumorigenic events, and do so stereospecifically and in a manner dependent on the duration of opioid receptor blockade. For example, administration of opioid antagonists at dosages which block the receptor for a short time each day, markedly inhibits tumor response and extends the survival of mice inoculated with tumor cells. It now appears that endogenous opioids may be involved in immunological response. To examine the role of immunity in opioid antagonist-induced modulation of cancer, the effects of opioid antagonist administration on tumor response in nude mice (which are immunologically compromised) were monitored.

Daily injections 0.1 mg/kg naltrexone (NTX), which invoked a receptor blockade for 6 to 8 hr/day (as determined by morphine-challenge experiments), were given to athymic nude (nu/nu) mice. Various dosages of S20Y neuroblastoma (2.5×10^4 to 10^6 cells/mouse) were inoculated into these nude mice. NTX-treated animals exhibited a 31-92% delay in latency time prior to the appearance of a measurable tumor, and a 27-49% increase in mean survival time; the magnitude of antitumor response was governed by tumor burden. Inoculation of neuroblastoma cells into control animals resulted in measurable tumors in 10-13 days, and mean survival times of 30 to 34 days.

In order to examine whether the transplanted neuroblastoma cells expressed endogenous opioid immunoreactivity and opioid receptors, tumors from control animals were excised when tumor size was 15 mm^2. Radioimmunoassay procedures showed that the tumor tissue contained immunoreactive analogues of β-endorphin (39.7 pg/mg protein). Opioid receptor binding assays for mu, delta, and kappa receptors were conducted. Radiolabeled ligands (^{3}H-DAGO, ^{3}H-DADLE, ^{3}H-EKC) were incubated with tumor tissue and selected blocking agents, and specific binding was determined in the presence of unlabeled DAGO, DADLE, and EKC. Receptor binding assays revealed specific saturable binding for delta and kappa receptors, but not for the mu receptor.

These results suggest that immunity may not play a major role in endogenous opioid regulation of oncogenesis. In addition, our study shows the feasibility of using the nude mouse as a valid model to explore the modulation of human neoplasia by endogenous opioids.

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320.3 IRREVERSIBLE OXYMORPHONE HYDRAZONE DERIVATIVES: ANALYSIS OF ANALGESIC POTENCY AND DURATION. R.J. Bodnar, E.F. Hahn*, J.A. Clark* and G.W. Pasternak. Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Ctr., Depts. of Neurology and Pharmacology, Cornell Univ. Medical Col., and Lab. of Biochemical Endocrinology, Rockefeller Univ., New York, NY 10021.

Oxymorphone phenylhydrazone (P=OXY) and oxymorphone p-nitrophenylhydrazone (PNP=OXY) irreversibly inhibit opiate binding in standard competition assays. The present study compared the analgesic potency and duration of action of these hydrazone derivatives with that of oxymorphone on the tail-flick test in mice. At 30 min following low (0.1-1.0 mg/kg, SC) doses, both hydrazone derivatives were significantly less potent than oxymorphone (P=OXY: 3-fold; PNP=OXY: 10-fold). All three dose-response curves appeared to be parallel. Different relative analgesic potencies and durations of action occurred at higher doses. At low doses, the duration of analgesia of the hydrazone derivatives and of oxymorphone were similar. As the doses increased (6-50 mg/kg), the duration of analgesia of the hydrazone derivatives increased far more than that of oxymorphone. For instance, at 25 mg/kg, no oxymorphone animals were analgesic by 6 h. Yet 50% of P=OXY and 70% of PNP=OXY mice displayed significant analgesia at this dose until 10 h and 12 h respectively. Similar patterns were observed in this entire dose range with the duration of analgesia increasing as the hydrazone derivative dose increased. Analgesia induced by PNP=OXY, like oxymorphone, was naloxone-reversible. Similar results were observed following quantal or graded analyses. PNP=OXY (50 mg/kg) decreased ^{3}H-DADL and ^{3}H-DAGO binding in whole-mouse brain 16 h after administration. At this dose, the inhibition was relatively mu-selective. These derivatives appear to be excellent candidates as long-acting analgesics and presumably act through their irreversible actions upon opiate binding sites. (Supported by ACS PDT 169).

320.4 **EVIDENCE FOR MU-DELTA OPIOID RECEPTOR INTERACTIONS IN VIVO.** Julius S. Heyman, Sheila A. Mulvaney & Frank Porreca (SPON: P. Monroe). Department of Pharmacology, University of Arizona, Tucson, AZ 85724

Previous work has suggested the possibility of interactions between classes of opioid receptors in vivo and in vitro. Vaught and Takemori (J. Pharmacol. Exp. Ther. 208: 86-90, 1978) have demonstrated that intracerebroventricular (i.c.v.) administration of subanalgesic doses of Leu-enkephalin (a delta opioid ligand) potentiated, while Met-enkephalin antagonized, morphine analgesia. Furthermore, instances of non-competitive displacement of labelled mu-ligands by the delta preferring enkephalins have led to the suggestion that mu and delta receptors exist in an opioid receptor complex (e.g. Rothman and Westfall, Mol. Pharmacol. 21: 538-547, 1982). This concept was further investigated in vivo using a selective delta receptor agonist (D-Pen^2, D-Pen^5)-enkephalin (DPDPE), the mu agonist morphine and a stabilized Met-enkephalin analogue (D-Ala^2, Met^5)-enkephalinamide (DAMA). Additionally, the selective delta opioid antagonist, ICI 174,864 (N,N-diallyl-Tyr-Aib-Aib- Phe-Leu-OH, where Aib is alpha-aminoisobutyric acid) and the mu antagonist, beta-funaltrexamine (beta-FNA) were used to aid in separation of receptor-mediated events.

The procedure was the mouse (male, ICR, 20-30 gm) warm water (55° C) tail-flick test; the latency to a rapid flick of the tail was taken as the endpoint and compared before and after graded i.c.v. doses of morphine. Testing occurred 20 min after injection. Following the establishment of i.c.v. dose-response lines for DPDPE and DAMA, threshold (subanalgesic) doses (1 and 0.1 ug, respectively) were given together with an i.c.v. ED50 dose of morphine (1 ug). Co-administration of DPDPE and morphine potentiated the morphine effect; this potentiation was antagonized by concurrent administration of ICI 174,864 (1 ug) given together with the morphine/DPDPE injection. When DAMA and morphine were co-administered, the morphine analgesic effect was significantly reduced; similarly, this reduction was reversed with concurrent ICI 174, 864 (1 ug) given together with the morphine/DAMA injection. Tolerance to morphine induced by a single subcutaneous morphine injection (100 mg/kg, 5 hr prior to testing, a time when no acute morphine effect could be seen) did not alter the ability of DPDPE to potentiate an ED50 dose (in tolerant mice) of morphine. Pretreatment with the non-equilibrium mu-antagonist, beta-FNA (10 ug, i.c.v. at -4 hr) while failing to produce any agonist effects alone, displaced the i.c.v. morphine, but not DPDPE, dose-response curve to the right. Administration of threshold analgesic doses of DPDPE or DAMA concurrently with morphine in beta-FNA pretreated mice failed to affect the response to morphine.

The present experiments support the concept of mu-delta interactions in vivo. The effects of the mu agonist morphine can be either potentiated or antagonized by stabilized enkephalins; these effects can be prevented by ICI 174,864, suggesting delta receptor mediation. Additionally, the proposed receptor complex may be inactivated by beta-FNA pretreatment. These data suggest that the enkephalins may play a modulatory role in analgesic processes. Supported by USPHS Grants NS 21193 and AM 36289.

320.5 EFFECTS OF (+)-N-ALLYLNORMETAZOCINE (SKF 10,047) AND PHENCYCLIDINE BINDING SITE LIGANDS ON VISUAL EVOKED RESPONSES IN THE RABBIT. G. F. Steinfels, G. P. Alberici and S. W. Tam, E. I. DuPont de Nemours & Co., Biomedical Products Dept., Wilmington, DE., 19898 U.S.A.

Recent biochemical and anatomical evidence indicate that (+)-N-allylnormetazocine (NANM) labels a binding site distinct from the phencyclidine (PCP) binding site. Despite these in vitro differences, in vivo studies generally show that (+)-NANM and PCP produce similar pharmacologic actions which are usually assumed to result from actions at PCP binding sites in the brain. Because PCP has been previously shown by investigators to produce electroencephalographic alterations and sensory disruptive effects, we hypothesized that examination of a sensory elicited evoked potential might discriminate between pharmacologic actions at the PCP or (+)-NANM binding site. Visual evoked potentials (VEP) were recorded in awake New Zealand rabbits from the primary visual cortex referenced to a midline electrode over the frontal cortex. For VEP recordings the rabbits were held in restraining cages with their eyes 28 cm from the photic stimulator. The visual stimulus consisted of a 15 μsec flash (4.75 x 10^6 lumens) delivered at a rate of 1/sec. Each VEP waveform was an average of 50 samples which were bandpassed at 5-100 Hz. Several components were identified on the VEP, however the most significant change was in the latency of the negative peak at 126 msec (N126). PCP (0.5-1.6 mg/kg, im) increased the latency of the N126 peak in a dose dependent manner to 150 msec. Similarly, (+)-NANM (1-4 mg/kg) also increased the latency of N126 to a maximum of 135 msec. Receptor binding studies in a separate group of rabbits indicated that PCP and (+)-NANM each had significant affinity at both PCP and (+)-NANM binding sites. Interestingly, (+)-pentazocine was more selective than (+)-NANM for the (+)-NANM vs. PCP binding site. However, (+)-pentazocine, unlike PCP or (+)-NANM, produced no change in the N126 latency at doses up to 10 mg/kg. Our data suggest that the N126 latency increases produced by (+)-NANM appear better correlated with its binding at the PCP site rather than at the distinct high affinity (+)-NANM binding site. The receptor binding data also indicate that (+)-pentazocine's greater selectivity for the (+)-NANM binding site make it a better choice as the prototypic (+)-NANM binding site ligand. In vivo studies with (+)-pentazocine might better characterize the functional relevancy of the (+)-NANM binding site.

320.6 EFFECT OF RECEPTOR SELECTIVE OPIOIDS ON BEHAVIORAL THERMOREGULATION: DIFFERENTIAL ALTERATION OF SET POINT. R.L. Spencer and T.F. Burks. Depts. of Psychology and Pharmacology, Univ. of Ariz., Tucson, Ariz. 85724

The ability of opioids to produce pronounced changes in body temperature (Tb) raises the possibility that endogenous opioids may be involved in the central control of thermoregulation. Tb measurements alone are not sufficient to determine at which point in the thermoregulatory process a drug acts. Examination of behavioral thermoregulatory responses to drugs allows determination of alterations of the thermoregulatory set point, reflecting modification of the central control of Tb. Receptor selective opioids were studied in a system which allowed monitoring of behavioral thermoregulatory responses of rats. Rats were placed in a thermocline - a 2 m long, 10 cm wide opaque hollow plexiglass tube with an internal linear temperature gradient ranging from 6-36 °C. The position of a rat in the thermocline (ambient temperature selected) was determined by a series of infrared LEDs and photocells. The Tb of the rat was monitored via a telemetry thermistor surgically placed in the peritoneal cavity. Rats were tested in the thermocline with (G+) or without (G-) the presence of a temperature gradient. Testing in the absence of a temperature gradient (22±1 °C) provided a measure of general activity levels. All compounds were injected i.c.v. The μ agonist [D-Ala^2,N-methyl-Phe^4,Gly^5-ol] enkephalin (DAGO, .3 μg) produced a mean (±SEM) increase in Tb of +2.56(.36) °C in G+ rats and an increase of +1.74(.09) °C in G- rats. The DAGO treated rats selected a warmer ambient temperature (Ta) (30.3±1.6 °C) compared to control rats (25±.4 °C) during the rising phase of the thermic response to DAGO. The δ agonist, [D-Pen^2, D-Pen^5]enkephalin (DPDPE, 30 μg) produced a decrease in Tb of -.82(.16) °C in G+ rats and a decrease of -.62(.16) °C in G- rats. The DPDPE treated rats selected a cooler Ta (13.9±1.4 °C) compared to control rats (19.7±1.1 °C) during the falling phase of the thermic response to DPDPE. The κ agonist, U50488H (100 μg) produced a decrease in Tb of -1.30(.13) °C in G+ rats and -1.13(.09) °C in G- rats. The U50488H treated rats selected a cooler Ta (13.3±2.2 °C) compared to control rats (20.4±1.0 °C) during the falling phase of the thermic response to U50488H. Thus, DAGO produced an increase in Tb which was facilitated by a behavioral selection of a warm Ta, while both DPDPE and U50488H produced decreases in Tb which were accompanied by selection of a cool Ta. These results indicate that different opioid agonists are able to shift the thermoregulatory set point in opposite directions and provides support for the hypothesis that endogenous opioids are involved in the central control of thermoregulation. (Supported by DA02163.)

320.7 DIRECT IN VIVO EVIDENCE FOR DELTA RECEPTOR MEDIATED ANALGESIA IN MU RECEPTOR DEFICIENT INBRED STRAINS OF MICE. J.R.Mathiasen* and J.L. Vaught* (SPON: B. Damiano). Dept. of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477.

Although several types of opioid receptors have been identified utilizing biochemical and pharmacological techniques, a prominent question still exists: can δ receptors mediate analgesia? Chaillet et al. (Eur. J. Pharmacol.,101:83,1984) have suggested that only μ receptors mediate analgesia. Consistent with this, analgesic naloxone pA_2 values for intracerebroventricularly (icv) administered morphine sulfate (MS), a prototypic μ receptor ligand, or [D-Pen^2,L-Pen^5]enkephalin (DPLPE), a prototypic δ receptor ligand, are the same suggesting a single, presumably μ, receptor type mediating analgesia [Heyman et al., Life Sciences (in press) 1986]. Paradoxically, DPLPE is not cross-tolerant to MS when administered icv to mice suggesting more than one receptor-mediated mechanism [Porreca et al., JPET (in press) 1986]. Despite classical means to determine the opioid receptors involved in antinociception the question remains unresolved. We have addressed this using a recombinant-inbred strain of mouse CXBK, the C57BL/6JbgJ and the progenitor strains of CXBK, C57BL/6By and BALB/cBy. We have compared all responses to our standard laboratory mice CRS-CD1. CXBK mice are deficient in μ opioid receptors (Baran et al., Life Sciences,17:633,1975). Autoradiographic analysis has shown δ opioid binding in the CNS of C57BL/6By and CXBK not to differ while μ binding was less in CXBK (Moskowitz and Goodman, Brain Research,360:108,1985). During the course of these studies, we discovered that C57BL/6JbgJ mice are also less responsive to MS (icv). MS and DAGO (icv) analgesia, as assessed in the tail flick test, was comparable in CRS-CD1 and progenitor strains of mice. However in the CXBK and C57BL/6JbgJ, a 10 to 30x decrease in analgesic potency was observed for MS and DAGO; consistent with μ receptor deficiency. However, DPLPE-induced analgesia was similar across all strains of mice. Given the observations of Moskowitz and Goodman that the δ receptor population is unaltered in CXBK mice, these data provide conclusive evidence that the δ receptor is capable of mediating analgesia. We also suggest that the CXBK or C57BL/6JbgJ strains of mice provide in vivo models for the examination of the physiological and pharmacological role of δ opioid receptors.

	MS	DAGO	DPLPE
CRS-CD1	0.3(0.1-0.5)	0.01(0.008-0.02)	3.0(2.1-5.0)
C57BL/6By	0.5(0.08-1.2)	50 ng=75%	3.2(2.0-6.5)
BALB/cBy	0.6(0.4-1.1)	.009(.004-.01)	1.8(1.1-3.2)
CXBK	~10	50 ng=0%	5.3(3.1-15.4)
C57BL/6JBgJ	3.1(1.1-15.7)	50 ng=25%	3.7(2.9-4.6)

[ED_{50} values in ug]

320.8 ENDOGENOUS OPIOID REGULATION OF CELL PROLIFERATION IN THE DEVELOPING BRAIN. I.S. Zagon and P.J. McLaughlin. Dept. Anatomy, The M.S. Hershey Med. Ctr., Penn State Univ., Hershey, PA 17033.

Studies in our laboratory have demonstrated that endogenous opioid systems regulate the growth of the developing nervous system (Science 221:1149, 1983; Life Sci. 35:2057, 1984). The underlying mechanisms are unclear, but investigations from our group and others suggest that modulation of mitotic events is involved. In the present study, 6-day old rats were injected with naltrexone (NTX), an opioid antagonist. Dosages of either 1 or 50 mg/kg NTX were utilized to block the receptor for 4-6 hr/day and 24 hr/day, respectively. Thirty min prior to sacrifice at 2 or 12 hr following NTX administration, rats received an injection of ^{3}H-thymidine. Brains were prepared for histology, sectioned in the sagittal plane, and processed for autoradiography. The labelling index (LI) for external germinal cells (EGL) was determined in the VIII lobule of the cerebellum.

At both 2 and 12 hr, a 32% LI in the EGL was recorded for control specimens. Animals given 1 or 50 mg/kg NTX had LI's of 37% and 41%, respectively; these elevated LI's were statistically greater than control levels. At 12 hr, the 50 mg/kg NTX group had an LI of 42%; this was significantly different from controls. The 1 mg/kg NTX group had a marked reduction ($p<0.01$) in their LI (23%) at 12 hr.

In order to confirm and extend these observations on the role of endogenous opioid development, a second experiment was designed to directly ascertain the effects of methionine-enkephalin (Met-Enk) on the EGL cells of 6-day old rats. Ninety minutes after injection of 1 mg/kg Met-Enk, animals received ^{3}H-thymidine and were sacrificed 2 hr after Met-Enk administration. Controls included a group given sterile water, as well as a group receiving Met-Enk and naloxone (1 mg/kg). Cerebella of rats in the Met-Enk group had an LI of 22% in comparison to a 32% LI for control animals; this difference was statistically significant. Rats given naloxone and Met-Enk were comparable to controls in regard to LI.

These results indicate that endogenous opioids play an important role in regulating cell proliferation in the developing brain. Based on these experiments, endogenous opioids appear to serve an inhibitory role on neural cell proliferation. Our results also extend previous findings in which an increase in cell number was recorded in developing animals subjected to a complete daily opioid receptor blockade, as well as a decrease in cell number observed in rats subjected to a temporary daily receptor blockade (J. Neurosci., in press, 1986; Devel. Brain Res., in press, 1986).

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320.9 INFLUENCE OF NALOXONE AND MORPHINE ON THE MONOAMINE AND CORTISOL RESPONSE OF INFANT SQUIRREL MONKEYS TO MATERNAL SEPARATION.
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It has been hypothesized that endogenous brain opioids inhibit brain circuits that mediate the distress of an infant separated from its mother. One measure of separation distress is an increase in infant vocalizations. A blockade of the opioid receptor by naloxone increases vocalization in a number of species. Conversely, administration of morphine inhibits vocalization. In addition to the behavioral response to separation, both the pituitary-adrenal and brain noradrenergic systems are activated following separation from the mother. In this study, independent groups of 3- to 5-month-old infant squirrel monkeys were treated i.v. with morphine, either of the two forms of naloxone (+naloxone or -naloxone), or saline prior to a 4-hr separation from their mothers. During the separation the number of species-specific "isolation peeps" emitted by the infant was recorded. Infants were separated under the respective drug treatments for 3-6 times at 10-day intervals. At the end of the separation, either cervical CSF samples were collected following brief ether anesthesia, or peripheral blood samples were taken under ether anesthesia. CSF samples were analyzed for the monoamine metabolites HVA, MHPG and 5HIAA. Blood samples were assayed for plasma cortisol levels. Between separations the infants were left undisturbed with their mothers in the home social group.

The results indicate that morphine treatment reduced infant vocalizations during the 4-hr separation. In contrast, the active form of naloxone increased infant vocalizations. The inactive form of naloxone did not change the rate of vocalization compared to the saline controls. Although the monoamine metabolite and cortisol response of the morphine and inactive naloxone subjects is in the process of analysis, the active naloxone and saline infants' response indicate that the elevations of MHPG and cortisol did not differ. Thus, vocalization may be a specific motor pattern emitted by an infant following separation which is modulated by the opioid system. However, the physiological indices of arousal are not influenced by administration of the active form of naloxone. Therefore, these data are not supportive of an opioid hypothesis of social bonding.

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320.10 ENDOGENOUS OPIOID REGULATION OF BRAIN GROWTH IS NOT DEPENDENT ON THE MU RECEPTOR. S. Ditty*, P.J. McLaughlin and I.S. Zagon (SPON: S. Prouty). Dept. Anatomy, The M.S. Hershey Med. Ctr., Penn State Univ., Hershey, PA 17033.

Previous studies in our laboratory have demonstrated that opioid agonists retard neural development in a stereospecific and naloxone-reversible fashion. Opioid antagonists also influence neurodevelopment in a stereospecific nature, and in a manner dependent on the duration of opioid receptor blockade. These results, gathered from extensive in vivo and in vitro studies, indicate that endogenous opioid systems (i.e., endogenous opioids and opioid receptors) subserve a novel and important function: growth regulation. In order to ascertain which receptor subtype(s) is(are) functionally involved in cell proliferation and differentiation, we examined the effects of β-funaltrexamine (β-FNA) on development; this compound binds selectively and irreversibly to mu receptors. Presumably, chronic administration of β-FNA, at concentrations which antagonize morphine-induced analgesia, should alter growth if mu receptors are involved.

Offspring of Sprague-Dawley rats were injected every 48 hr from birth with 5 mg/kg β-FNA or sterile water. A group of animals injected daily with 50 mg/kg naltrexone, a dosage known to block the receptor the entire day and which markedly influences growth, was also included and served as "positive" controls. Animals were sacrificed on day 22 and evaluated for body, brain, and organ weights, brain dimensions, and morphometric analysis of total cerebellar area.

β-FNA had little effect on body weight throughout the preweaning period, nor were body or organ weights altered on day 22. Brain and cerebellar weights and measurements, as well as total cerebellar area, of β-FNA rats were comparable to controls. In contrast, 50 mg/kg naltrexone-treated rats exhibited 10-42% increases in body weight and organ weights, and 6-17% increases in brain and cerebellar weights and measures. These data indicate that at concentrations which block morphine-induced analgesia, β-FNA had little effect on growth processes. This would suggest that mu receptors, selectively antagonized by β-FNA, are not involved in the modulation of growth by endogenous opioid systems.

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320.11 THE ROLE OF SEROTONIN IN OPIATE-INDUCED PROLACTIN SECRETION AND ANALGESIA IN THE DEVELOPING RAT. L.A. Bero and C.M. Kuhn
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The anterior pituitary hormone prolactin (PRL) plays a role in sexual function and the stress response in both rats and humans. Because normal PRL secretion is necessary for sexual maturation in both males and females, an understanding of PRL regulation in the developing animal is particularly important. Our laboratory has demonstrated previously that opiates stimulate PRL release before the PRL response to serotonergic drugs is functionally mature. The present study demonstrates that opiates stimulate PRL secretion through a serotonergic mechanism in adult rats, but a non-serotonergic mechanism in neonatal rats.

Adult (50 day old) and neonatal (10 day old) rats were pretreated with the serotonin antagonist, cyrpoheptadine (CYPRO), or the neurotoxin, 5,7-dihydroxytryptamine (5,7DHT). Both CYPRO (10 mg/kg, i.p.) and 5,7DHT (50 µg, ivt. in 10 day olds, 100 µg in adults 4 days before morphine) attenuated the PRL response to morphine in adult, but not neonatal, animals. 5,7DHT decreased serotonin and 5-hydroxy-indoleacetic acid substantially in the hypothalamus (60% and 70% depletion, respectively, in neonates and adults). Interestingly, when rats were pretreated with 5,7DHT at 3 days of age and injected with morphine at 25 days of age, serotonin depletion was more pronounced, but the PRL response to morphine was not blocked. In addition, the PRL response to 5-hydroxytryptophan was greatly potentiated, suggesting that functional supersensitivity developed in the 5,7DHT treated animals.

The ability of CYPRO and 5,7DHT to block the serotonergic component of a different morphine-induced behavior in the neonate was tested using the tail immersion test for analgesia. Morphine produced profound analgesia in the 10 day old rat pup. When the same treatment paradigms used in the hormone studies were given, CYPRO and 5,7DHT attenuated morphine-induced analgesia markedly in the 10 day old rat.

In summary, opiates appear to mediate their stimulatory effects on PRL release, at least in part, through a serotonergic mechanism in adult rats. In contrast, opiates do not stimulate PRL release through a serotonergic mechanism in neonatal rats, which lack a PRL reponse to serotonin agonists. This functional deficit appears to be specific to the serotonin neurons regulating PRL secretion because the serotonergic mechanisms involved in morphine-induced analgesia are functional in the neonate. Our findings suggest that the neural mechanisms by which opiates alter PRL secretion may change during development, making these pathways particularly susceptible to disruption with long-lasting consequences in adults.

320.12 ROLE OF OPIOID PEPTIDES IN AUTISM: EFFECTS OF ACUTE ADMINISTRATION OF NALTREXONE. B.H. Herman, M.K. Hammock*, A. Arthur-Smith*, J. Egan*, I. Chatoor*, N. Zelnik*, M. Corradine*, K. Appelgate*, R.L. Boeckx* & S.D. Sharp*(SPON: V.P. Bingman). Brain Res. Cen. & Depts. Psychiat., Neurosurg. & Clinical Chem. of Children's Hosp. Natl. Med. Cen. (CHNMC) & Depts. Psychiat., Neurosurg. & Child Health & Develop. of George Washington Univ. Sch. Med., CHNMC, 111 Michigan Ave., N.W., Washington, D.C. 20010.

The hypothesis that some of the symptoms of autism including a failure to seek social attachments may be due to overactivity in some opioid system in the brain was tested. Animal research suggests that opioids are important for the maintenance of infant-maternal social behavior (cf. Panksepp et al., Neurosci. Biobehav. Rev. 4:473, 1980). There is also recent data demonstrating significant elevations in the concentrations of opioid peptides in the cerebrospinal fluid of autistic versus control children (Gillberg et al., Arch. Gen. Psychiat. 42: 780, 1985).

The acute effects of the orally administered, opioid receptor antagonist-naltrexone HCl (NLT)-was evaluated in five autistic children (4 male, 1 female; 4 to 12 years of age). As required, subjects (Ss) were given NLT (0.5-, 1.0-, 1.5- and 2.0-mg/kg) once per week according to an ascending dose regimen with baseline and placebo surrounds. Pre- and post-drug (2h) sessions were conducted. Ss were evaluated on the BRC Social Proximity test (BRC Prox; 10 min test designed to determine social interactions of a child toward a familiar adult volunteer (V) in a playroom), the BRC Autism scale (parent questionnaire) and the BOS Scale. All sessions were videotaped for blind analysis. Means ± standard error of the means are reported. Significance is $p < .05$.

No significant effects of NLT were obtained on heart rate, blood pressure or axillary temperature. The results of the BRC Prox tests were as follows. Consistent with a diagnosis of autism, under placebo, Ss spent the least percent of time in a quadrant near the V (7.2 ± 3.7) as compared with the three other empty quadrants (range from 20.6 ± 13.3 to 51.0 ± 20.2). This pattern was not significantly altered by NLT. Locomotor activity (number of squares crossed) was not significantly influenced by NLT, although a trend toward a decrease with the 2.0 mg/kg dose was suggested (Pre = 33.4 ± 6.6, Post = 22.4 ± 1.0). Under placebo, autistics spent only 7.3 % of total test time (44 out of 600 sec) in eye contact with V, whereas this value is several fold higher in normals. Duration of eye contact was not significantly changed by NLT. Number of spontaneously uttered words was also not significantly affected by NLT.

In contrast with the above, parents observed some beneficial effects of NLT(decrease abnormal motor behavior, increase "enjoys hug") as determined by results of the BRC Autism Scale. As measured by radioimmunoassay, concentrations of β-endorphin-like immunoreactivity were similar in autistics and age-matched controls.

321.1 EFFERENT PROJECTIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA (CNA): A RE-EXAMINATION USING THE PHA-L ANTEROGRADE TRACING METHOD. T.S. Gray, M.M. Moga and D.J. Magnuson*. Dept. Anat., Loyola Stritch Sch. Med., Maywood, IL 60153.

Physiological studies have demonstrated that the CNA functions in the expression of autonomic responses associated with defensive or "stress-like" behaviors. These responses are mediated through the CNA's efferent connections with subregions of the hypothalamus and brainstem that are also involved in central autonomic regulation. In order to more precisely define the organization of the efferent subzones innervated by the the CNA, we have employed the highly-sensitive anterograde tracing technique developed originally by Gerfen and Sawchenko (1984). Injections of Phaseolis vulgaris-leucoagglutinin lectin (PHA-L) were applied iontophoretically into the CNA of Long-Evans black-hooded rats using standard stereotaxic technique. Animals were overdosed with sodium pentobarbital and their brains were fixed through vascular perfusion of buffered paraformaldehyde. PHA-L immunoreactivity was visualized by processing 20um thick sections using the avidin-biotin immunoperoxidase procedure. Injections of PHA-L confined within the boundaries of the CNA produced labeling of axons and terminal varicosities within all regions previously reported to receive CNA afferents. These include the bed nucleus of the stria terminalis (BNST), paraventricular and lateral hypothalamic nuclei, the thalamus, substantia nigra-ventral tegmental area, midbrain central gray, parabrachial nucleus, nucleus tractus solitarius and dorsal motor nucleus of the vagus. Labeled axons exited the CNA by traveling through 1) the ventral pathway over the optic tract, 2) the substantia inominata and 3) the stria terminalis. PHA-L immunolabeled beaded axons and terminal boutons formed a continuum from the CNA through the substantia inominata and anterior amygdaloid area coursing dorsally to the lateral BNST. Fibers also entered the lateral BNST through the stria terminalis. PHA-L immunolabeled terminals were found within the caudal ventrolateral periaqueductal gray. And, terminals were distributed selectively within a number parabrachial subnuclei including the external lateral, ventral lateral, central lateral and medial subnuclei (as defined by Fulwiler and Saper, '84). In addition, the PHA-L technique revealed several new findings not observed in previous studies using autoradiographic and/or HRP anterograde tracing methods. For example, labeled axons and terminal boutons appeared to innervate the periventricular region of the hypothalamus and the ependyma of the cerebral aquaduct in the midbrain. The present results will be further discussed with respect to possible difference between the projections of the medial and lateral CNA. (Supported by NS 20041).

321.2 COEXISTENCE OF NEUROPEPTIDES IN SYMPATHETIC PREGANGLIONIC NEURONS OF THE CAT. T.L. Krukoff and L. Bremner*. Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Canada. T6G 2H7.

A recent study (Krukoff et al., J. Comp. Neurol. 240: 90-122, 1985) has shown that sympathetic preganglionic neurons (SPN) in the cat contain at least four neuropeptides. The numbers and distribution of SPN containing these peptides in the autonomic areas of the spinal cord suggested that individual SPN contain more than one peptide. To investigate this possibility, 10 male cats received intrathecal injections of colchicine (600 µg in saline) under ketamine anesthesia (35 mg/kg i.m.). After 24-30 hr, cats were reanesthetized and perfused with Zamboni's fixative. Frozen transverse and longitudinal sections (50 µm) were cut from the thoraco-lumbar (T-L) spinal cord. In order to determine which peptides coexist in SPN, immunohistochemical double-labelling was done using antibodies (Immuno Nuclear Corp., Stillwater, MN) for leucine-enkephalin (ENK), neurotensin (NT), somatostatin (SS), and substance P (SP) in all combinations. Vecta Stain Kits for ABC-peroxidase and ABC-GO (Vector Labs, Burlingame, CA) were used to demonstrate immunoreactivity as brown and blue reaction products in the same tissue, respectively.

The intermediolateral nucleus (IML), nucleus intercalatus (IC), and central autonomic area (CA) of the T-L cord were analyzed for SPN which contained only one peptide or two coexisting peptides. It was found that SP coexists with all three other peptides, SS coexists with NT and SP, and ENK coexists only with SP. In all cases of coexistence, SPN which contained two peptides were found in the IML in almost all levels of the T-L cord. Much smaller numbers of SPN which contained two peptides (same combinations) were found in the IC and not all segments contained such neurons. In the CA, only one neuron was found which contained two peptides (SP/SS). The distribution of SPN containing two peptides in the combinations observed in this study suggests that these particular neurons may participate in more general functions of the autonomic nervous system and that they are not likely involved in the innervation of specific visceral organs.

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321.3 THE EFFERENT ROLE OF SENSORY AXONS IN NERVE-EVOKED BLADDER CONTRACTION. L. Kolton* and C.W. Bowers (SPON: Herman Gordon) Howard Hughes Medical Institute Laboratory and the Department of Physiology, University of California, San Francisco, 94143.

Electrical stimulation of the bullfrog's bladder nerve near its entry to the bladder neck produced a biphasic response consisting of a relatively fast contraction lasting 10-20 s with a response latency of approx. 2 s and a slower contraction of comparable amplitude lasting 30-60 s with a latency of approx. 4 s. The fast contraction was completely and specifically blocked with either atropine (3×10^{-7}M) or the nicotinic antagonist dihydro-ß-erythroidine (DHßE; 10^{-5}M) and could be elicited specifically by stimulation of the distal cut end of the 10th ventral root. The slower contraction was not blocked by atropine (10^{-5}M), DHßE (10^{-4}M), methysergide (10^{-5}M), cimetidine (10^{-5}M), chlorpheniramine (2×10^{-5}M), phentolamine (10^{-5}M), propranolol (10^{-6}M) or indomethacin (10^{-5}M) and could be specifically elicited by stimulation of the distal cut end of the 10th dorsal root at frequencies as low as 2Hz. Serotonin (10^{-8} - 10^{-6}M) specifically blocked the slow contraction without affecting the fast contraction; methysergide did not affect this blockade by serotonin.

Immunocytochemical techniques demonstrated a somatostatin (SS)-like antigen in parasympathetic neurons and their terminals as well as a substance P(SP)-like antigen in a putatively sensory network of fibers in the bladder wall. SS(10^{-5}M) had no effect on the bladder nor its neurally evoked contractions, while SP(10^{-9}M) produced prolonged bladder contractions. The effects of SP did not desensitize nor did the following antagonists (10^{-5}M) affect nerve-evoked or SP-induced contractions; [D-pro^2, D-phe, D-trp^9]-SP; [D-arg, D-pro^2, D-trp^{7,9}, leu^{11}]-SP; [D-pro^2, D-trp^{7,9}]-SP; and [D-arg^1, D-trp^7, D-leu^{11}]-SP (Spantide). ATP (10^{-5}M) also produced contraction of the frog bladder but α,ß-methylene ATP did not block this purinergic response.

These results demonstrate that a large portion of the bladder contraction produced by stimulation of the bladder nerve is due to "antidromic" stimulation of sensory axons. It is therefore likely that a substance in peripheral sensory terminals (perhaps SP or ATP) can be released as an effective agonist on bladder smooth muscle receptors. Because these sensory contractions can be elicited at relatively low frequencies and because it is known that there are stretch-sensitive sensory axons in the frog bladder (Stella, G. (1934) J. Physiol. 82:22P), it is plausible that these sensory terminals can act as a local efferent reflex system in vivo.

321.4 SYMPATHETIC NERVE FIBERS IN THE RAT CORNEA DEMONSTRATED BY THE HORSERADISH PEROXIDASE-WHEAT GERM AGGLUTININ (HRP-WGA) ANTEROGRADE TRANSPORT TECHNIQUE. Carl F. Marfurt and Debora A. Huseman. Northwest Center for Medical Education, Indiana University, Gary, IN

Small numbers of adrenergic fibers have been demonstrated using fluorescent techniques in the corneal stromas and epithelia of most mammals. However, the 3-dimensional distribution, termination sites, and functional significance of these fibers remain unclear.

In the present study, we investigated the sympathetic innervation of the rat cornea at the light microscopic level using retrograde and anterograde HRP-WGA tracing techniques. In the first series of experiments, 2% solutions of HRP-WGA in saline were applied to the scarified corneal surfaces of 12 adult rats. The tracer was taken up by the sensory and sympathetic nerve fibers of the cornea and transported retrogradely to their cell bodies of origin in the ipsilateral trigeminal (TG) and superior cervical (SCG) ganglia. The tissues were then perfusion-fixed and processed for HRP histochemistry according to the tetramethylbenzidine (TMB) technique. HRP-labeled TG cells outnumbered greatly HRP-labeled SCG cells by an average of 330:1. The number of HRP-labeled neurons in the TG ranged from 139-444; the number of HRP-labeled cells in the SCG ranged only from 0-3.

In a second set of experiments, the superior cervical ganglia of 32 rats were injected with 0.5-1.0µl of 2% HRP-WGA. The tracer was taken up by the neuronal somas and transported intraxonally into the peripheral fibers in the cornea. Forty-eight to 72 hours later, the corneas were immersion-fixed and processed for TMB histochemistry as whole mount preparations. The HRP-WGA-labeled nerve fibers were identified readily by the presence within their axoplasms of linear arrays of crystalline HRP-TMB reaction product. The majority of labeled fibers seen in our study (45-75 per animal) formed delicate plexuses around limbal blood vessels; a smaller population of fibers (0-9 per animal) entered the cornea proper. Most of the corneal sympathetic fibers entered the stroma in conjunction with prominent bundles of (unlabeled) trigeminal sensory fibers; a few, however, followed independent pathways into the cornea. The sympathetic fibers branched only once or twice, or not at all, before terminating. The vast majority distributed to the central half of the cornea, with only a sparse innervation provided to the peripheral cornea. HRP-filled expansions, suggestive of boutons en passant and terminal boutons, were found only in the most superficial laminae of the corneal stroma. HRP-labeled fibers or boutons were never observed in the corneal epithelium.

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321.5 PREGANGLIONIC AND SENSORY NEURONS OF THE RAT GREATER SPLANCHNIC NERVE: DISTRIBUTION AND MORPHOLOGY. L. Sepulveda* and I. Nadelhaft (SPON: P.J. Jannetta), VA Med. Ctr. and Depts. of Neurol. Surgery and Pharmacol., Univ. of Pittsburgh Med. Sch., Pittsburgh, PA 15261.

Previous reports on the locations of sympathetic preganglionic neurons (PGN) in the rat have shown them to be found predominantly in the intermediolateral nucleus (IML) and ipsilateral to the nerve labelled. In these studies, horseradish peroxidase (HRP) was applied to various nerves: cervical sympathetic trunk, adrenal gland, abdominal sympathetic trunk. Approximately 95% of th PGNs were located in the IML. A striking exception to this distribution occurred when HRP was applied to the hypogastric nerve. In that case 75% of the labelled PGNs were found in the dorsal gray commissure and the remaining 25% were divided about equally between the IMLs. To see if there were any other medially-located PGNs, HRP was applied to a major nerve not yet examined in rat: the greater splanchnic nerve.

Several hundred preganglionic neurons were found in segments T4 through T13. They were located exclusively ipsilateral to the experimental side and restricted overwhelmingly to the intermediolateral column (90%). Only 3 cells were found in the dorsal gray commissure. Several thousand sensory neurons were found in the ipsilateral dorsal root ganglia T4 through T13. Central afferent projections were noted throughout Lissauer's tract and in the medial and lateral marginal zones. The density in the medial collateral pathway was greater than in the lateral collateral pathway. A projection was also noted to the spinal nucleus of the dorsolateral funiculus. A few longitudinal fibers were found medially just below the central canal.

321.6 SEXUAL DIMORPHISM IN SYMPATHETIC PREGANGLIONIC NEURONS OF THE RAT HYPOGASTRIC NERVE. I. Nadelhaft and K.E. McKenna. VA Med. Ctr. and Depts. of Neurol. Surgery and Pharmacol., Univ. of Pittsburgh Med. Sch., Pittsburgh, PA 15261.

Horseradish peroxidase applied to the hypogastric nerve on one side labelled sensory and preganglionic neurons associated with segments T11 to L3. More than 94% of the preganglionic neurons were found in segments L1-L2. Most of these were located in the dorsal commissural nucleus (75%) with the balance divided about equally between the intermediolateral nuclei of both sides. There also were a small number of cells labelled in the intercalating region and a very few in the lateral funiculi. In the male an average of 415 preganglionic neurons were labelled per animal while in the female only 110 neurons were labelled.

More than 92% of the labelled sensory neurons were found in the L1-L2 dorsal root ganglia. Most of these neurons were of the smaller type. An average of 75% were ipsilateral to the labelled nerve and the remaining 25% were in the contralateral ganglia. There was no significant difference in the number of sensory neurons labelled in the two sexes; an average of 130 neurons were labelled per animal. Central afferent projections were noted in Lissauer's tract and in the lateral and medial collateral pathways around the dorsal horns.

In summary, a strong sexual dimorphism was found in the hypogastric preganglionic neurons but this was not shown in the sensory components.

321.7 THE ACUTE SURVIVAL NETWORK: CONNECTIONAL EVIDENCE FOR INTEGRATION OF CENTRAL AUTONOMIC, ANALGESIC, AND PAIN TRANSMISSION SYSTEMS IN THE RAT BRAIN. Edward Tongju Koh* and Gary D. Hammer* (SPON: K. Hamilton) Departments of Anesthesiology and Neuroscience, Tufts University School of Medicine, Boston, Mass. 02111.

The role of autonomic responses in "fight or flight" reactions has long been recognized. Over the last decade, the central structures governing these responses have been identified. Concurrently, a central analgesic system has also been delineated. Based on interconnections between the pathways, we postulate that they, together with a portion of the spinothalamic projection, may act as a unit; we will refer to this unit as "the acute survival network."

The autonomic control system originates in the caudal part of the nucleus of the solitary tract (nts), ascends to the parabrachial region of the pons (pb), and involves, in the forebrain, the bed nucleus of the stria terminalis, the central amygdaloid nucleus (ac), and the paraventricular nucleus of the hypothalamus (pvn; Brain Res 153:1). This axis regulates sympathetic and parasympathetic output via descending fibers from ac and pvn, and anterior and posterior pituitary hormones via fibers from the pvn.

The central analgesic system comprises the central gray substance (cgs), nucleus raphe magnus (rm) of the medulla, and the superficial layers of the spinal cord (Ann Rev Nsci 11:1180). Major inputs to the cgs include two cortical inputs, from medial prefrontal and sulcal (dorsal to the rhinal sulcus) cortices (Nsci 7:133).

Several projections crosslink these two systems. The autoradiographic studies which revealed the raphe-spinal tract also showed that rm projects heavily to the parabrachial area. Other such connections include ac to cgs, pb to cgs, rm to pb, and nts to rm. Furthermore, only two cortical areas have been implicated in visceral pathways: medial prefrontal and sulcal cortices, which project to pb and nts, and receive input from pb (SNS 11:1275).

The classical spinothalamic path, which affects somatosensory cortex via ventrobasal thalamus, does not intersect with analgesic or autonomic pathways. However, Craig et al. have described a spinothalamic tract which involves nucleus submedius. The projection zone of nucleus submedius is sulcal cortex (JCN 206:28).

Functional integration of the systems is implied by findings that 1) stimulating the nts leads to analgesia (SNS 11:637), 2) pb stimulation blocks reaction to aortic cross-clamping (SNS 11:1180), and 3) baroreceptor activation leads to analgesia (Nsci B 8:343).

The physiologic action of this network may be seen at times of dire threat followed by tissue injury. Autonomic responses prepare an animal for combat or escape, analgesia allows it to act despite injury, and the subsequent pain triggers recuperative activity. A similar formulation has already been put forward on behavioral grounds (Behav Brain Sci 3:291); as reviewed above, there is ample neuroanatomical evidence to lend these notions further credence.

321.8 LESIONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS ATTENUATES THE PRESSOR AND DRINKING RESPONSE TO ANGIOTENSIN II ACTING AT THE SUBFORNICAL ORGAN. M.B. Gutman*, D.L. Jones and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

Angiotensin II (AII) has been reported to act at the subfornical organ (SFO) to increase arterial pressure, water intake and vasopressin secretion. Neuroanatomical and electrophysiological techniques have been used to demonstrate direct projections from the SFO to the paraventricular nucleus of the hypothalamus (PVH). In addition, lesions of the PVH have been shown to attenuate the pressor response elicited by electrical stimulation of the SFO in anesthetized rats. Furthermore, the PVH has also been implicated in the regulation of ingestive behavior. Taken together these observations suggest that the PVH may be involved in the pressor and drinking responses to AII acting at the SFO. To investigate this possibility, the effect of kainate lesions of the PVH on the pressor and drinking responses elicited by microinjection of AII directly into the SFO was tested. Male Wistar rats were implanted with guide cannulae above the SFO and the PVH (bilaterally) and with aortic catheters via the femoral artery for chronic monitoring of arterial pressure. Following recovery (1 day) from the surgery, drinking and cardiovascular responses were elicited by injection of 5 ng (0.2 µl) AII into the SFO of unrestrained animals. Pressor responses always preceded the drinking responses and were not accompanied by behavioral changes. Both responses occurred within 2 min following injection. Following this initial test kainic acid (0.5 µg in 0.2 µl) was injected bilaterally into the PVH of the rats under pentobarbital anesthesia (60 mg/kg) and the animals were allowed to recover for 1 day. Kainate lesions of the PVH significantly attenuated the pressor response (15 ± 1 mmHg before and 5 ± 2 mmHg after the lesion, n = 5) and abolished the drinking response (7.7 ± 1.8 ml/hr before and 0 ml/hr after the lesion, n = 6). The effects of PVH damage was specific since lesions of adjacent areas such as the zona incerta, lateral hypothalamus, nucleus reuniens and medial preoptic area did not effect the responses. These observations suggest that the PVH plays an important role in mediating the pressor and drinking responses to SFO activation by AII. (Supported by Medical Research Council of Canada and Heart and Stroke Foundation of Ontario).

321.9 PROGRESSIVE AUTONOMIC FAILURE: MICRONEUROGRAPHY OF THE DECENTRALIZED POSTGANGLIONIC SYMPATHETIC EFFERENT UNIT. R. Dotson*, P. Marchettini*, M. Cline*, J. Ochoa. (SPON: R. Sufit). Department of Neurology, University of Wisconsin Hospital, Madison, Wisconsin 53792, USA.

Patient with chronic autonomic failure had loss of functions requiring sympathetic effector activation: 1) Vasomotor: postural hypotension, a blocked Valsalva response monitored with intra-arterial blood pressure recording, and absent cold-or-pain-induced skin vasoconstriction monitored by pulse plethysmography and thermography.
2) Sudomotor: absence of spontaneous and of heat-induced sweat.

Such manifestations might equally result from preganglionic, postganglionic, or combined dysfunction, the different forms having different outcomes. For prognosis and to investigate the basis of both anticipated and paradoxical clinical features (spontaneous episodic piloerection and cold skin), we invasively tested the status of postganglionic sympathetic neurons.
a) Catecholamine (epinephrine and norepinephrine) and dopamine levels (supine): normal, indicating functional postganglionic sympathetic neurons.
b) Skin nerve biopsy: normal numbers, caliber spectrum and ultrastructure of unmyelinated axons, implying intact subpopulation of sympathetic efferent fibers.
c) Microneurography (median and ulnar skin nerve fascicles): absent spontaneous and reflexly induced typical multi-unit sympathetic postganglionic discharges (nine different occasions: highly improbable false negative from sampling). Findings were consistent with sympathetic failure but could not discriminate pre - vs. postganglionic lesion.

Strikingly, on three occasions, episodes of otherwise typical spontaneous sympathetic discharges were recorded. However, they were not influenced by sympathetic reflex maneuvers. In possible relation to such discharges the patient volunteered episodic spontaneous cold feeling and obvious piloerection was witnessed in the limb.

We interpret these discharges as due to "spontaneous", automatic, mass activation of decentralized, biologically viable postganglionic sympathetic neurons. We speculate on their possible triggering mechanisms and unwanted physiological consequences, while foreseeing their diagnostic value.

321.10 **A Versatile Method for Analyzing Autonomic Nerve Connectivity.** J.C. Prechtl and T.L. Powley. Lab. Regulatory Psychobiology, Purdue Univ., West Lafayette, IN 47907.

A common problem encountered in experimental analyses of the course and distribution of autonomic (or other peripheral) nerves is that complex pathways and plexuses can make reconstructions from serial sections problematic. In what follows we describe an osmium tetroxide-vapor (OsO_4) whole mount method which takes only a few hours for processing and can be used to analyze intact nerves and plexuses as well as the results of acute nerve manipulations (e.g. ablations, electrode placements, etc.). Applied as a vapor, the penetration and accumulation of OsO_4 can be efficiently metered to produce a fine staining.

After an animal is exsanguinated by saline perfusion, a tissue block containing the nerve or plexus is removed, and the tissue surface is exposed to osmium vapors for 20-50 minutes by suspending the block above 2ml of 2% osmium tetroxide in a 5cm wide, 110ml specimen jar. For this exposure, the tissue block is loosely pinned to a layer of cured silicone rubber (Dow Corning Sealant) adhered to the inner surface of the lid of the jar. The solution is handled under a functioning hood, and is prepared by breaking the ampoule of OsO_4 within a glass stoppered reagent bottle already containing the water. Following OsO_4 exposure, the tissue is rinsed in saline and pinned onto a layer of clear silicone rubber in a petri dish filled with saline. The stained nerve can then be dissected free of any obscuring tissues by using alternately rear and epi-illumination. Palely stained specimens can be reexposed to vapors, and excess staining can be removed with an oxidant (1% H_2O_2). Nerves deeply embedded in fat often require osmication and dissection in stages, since heavily osmicated lipids obscure dissection. The tissue is then dehydrated in graded alcohols and cleared in xylene and mounted in permount.

The method described is highly sensitive, readily permitting identification of individual myelinated axons in autonomic nerves. The method is also versatile: 1) It can be adapted for EM processing (tissue cleared by embedding medium). 2) It is compatible with Microfil rubber vascular endocasts (OsO_4 after endocast curing and tissue clearing as recommended by manufacturer: Canton Bio-medical Products). 3) Different endoneurial components can be distinguished, including myelinated and unmyelinated populations. Further, the method is also characterized by its ease and convenience: 1) Processing is very rapid, being as short as a few hours, depending on the amount of dissection necessary. 2) Few materials are needed (OsO_4, dehydrant, and clearing agent). NIH Grant AM27627.

NEUROENDOCRINE CONTROL: PITUITARY V

322.1 **STEROID REGULATION OF mRNA LEVELS ENCODING PRECURSOR FOR GONADOTROPIN-RELEASING HORMONE (GnRH) AND GnRH-ASSOCIATED PEPTIDE (GAP).** R.T.Zoeller*, A.J.Mason*, P.H. Seeburg* and W.S.Young III (SPON:P.Brownell). Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

Gonadotropin-releasing hormone (GnRH) and an associated peptide (GAP) are synthesized as parts of a single precursor. Neuronal cell bodies containing this precursor are distributed throughout the septum and preoptic area of the rat and mouse brain. The concentration of immunoreactive GnRH (and presumably GAP) in specific brain areas changes during the estrous cycle of the rat and in response to sex steroid manipulation. The nature of these changes implicates sex steroids in the regulation of the mRNA encoding this precursor. To examine whether sex steroids can influence the cellular level of this mRNA, we ovariectomized and implanted (sc) estradiol or empty Silastic capsules in female Sprague-Dawley rats. GnRH/GAP precursor mRNA was detected and levels quantitated in brain by *in situ* hybridization histochemistry (Young et al. Neurosci. Lett.,1986).

Although our probes (synthetic 48-mers complementary to coding regions for both GnRH and GAP) labelled cells in a distribution similar to that observed with antibodies for GnRH and GAP, we focused our attention on the organum vasculosum of the lamina terminalis (OVLT) because we observed the greatest density of cells there and because the OVLT has been implicated in the control of gonadotropin secretion. GnRH/GAP precursor mRNA was nearly two-fold lower in rats implanted with estradiol than in control rats. However, this effect was observed only when comparing medially-located cells (within 3 µm of the midline); GnRH/GAP precursor mRNA levels in laterally-located cells were not affected by steroid manipulation. To examine whether steroid effects on GnRH/GAP mRNA are a feature of normal female rats, we also correlated GnRH/GAP mRNA levels with plasma steroid levels throughout the normal 4-day estrous cycle.

The relationship between sex steroids and immunoreactive GnRH(+GAP) in brain is sexually dimorphic; the development of this dimorphism is dependent in part on the steroid milieu during development. Thus, the results of experiments with normal rats are being compared to similar studies in androgen-sterilized females. In addition, we have investigated the regulation of the GnRH/GAP precursor mRNA in the hypogonadal mouse (hpg/hpg) which possesses an aberrant GnRH gene (Mason et al., 1986) and apparently lacks the GnRH peptide. Cells containing the GnRH/GAP precursor mRNA are distributed normally throughout the brain of the Hpg mouse, but levels of the transcript are reduced from 5-15 fold. Thus, GnRH-containing neurons appear to develop normally and express the aberrant GnRH/GAP precursor mRNA even in the absence of sex steroids.

322.2 REDUCTION IN NUMBER OF CELLS CONTAINING GONADOTROPIN-RELEASING HORMONE mRNA FOLLOWING CASTRATION IN THE BRAIN OF THE ADULT MALE MONKEY, MACACA FASCICULARIS. L.J. Standish, L.A. Adams,* L. Vician,* D.K. Clifton,* and R.A. Steiner. Depts. of OB/GYN and Physiology & Biophysics, University of Washington, Seattle, WA 98195.

Gonadotropin-releasing hormone (GnRH) regulates the activity of the reproductive system. Gonadal hormones act at the hypothalamus to influence the release of GnRH, but the cellular mechanism of this feedback action is unknown. In particular, it is not clear whether or how gonadal hormones influence GnRH gene expression. Using in situ hybridization, we measured the number and distribution of cell bodies containing the messenger RNA (mRNA) coding for GnRH production in the basal forebrain and hypothalamus of intact (n=2) and castrate (n=2) adult male monkeys, Macaca fascicularis. Monkeys were anesthetized, then perfused with 4% paraformaldehyde. Frontal sections were cut from a frozen block of tissue containing the hypothalamus and preoptic/septal area. Sections 400 micra apart were incubated overnight at 37 C with an 35-S-labeled synthetic oligonucleotide probe (DNA*) complementary to the proGnRH mRNA. After incubation, sections were washed, dipped in photographic emulsion, and exposed for 2 weeks. The slides were then developed, counterstained with cresyl violet, randomized, and read blindly by dark-field illumination. Cells containing GnRH mRNA/DNA* hybrids were identified in a variety of hypothalamic and forebrain regions, and were most abundant in the septal nuclei, diagonal band of Broca (DBB), medial preoptic area (MPOA), suprachiasmatic and supraoptic nuclei, and ventromedial hypothalamus (VMH). There was a 40% reduction in the total number of labeled cells in castrate compared to intact animals, and this reduction was accounted for largely by a decrease in the number of labeled cells in the areas listed in the table below.

Region	Number of labeled cells	
	Intact (n=2)	Castrate (n=2)
Septal	27	13
DBB	64	43
MPOA	101	54
VMH	127	75

This study documents the distribution of cells containing GnRH mRNA in the brains of castrate and intact male monkeys, and suggests that removal of gonadal hormones by castration results in a reduction of the number of cells containing detectable levels of GnRH mRNA in the basal forebrain and hypothalamus of the adult male macaque. Conclusion: The expression of the GnRH gene in certain brain areas may depend upon the trophic action of gonadal hormones.
(Supported in part by NIH Grant HD12625)

322.3 EVIDENCE THAT ESTROGEN INHIBITION OF LH RELEASE IS NOT MEDIATED BY LHRH AND OPIOID PEPTIDE NEURONS. J.E. Levine, D.G. Karahalios*, M.B. Sholand*, P.C. Fallest*, and J.F. Azzarello*. Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201.

The mechanism(s) by which estrogen (E_2) exerts its negative feedback actions on LH release are not well understood. It is generally believed that the post-ovariectomy increase, and E_2-induced decrease in LH secretion reflect changes in antecedent hypothalamic LHRH release. It has also been proposed that E_2 may indirectly inhibit LH release via intermediate actions on endogenous opioid peptide (EOP) neurons, i.e. E_2 may suppress LH release by increasing EOP inhibition of LHRH release. These studies were conducted to determine if E_2 negative feedback actions on LH are a consequence of (i) direct E_2 effects on LHRH release, (ii) indirect effects of E_2 on LHRH release via EOP neurons, or (iii) E_2 effects on a LHRH-independent mechanism. The push-pull perfusion (PPP) method was combined with a blood sampling and replacement procedure to measure hypothalamic LHRH release and plasma LH in conscious, freely-moving animals. Female rats were ovariectomized (Ovx) or laparotomized on diestrus I (DI) 4d or 8d before PPP experiments. Rats received stereotaxic push-pull canula (PPC) implants into the mediobasal hypothalamus (MBH) at five days, and jugular catheters at one day, prior to PPP. In each rat, MBH perfusates were collected for 5-8hrs and stored for LHRH RIA. Plasma from blood samples obtained during PPP intervals were stored for LH RIA. After 2-3h of sample collection, animals received estradiol benzoate (E_2B, 1ug) or oil injections, s.c.. In a second series of experiments, the opiate antagonist naloxone (2.5mg/kg or 10mg/kg) or saline was injected. Baseline (pre-injection) LHRH release rates were highest in DI rats (3.10 + 0.86 pg/10min), and significantly lower in Ovx + 4d (1.10 + 0.21 pg/10min) and Ovx + 8d (0.80 + 0.50 pg/10min) rats ($p<.05$). Injections of E_2 suppressed LH below, or nearly below detectable levels within 3h, while having no significant effect upon mean LHRH release or LHRH pulse amplitude. In the second study, DI, Ovx + 4d, and Ovx + 8d animals responded naloxone injections with equally robust, dose-dependent increases in LHRH release. We conclude that (i) LHRH release is not increased at 4d or 8d following Ovx; rather, it is decreased, (ii) acute E_2 administration suppresses LH, but not LHRH release, and (iii) EOP inhibitory tone is not diminished following E_2 removal. On the basis of these data, we propose that E_2 negative feedback on LH release is mediated by a direct effect of E_2 on pituitary gonadotropes, or by a neural, LHRH-independent mechanism. The decline in LHRH release following Ovx may be a consequence of increased "short-loop" feedback inhibition of LHRH by rising levels of LH.

322.4 INTRACELLULAR RECORDINGS FROM SUPRACHIASMATIC (SCN) AND MEDIAL PREOPTIC NUCLEUS (MPN) NEURONS: EFFECTS OF 17β-ESTRADIOL, ACETYLCHOLINE, AND NOREPINEPHRINE. O.K. Ronnekleiv and M.J. Kelly. Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201.

Results from our laboratory have further established that the SCN and MPN are involved in the control of the proestrous surge of LH and prolactin (PRL) in the female rat. Lesions of these nuclei remove a critical input signal necessary for the release of LHRH on proestrus (Ronnekleiv and Kelly, Neuroendocrinology, 1986). Therefore, we decided to use the in vitro hypothalamic preparation to study the endogeneous electrical activity of SCN/MPN neurons and look at the effects of 17β-estradiol (E_2) and neurotransmitters on these neurons. Coronal hypothalamic slices (400 μm) were prepared from cycling female rats during the light-on phase (morning) of proestrus. Extracellular and intracellular recordings of MPN and SCN neurons were made using conventional recording techniques (Kelly et al, Brain Res Bull. 12:399, 1984). E_2 (10^{-10}M), norepinephrine (NE; 10^{-6}M), and acetylcholine (Ach; 10^{-3}M) dissolved in artificial CSF were applied via a pressure pipette to the slice. Intracellular recor dings from 12 MPN and 18 SCN neurons revealed a high degree of spontaneous activity with a mean firing frequency of 3.92 Hz, spikes over 100 mV, R_m's of 300 MΩ and pronounced afterhyperpolarzations. Two cells exhibited phasic activity, and 31% of the cells could be driven orthodromically by lateral hypothalamic stimulation. E_2 hyperpolarized and inhibited the spontaneous activity of 7 out of the 13 SCN/MPN neurons. These pronounced inhibitory effects were similar to its effects on guinea pig mediobasal hypothalamic neurons (Kelly et al., 1984) NE depolarized and increased the firing of 3 SCN and 1 MPN neuron, and 1 MPN neuron was inhibited. ACh depolarized and increased the firing in two MPN, but hyperpolarized and inhibited two SCN neurons. 30% of the cells recorded were injected with procion yellow, and processed through immunocytochemistry (Kelly et al., 1984). The SCN neurons showed a high degree of dye coupling, and one of the SCN neurons which responded to E_2, NE and Ach was identified as a VIP-containing neuron. Therefore, SCN and MPN neurons not only show endogenous spontaneous activity, but are sensitive to E_2, NE and Ach. These findings indicate that the SCN and MPN neurons exhibit features that are essential for the control of LHRH neurons and the reproductive cycle of the rat.
(Supported by NIH grants HD 19905, HD 16793)

322.5 EFFECTS OF 17β-ESTRADIOL ON THE ELECTRICAL COUPLING OF MEDIOBASAL HYPOTHALAMIC NEURONS. M.J. Kelly, T.P. Condon and O.K. Ronnekleiv, Department of Physiology, Oregon Health Sciences U., Portland, OR 97201.

Luteinizing hormone and its releasing factor, luteinizing hormone-releasing hormone (LHRH) are known to be released in a pulsatile manner in the mammal. We have found that LHRH neurons in the in vitro slice preparation release LHRH in a "pulsatile" manner (Kelly et al., Brain Res 345:264, 1985), and therefore sought to identify the electrophysiological properties of mediobasal hypothalamic neurons in the slice. Sagittal hypothalamic slices were prepared from intact, ovariectomized and ovariectomized, estradiol benzoate treated female guinea pigs as previously described. Arcuate (ARC) and cell-poor zone (CPZ) neurons were recorded over a 12 hr period with KCL/KCitrate- and procion yellow (PY, 4%)-filled electrodes. The effluent medium was collected in about 50% of the experiments and assayed for LHRH to verify that LHRH was released in a physiological manner. With KCL/KCitrate-filled electrodes (R=100 MΩ) we were able to obtain good penetration of the small parvocellular ARC and CPZ neurons. The characteristics of these recordings included RMP's of 55 - 75 mV, spikes over 100 mV and R_m's of 200 - 400 MΩ. 11% of the ARC-CPZ neurons were antidromically (AI) driven by median eminence stimulation. In neurons which showed robust intracellular records, double spike complexes were observed spontaneously or following AI stimulation. We observed these presumed coupling potentials in 40 such neurons of the approximately 400 which were recorded. For statistical analysis, we divided the three groups of guinea pig into two categories based on 17β-estradiol (E_2) levels measured in the trunk blood. The low estrogen group had E_2 levels less than 30 pg/ml (follicular/ovariectomized levels) and the high estrogen group had E_2 levels greater than 30 pg/ml (periovulatory/ovariectomized, E_2-treated). A Chi-square analysis revealed that significantly ($p<0.01$) more coupling events occurred in the high E_2 group (N = 31) versus the low E_2 group (N = 9). Interestingly, very little dye coupling (<2%) was observed following injection of procion yellow into ARC-CPZ neurons recorded in both high and low E_2 groups. However, our findings indicate that ARC-CPZ neurons show a higher incidence of coupling in the presence of estrogen and therefore suggest that the "pulsatile" release of LHRH might be due to the synchronous firing of LHRH neurons during periods of elevated estrogens.
(Supported by NIH grants HD 19905 and HD 16793)

322.6 LHRH-RECEPTOR REGULATION OF Ca^{++}ATPASE ACTIVITY IN MURINE PITUITARY GLAND. S.L. Dalterio, C.R. Esquivel* and S.A. Bernard*, Division of Molecular Pharmacology, University of Texas Health Science Center, San Antonio, Texas 78284.

The interaction of luteinizing hormone releasing hormone (LHRH) with pituitary receptors results in luteinizing hormone (LH) release. The role of Ca^{++} as a messenger in this process has been suggested by reports that agents which increase intracellular Ca^{++} also stimulate LH release. Recent studies utilizing intracellular fluorescent probes (Quin-2) determined that LHRH stimulation produces an increase in Ca^{++} maximal in 10 sec. To assess the indirect effect of LHRH on cytosolic Ca^{++} we have examined the interaction of LHRH directly on Ca^{++}-dependent ATP hydrolysis (Ca^{++}ATPase) in pituitary membrane preparations. The following data was derived from LHRH receptor regulation of Ca^{++}ATPase and correlation with release of pituitary LH.

Addition of LHRH in vitro ($5x10^{-5}$-10^{-9}M) to murine pituitary membranes resulted in a dose-related decrease in Ca^{++}ATPase activity within 15 min. Inhibitory effects of LHRH (10^{-7}M) occurred after 90 sec and were maximal within 120 sec. Eadie Hofstee analysis at 10^{-7}M LHRH at varying Ca^{++}_{free} resulted in a K_m of 0.97 μM and V_{max} = 19.2 nmoles/mg/2 min compared to controls of K_m = 0.72 μM and V_{max} = 33.6 nmoles/mg/2 min. Preincubation (5 min) with LHRH antagonist (LHRH-A) (10^{-6}M) significantly reversed ($P<.01$ by ANOVA) the inhibitory effects of LHRH (10^{-7}M) on pituitary Ca^{++}ATPase. Addition of LHRH (10^{-7}M) to pituitary homogenates significantly increased LH release at 10 - 40 sec, compared to basal LH release.

Systemic administration of 50 ng LHRH (i.p.) significantly ($P<.05$) reduced pituitary Ca^{++}ATPase activity after 30, 60 and 90 min with return to control at 120 min. Pituitary LH content was slightly increased at 15 min with significant incrases at 90 and 120 min post-treatment. Plasma LH levels were elevated at 5 and 15 min, returning to control within 60 min post-LHRH. These results demonstrate that LHRH receptor activation may modulate intracellular Ca^{++} levels via inhibition of Ca^{++}ATPase activity. Furthermore, these effects correlate with changes in LH activity.
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322.7 FURTHER STUDIES OF THE GNRH RELEASING ACTIVITY OF N-METHYL-DL-ASPARTATE (NMA) IN THE PREPUBERTAL RHESUS MONKEY. T. M. Plant, V. L. Gay* and R. P. Hoffman*, Departments of Physiology and Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Previous studies with prepubertal male rhesus monkeys, in which pituitary gonadotropes were first primed with a chronic intermittent infusion of GnRH (see below), have shown that an iv bolus injection of NMA (15 mg/kg BW) stimulates the release of a pulse of LH and FSH. That this action of NMA is mediated indirectly via the discharge of hypothalamic GnRH was concluded from the concomitant observation that pretreatment with a potent GnRH antagonist ([AcD2Nal[1], 4ClDPhe[2], DTrp[3], DArg[6], DAla[10]]-GnRH • HOAc) abolished the gonadotropin response to administration of the excitatory amino acid. The purpose of the present study was to determine 1) the NMA dose - LH response relationship, 2) the effects of excitatory amino acid receptor blockade with 2-amino-5-phosphonovaleric acid (2APV, a specific NMA receptor antagonist) and γ-D-glutamylglycine (γ-DGG) on the LH response to NMA stimulation, and 3) the ability of repetitive NMA stimulation to sustain GnRH secretion from the hypothalamus of the immature primate. For this purpose, 8 orchidectomized rhesus monkeys were implanted with double indwelling cardiac catheters and housed in remote sampling cages that permitted simultaneous infusion of saline and the withdrawal of blood without anesthesia or restraint. In order to utilize pituitary LH secretion as an index of hypothalamic GnRH release, the responsivity of the gonadotropes of the prepubertal pituitary to GnRH was increased to adult levels by a chronic intermittent infusion of GnRH (0.1 μg/min for 3 min every h). The GnRH infusion was interupted to examine dose response relationships. Four doses of NMA (1.5, 4.7, 15, and 47 mg/kg BW) were administered to each of 4 animals as a brief iv infusion. Increasing doses of NMA resulted in progressively larger discharges of GnRH as reflected by plasma LH levels with the exception of the highest NMA dose which appeared to be supra-maximal. On the basis of these results, the 4.7 mg/kg dose of NMA was selected for further experiments. Administration of 2APV (9 mg/kg BW; iv in saline; N=4) 1 min before the NMA injection abolished the LH response. γDGG (18 mg/kg BW; iv in saline; N=4) produced a severely truncated LH response to NMA. Neither receptor antagonist compromised the pituitary response to exogenous GnRH. Following an additional week of control GnRH priming the iv infusion of GnRH was terminated and a chronic intermittent iv infusion of NMA (4.7 mg/kg BW as a 1 min pulse every 1 or 2 h) was immediately initiated. This intermittent stimulation with NMA sustained an adult-like pattern of pulsatile GnRH release from the hypothalamus of the castrated prepubertal monkey for as long as 48 h. Taken together, these findings suggest that repetitive activation of specific NMA receptors, presumably located within the hypothalamus of the prepubertal primate, should result in a premature reawakening of the hypothalamic-pituitary-gonadal axis.
Supported by NIH grants HD13254 and HD08610

322.8 PULSATILE LHRH PATTERNS FROM FETAL BABOONS AT MIDGESTATION. D.C. Meyer and G.J. Pepe, Department of Physiology, Eastern Virginia Medical School Norfolk, Va. 23501

Since there is little research dealing with the nature of in vitro LHRH secretion in prenatal primates, information on the profile and mechanism of LHRH secretion would be of critical importance in understanding the role of this brain peptide in controlling LH release and/or other neuroendocrine parameters. Pulsatile LHRH secretion was measured from the mediobasal-suprachiasmatic-preoptic (MBH-SCN-POA) region of the hypothalamus from fetal baboons (Papio anubis) at midgestation. The entire MBH-SCN-POA (47.68 ± 5 mg.) was obtained between 1100 and 1200 hours and immediately placed in ice cold phosphate buffer (pH 7.4). The MBH-SCN-POA units were halved at the midline, superfused in parallel at 37.5 C^{o} for 5 hours, and 500 ul. of superfusate collected at 10 minute intervals. LHRH was measured by radioimmunoassay using the Chen-Ramirez antibody. Control baboons (n=3) had an average LHRH pulse amplitude of 16.1 ± 4.19 pg., interpulse interval of 33.0 ± 0.89 minutes and an average 10 minute output of LHRH of 9.49 ± 2.03 pg. In contrast both midgestation (n=2) fetal baboons from mothers with high E_2 from androstenedione exposure or term fetal baboons (n=2) from mothers with high endogenous E_2 had an average LHRH pulse amplitude of 2.63 ± 0.42 pg, interpulse interval of 37.0 ± 5.33 minutes, and an average 10 minute output of LHRH of 1.87 ± 0.42 pg. These data suggest that as early as midgestation fetal baboons display a periodicity of 33-37 minutes in their pulsatile LHRH output. The significant decrease in LHRH pulse amplitude and decrease in the average 10 minute LHRH output ($p<.001$) in the fetal baboons from mothers with high steroid levels suggest that maternal steroids can inhibit the output but not the pulsatility of LHRH systems as early as midgestation in the fetal baboon. This study was supported by NICHD grant 20535 to DCM and 13294 to GJP.

322.9 SIMULTANEOUS MEASUREMENT OF LHRH, LH AND FSH IN INTACT AND CASTRATED MALE RATS. M.T. Duffy* and J.E. Levine (SPON: M. Lidow). Dept. of Neurobiology & Physiology, Northwestern University Evanston, IL 60201.

Previous work demonstrated a close temporal relationship between pulsatile LHRH and LH release in ovariectomized sheep (Endo. 111:1449) and monkeys (Endo. 117:711). In the present study the push-pull perfusion (PPP) method was combined with a blood withdrawal and replacement procedure to simultaneously measure LHRH release and plasma LH and FSH levels in conscious, freely-moving male rats. Our aims in these experiments were three-fold: (i) to determine if pulsatile LH release is associated with pulsatile LHRH release in male rats, (ii) to test the hypothesis that acute changes in gonadotropin release following castration are associated with alterations in the pattern of LHRH release, and (iii) to directly assess the role of LHRH in the stimulation of pulsatile FSH release. Male rats received unilateral stereotaxic push-pull cannula (PPC) implants into the mediobasal hypothalamus (MBH) and were allowed 5-7 d recovery. At 24h following castration or sham surgery, and at least 6h after jugular catheterization, rats underwent PPP experiments. A stylette was removed from the PPC and an inner cannula assembly (33ga) was inserted. The MBH was perfused with artificial CSF at 15ul/min for 4-6h. Ten-minute perfusate fractions were collected on ice, acidified and stored for subsequent LHRH RIA using EL-14 LHRH antiserum. Plasma from 400ul blood samples obtained during each 10-minute PPP interval were stored for LH and FSH RIA. During experiments, 800ul of a blood replacement mixture were injected into the animal after alternate blood sample withdrawals. For data analysis, pulses of LHRH, LH and FSH were identified if % increases from nadir to peak were >5x c.v. in respective assays. LHRH release profiles were pulsatile, and mean LHRH release rates were similar in intact (0.62 ± .17 pg/10min) and castrate animals (0.45 ± .07 pg/10min). Mean frequency of LHRH pulses was higher ($p<.01$) in castrate (1.85 ± .14 pulses/h) compared to intact animals (0.98 ± .12 pulses/h). Plasma LH levels were increased 6-fold in castrate versus intact rats. Of a total of 23 LH pulses observed in 10 rats, 19 were temporally associated with LHRH pulses (83%). In contrast, only 6 of 19 FSH pulses (32%) showed this relationship. We conclude from these studies that (i) the LHRH pulse generator appears to stimulate pulsatile LH in male rats, (ii) increased LHRH pulse frequency, but not amplitude, occurs during acute post-castration increases in LH and FSH, and (iii) pulsatile FSH secretion is not strongly correlated with pulsatile LHRH release. The latter finding suggests that a physiological relationship between pulsatile LHRH and FSH secretion, if it exists, is not easily discerned in male rats. Alternatively, another neural factor (FSH-RF) may operate as the primary neural stimulus for pulsatile FSH secretion.

322.10 IN VITRO DEVELOPMENT OF LHRH CELL SUBTYPES. S.Wray, B.Gahwiler[1], and H.Gainer. Laboratory of Neurochemistry and Neuroimmunology, NICHD, Bethesda, MD 20892, [1]Preclin. Res., Sandoz Ltd, CH-4002 Basel, Switzerland.

Luteinizing hormone releasing hormone (LHRH) cell subtypes in male and female neonatal rats include: 1) smooth, aspinous LHRH cells (sLHRH) and 2) irregular LHRH cells (iLHRH) with either knob-like spinous processes (sessile) or pedunculated spinous processes (thin protuberances with bulbous endings). At birth, sLHRH cells predominate. During postnatal development, a subpopulation of sLHRH cells appears to transform into pedunculated iLHRH cells, which predominate in the adult (Wray and Hoffman, Neuroendocrinology, in press). In order to study the intrinsic and extrinsic factors which influence the development of specific LHRH cell subtypes, an in vitro organotypic culture system similar to that described by Gahwiler (JCN,4:329-342,1981) was used.

400 um diencephalon sections from 3-6 day old rats were plated on glass coverslips, inserted in test tubes (containing 25% horse serum, 50% Eagle basal medium, 25% Earles' BSS, 6.5 mg/ml glucose and phenol red) and placed in a roller drum at 36°C. At various times (1-7 weeks), the cultures were fixed with 4% paraformaldehyde/0.2% picric acid in 0.1M sodium cacodylate buffer (pH 7.4). After fixation, the cultures were rinsed in phosphate buffered saline (pH 7.4), treated with 0.2% triton X-100, blocked in 10% normal goat serum, and then placed in the primary antiserum (anti-LHRH, 1:5000) overnight at 4°C. The cultures were immunocytochemically processed using an avidin-biotin-peroxidase complex.

Immunoreactive LHRH cells and numerous fibers were visualized in cultures from male and female animals. The majority of immunoreactive LHRH cells were located within the rostral sections, and were found to have a distribution similar to that observed in vivo. 637 immunoreactive LHRH cells were counted and classified, based on their cellular morphology. Approximately 55% of the cells counted were categorized as sLHRH cells while 40% were classified as iLHRH cells with sessile spines. The remaining 5% mainly consisted of cells whose morphology was uncertain and thus these cells were not categorized. Less than 1% were classified as iLHRH cells with pedunculated spines. Even after 7 weeks in culture few iLHRH cells with pedunculated spines were observed.

Although both immunoreactive LHRH cells and fibers are maintained in this in vitro system, iLHRH cells with pedunculated spines do not develop. We are currently examining whether the addition of target tissue and/or afferent systems (e.g. A1 and A2) in a co-culture configuration will induce the development of both iLHRH cell subtypes in this in vitro model.

322.11 PHOTOPERIODIC CONTROL OF PULSATILE LHRH AND SERUM GONADOTROPIN LEVELS IN THE MALE GOLDEN HAMSTER J.E.Joy*, J.E.Levine, A.E.Jetton* and F.W.Turek (SPON:V.Carr) Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

The reproductive system of the golden hamster is tightly regulated by seasonal changes in daylength. The effects of photoperiod on reproductive function depend on the regulation of serum gonadotropin levels (LH and FSH), which are elevatedin animals exposed to long days and low in animals exposed to short days. Both LH and FSH are released by the pituitary gland in a pulsatile fashion and are under control of hypothalamic LHRH which has been shown in other species to be also released in a pulsatile fashion. It has frequently been concluded that a central feature of photic regulation of reproduction involves regulation of the hypothalamic neural pulse generator. And yet, until now, no measurements of pulsatile release of LHRH have been made in photoperiodic animals under different photic conditions.

Using the push-pull perfusion system, we have successfully measured hypothalamic LHRH release in vivo in hamsters with large testes exposed to LD 14:10 and in hamsters with regressed testes exposed to LD 6:18. Hypothalamic perfusates and blood samples (obtained via jugular cannulae) were collected every 10 minutes over a 6-hour period in unanesthetized, unrestrained animals. LHRH release was clearly pulsatile and LHRH pulses occurred approximately once per hour under both lighting conditions. However, short day animals showed much higher peak levels (≥100 pg LHRH/10 min) than the long day animals (approx. 10 pg LHRH/10 min). The total amount of LHRH release was also much higher in short day animals than in long day animals (mean release ±SEM pg LHRH/10 min: 38.0 ±13.7 and 4.9 ±2.3, reapectively). Serum gonadotropin levels were pulsatile in both long and short day groups and were within previously reported ranges for their respective groups. Thus animals exposed to short days have extremely high levels of LHRH release associated with low serum gonadotropin levels, whereas animals exposed to long days have low levels of LHRH release associated with high serum gonadotropin levels.

This surprising finding raises a number of novel questions related to how the hypothalamus mediates photic-induced changes in pituitary gonadotropin release. One possibility is that an increase in hypothalamic LHRH release, induced by exposure to short days, results in a reduced sensitivity of the pituitary to LHRH. Alternatively, high LHRH release in short day animals may reflect the removal of pituitary or gonadal feedback.

322.12 CHANGES IN BRAIN BIOGENIC AMINES ASSOCIATED WITH SHORT PHOTOPERIOD-INDUCED GONADAL REGRESSION IN THE WHITE-FOOTED MOUSE. J.D. Glass*[1], D. Deaver*[2] (SPON: J.A. Garner). Dept. of Biological Sciences Kent State Univ. Kent, OH 44242[1]. Dept. Dairy and Animal Science Penn. State. Univ. University Park, PA 16802[2].

Photoperiod-induced gonadal regression is thought to be mediated by the action of brain biogenic amine systems on the secretion of gonadotropin-releasing factor from the hypothalamus and/or prolactin from the pituitary gland. In previous studies, tyrosine hydroxylase inhibitor was used to assess the effects of short photoperiod on central turnover of dopamine (DA) and norepinephrine (NE). In this study, the concentrations of DA, NE and 5-hydroxytryptamine (5-HT) and their respective metabolites were measured as an alternative approach to assess the effects of short photoperiod-induced gonadal regression on the metabolism of biogenic amines in the preoptic area (POA), anterior hypothalamus (AH) and posterior hypothalamus (PH). Sexually mature female white-footed mice (Peromscus leucopus) maintained on a long (16L:8D; n=5) or short photoperiod (8L:16D; n=5) for 7 weeks were killed, and their POA and hypothalamic regions dissected and stored in liquid nitrogen. Amines were separated by HPLC and quantified by electrochemical detection. The concentrations of amines are expressed as ng/mg protein.

Mice exposed to short photoperiod had a greatly reduced reproductive tract weight (40.5 ± 5.0 mg) compared to mice under long photoperiod (112.5 ± 4.0 mg; $p < 0.01$). For mice under short photoperiod, the concentrations of DA (8.5 ± 0.5 ng/mg), and its metabolites homovanillic acid (HVA;2.5 ± 0.3 ng/mg) and 3,4-dihydroxyphenylacetic acid (DOPAC;2.4 ng/mg), 5-HT (20.5 ± 0.9 ng/mg) and its metabolite 5-hydroxyindolacetic acid (5-HIAA;4.4 ± 0.3 ng/mg) and NE (64.4 ± 4.5 ng/mg) in the PH were significantly greater than in mice under long photoperiod (DA, 6.5 ± 7 ng/mg; HVA, 1.8 ± 0.2 ng/mg; DOPAC, 1.5 ± 0.2 ng/mg; 5-HT, 13.9 ± 1.7 ng/mg; 5-HIAA; 3.0 ± 0.4 ng/mg; NE, 43.0 ± 5.4 ng/mg; all $p < 0.05$). Within the AH, the concentration of DA (7.0 ± 0.4) was lower, and the concentration of 5-HT (18.1 ± 1.1) higher in mice under short compared to long photoperiod (10.4 ± 1.9 ng/mg and 14.4 ± 0.8 ng/mg, respectively; both $p < 0.05$). There was no effect of photoperiod on the concentrations of amines in the POA.

These results are evidence that in response to exposure to short photoperiod, there may be increased metabolism of DA, NE and 5-HT in the PH, and decreased DA, but increased 5-HT metabolism in the AH. The significance of these changes is unknown, but they may reflect seasonal reproductive and/or thermoregulatory neuroendocrine adjustments.

CHEMICAL SENSORY SYSTEMS: OLFACTION

323.1 THE ODORANT-SENSITIVE ADENYLATE CYCLASE OF OLFACTORY RECEPTOR CELLS: DIFFERENTIAL STIMULATION BY DISTINCT CLASSES OF ODORANTS. Pamela B. Sklar, Robert R.H. Anholt*, and Solomon H. Snyder. The Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

We have characterized odorant-stimulated adenylate cyclase activity in isolated chemosensory cilia prepared from frog and rat olfactory epithelium. Olfactory receptor cells extend a dendrite toward the nasal lumen which carries at its apex a group of chemosensory cilia. These cilia are thought to be the sites where the initial recognition and transduction events in olfaction take place. Cilia prepared from both frog and rat exhibit high levels of adenylate cyclase activity. Basal activity in the frog and rat is 2.0 nmol/mg/min and 3.6 nmol/mg/min, respectively, and is stimulated approximately 2-fold by GTP and approximately 5-fold by GTPγS (guanosine-5'-O-(thiotriphosphate)) and forskolin. Odorants augment enzyme activity 30-65%/• above the basal level in a tissue-specific and GTP-dependent manner. Calcium reduces GTP-stimulated activity with a half-maximal effect at 10 μM.

Odorants vary in their influence upon olfactory adenylate cyclase activity. In general, fruity, floral, minty, and herbaceous odorants enhance activity. Citralva, a substituted terpenoid odorant having a fruity odor quality, is one of the most potent cyclase stimulators. Many odorants which are structurally unrelated to citralva, such as menthone, D-carvone, L-carvone, 3-hexylpyridine, 2-hexylpyridine, hedione, helional, and coniferan, are also potent stimulators of the olfactory adenylate cyclase. Several fruity, floral, minty, and herbaceous odorants stimulate activity to an intermediate level. Examples include amylsalicylate, dimethyloctanol, eucalyptol, eugenol, and cinnamic aldehyde. Interestingly, the classes of fruity, floral, minty, and herbaceous odorants also contain some non-stimulating odorants such as limonene, lyral, phenylethylalcohol, lilial, and ethylvanillin. Citralva, menthone, D-carvone, L-carvone, and 2-isobutyl-3-methoxypyrazine display similar potencies in activating the adenylate cyclase with maximal effects at 100 μM. Putrid odorants and chemical solvents such as isovaleric acid, triethylamine, pyridine, thiazole, and methoxypyrazine do not stimulate enzyme activity. In homologous series of pyrazine, thiazole and pyridine odorants, compounds with the longest hydrocarbon side chains are best able to enhance enzyme activity. The failure of certain odorants to affect adenylate cyclase activity suggests that additional transduction mechanisms besides cAMP are involved in olfaction. Investigations of odorant-stimulated phosphatidylinositol turnover in frog olfactory cilia are currently in progress.

323.2 ACTIVATION BY ODORANTS OF A VOLTAGE DEPENDENT, CATION-SELECTIVE CHANNEL IN CILIARY MEMBRANES FROM THE OLFACTORY EPITHELIUM OF THE BULLFROG. P. Labarca*, S.A. Simon* and R.R.H. Anholt*(Spon: R. Erickson). Department of Physiology, Duke University Medical Center, Durham, N.C. 27710.

We have detached chemosensory cilia from the olfactory epithelium of the bullfrog, Rana catesbeiana, and fused the isolated ciliary membranes with preformed planar lipid bilayers. Single channel records revealed spontaneous openings of at least three different cation selective channels with conductances of 40 pS, 100 pS and 230 pS, respectively, in 200 mM KCl. Only the 100 pS conductance channel was activated (opened) by odorants in a concentration dependent manner. Activation was observed at nanomolar concentrations of the potent bell pepper odorant, 3-isobutyl,2-methoxypyrazine, and the citrus odorant, citralva (3,7-dimethyl-2,6-octadienenitrile). The lifetime of the open state of the 100 pS channel in the presence of odorants was in the msec range. Channel activation was reversible upon removal of the odorant from the bilayer chamber via perfusion. The odorant channel exhibited voltage dependence between -70 mV and +70 mV. The probability of the closed state increased significantly at negative applied voltages. The channel was cation-selective with similar permeability to sodium and potassium ions. We postulate that channel activation by odorants may mediate excitation of the olfactory receptor cell, whereas the generation of cAMP via an olfactory adenylate cyclase observed at higher odorant concentrations over a longer time scale may reflect odorant desensitization.

323.3 MEMBRANE CONDUCTANCES IN DISSOCIATED OLFACTORY RECEPTOR NEURONS FROM NECTURUS. E. Potter* and V.E. Dionne* (SPON: J.H. Brown). Division of Pharmacology, Department of Medicine, University of California, San Diego, CA 92093

Olfactory receptor neurons were dissociated from the olfactory epithelium of adult Necturus and studied using whole-cell patch-clamp methods. Single cells were isolated by incubation for one hour at room temperature in Ca^{++}-free amphibian saline with papain (2 mg/ml), collagenase (2 mg/ml) and DNAse (0.5 mg/ml); isolated neurons retained their unique morphology and were readily identified. Whole-cell membrane currents were digitally recorded from neurons bathed in normal amphibian saline [mM: 112 NaCl, 2 KCl, 8 $CaCl_2$, 3 HEPES; pH 7.2] or in elevated Ca^{++} saline [mM: 56 NaCl, 2 KCl, 44 $CaCl_2$, 3 HEPES; pH 7.2]. The recording electrode contained Cs^+ to block outward K-currents [mM: 120 CsCl, 0.500 $CaCl_2$, 2.5 $MgCl_2$, 1.000 BAPTA, 3 HEPES, 0.1 GTP, 5 ATP; pH 7.2, pCa^{-8} 7.0].

With 120 mM K^+ in the pipet, whole-cell currents exhibited both inward and outward components in response to depolarizing voltage steps from a holding potential of -80 mV (V.E. Dionne, ISOT abst. 1986). When Cs^+ replaced K^+ in the pipet, the inward current was unaffected while the outward current was reduced by ~80%. The inward current, which had a rapid onset and was transient, consisted of two components: 1. a Na-current which could be blocked by 100 nM tetrodotoxin and was totally inactivated at holding potentials >20 mV; 2. a Ca-current which was weakly sensitive to Cd^{++} (partial block with 1 mM) and which was fully inactivated at holding potentials >40 mV. The Ca-current was insensitive to tetrodotoxin and was increased 5-fold or more in the elevated Ca^{++} bath. The outward current, both with and without Cs^+ in the pipet, was non-inactivating. The component of outward current which persisted with Cs^+ in the pipet consisted of two components, one of which was Ca^{++}-sensitive. The number of components which might comprise the Cs^+-blocked outward current has not been determined. In these neurons no detectable currents were evoked by hyperpolarizing voltage pulses.

In summary, at least five different conductances appear to contribute to the membrane currents of isolated olfactory neurons. To what extent their currents contribute to the change in neuronal firing that accompanies the response to odorants by such neurons remains uncertain. [Supported by NINCDS grant NS20962]

323.4 THE EFFECT OF AMITRIPTYLINE ON DIFFERENTIATION OF OLFACTORY EPITHELIUM IN TISSUE AND ORGAN CULTURE. A. I. Farbman, M.-I. Chuah*, and F. Gonzales*. Dept. of Neurobiology, Northwestern Univ., Evanston, IL 60201 and Dept. of Anatomy, Chinese Univ. of Hong Kong, Shatin, N.T., Hong Kong.

In previous studies, we have shown that the olfactory bulb exerts an effect on the differentiation of the olfactory epithelium. The co-culture of fetal rat olfactory bulb with olfactory mucosa from fetuses of the same age results in a doubling of the amount of olfactory marker protein (OMP) synthesized by the epithelium and a doubling of the number of multiciliated receptor epithelial cells, after one week in culture (Chuah & Farbman, 1983; Chuah et al., 1985). This effect appeared to be specific to the bulb: substitution of other parts of the nervous system, including embryonic cerebrum, cerebellum or spinal cord for the bulb had no effect in the organ culture system. In the present study we report that small doses of a commonly used anti-depressant drug, amitriptyline, inhibits olfactory receptor neuron differentiation. In one set of experiments, we explanted olfactory bulbs in combination with olfactory mucosa from E15 fetal rats, and grew them for seven days in the presence of medium containing amitriptyline in doses of 1 - 10 µg/ml medium. The amount of OMP in the cultures was measured by radioimmunoassay, and compared to control cultures grown in the absence of the drug. A dose response curve showed that as little as 2 to 3 µg/ml of the drug had a significant effect in reducing the amount of OMP synthesis to less than one half of that of control cultures. The results suggested that either 1) the enhancing influence of the bulb was effectively abolished, or 2) the drug might have a direct effect on differentiation of the epithelium. Using another culture system, we explanted fragments of olfactory mucosa from 5 day embryo chicks in the presence of 1 - 10 µg/ml amitriptyline. In control cultures, axon outgrowth from these tissue fragments could be visualized as early as two days after explantation. By seven days, axons grew as long as 1 - 2 mm (Gonzales et al., 1985). In cultures containing the drug, we noted that no axonal outgrowth occurred in cultures containing 5 or 10 µg/ml. At lower doses, some axonal outgrowth does occur, but significantly less than that seen in control cultures.

These experiments show that amitriptyline exerts a toxic effect on the development of olfactory epithelium in cultured explants of two species of vertebrate embryos. The effective doses of the drug in both animals are approximately equivalent. The mechanism of toxic action of the drug is unknown.

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323.5 TRANSCELLULAR EFFECTS OF OLFACTORY NERVE SECTION ON THE SECRETORY CELLS OF THE SALAMANDER OLFACTORY MUCOSA. M.L. Getchell*, B. Zielinski* and T.V. Getchell. Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48236.

Histological and morphometric examination of the olfactory mucosa 7 d after unilateral section of the olfactory nerve adjacent to its entry into the olfactory bulb revealed both ipsi- and contralateral effects. In addition to the ipsilateral degeneration of the olfactory receptor neurons, changes were noted in the glial-like epithelial sustentacular cells and in the olfactory glands. Secretion of mucopolysaccharides into the overlying mucus layer and vacuolation, indicative of water and electrolyte transport, were induced in sustentacular cells in the ipsilateral and, to a lesser extent, in the contralateral epithelium. In superficial Bowman's glands (sBG), the secretory granule content, measured as area of the acinar cells occupied by secretory granules, was reduced by 16% ($P<0.05$) ipsilaterally and 28% ($P<0.001$) contralaterally compared to that of glands in non-nerve-sectioned tissue; vacuoles, which rarely were observed in control tissue, were evident throughout the acinar cells on both sides. In deep glands (dG), secretory granule content was increased by 34% ($P<0.01$) and 27% ($P<0.05$) ipsi- and contralaterally, respectively. These increases were due in large part to secretory granule swelling as a result of water and electrolyte uptake as indicated by the presence of large vacuoles throughout the cells of dG. Differences in the responsiveness of the glands to pharmacological agents also were observed. Topically applied methacholine, a cholinergic agonist, caused significantly ($P<0.001$) less reduction in the secretory granule content of sBG both ipsi- and contralaterally from nerve-sectioned tissue than from non-nerve-sectioned tissue. In dG, there was no significant difference between the effects of methacholine on nerve-sectioned and non-nerve-sectioned tissue; however, the analysis was complicated by the fact that the secretory granule content of nerve-sectioned controls increased due to water and electrolyte uptake induced by nerve section alone. Isoproterenol, a β-adrenergic agonist, injected i.p. caused significantly ($P<0.001$) less reduction in secretory granule content in nerve-sectioned than in non-nerve-sectioned tissue ipsi- and contralaterally in both sBG and dG.

These results suggest that degenerating receptor neurons trigger secretory and transport processes in neighboring sustentacular cells and in sustentacular cells in the contralateral epithelium, and that section of the olfactory nerve affects cholinergic and β-adrenergic responses of the olfactory glands.

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323.6 COLLATERAL SPROUTING OF AFFERENT NERVE AXONS IN THE TONGUE: A MORPHOLOGICAL STUDY IN ADULT RATS
Erik Kinnman* and Håkan Aldskogius. Dept. of Anatomy, Karolinska Institutet, Stockholm, Sweden.

Previous studies have shown that taste buds in mammals degenerate when deprived of their gustatory innervation. Regeneration of appropriate nerves is followed by reappearance of the taste buds. In the present study transganglionic transport of horseradish peroxidase (HRP) from the brain stem to the tongue was used to study reinnervation by neighbouring afferent nerve axons after chorda tympani or lingual nerve lesions.

In a first series of experiments certain methodological aspects on labeling of afferent fibers in the epithelium and the taste buds were analysed, namely the type of tracer used and the influence of postinjection survival time. Wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) horseradish peroxidase (HRP) or a combination of both was injected in the central primary projection areas of the afferent nerves of the tongue. The animals were aldehyde perfused 20-48 hours after the injection and the tongue was serially sectioned transversely or longitudinally and processed according to the tetramethyl benzidine method of Mesulam (1978). Optimal labeling of axons in the tongue papillae was obtained when 2 µl of 20% HRP was injected in the brain stem.

In a second series of experiments the normal territory of single nerves was examined by using this injection procedure in combination with transections of other afferent nerves to the tongue. Glossopharyngeal nerve axons were found in the circumvallate papilla bilaterally as well as in foliate papillae and the epithelium of the posterior 1/4 of the tongue. Axons from the chorda tympani nerve were present in taste buds in the fungiform papillae in the anterior 2/3 of the tongue and in a few of the most anteriorly situated foliate papillae. Lingual nerve fibers were found in the extra gemminal epithelium of the anterior 3/4 of the tongue.

In a third series of experiments the nerve territories of intact nerves in the tongue were examined with the same methodology 2-12 weeks after chronic chorda tympani or lingual nerve lesions. 8 weeks after the chorda tympani lesion ipsilateral trigeminal nerve fibers were found in the taste buds of fungiform papillae in the anterior 2/3 of the tongue. 12 weeks after the lingual nerve lesion, contralateral trigeminal nerve fibers were found in the taste buds of a few fungiform papillae. These findings indicate that trigeminal nongustatory axons can reinnervate and reform fungiform taste buds and that these axons can extend across the midline of the tongue.

323.7 FOCAL INJECTIONS OF WGA-HRP INTO OLFACTORY EPITHELIUM PRODUCE DIFFUSE AND FOCAL LABELLING OF OLFACTORY BULB GLOMERULI. W.T. Nickell, Gloria Adamek and M.T.Shipley.
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An understanding of the precise nature of the projection between the olfactory epithelium (OE) and the olfactory bulb (OB) may provide clues to the way in which olfactory information is coded. We have studied this projection using very small, localized iontophoretic injections of the tracer WGA-HRP into the OE.

The dorsal aspect of the epithelium was exposed by removal of part of the nasal bone. In some cases, iontophoretic injections were placed in this region after advancing the pipette through the nerve layer. In most cases the receptor surface on the medial septum was exposed through a window made in the dorsal epithelium. The injection pipette was advanced 80 micron beyond the point where electrical contact with the epithelial surface was established.

Pipettes (2-5 micron) were filled with 1% WGA-HRP in 0.9 % NaCl and the tracer was injected by an intermittent current of 1-3 uA applied for 5 - 10 minutes. Animals survived 24 hours and were perfused and processed for the TMB reaction. To maintain their structure, the epithelia were embedded in albumen gelatin and exposed to formalin vapors for 1 week before sectioning.

Transport from the epithelium was very efficient; small (1 mm diameter), focal injection sites produced heavy labelling of individual glomeruli which was visible in bright field illumination. This labelling appeared to represent fascicles of fibers and terminal arbors distributing within individual glomeruli. Heavily labelled glomeruli were scattered among glomeruli containing little label. Often, label was confined to part of a glomerulus.

Surprisingly, these localized injections produced significant diffuse labelling distributed throughout the glomerular layer. This labelling appeared as small speckles of reaction product. There was considerable variability in intensity of this diffuse labelling among glomeruli. In some cases individual glomeruli on the side opposite the zone of focal projection showed relatively heavy labelling.

These results demonstrate that small regions of the OE, comprising only a fraction of the receptive surface, send projections to much of the glomerular surface. Even the focal part of this projection fills a significant portion of the OB surface. Thus, within any small, contiguous, population of primary olfactory neurons (PONs) there are two sub-populations: one which projects diffusely throughout the bulb and another which projects more focally to a few glomeruli. Since there is continual neuronal turnover in the olfactory epithelium, it is tempting to speculate that immature and relatively non-selective PONs generate the diffuse label and more odorant selective, mature cells have a dense, focal projection.

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323.8 **DIFFERENTIAL INFLUENCE OF PERIPHERAL DEAFFERENTATION ON EXPRESSION OF GABA AND DOPAMINE IN JUXTAGLOMERULAR NEURONS OF MOUSE MAIN OLFACTORY BULB.** H. Baker, A.C. Towle* and F.L. Margolis[1]. Dept. Neurology, Cornell Univ. Med. Col., New York, NY 10021 and [1]Roche Inst. Molec. Biol., Nutley, NJ 07110.

Peripheral deafferentation of the rodent main olfactory bulb produces profound alterations in intrinsic dopamine neurons. Specifically, in the juxtaglomerular neuronal population, enzyme activity and immunoreactivity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, was reduced to 25% of control. In the hamster bulb similar loss of immunoreactivity was noted for both intrinsic substance P- and tyrosine hydroxylase-containing neurons. The heterogeneous juxtaglomerular neuronal population also includes a large group of GABA-containing cells. In these studies, we focused on possible alterations in bulbar GABA synthesis produced by chemical destruction of the olfactory receptor epithelium. Mice were analyzed 3 weeks following intranasal irrigation with 0.17M $ZnSO_4$. Animals were perfused with fixative containing acrolein (3.5%) and formaldehyde (2%) in 0.1M phosphate buffer and brains were processed for immunocytochemistry. Tyrosine hydroxylase and GABA were localized in adjacent sections by the indirect peroxidase antiperoxidase (TH) or ABC (GABA) techniques using 3,3'-diaminobenzidine as the chromogen. In agreement with previous studies, the number of immunoreactive tyrosine hydroxylase containing neurons was reduced substantially. In contrast, the number of GABA containing neurons did not appear to change significantly. However, the distribution of neurons stained with GABA was altered as a consequence of the degeneration of the axonal processes of the receptor cells and subsequent collapse of the glomeruli. The decrease in tyrosine hydroxylase staining and relative lack of change in GABA immunoreactivity are consistent with previous biochemical studies. The specific activity of the GABA synthetic enzyme, glutamic acid decarboxylase (GAD) was unchanged in $ZnSO_4$ treated mice. However, GAD activity was reduced by about 30% when expressed per bulb reflecting shrinkage of the deafferented bulb. In similar experiments tyrosine hydroxylase specific activity was reduced by 66% and by 75-80% of total activity when expressed per bulb. Unlike tyrosine hydroxylase, the changes in the GABAergic system are similar to those observed for the enzyme dopa decarboxylase (DDC), the second enzyme in the catecholamine synthetic pathway. In fact, the continued presence of DDC activity and immunoreactivity constituted evidence for the survival of dopamine neurons despite the absence of tyrosine hydroxylase. In conclusion, these studies show that while expression of some neurotransmitter phenotypes is highly dependent on peripheral afferent innervation others are not.

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323.9 ODOR INTEGRATION IN THE OLFACTORY BULB; CORRELATIVE EVIDENCE FROM INTRACELLULAR RECORDING AND ENHANCED RESOLUTION 2-DEOXYGLUCOSE (2DG) MAPPING. J.S. Kauer and K.A. Hamilton (Depts. of Neurosurgery, Anatomy and Cell Biology, Tufts-N.E.M.C., Boston, MA. 02111).

Intracellular recordings from salamander mitral/tufted [M/T] cells (Hamilton & Kauer, Br. Res. 338:181, 1985) have shown that hyperpolarization is the most common initial event in response to square pulse odorant stimulation at moderate concentrations. Categorization of the responses into 'E' and 'S' types previously seen in extracellular recordings, can be made by observing the latencies of depolarization and excitatory impulse activity and whether excitation precedes or follows hyperpolarization at the highest odorant concentrations.

At high intensity stimulation 'E' responses are characterized by short latency (60-90 msec.) depolarizations and spike bursts followed by hyperpolarization, and 'S' responses are characterized by longer latency (230-450 msec.) depolarizations and spike bursts preceded by hyperpolarization. Pooled extracellular data from many animals show that each of these M/T response types can be elicited by any of the odorants tested. This suggests that all the response types are elicited simultaneously, in parallel, across the M/T population upon stimulation with a single pulse of any one odorant.

Since the coordinated activity of all the M/T cells in the bulb cannot be simultaneously examined using conventional electrophysiological methods, to examine the ensemble response to odor stimulation, we have used enhanced resolution, 2DG autoradiography. By superimposing the 2DG autoradiograph on its histological section, precise comparisons between functional activity and bulbar cell laminae have been made. Using this method, we have found that, at moderate concentrations, the highest levels of odor-induced, 2DG uptake in glomerular foci occur over periglomerular(PG) cell somata. We hypothesize that the actvity observed in the PG cell population using 2DG may be the source of the hyperpolarization which we have recorded preceding the onset of spikes in M/T cells.

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323.10 **THE MODIFIED GLOMERULAR REGION AND PATTERN OF 2-DG UPTAKE IN THE OLFACTORY BULB OF RABBIT PUPS EXPOSED TO NIPPLE-SEARCH PHEROMONE** H. Distel*, H. Martin* and R. Hudson*. (SPON: H. Holländer)
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Rabbit pups are only nursed for about 3 minutes once a day. To locate nipples in this short time they are totally dependent on a short ranging pheromone on the mother's belly which releases and guides a highly stereotyped nipple-search behavior. This is the case even for pups delivered by caesarian section, and thus without postnatal experience of the pheromone. Newborn rats also depend on odor cues to locate nipples, and 2-deoxyglucose studies of activity in the olfactory bulb have indicated a modified glomerular region adjacent to the accessory bulb to mediate these cues (Teicher et al., Brain Res., 194:530, 1980). It was thus the purpose of this study to examine whether a similar region is present in the newborn rabbit and, using the 2-DG method, whether it is specifically activated during exposure to the pheromone.

Five-day-old rabbit pups were injected intracardially with 300 µCi of 14C-labeled 2-deoxy-d-glucose and then placed for 45 min on either the belly of an anesthetized, pheromone producing female rabbit, on a non-pheromone producing female or on a non-pheromone producing female scented with the odor of citral, to which pups had been conditioned to respond with nipple-search behavior (Hudson, Dev. Psychobiol., 18:575, 1985).

The autoradiograms revealed loci of high 2-DG uptake adjacent to the unlabeled accessory olfactory bulb, which did indeed correspond, when compared to the Nissl sections, to a morphologically distinct region. In the rabbit, this region appears to form a crescent around the anterior rim of the accessory olfactory bulb and is characterized by a fusing of glomeruli, a much reduced external plexiform layer, and a lose arrangement of numerous mitral cells below. However, 2-DG-labeling of this modified glomerular region was not confined to animals exposed to nipple-search pheromone but was also clearly seen in both control conditions. Further, exposure to the pheromone resulted in patches of strong labeling distributed throughout the glomerular layer of the main bulb. A strong but not so widespread labeling of glomeruli was observed in the citral condition, while a much reduced pattern of activity was obtained for the control pups on the unscented non-pheromone female. Thus, although pheromone exposure resulted in the strongest labeling, the actual pattern of activity could not be regarded as distinctive, particularly with respect to activation of the modified glomerular region.

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323.11 **REGIONAL AUTONOMY IN THE PERIPHERAL PROCESSING OF NIPPLE-SEARCH ODORS.** R. Hudson* and H. Distel*. (SPON: A. Hofbauer)
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Newborn rabbits are completely dependent on pheromonal cues on the mother's belly for the release of nipple-search behavior, but can be rapidly conditioned to respond with the same search behavior to other odors when these are paired with suckling (Hudson, Dev. Psychobiol. 18:575, 1985). As reported in the accompanying abstract, 2-DG labeling is found throughout the olfactory bulb following exposure to such nipple-search odors. This raises the question whether the entire bulb is necessary for mediating these, and if not, whether specific areas, such as the modified glomerular region, are essential.

To test this question, extensive bilateral lesions of the medial, lateral or dorsal bulb were performed on day 4 after pups had been conditioned to the odor of citral or isopropyl alcohol. The next day pups' responsiveness to both the pheromone and the conditioned odor was tested, as well as their ability to obtain milk in the normal nursing situation in competion with unoperated pups. Lesioned pups were able to suckle in the nest, and searched vigorously on a test female scented with the conditioned odor, even after one nare had been temporarily closed. Only in a few severely lesioned cases was a decrement in nipple-seach performance observed.

Similar lesions were also performed on naive pups 2 hours after birth. They, too, could respond to the pheromone and suckle when tested the following day, and furthermore, could be conditioned to the novel odors. Reconstruction of the lesion sites showed them to overlap fully so that no part of the bulb had been completely spared, and that even pups with less than 30% of the bulb remaining intact could still respond with search behavior to the various odors.

These results not only confirm the earlier finding that the vomeronasal organ is unimportant for the mediation of such nipple-search odors (Hudson & Distel, Physiol. Behav. 37: in press), but also show the modified glomerular region not to be essential. Infact it would seem that any part of the main olfactory bulb of newborn rabbits is capable of autonomously processing the pheromone or a postnatally acquired artificial odor. By implication, this also seems to be true for the olfactory epithelium as this is known to project in a broadly topological manner to the bulb. However, whether these findings hold for all odors, and for the adult animal remains to be investigated.

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323.12 ULTRASTRUCTURAL SIGNATURES OF THE NEURONAL CLASSES WITHIN THE GUSTATORY ZONE OF THE NUCLEUS OF THE SOLITARY TRACT IN THE HAMSTER. B.J. Davis and T. Jang*. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

Previous quantitative light microscopic morphometric studies of the gustatory zone of the nucleus of the solitary tract (NST) in the hamster demonstrated four morphologically distinct classes of neurons and characterized these classes according to their somal areas, nuclear profiles and distributions. Ongoing ultrastructural analyses attempt to identify additional morphological features that serve to further characterize these four classes prior to relating such structural features with functional attributes (e.g., output vs. interneurons and associated putative neurotransmitters). The strongest predictors of class are somal areas and the ratio of somal area:nuclear area. The previously defined X1, X2, X3, and X4 classes of NST neurons each possess a characteristic and significantly different somal area: nuclear area ratio because each class differs in average somal and nuclear sizes. The average ratio values are: X1: 1:2.3 (N=84); X2: 1:2.6 (N=54); X3: 1:2.0 (N=62); and X4: 1:1.6 (N=59). A 0.72-0.99 range of correlations between the area of the soma and area of the nucleus among the four neuronal classes contributes to the reliably of these ratio values. Morphologically, these ratio values reflect the relative amounts of perinuclear cytoplasm and nuclear area in each of the four classes of neurons. At one extreme, X2 neurons possess much perinuclear cytoplasm that is loaded with various membraneous organelles and the largest ratio of somal area:nuclear area. On the other hand, the X4 class is noted for its near absence of perinuclear cytoplasm and the close opposition of the nuclear and outer cellular membranes and therefore, its ratio value approaches 1:1. The calculation of such ratio values allows us to examine a neuron ultrastructurally and assign it with confidence to one of four classes which are being more fully characterized by Golgi and correlated Golgi-EM methods. These data allow us to infer the relative prevalence and distribution of any one type of morphologically distintive Golgi or functional class of neuron throughout the dorsal, intermediate and ventral levels of the gustatory zone from both light and electron microscopic material and add to our knowledge about the make-up, lay-out and intrinsic organization of the neuronal constituencies of the gustatory NST.

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VISUAL CORTEX IV

324.1 **RETINOTOPIC ORGANIZATION OF HUMAN VISUAL CORTEX USING A PET ANALYSIS STRATEGY THAT IMPROVES SPATIAL RESOLUTION** P.T. Fox, F.M. Miezin, J.M. Allman, M.A. Mintun, D.C. Van Essen, & M.E. Raichle. Depts. of Neurology and Radiology, Washington U. Med. Sch., St. Louis, MO and Div. of Biology, California Inst. of Technology, Pasadena, CA

Annular, red:black checker-board stimuli were used to induce focal cerebral blood flow (CBF) increases in human striate cortex. Cortical responses were mapped for three retinal eccentricities: Ø.7 deg. (macular; Ø.1-1.5 deg); 3.5 deg. (perimacular, G.5-5.5 deg); and 1Ø.5 deg. (peripheral; 5.5-15.5 deg).

Seventeen control:stimulus pairs of CBF images were obtained from six volunteers using intravenous boluses of oxygen-15 labeled water and positron emission tomography (PET). Response foci were imaged by subtracting a control-state measurement (fixation point only) from each stimulated-state measurement. Responses were identified by a roving, cubic (1Ø cc) region of interest and a center-of-mass algorithm. Response locations were plotted in stereotactic coordinates (J. Comput. Assist. Tomog. 9:141-153, 1985) as mm from the center of the AC-PC line.

Response locale varied systematically with stimulus field. In all trials the primary response was on the medial occipital surface. Macular responses (Ø.7 deg.) were slightly anterior to the pole. Perimacular responses (3.5 deg.) lay 6 mm anterior and 4 mm superior to macular. Peripheral responses (1Ø.5 deg.) lay 3 mm anterior and 5 mm superior to perimacular. The same organization was seen in all subjects. The linear extent of striate cortex to which each degree of retinal field projects (magnification factor) was calculated from these data for 3 eccentricities: 3.4 mm/deg. at 2.1 deg.; 1.6 mm/deg. at 5.6 deg.; Ø.9 mm/deg. at 7.Ø deg.

Cortical zones separated by as little as 3 mm were distinguished using images with a resolution of > 15 mm. Improved effective spatial resolution was obtained by localizing each response focus independently in subtraction format. Response localization is not limited by the conventional definition of spatial resolution, i.e., full width at half maximum. Simulations of focal brain activation using brain-phantom images indicate that PET can discriminate among sequentially imaged responses less than 1 mm apart.

324.2 **ORGANIZATION OF EXTRASTRIATE CORTEX IN THE OWL MONKEY.** M.I. Sereno, C.I. McDonald*, and J.M. Allman. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

Detailed electrophysiological mapping studies of owl monkey extrastriate cortex had previously revealed a number of visuotopically organized areas beyond V-I and V-II. The present experiments concentrated on the rostral parts of visually responsive cortex. This region was mapped in detail in chronic experiments. In each session, a row of penetrations spaced at 1 mm or 0.5 mm intervals was made. We plotted 1200 different receptive fields from the most densely mapped hemisphere.

The organization of several previously described extrastriate areas-- VP, VA, MT, and the medial half of DL (representing the lower visual field) --was confirmed. Several new areas were found. They will be described in a medial to lateral sequence. Medial and rostral to MT and DL, there is a topographically organized representation of most of the visual field in the temporoparietal region (TP). Directly rostral to MT and DL in the superior temporal region (ST), receptive field size increases dramatically and although an orderly sequence of receptive field centers usually occurred in a given penetration, it was difficult to discern large scale order in this area.

A strip of cortex about 1 mm wide adjoining the lateral border of MT (the zero vertical meridian of the upper visual field) contains a representation of part of the upper visual field but unlike medial DL, this lateral strip contains no representation of azimuths past 15 degrees from the vertical midline. Lateral to the rostral part of this strip in posterior inferotemporal cortex, there is a topographic representation of the lower (to 20 degrees eccentricity) and most of the upper visual field. Finally, rostral to the representations of the upper visual field in VP and VA on the ventral surface, there is a region with an orderly representation of the center of vision (to 15 degrees eccentricity).

These results suggest that there are several topographically organized visual areas in extrastriate cortex of the owl monkey beyond those already described. Thus, most of the visually responsive cortex in this animal shows some degree of retinotopic order.

This work was supported by NIH grants F32 EY05887 and EY03851.

324.3 MULTIPLE TOPOGRAPHIC AND NON-TOPOGRAPHIC SUBDIVISIONS OF THE TEMPORAL LOBE REVEALED BY THE CONNECTIONS OF AREA V4 IN MACAQUES. D.J. Felleman, J.J. Knierim, and D.C. Van Essen. Div. of Biol. 216-76, Calif. Institute of Technol., Pasadena, CA 91125.

Inferotemporal cortex (IT) of macaque monkeys is a large expanse of visual cortex situated between anterior prestriate cortex (V4) and the temporal pole. Posterior (PIT) and anterior (AIT) subdivisions of IT have previously been suggested on behavioral and anatomical evidence. Physiological studies have heretofore not revealed any discernable topographic organization within IT. In order to address the issues of possible subdivisions and topographic organization in IT we have used anterograde (^{3}H-proline) and retrograde (HRP and multiple fluorescent dye) tracers to investigate the cortical connections of area V4, the major source of cortical input to posterior IT.

We examined the cortical connections of both dorsal (V4d) and ventral (V4v) portions of V4, corresponding to the representations of the inferior and superior visual fields, respectively. Tracer injections were made into V4d (n=10) and V4v (n=4) either alone or in various combinations in 9 hemispheres. The distribution of label was analyzed using 2-dimensional, unfolded maps of extrastriate cortex and 3-dimensional computer graphic reconstructions.

We used the laminar pattern of labeling and the segregation or convergence of inputs from the upper (V4v) and/or lower (V4d) visual field to distinguish 3 pairs of subdivisions located respectively in posterior, anterior and medial portions of the temporal lobe. Topographically organized (segregated) connections were observed with two separate regions within posterior IT: (1) lateral to area MT and ventral to V4d in the STS; (2) ventrolateral cortex from the crown of the STS to the lateral OTS, ventral to V4d and anterior to V4v. These zones were characterized by strong retrograde labeling in supra- and infragranular layers following fluorescent dye injections into V4. A second pair of subdivisions were located in anterior IT: (3) anterior and ventral to MT in the floor of the STS; and (4) anterior to region 2, from the crown of the STS to the OTS. The third pair were located in medial cortex: (5) just medial to the fundus of the OTS (perhaps area TF) and (6) further medial in neocortex (perhaps area TH). Both the anterior IT and medial subdivisions were characterized by infragranular retrograde labeling and non-topographic (convergent) inputs from V4.

While not all of these subdivisions were observed following every injection, some variability could be attributed to differential tracer sensitivities. The most variable components were located in most medial neocortex and anterolateral IT. Finally, we found the topographic organization of the posterior lateral subdivision to be variable in that the relative locations of upper field and lower field inputs differed across individuals.

324.4 ASYMMETRY OF CENTRAL AND PERIPHERAL FIELD INPUTS FROM AREA V4 INTO THE TEMPORAL AND PARIETAL LOBES OF THE MACAQUE. L.G. Ungerleider, R. Desimone and J. Moran*, Lab. Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.

The cortical connections of area V4 were examined by injecting multiple anterograde and retrograde tracers (tritiated amino acids, WGA-HRP, and fluorescent dyes) into physiologically identified V4 sites on the prelunate gyrus in seven cynomolgus monkeys. Visual field representations at the injection sites included the center of gaze and positions ranging from 2° to 30° in the lower visual field.

As previously reported by several investigators, V4 receives topographic projections from areas V2 and V3 and projects in turn to a widespread region in the inferior temporal (IT) cortex. Following V4 injections, anterogradely labeled terminals in the temporal lobe were found predominantly in layer IV, and retrogradely labeled cells were largely in layers V and VI but also in layer III. Within posterior IT cortex, or architectonic area TEO, label was found only after V4 injections involving the representation of about the central 10° of the visual field, whereas within anterior IT cortex, or architectonic area TE, label was found after injections of any part of V4. Thus, central but not peripheral field representations in V4 project to TEO, while both central and peripheral field representations in V4 project to TE. There was, in addition, a projection to the lateral portion of area TF, which was most extensive from V4 sites representing the peripheral visual field.

Besides the widespread projections into the temporal lobe, V4 was also found to project, though less extensively, into the parietal lobe. The laminar distribution of both anterograde and retrograde label in the parietal lobe resembled that found in the temporal lobe. Following injections of central visual field representations in V4, label in the parietal lobe was largely confined to a portion of area POa on the posterior bank of the caudal intraparietal sulcus, whereas following injections of peripheral field representations, the projection also included V3A, DPL, VIP, PO, and as yet unidentified posterior parietal areas. Thus, while V4's major outputs are into the temporal lobe, of those that are directed into the parietal lobe, the ones representing the peripheral visual field predominate.

The predominance of central field inputs to area TEO in the temporal lobe and of peripheral field inputs to several areas in the parietal lobe presumably reflects the importance of these inputs for object vision and spatial vision, respectively. The predominance of peripheral field inputs to area TF suggests that this temporal lobe region, like posterior parietal cortex, may have chiefly visuospatial functions.

324.5 DISTRIBUTION OF PURSUIT CELLS IN THE MEDIAL SUPERIOR TEMPORAL AREA (MST) OF THE MONKEY H. Komatsu and R.H. Wurtz Lab. Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20892 USA.

Cells discharging in relation to pursuit eye movements have been previously identified within the superior temporal sulcus (STS) of the awake macaque monkey. In the present experiment, we determined the distribution of these pursuit cells in relation to the visual properties of cells in specific areas of the STS: the middle temporal (MT) and the medial superior temporal (MST) areas.

We first made a series of vertical electrode penetrations every 1-2 mm, recorded multiple cell activity, and determined the visual properties of the cells. MT and MST were distinguished from the surrounding cortex by their high proportion of directionally selective cells, and from each other by the larger receptive fields (RF) in MST. Cell discharge during pursuit eye movements was tested, and about 15% of the cells tested were activated. The pursuit cells were found primarily in three areas: on the posterior bank of the STS in foveal MT and the lateral area of MST adjacent to foveal MT; on the anterior bank in dorsal MST, on the fundus of the STS outside of MST and anterior to foveal MT. Very few pursuit cells were found in the intermediate area of MST between the lateral and dorsal MST areas. Visual RF of both the lateral and dorsal MST usually included or were close to the fovea.

We then recorded single cell activity in selected areas: foveal and extrafoveal MT, lateral, dorsal and intermediate MST. Consistent with the results described above, most of the pursuit cells were obtained from foveal MT, lateral MST and dorsal MST, but the properties of the pursuit cells were different among these areas. The cells in foveal MT had small RFs and were sensitive to the movement of small, well-localized visual stimuli. Activity of most cells decreased during pursuit when visual stimulation was reduced by blinking off the pursuit target indicating the visual nature of the pursuit response. The cells in dorsal MST had large RF (>20°) that usually included the fovea. These cells tended to respond better to large field random dot movement rather than to movement of small, well-localized visual stimuli, and many cells had a RF organization such that preferred direction of the dot motion changed according to the size of the field of dots. Most cells did not change activity during blink of the pursuit target. In lateral MST, some pursuit cells had the same properties as in foveal MT, but others had the same properties as in dorsal MST.

We conclude that pursuit cells lie in some but not other areas of MST. This suggests that MST consists of several functional subregions distinguishable by their visual and oculomotor properties.

324.6 PURSUIT AND OKN DEFICITS FOLLOWING IBOTENIC ACID LESIONS IN THE MEDIAL SUPERIOR TEMPORAL AREA (MST) OF MONKEY. M. R. Dürsteler, R. H. Wurtz, D. S. Yamasaki. (SPON: F. Miles). Lab. of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20892 USA.

In previous experiments we found that pursuit eye movements were dependent upon visual motion processing in the middle temporal area (MT) of macaque monkey cerebral cortex. In the present experiments we determined whether the integrity of a visual area to which MT projects, the medial superior temporal area (MST), is necessary for generation of pursuit eye movements.

Monkeys were trained to fixate on a small target spot and then to pursue it as the spot stepped to a point on the horizontal meridian and then ramped away or toward the fixation point. Following injection of a neurotoxin, ibotenic acid, into MST we found two deficits. A retinotopic deficit was observed with initiation of pursuit, to a target moving in any direction in the affected area of the contralateral visual field. This deficit was similar to the deficit following damage to extrafoveal regions of MT (Newsome, et al., J. Neurosci. 5:825, 1985). A directional deficit was observed with maintenance of pursuit when the target moved toward the side of the lesion, regardless of the visual field in which the target fell. This deficit was similar to that seen following injections of the foveal areas of the STS which included areas of MT and MST (Dürsteler, et al., Neurosci. Abst. 10:475, 1984). The relative prominence of these two deficits varied with the location of the lesion. The retinotopic deficit was largest following injections which included the anterior bank of the STS. The directional deficit was more prominent when the lesion included the floor of the STS adjacent to MT.

Optokinetic nystagmus (OKN) in these monkeys showed two deficits. One was a reduced initial rapid rise in OKN thought to result from activation of the pursuit system. The second was a reduction in the maximum speed obtained by the slow buildup of OKN. The reduced rapid rise was associated with the retinotopic pursuit deficit and occurred for both directions of horizontal OKN. The reduced slow buildup was larger for OKN stimulation moving toward the side of the lesion and was associated with the directional pursuit deficit.

We conclude that: 1) MST must lie on an output pathway from visual cortex to brainstem oculomotor areas; direct projections from MT are intact after MST lesions but a pursuit deficit still is present. 2) the directional pursuit deficit can be produced by lesions that spare MT, 3) Damage to MST alters not only the initial fast phase of OKN but surprisingly the slower buildup as well. 4) Regions within the STS are related differentially to the retinotopic and directional pursuit deficits and the fast and slow phases of OKN respectively.

324.7 TWO- AND THREE-DIMENSIONAL STRUCTURE FROM MOTION SENSITIVITY IN MONKEYS AND HUMANS. R.A. Andersen and R.M. Siegel. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

In these experiments aspects of the perception of structure from motion have been examined in psychophysical tasks using novel motion displays. Subjects in these experiments were one monkey (Macaca mulatta) and two humans. This is the first study to our knowledge demonstrating that monkeys perceive structure from motion. These experiments are part of a larger study to determine stages in motion processing in extrastriate visual cortex using a combination of psychophysical, recording, and lesion techniques. In the accompanying abstract (Siegel and Andersen, Neurosci. Abstr., 1986) we report deficits in motion perception after lesions to the middle temporal area (area MT) that were accessed using some of these psychophysical tasks.

The psychophysical tasks are designed to determine thresholds for detecting the transition from unstructured velocity fields to velocity fields with various degrees of structure using a reaction time task. Stimuli are generated by a real-time computer graphics system and displayed on a large CRT screen. The two-dimensional structured motion stimuli are a surface rotating in the fronto-parallel plane and a linearly expanding dot field. The three-dimensional structure is a revolving hollow cylinder. The displays are composed of moving random dot fields with short-lived dots and are designed to remove familiar position cues and shape-change cues.

The structure of the displays is perturbed by displacing each motion trajectory some randomized distance within limits defined in screen parameters. Different degrees of structure are achieved by changing the limits for displacing the motion vectors. Psychometric curves were obtained by plotting the percent hits as a function of the amount of structure present in the displays. Fifty percent hit thresholds were determined using probit analysis.

The most significant finding was that the monkey could perform these tasks with ease. The monkey 50% hit thresholds were slightly higher than the human thresholds. Further data will be required from additional subjects to determine whether this is a difference between species or simply variability among individuals within a species. The effects on thresholds of velocity, point density, display size, and position in the visual field will be discussed.

To investigate whether local cues such as parallelism were a factor the tasks were also done with only a limited portion of the screen visible. As would be predicted thresholds were then dramatically raised for the 2-D tasks and it was impossible for the subjects to do the 3-D task. Since the availability of the whole structure of the motion field is necessary for optimal thresholds it would appear that the subjects were using global percepts of structure from motion in accomplishing these tasks.

Interestingly, the 900 msec reaction times for the 3-D task were about 300 msec longer than those recorded for the 2-D task. This increased reaction time could not be accounted for by the difficulty of the task, since the percentage structure in the tasks had little effect on reaction times. Thus the much longer reaction times probably reflect the longer computation times required to extract 3-D information.

324.8 MOTION PERCEPTUAL DEFICITS FOLLOWING IBOTENIC ACID LESIONS OF THE MIDDLE TEMPORAL AREA (MT) IN THE BEHAVING RHESUS MONKEY. R.M. Siegel and R.A. Andersen. The Salk Institute, La Jolla, CA 92037.

The macaque extrastriate area MT predominantly contains neurons that respond to visual motion. In order to examine the necessity of MT for the perception of motion, a reaction time task was used to assess a monkey's visual motion detection before and after an ibotenic acid lesion of MT. The detection of shear motion was tested by presenting a stationary, 6° square random dot field whose elements were then moved according to $\dot{x} = A\sin(\omega_t t)\sin(\omega_y y)$. The animal was trained to release a response key following the onset of shear while fixating a 0.3° LED. The amplitude of the shear and location of the test stimulus relative to the LED were varied in a random block design to determine the shear motion threshold at different locations in the visual field. Multiunit recordings were used to map the retinotopic representation of MT; ibotenic acid ($1\mu l$ of $15\mu g/\mu l$) was then injected to destroy a selected part of this representation. Lesion were anatomically verified to be in MT.

One day after the lesion, shear motion thresholds were elevated up to eight-fold from a prelesion value. Following this large increase, the motion thresholds returned to within 130% of the control levels after four days. The thresholds did not decrease further for the remaining nineteen days tested. The deficit was greatest close to the lesion center in retinotopic coordinates. The monkey's ability to detect contrast was not changed, as assessed using a step change from a uniform illuminated 6° field to a 2 cycle/deg sine wave contrast grating. These results, found in two hemispheres of one animal, indicate the presence of a deficit in the monkey's ability to detect shear motion. The fast recovery of shear thresholds suggests that simple perceptual motion analysis can recover by either rearrangement of receptive fields within MT or by utilization of other parallel motion pathways.

A three dimensional structure from motion task, which requires global processing of motion information, was also studied in one of the hemispheres used for the shear experiments. The animal was required to detect a change from an unstructured motion velocity field to a structured motion velocity field. The object underlying the structured motion velocity field was the rotating surface of a cylinder covered with short lived points. The animal was able to perform this task parafoveally prior to the ibotenic lesion. Following the lesion, the animal performed at chance at a location within the lesioned MT field. However, he could still correctly do the task at a second location not in the lesioned field. This localized deficit lasted 23 days at which time the animal was sacrificed. The structure for motion task was only used three times after the lesion whereas the shear task was run daily. If the persistence of the structure from motion deficit is not due to lack of post-lesion training, then its long time course suggests that MT is necessary for this particular motion analysis and that parallel pathways cannot compensate for its loss.

324.9 MT LESIONS IMPAIR DISCRIMINATION OF DIRECTION IN A STOCHASTIC MOTION DISPLAY. W.T. Newsome and E.B. Pare.* Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Because a majority of its neurons are direction and speed selective, extrastriate area MT of monkeys is thought to play a role in the cortical analysis of motion. To identify the perceptual functions to which neural processing in MT contributes, we are examining the effects of MT lesions in monkeys trained on appropriate psychophysical tasks.

We trained rhesus monkeys to discriminate upward from downward motion in a dynamic random dot display. The display consists of a stream of randomly positioned dots forming a dynamic image like that of "snow" on a TV screen. A specified proportion of the dots may be plotted nonrandomly ("correlated") so that they move with a uniform direction and speed. By varying the percentage of "correlated" dots, the intensity of the motion signal can be controlled. We measured the percentage of correlated dots for which monkeys successfully discriminated the direction of motion. In each trial, the monkey fixated a point of light for two seconds while viewing the dot pattern perifovesally (10° diameter, centered 7° eccentric on the horizontal meridian). When the fixation point was extinguished, two points of light appeared simultaneously, one above the patch of dots and one below. The monkey made a saccadic eye movement to the light toward which the motion was directed. Thresholds were measured by a staircase procedure in which a correct response resulted in a 37% chance of a more difficult discrimination on the next trial (fewer correlated dots), and an incorrect response resulted in a simpler discrimination on the next trial (more correlated dots).

The monkeys' pre-lesion performance on this task was similar to that of humans. For optimal conditions, the monkeys reliably discriminated the direction of motion when fewer than 10% of the dots were correlated. An ibotenic acid lesion of MT in the left hemisphere caused deficits in the right hemifield while the left hemifield was unaffected. In an acute phase, lasting three days post-lesion, psychophysical thresholds were dramatically elevated: the monkey was impaired for correlations as high as 100%. In a chronic phase, lasting 1-4 weeks, psychophysical thresholds gradually approached pre-lesion levels. In a second round of lesions in the same hemisphere, we attempted to destroy all of MT and a substantial portion of MST as well. The acute phase was virtually identical to that which followed the first lesion of MT. Substantial recovery has occurred during the chronic phase (now 2 weeks), but thresholds remain elevated above pre-lesion levels. These observations suggest that motion-related visual areas in the STS play a role in the perception of motion signals embedded in visual noise.

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324.10 VELOCITY DISCRIMINATION IN THE MONKEY. E. Vandenbussche* (SPON: J. Duysens), G.A. Orban and H. Maes*. Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

In humans the velocity discrimination curve plotting JND in velocity, expressed as Weber fraction, as a function of velocity is a U-shaped curve with minimal JNDs of 5-6% measured at velocities ranging from 4 to 64°/sec (Orban et al., Vision Res., 24: 17, 1984). The cat's discrimination curve is similarly U-shaped but the level of performance reached at optimal speeds is much lower (40-70% Weber fraction) (Vandenbussche et al., Vision Res., in press, 1986). The present experiments were undertaken to test 1) whether the velocity discrimination curve is U-shaped in monkeys as in man and cats and 2) whether the levels of performance in monkeys are closer to humans than those of cats. We trained four monkeys (Macaca Fuscata) to discriminate between two sequences of stimuli : one in which the reference velocity (at which performance is measured) was repeated and one in which the reference velocity was followed by a velocity differing by an amount Δv from the reference. Such a same-difference design yields thresholds which are double those obtained with the method of single stimuli (Vogels and Orban, J.exp.Psychol : Human Perception and Performance, in press, 1986). The average duration of stimulus movement was shorter than 200 msec and the duration of motion varied randomly around the average (maximum variation by a factor 2) to avoid the use of motion rather than speed as cue for discrimination. The moving stimuli were long narrow slits (0.6° wide) of moderate contrast (slit luminance 25 cd/m^2, background illumination 22 cd/m^2). In these standard conditions the velocity discrimination curve of all four monkeys was indeed U-shaped with minimal JNDs of 10 to 20% for velocities ranging from 8 to 64°/sec. By increasing the contrast, the width of the discrimination curve increases, exactly as has been reported for humans.

These results suggest that indeed velocity discrimination of monkeys bears an even greater similarity with human velocity discrimination than that of cats.

324.11 EYE MOVEMENT RELATED RESPONSES OF NEURONS IN SUPERIOR TEMPORAL POLYSENSORY AREA OF MACAQUE C. L. Colby and E. K. Miller*
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The superior temporal polysensory area (STP) in macaque is a polymodal area in which visual responses predominate. We have recorded from single cells in STP in the alert rhesus monkey trained to fixate and to perform saccadic and smooth pursuit eye movements. We have examined both the visual response properties and the eye movement related responses of these neurons.

During fixation, many STP neurons:
1) respond vigorously to small, rapidly moving spots
2) have very large, bilateral receptive fields extending across the tangent screen (100 x 100 deg)
3) are selective for the direction of stimulus motion, often for stimuli moving towards or away from the fovea
4) respond well to stimuli in the extreme periphery.

Many cells in STP discharge in relation to saccadic eye movements. These cells respond before and during saccadic eye movements with an increase in firing rate. Several tasks were used to determine whether presaccadic responses were primarily visual or primarily motor. In the standard saccade task, the trial is initiated when the monkey achieves fixation of a 0.1 deg fixation point (FP). One sec later, the FP goes off and the saccade target appears. The monkey is rewarded 1 sec after achieving fixation of the target. In order to rule out the possibility that the presaccadic response is due to the offset of the FP, a saccade-gap task is used. In this task, the FP is turned off 600 ms before the target appears but the monkey must not make a saccade during the gap. Finally, in order to determine whether the response is due to the onset of the saccade target or to the performance of the saccade, a saccade-overlap task is used. In this task, the saccade target appears 500 msec before the offset of the fixation point, allowing visual and motor responses to be segregated. Using this set of tasks, we have identified three classes of cells with saccade-related activity:
1) visual cells, whose firing is time-locked to the onset of the target stimulus
2) motor cells, whose firing is time-locked to the initiation of the saccade
3) visuomotor cells, which fire throughout the interval between the appearance of the target and the execution of the saccade.

Some neurons in STP responded vigorously, or were inhibited, during smooth pursuit eye movements, often in a directionally-selective manner.

In conclusion, neurons in STP respond not only in relation to sensory stimuli, as described previously, but also in relation to the performance of eye movements.

324.12 "VANISHING" OF FOVEAL TARGETS: A FAILURE TO SUSTAIN VISUAL ATTENTION DUE TO BILATERAL ASSOCIATION VISUAL CORTEX LESIONS IN MAN. Matthew Rizzo, MD, Richard Hurtig PhD*, Antonio Damasio, MD, PhD., Division of Behavioral Neurology, Department of Neurology, University of Iowa College of Medicine, Iowa City, Iowa 52242.

Attention is the broad term applied to the neural network that controls information processing by enhancing and filtering both internal and external signals. It consists of a "state" function to sustain attention and "vector" function to shift the finite CNS processing capacity. This model offers the possibility that various disorders of higher nervous function result from defects of attention at different CNS levels. We studied three subjects who reported the apparent disappearance from direct view of stationary objects in the visual panorama. They had CT defined bilateral lesions of the superior visual association cortices. We tested the hypothesis that their disturbance was due to a specific inability to sustain visual attention. They were alert, cooperative and had no abnormalities of visual acuity or fields to explain their defect. Language, verbal intellect, single word reading and visual recognition of objects and faces were also intact. Visual disappearance was not due to binocular rivalry or to internal or nonvisual external signal interference related to listening, hearing, speaking, motor activity or thinking during problem solving.
Eye movements were recorded using standard EOG technique with a minimum resolution of 0.5°. Signals were digitized for computer analysis. The subjects showed normal pursuit velocity and gain, saccadic latency, accuracy and velocity. Compared to normal controls, they exhibited appropriate scanning of picture faces and scenes and showed the ability to carry out goal-directed scanning to answer specific questions about pictures. But the most important observation was that the subjects reported the disappearance of a light-emitting diode target during EOG verified fixation. They continued to process information sufficiently to drive the eyes accurately on fixation targets, but the level of processing was insufficient to maintain conscious experience of the stimulus. Thus the subjects were looking but not seeing. The results show that visual attention can be separated from foveation as the result of selective lesions in the superior visual association cortices. Findings are consistent with normal human studies showing non-obligatory linkage of attention and foveation, and with microelectrode studies in primates reporting alteration of firing by prestriate but not striate cells with changes of attention during fixation. The results also support the notion that the "state" function for sustained awareness, and "vector" function which shifts gaze and drives visual search, have relatively separate anatomical underpinnings.

324.13 LONG TERM VISUAL AFTEREFFECTS:
STRUCTURAL CHANGE IN THE HUMAN VISUAL CORTEX?
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Looking at one stimulus can change the appearance of a subsequent stimulus. For example, lines tilted to the left of vertical will make vertical lines appear to be tilted right. These visual aftereffects (AEs) exist for orientation, motion, depth, size (or spatial frequency), color, and perhaps for phase and vernier offset. Each of these AEs comes in two forms: short term and long term. Short term AEs last for a time roughly equal to the length of time that a subject viewed the adapting pattern. Thus, a short term AE will be gone one minute after the end of one minute of adaptation. Long term AEs are defined as AEs that last at least 10 times longer than the adaptation period and can last for weeks. For example, a long term orientation AE can last at least 2 weeks after just 4 minutes of adaptation (Wolfe & O'Connell, Invest. Ophthal. & Vis. Sci. 1986, 27, 538-543). Psychophysical experiments suggest that the loci of both forms of AE are in visual cortex, probably VI.

The mechanism of short term adaptation is probably neural fatigue. Consider orientation. When viewing a vertical contour, neurons tuned for deviations to the left and right will be equally activated. After adaptation to lines tilted left, neurons tuned for deviations to the left will be fatigued. A truly vertical line will produce more activity from right than from left tuned neurons and that line will appear to tilt to the right. Given their time course, fatigue is an unlikely explanation for long term AEs. Instead, they may be due to structural changes in visual neurons. Consider the previous example. Before adaptation, vertical was "defined" by equal activity in left and right neurons. In long term adaptation, suppose that exposure to left increases left's contribution to the definition of vertical perhaps by strengthening the connections between a left tuned neuron and subsequent units that embody the definition of vertical. After adaptation, a contour would need to tilt slightly to the left in order to appear vertical. A physically vertical stimulus, producing merely equal activation of left and right neurons, would now appear tilted to the right. The underlying mechanism could be synaptic facilitation as seen, for example, in long term potentiation (LTP). The time course of long term AEs is comparable to that reported in studies of LTP. Long term AEs may represent a simple mechanism of learning that fine-tunes the calibration of human vision.

325.1 MORPHOLOGICAL CLASSIFICATION OF INTRACELLULARLY HRP INJECTED EFFERENT NEURONS OF THE DEEPER LAYERS OF THE PRIMATE SUPERIOR COLLICULUS. A.B. Karabelas*, A.K. Moschovakis*, and S.M. Highstein (SPON: D.C. Zuazaga de Ortiz). Dept. Otolaryngology and the McDonnell Center for Studies of Higher Brain Function, Washington University, St. Louis, MO. 63110.

Intrasomatic recording and injection of horseradish peroxidase (HRP) was utilized to study neurons of the superior colliculus responding to electrical stimulation of the predorsal bundle (PDB), contralateral superior colliculus, substantia nigra pars reticulata and cerebral peduncle, in anesthetized paralyzed squirrel monkeys. Recovered efferent neurons of the deeper tectal layers can be classified into two major classes.

a) X Neurons. These have medium to large size (soma cross-sectional area (Sa): 700-1600μm^2) multipolar cell bodies located in the stratum griseum intermediale and vertical or radiating dendritic trees that may extend 1.4mm in one or more directions. The axonal system of X neurons includes: a) a relatively thick fiber, 3-6 μm in diameter, that joins the contralateral predorsal bundle (PDB). b) ventral ascending collaterals, that run rostrally ipsilaterally to the cell body towards the parafascicular nucleus of the thalamus and the zona incerta, and c) a heavy projection to the ipsilateral central mesencephalic reticular formation (cMRF). The axonal system of X neurons may also include thin recurrent collaterals.

b) T Neurons. These are located in the stratum opticum and stratum griseum intermediale and have a small to medium-sized (Sa: 200-600μm^2) quadrangular or ovoid cell body. Their relatively thin axons, 1.5-3.5 μm in diameter, give rise to an axonal system that always includes: a) Thin (1-2 μm in diameter) recurrent collaterals, b) a tectal commissural fiber. There may also be either a PDB and an Av fiber together with a heavy projection to the cMRF, or an ipsilaterally descending fiber.

These findings, demonstrate that the major neuronal types of the squirrel monkey superior colliculus are, with minor differences, similar to those of the cat (Moschovakis and Karabelas, J. Comp. Neurol., 239:276, 1985).

325.2 MORPHOPHYSIOLOGY OF SUPERIOR COLLICULUS PRESACCADIC NEURONS IN THE ALERT SQUIRREL MONKEY. A.K. Moschovakis*, A.B. Karabelas* and S.M. Highstein, (SPON:S. Goldring). Dept. Otolaryngology, Washington University, St. Louis, Missouri 63110.

Intraaxonal recording and injection of horseradish peroxidase (HRP) were employed in alert naive, squirrel monkeys to study superior colliculus (SC) neurons that fire before spontaneous saccades. Saccade-Related-Burst-Neurons (SRBNs) have little if any spontaneous activity and burst before spontaneous saccades with a latency of about 20msec (10-60msec, x=23, S.D.=17) and an instantaneous firing frequency that may reach 1250 spikes/sec. These neurons have movement fields similar to those of the movement-related neurons of Sparks, Holland and Guthrie (Brain Res., 113:21, 1976). The mediolateral and anteroposterior location of the cell bodies together with their movement fields are consistent with the motor map of the deeper tectal layers. Medially located SRBNs fire before upward saccades and laterally located SRBNs fire before downward saccades. Rostrally located SRBNs fire before smaller saccades than caudally located SRBNs.

SRBN somata recovered to date are located in the stratum opticum (n=5). They are small to medium in size (soma cross sectional area: 265-648μm^2) and ovoid in shape. They give rise to 4-6 primary dendrites that generate a vertically biased dendritic tree that may extend well within the stratum griseum superficiale. The axons, 2.5-3.5 μm in diameter, originate from the cell body or a primary dendrite, run ventrally towards the periaqueductal gray, turn laterally in the stratum album profundum, circumscribe the periaqueductal gray and cross the midline in the dorsal tegmental decussation to join the predorsal bundle (PDB). Consistent morphological axonal features include: a) Thin (1-2 μm in diameter) recurrent collaterals, about four in number, that ramify in both the deeper and superficial layers of the SC, near the parent soma, b) a tectal commissural fiber, c) ventral ascending collaterals (Av), that run rostrally ipsilaterally to the cell body towards the parafascicular nucleus of the thalamus and the zona incerta and ramify within the interstitial nucleus of Cajal, and d) a heavy projection to the ipsilateral central mesencephalic reticular formation (cMRF).

These morphophysiological findings demonstrate that tectal SRBNs are a specific subset of T neurons (cf. accompanying abstract by Karabelas et.al., Soc. Neurosc. Abstr., 12, 1986). Thus a vectorial presaccadic signal is relayed by each tectal SRBN to reticular targets of the PDB, contralateral superior colliculus and ascending targets of the Av as well as potentially participating in intratectal information processing. (From the Department of Otolaryngology and the McDonnell Center for Studies of Higher Brain Function).

325.3 SACCADIC EYE MOVEMENTS EVOKED BY MICROSTIMULATION OF OPTIC TECTUM OF THE CHICKEN. L. Z. Wise*, J. C. Letelier* and J. Wallman. (SPON: S. Springfield) Dept. of Biology, City College of City University of New York, New York, NY 10031.

Most evidence on which current theories of saccadic eye movement control are based comes from studies of the neurophysiology and behavior of primates, which have frontally placed foveate eyes which move in a strictly conjugate manner. Since saccadic targets in the contralateral visual field are represented in each superior colliculus, and a pathway exists for yoking of the eyes at the motoneuronal level, an attractively simple model of saccadic eye movement control can be constructed. In birds, on the other hand, saccades, although simultaneous, are not conjugate; rather each eye generally moves in a different direction. This situation would seem to require a different scheme for saccade generation.

We measured horizontal and vertical eye movements of chickens with scleral search coils in each eye. The optic tectum (the avian homolog of the superior colliculus) was stimulated via a microelectrode (typical stimulus parameters: 0.5 ms, 200 Hz, 30 uA pulses in 200 ms trains).

Tectal microstimulation elicited synchronous saccadic eye movements in each eye which were indistinguishable from spontaneously occurring saccades. They had a latency of approximately 180 ms, and had the prominent oscillatory component (25-30 Hz) typical of avian saccades. The final position of the contralateral eye was largely dependent on the site of stimulation ("goal-directed" saccades), but was also influenced to some extent by initial eye position, such that large saccades tended to fall short of the "goal." In constrast, the saccades of the eye ipsilateral to the stimulation site generally appeared unaffected by the stimulation, although at one site in which upward, nasalward saccades were elicited in the contralateral eye, the ipsilateral eye also made goal-directed, upward, nasalward saccades. These data imply that in birds each tectum controls the trajectory of the saccades of the contralateral eye, but in addition, exerts some degree of control over the yoking of saccades. (Supported by NSF BNS-8510945)

325.4 A MAP OF HEAD MOVEMENTS IN BARN OWLS IS REVEALED BY ELECTRICAL STIMULATION OF THE OPTIC TECTUM. Sascha du Lac and Eric I. Knudsen, Department of Neurobiology, Stanford University, Stanford, California, U.S.A.

The optic tectum of barn owls (Tyto alba) contains bimodal neurons which respond selectively to visual and auditory stimuli in particular regions in space. These neurons are arranged to form aligned maps of visual and auditory space such that neighboring neurons are activated by stimuli in adjacent spatial locations. Barn owls respond to an interesting sensory stimulus with a rapid shift of gaze toward the stimulus: since their eyes and ears are immobile in the head, they employ a rapid head movement to achieve this gaze shift. We hypothesized that the bimodal space map in the optic tectum is used to direct orienting head movements toward stimulus sources and have begun to test this idea by electrically stimulating focal tectal regions.

Electrodes were placed into the tecta of anesthetized owls, visual and auditory receptive fields were mapped, and the electrodes were fixed in place. After 24-48 hours, unanesthetized owls were placed in a restraining tube which allowed unrestricted head movements and pulse-train stimuli were delivered through the implanted electrodes. Head movements were monitored using a search coil system.

Control head movements were studied in owls trained to direct their gaze toward noise bursts or light flashes in a darkened sound attenuated chamber. Tectal stimulation elicited head movements resembling those evoked by sensory stimuli. At a given stimulus site, the magnitude of the movement increased with both stimulus duration and intensity until it reached a plateau value. Initial head position had some effect on movement size and direction (movement vector), but movements were not goal directed. In general, the movement vector matched the sensory coordinates of the electrode site. Thus, in the barn owl, the optic tectum contains a motor map specifying head movement vectors that is aligned with the visual and auditory space maps.

(Supported by NIH grants R01 NS 16099-06 and ST32 NS 07158-07.)

325.5 AN ADAPTIVE NEURAL NETWORK MODEL FOR INVARIANT MAPPING OF VISUAL TARGET POSITION. M. Kuperstein. Wellesley College, Wellesley, MA 02181.

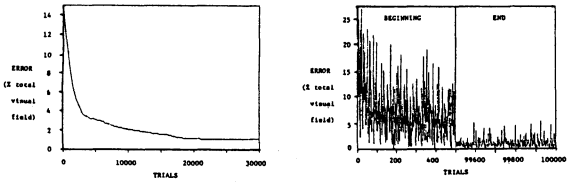
The position of a visual target is perceived as the same when measured by any combination of its registration on the retina and the orbital position of the eye in the head when the target is presented. This study derives and simulates a neural network model that learns to represent visual targets as invariant with respect to any combination of these target measures. Computer simulations of the model show how it is adaptive to changes of the eye and muscles as well as internal system parameters. It even works well with partial damage to the networks. The model learns and maintains precise sensory-motor calibrations starting with only loosely defined relationships.

There are three key aspects to the model: 1) A new network architecture is used to combine the sensory measures of a target. The visual and motor inputs in the model are organized into columns that have a global retinal topography but a local muscle topography. Each column contains representations from all eye muscles. 2) Local computations, that vary across the model's topography, reflect local, linear approximations of the target position. However, convergence of all the local computations yields the precise global position for any target. 3) Learning is achieved by modifying the distributions of all input weights over many performance trials. Incremental changes in the weights are made in each trial and are shared for both visual and motor inputs in the model.

Computer simulations show that the average error in target orientation after learning is as low as 1% of the total visual field. The model is shown to maintain good accuracy across choices in 12 different parameter families.

The model is most related to the function of the posterior parietal cortex. Testable physiological predictions are made for the columnar topography and learning in that brain structure.

Because these results are not constrained by the number of network elements or the number of multimodal inputs or the number of networks, they can be generalized to adaptive multimodal, multijoint coordination.



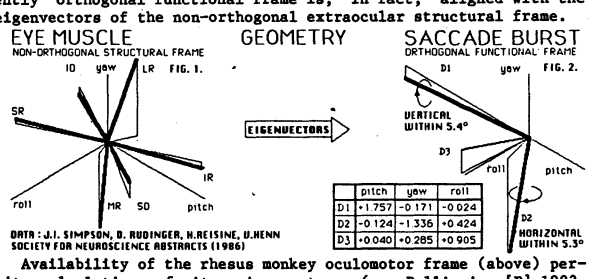
325.6 GEOMETRY OF EXTRAOCULAR MUSCLES OF THE RHESUS MONKEY. J.I. Simpson, D. Rudinger*, H. Reisine and V. Henn (SPON: H. S. Cohen). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016 and Dept. Neurology, University Hospital, Zurich, Switzerland.

Interest in three dimensional aspects of compensatory eye movements has recently experienced a revival with a decidedly quantitative bent. As a result, measurements of the spatial arrangement of the semicircular canals and the extraocular muscles have been made to determine intrinsic reference frames useful in investigating the vestibuloocular reflex as a multidimensional sensorimotor transformation (Pellionisz & Llinas, 1980; Simpson & Graf, 1981; Robinson, 1982). Although the rhesus monkey has featured prominently in studies of compensatory eye movements, its eye muscle reference frame has not been available. The orientation of the rotation axis for each eye muscle acting alone was determined in three rhesus monkeys, two of which were used by us previously (Reisine, et al., 1985) in determining anatomically and physiologically the semicircular canal orientations. Each muscle axis orientation is given in terms of direction cosines in a right-hand Cartesian reference frame fixed to the head, which was placed in the Horsley-Clark stereotaxic position. The eye was in the anatomical position of rest. The Z=0 plane is horizontal; the Y=0 plane is the mid-sagittal plane. Anterior, left and dorsal are positive. The average (n=3) direction cosines (X,Y,Z coordinates) of the rotation axis for contraction of each of the left eye muscles are as follows: MR, .208, .086, -.974; LR, -.292, .122, .949; SR, .437, -.899, .035; IR, -.425, .901, -.086; SO, .525, .831, .186; IO, -.473, -.879, -.052; Accessory LR, .090, -.719, .690. These values were obtained assuming that the center of the globe is the center of rotation (CR). Since the location of the CR of the rhesus monkey eye is not known, the orientations of the rotation axes were calculated for each of eight possible CR locations; the maximum deviation between these rotation axes for a given muscle ranged from 2° to 9°. Comparison of the monkey data with human data obtained by Volkmann (1869) reveals several noteworthy differences. First, in humans the superior and inferior recti axes depart from collinearity by 35°, while in monkeys they depart from collinearity by 2° to 20°, depending on CR location. The range of this measure for rabbit and cat (Simpson, 1983; Ezure & Graf, 1984) falls within that found for the monkey. Second, the measurements indicate that the oblique muscles in monkey produce more vertical than torsional eye movement, in contrast to their actions in humans. Because of these differences, contemporary data on the geometry of human extraocular muscles would be welcome. Supported by NS-13742 and Swiss Fnd. Sci. Res.

325.7 TENSORIAL RELATIONSHIP FOUND FOR STRUCTURAL & FUNCTIONAL REFERENCE FRAMES OF BRAIN FUNCTION: SACCADE NEURONS IN MONKEY UTILIZE FRAMES COMPOSED OF THE EIGENVECTORS OF THE FRAME OF EXTRAOCULAR MUSCLES A. Pellionisz, Physiol. & Biophys, NYU Med. Ctr. N.Y., N.Y., 10016

Brain function is expressed by multidimensional intrinsic coordinates. Neurons connected to the peripheries of sensorimotor systems (e.g. vestibular canals & extraocular muscles) must use structural frames. Their rotational axes have been quantitatively established for several species. Typically, they are non-orthogonal overcomplete multidimensional frames (Fig. 1). Since central neurons of this system use an orthogonal frame, as claimed by Luschei & Fuchs 1972, Keller 1974, Buttner et al. 1977, King & Fuchs 1979, Hepp & Henn 1982, Gisbergen et al. 1985: there is, then, this paradox that the paramedian pontine reticular formation reportedly contains medium-lead burst cells, whose firing rate is tightly related to eye velocity in either horizontal or vertical saccades.

Since tensor network theory of the CNS centers around the general question of transformations among intrinsic coordinates plus the more profound problem of how structural and functional geometries relate, the question emerges as to whether the apparently orthogonal functional frame is, in fact, aligned with the eigenvectors of the non-orthogonal extraocular structural frame.



	pitch	yaw	roll
D1	+1.757	-0.171	-0.024
D2	-0.124	-1.336	+0.424
D3	+0.040	+0.285	+0.905

Availability of the rhesus monkey oculomotor frame (above) permits calculation of its eigenvectors (see Pellionisz [P] 1983, Simpson & [P]. 1984, Graf & [P] 1985), that gives an affirmative answer (see Figs. 1-2): Functional and structural geometries are connected in a manner that the frame in one utilizes the eigenvectors of the frame in the other. This finding may have both important theoretical and experimental implications. In the meta-organization of networks ([P] & Llinas 1985), neurons must utilize the eigenvectors of the metric tensor of the motor frame to enable independent adaptive modifications, since eigenvectors form an orthogonal set. Experimentation could help by providing further data for A) other species (that have different motor frames and eigenvectors) and for B) adaptive coordination along eigenvectors.

325.8 MORPHOPHYSIOLOGY OF VERTICAL MEDIUM LEAD BURST NEURONS IN THE ALERT SQUIRREL MONKEY S.M. Highstein, A.K. Moschovakis* and J.D. Warren*. Dept. Otolaryngology, Washington University, St. Louis, MO. 63110.

The morphology and physiology of vertical burst neurons (VBN) in the oculomotor system of the squirrel monkey have been investigated utilizing intraaxonal recording and injection of horseradish peroxidase (HRP) in alert, behaving animals. These neurons emit a burst of impulses between 4.6 and 12.8msec before the onset of saccadic eye movements with a component in their on-direction. The on-directions of the VBNs studied were either upward (85-121°) or downward (242-292°). The characteristics of the burst (duration and number of spikes) were highly correlated with parameters of the saccadic eye movements (duration and amplitude of the saccade component in the on-direction).

Following physiological characterization, axons of VBNs were injected iontophoretically with HRP for the subsequent visualization of their somata and axonal projections. Their triangular or quadrangular, small to medium sized (soma cross-sectional area: 250-450 μm^2) cell bodies were recovered in the rostral interstitial nucleus of the medial longitudinal fasciculus (rIMLF). In the rIMLF, VBN somata do not appear to be segregated according to their on-direction. The medium-sized (2.5-4.5μm in diameter) axons of VBNs originate from the cell body, travel caudally within the rIMLF, the interstitial nucleus of Cajal (NIC) and the medial longitudinal fasciculus (MLF) ipsilaterally. All VBNs have terminal fields within the NIC bilaterally, as well as vertical motoneuron pools of the III nucleus bilaterally, and, in the case of down-on VBNs, the trochlear nucleus. The axons of VBNs then continue within the ipsilateral MLF to as yet undisclosed targets caudal to the trochlear nucleus.

In conclusion, the morphology and physiology of the VBNs here-in presented suggest that these cells could be the medium-lead burst neurons which are responsible for the generation of vertical saccadic eye movements. The bilateral output to the NIC could play a role in providing this structure with necessary signals to perform an integration of the rapid eye movement signal. (Supported by NIH EY 05433).

325.9 VERTICAL AND TORSIONAL RAPID EYE MOVEMENT GENERATION IN THE RIMLF. K.Hepp*,T.Vilis and V.Henn. Physics Dept. ETH and Neurology Dept. Univ., 8093 Zuerich, Switzerland.

The common pathway from visual and vestibular input to rapid eye movement output passes through the population of the short-lead burst neurons (SBN) in the PPRF for horizontal saccades and the riMLF for vertical saccades. We have examined the hypothesis that two subpopulations of vertical SBN exist in the riMLF with on-directions in approximately the planes of the two vertical semicircular canals. Rhesus monkeys were seated in a 3-axes turntable and sinusoidally oscillated about four vertical planes: vertical (sagittal), torsional (Listing's plane), RALP (right anterior - left posterior canals) and LARP (left anterior - right posterior canals).

During visually elicited saccades with the head stationary, SBN with vertical on-directions, either upwards or downwards, were observed bilaterally (as in Büttner et al, Brain Res (1977) 130:239). During sinusoidal head rotation in the dark two further subpopulations could be distinguished. Downward SBNs in the right riMLF and upward SBNs in the left exhibited a more directionally specific firing pattern during head rotation in the RALP plane. Thus, relative to spontaneous eye movements in the light, the units had higher average firing rates in their on-directions and lower rates in their off-directions for a given vertical eye displacement. The degree of modulation decreased with sagittal and torsional planes of head rotations and the units became most isotropic with a LARP or a horizontal plane of head rotation. Upward SBNs in the right riMLF and downward SBNs in the left exhibited the mirror image directional preference. During VOR in the light the directionally specific modulation became smaller.

As noted previously (Henn et al, Exp. Brain Res. (1984) 54: 166) during light sleep rapid eye movements are abolished and SBNs exhibit a tonic activity which can be modulated by vestibular stimulation. In this condition, each of the four subpopulations of SBN in the riMLF behaved like one of the four vertical type I vestibular neuron populations. Thus for example the tonic activity of downward acting SBN in the right riMLF increased during downward head rotation in the plane of the right anterior canal. This modulation decreased for head rotation in the sagittal or vertical planes with the least modulation occurring in the LARP plane.

The findings suggest that the on-directions of SBNs in the riMLF are approximately coplanar with ipsilateral vertical motoneurons consistent with an ipsilateral projection. It also suggests a close functional relation with the ipsilateral anterior and posterior semicircular canals.

325.10 UNILATERAL riMLF LESIONS IMPAIR SACCADE GENERATION ALONG SPECIFIC VERTICAL PLANES. T.Vilis,K.Hepp*,U.Schwarz*,V.Henn and H.Haas*. Neurology and Neurosurgery Dept. Univ. and Physics Dept ETH, 8091 Zuerich, Switzerland.

The goal of this study was to characterize saccadic deficits produced by reversible lesions of the vertical saccade generator. The technique involved first localizing and characterizing saccade related neurons in the rostral iMLF, details are given in the companion abstract of Hepp et al. Secondly a reversible lesion of this same region was produced by means of a microinjection of Muscimol, a GABA agonist. Thirdly the characteristics of both visually elicited saccades and the fast phase of nystagmus during sinusoidal head rotation in different planes were compared pre and post injection using chronically implanted EOGs. Injections of as little as .3 ul of .1% Muscimol produced saccadic deficits within 5 to 10 minutes. The deficits persisted for 1 to 2 hours and then gradually subsided.

Bilateral riMLF injections of Muscimol abolished all vertical visually elicited saccades and the vertical fast phase of nystagmus. Horizontal saccades were preserved as were horizontal and vertical VOR and pursuit. The initial or transient effect of such lesions was the appearance in the vertical plane of a series of hypometric visually elicited saccades resembling a staircase and shimmering nystagmus during VOR.

Unilateral injections did not produce a vertical gaze palsy. However it did disrupt saccades along specific planes. Right injections impaired the generation of visually elicited saccades up to the left and down to the right. Conversely left injections impaired saccades up to the right and down to the left. Such a deficit would be consistent with an ipsilateral projection from burst neurons in the riMLF to vertical motoneurons. A right riMLF lesion would result in an impaired saccadic drive to one of the upward acting pair of muscles in each eye, the right inf. oblique and the left sup. rectus, and to one of the downward acting pair, the right inf. rectus and the left sup. oblique. Such a segregation was confirmed in terms of deficits in the fast phase of nystagmus produced during sinusoidal VOR about various vertical planes. After a right injection, the generation of downward saccades was most impaired during head rotation in the plane of the right anterior canal, and less impaired in other planes of head rotation.

The results are consistent with the suggestion that the eye can be moved vertically in two modes. During visually elicited saccades, burst neurons in the riMLF are bilaterally activated, thus preserving Listing's law. In contrast vestibularly elicited fast phase nystagmus can be generated in the plane of head rotation, a plane whose axis is not necessarily in Listing's plane, by a preferentially unilateral activation of riMLF burst neurons.

325.11 Broad-band Gain Plasticity of the Human Vestibulo-ocular Reflex (VOR). J.L. Demer, J. Goldberg, F.I. Porter*, and H.A. Jenkins*. Cullen Eye Institute and Clayton Foundation for Research Neurotology Laboratory, Baylor College of Medicine, Houston, Texas 77030.

It has been demonstrated by Lisberger et al. (J Neurosci 3:1234, 1983) that the mechanism underlying adaptive control of the VOR of the rhesus monkey is frequency selective. We now report that this may not be the case for the human VOR, where short duration telescopic spectacle viewing at one frequency of head rotation induces similar adaptive VOR gain changes at higher and lower frequencies.

Seven adult volunteers underwent passive sinusoidal whole-body oscillation. Horizontal eye movements were measured by DC coupled electro-oculography. Slow phase eye velocity was extracted by digital filtering, and the sinusoidal envelope of slow phase eye velocity was estimated by least squares curve fitting. VOR gain was estimated by dividing slow phase eye velocity amplitude by head velocity amplitude at each frequency. Calibration was obtained by numerous saccade trials to targets at known eccentricities, and was performed with unmagnified vision.

The VOR was observed in darkness at pure sinusoidal frequencies 0.1 and 0.2 Hz (amplitude 60 deg/sec) prior to the wearing of telescopic spectacles. Two subjects were also tested at 0.8 Hz, amplitude 30 deg/sec. Responses to a sum of sinusoidal frequencies of head rotation were also observed; these were harmonics (1, 2, 5, 9, 17) of 0.025 Hz having no common sums or differences and ranging up to 0.425 Hz. The amplitude of each component frequency in the sum of sines stimulus was 15-30 deg/sec.

VOR gain in the light was highly reproducible, both during unmagnified vision (gain 1.00-1.10) and during wearing of 2X binocular telescopic spectacles having a field of view limited to 18^0 (gain 1.60-1.80). After initial VOR measurements, subjects wore telescopic spectacles to view a distant video monitor while undergoing rotation at 0.2 Hz, amplitude 20 deg/sec for 15 min. Observation of the VOR was then repeated. VOR gain in the light did not change significantly. Three of seven subjects exhibited significant VOR gain increases in darkness of 0.07 to 0.18; the remaining four exhibited no consistent gain increases. Gain increases were similar at all frequencies tested. This suggests that there is minimal if any frequency selectivity of the adaptive gain mechanism operating during short term VOR gain plasticity in the human. However, it is possible that larger gain changes induced by hours of adaptation may exhibit frequency selectivity.
(Supported by EY06394 and the Clayton Foundation for Research.)

325.12 CELLULAR ACTIVITY IN THE MONKEY SUPERIOR COLLICULUS DURING SHORT TERM SACCADIC ADAPTATION. E. J. FitzGibbon and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD 20892 USA.

The gain of the saccadic system is under adaptive control and changes when extraocular muscle activity is inappropriate for target acquisition. We used an experimental model of saccadic adaptation in which a rhesus monkey fixates a central target and then makes a saccade to a peripheral target. During the monkey's saccade the peripheral target is moved either further from central fixation, causing the monkey to undershoot the target (lengthening paradigm), or closer to central fixation, causing the monkey to overshoot its target (shortening paradigm). With training, the monkey learns to make saccades larger or smaller than those dictated by the peripheral target's original position.

We have previously shown that during short-term saccadic adaptation the amplitude of saccades evoked by electrical stimulation of the intermediate grey layers of the superior colliculus is unaffected by the adaptation process. This result had several possible interpretations: the adaptation could occur before the colliculus, in which case the collicular sensory discharge would be appropriate to the stimulus and the collicular movement discharge would be appropriate for the adapted saccade. Alternatively, the adapted saccade might arise as the sum of several active sites in the colliculus, including a movement discharge appropriate to the retinal location of the target and movement activity appropriate for the difference between the original target location and the actual adapted saccade amplitude.

To choose between these alternatives we recorded cellular activity in the superior colliculus during lengthening and shortening paradigms. In all cases the visual activity was appropriate for the real retinal location of the target and the movement activity appropriate for the saccade actually performed. For example, in the lengthening paradigm the monkey began by making a saccade to the original target position and then a corrective. Visual and movement activity occurred appropriate for this first saccade and then for the corrective saccade. When the monkey made the adapted saccade, the activity associated with the corrective saccade ceased, the visual activity was appropriate for the original target position but the movement activity was appropriate for the new saccade.

These results argue that saccadic adaptation does not occur in the superior colliculus: the cellular activity in the colliculus occurs at the single loci appropriate for where the stimulus is and what the saccade will be. Neural events associating the original stimulus with the adapted saccade must occur in structures active before the superior colliculus in the neural events associated with the generation of saccadic eye movements.

326.1 ENHANCED APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR AFTER SPECIFIC D-2 ANTAGONIST PRETREATMENT IN MICE WITH UNILATERAL STRIATONIGRAL LESIONS R.J. Mandel and P.K. Randall Depts. Psychology and Physiology and Biophysics, USC, Andrus Gerontology Ctr., L.A., CA 90089-0191

Recent observations indicate that stimulation of specific dopamine receptor (DAr) subtypes in striatum can lead to simultaneous stereotypic gnawing and catalepsy (Yurek and Randall, Life Sci., 37:1665, 1985). In addition, Herrera-Marschitz and Ungerstedt (Br. Res. 323:269,1985) have reported that different DAr subtypes are related to different striatal efferents with D-1 stimulation apparently being more prevalent in the striato-nigral efferent. These data suggest that the simultaneous hypo-and hyper-dopaminergic behaviors noted by Yurek and Randall are being subserved by different striatal efferents. Biochemically, Stoof and Kebabian (Nature 294:366, 1981) have demonstated that there is a functional antagonism between the DAr subtypes in striatum related to stimulation of adenylate cyclase . Taken together, these data suggest that if the behavioral output of the striatonigral efferent can be isolated, then blockade of D-2 receptors may enhance D-1 stimulated behavior resulting from a non-specific DA agonist. This experiment utilized a discrete unilateral electrolytic lesion in the striatonigral pathway to examine the effect of a low dose of spiperone (SPIP) on apomorphine (APO) -induced rotation in the C57BL/6J mouse.

Fifteen mice were adminstered electrolytic lesions (1mA for 5 sec) in the striatonigral tract, using coordinates -1.6mmAP, ± 1.7mmLAT, and -4.9mm D-V from bregma. The 8 mice that showed robust rotational behavior to amphetamine (2.0 mg/kg, IP) were used in a dose-response experiment in which APO (7.7, 3.75, 1.875, .9375, and .46875 mg/kg, IP) was administered after pretreatment (30 min) with either 0.01 mg/kg spiperone or the 1% lactic acid vehicle. Rotational behavior ipsilateral to the striato-nigral lesion was the main dependent measure.

SPIP pretreatment significantly elevated apomorphine-induced rotational behavior ($F(4,63)=8.7; p<0.01$), nearly doubling it at the highest doses. No shift to the left in the dose response curve was observed (common ED50=1.5mg/kg:non-linear least-squares estimate), but the maximum response parameters were quite different in the two conditions (SPIP pretreatment max response=117.8;vehicle max response=65.7).

These results indicate that there is a demonstrable functional interaction between D-1 and D-2 receptors in striatum, especially in relation to the striatonigral pathway. These data are not explained by the effect of SPIP on a DA autoreceptor since the largest behavioral effect occured at the highest doses of APO.

326.2 BLOCKADE OF DOPAMINE D-1 RECEPTORS BY SCH 23390 PREVENTS L-DOPA-INDUCED EEG AND BEHAVIORAL ACTIVATION IN THE RABBIT. E. Ongini, M.G. Caporali* and M. Massotti. Lab. di Farmacologia, Istituto Supoeriore di Sanità, 00161 Roma, Italy.

Electroencephalographic (EEG) studies in the rabbit have clarified the involvement of dopamine (DA) receptor subtypes in the generation of the central stimulatory effects of DA agonists. On the one hand, both the EEG desynchronization and behavioral arousal elicited by the D-1 agonist SKF 38393 (10 mg/kg iv) are selectively prevented by the D-1 antagonist SCH 23390 (0.003-0.01 mg/kg iv), but not by the D-2 antagonist, 1-sulpiride (25 mg/kg iv) (Ongini et al., Life Sci, 37:2327, 1985). On the other hand, the behavioral stimulation elicited by the D-2 agonist LY 171555 (0.5 mg/kg iv) is selectively blocked by 1-sulpiride (6-12.5 mg/kg iv) (Ongini et al., submitted).

The observation that L-DOPA induces in the rabbit central stimulatory effects (EEG desynchronization and behavioral alert: Florio and Longo, Neuropharmacol., 10: 45, 1971), prompted us to explore whether these responses imply activation of either D-1 or D-2 receptors.

Groups of rabbits implanted for EEG recording received CARBIDOPA (2 mg/kg iv) followed 10 min later by L-DOPA (20 mg/kg iv). After the latter, a desynchronized pattern emerges, lasting about one hour. Pretreatment, 15 min before, with SCH 23390 (0.003-0.01 mg/kg iv) causes a dose-related decrease of the duration of the EEG desynchronization. On the contrary, haloperidol (0.3 mg/kg iv) and 1-sulpiride (6 mg/kg iv) were unable to prevent L-DOPA effects on EEG. Both neuroleptics, at the same doses, inhibited LY 171555-induced stimulation, but did not interact with SKF 38393.

These results suggest that the D-1 receptor subtype mediate the EEG activation induced by L-DOPA.

326.3 PRESYNAPTIC NEUROMODULATION OF A CATECHOLAMINE TRANSMITTER SYSTEM BY ANAPHYLATOXIN (C5a) IN THE RAT HYPOTHALAMUS. C.A.Williams, N.Schupf, A.Berkman* and W.S.Cattell*. Division of Natural Sciences, SUNY Purchase, Purchase, NY 10577.

An experimental animal model has been developed to elucidate the mechanisms by which infectious, allergic or autoimmune reactions in the brain can alter behavior. Immune complexing systems (ICS) injected via implanted cannulae into the perifornical hypothalamus have been reported to stimulate eating in sated rats (N.Schupf and C.A. Williams, J. Neuroimmunol., 9:1-13, 1985), the same effect which is seen after the injection of norepinepherine (NE). The complement cascade, when activated by immune complexes, produces a pharmacologically active peptide, C5a anaphylatoxin, which is also able to induce eating at the same brain site (C.A.Williams, N.Schupf and T.E.Hugli, J. Neuroimmunol., 9:14-29, 1985). Increased food intake induced by either C5a or NE is blocked by phentolamine (PHT), indicating that an alpha-adrenergic receptor system is being activated in both cases. The ICS effect can be blocked by stopping the complement cascade at a step prior to the production of C5a (in press), suggesting that it could be the anaphylatoxin which mediates the effect of the ICS at this site. The experiments we describe in this report lead us to propose the neuropharmacological mechanism by which C5a induces the behavioral response in the rat. Pre-injection of the inactive desarginated form of human C5a (kindly supplied by Dr. T. E. Hugli) blocks C5a- but not NE-induced eating, indicating that C5a and NE do not act at the same postsynaptic receptor. However, since the common pathway appeared to be the NE receptor system in this site, a presynaptic modulatory mechanism for C5a was proposed. A group of 15 rats were pretreated focally with alpha-methyl-p-tyrosine (AMPT) to block the biosynthesis of the catechol neurotransmitters in the site and thus any presynaptic mechanism. Under these conditions C5a no longer induced eating, but the response to injected NE was unimpaired. Although the AMPT experiment confirms the presynaptic action of C5a, it does not distinguish between NE release and NE re-uptake blockade as possible mechanisms. Preliminary experiments with crude nerve terminal preparations (synaptosomes) show that C5a causes the release of preloaded tritiated NE and dopamine (DA), but not of acetylcholine. Endogenous DA release is detected by HPLC. We tentatively conclude, therefore, that anaphylatoxin C5a is a catecholergic secretagogue in the rat brain.

326.4 D-DOPA AND L-DOPA SIMILARLY INDUCE ROTATIONAL BEHAVIOR IN RATS WITH UNILATERAL LESIONS OF THE SUBSTANTIA NIGRA INDUCED BY 6-HYDROXYDOPAMINE. F. Karoum, H.E. Spoor*, W.J. Freed, L-W. Chuang*, R.J. Wyatt and E. Costa. Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032 and FGIN, Washington, D.C.

We have recently compared the ability of D- and L-DOPA to form dopamine and its metabolites in rats. Both amino acids increased hypothalamic and striatal dopamine by about the same extent. This unexpected result led us to assess the ability of these two amino acids to produce rotational behavior in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. The right substantia nigra was lesioned by stereotaxic injection of 6-hydroxydopamine and after at least four weeks all rats were tested for apomorphine-induced rotational behavior to insure that the lesions were essentially complete. D- and L-DOPA, 50 mg/kg, were coadministered with 5 mg/kg carbidopa intragastrically and the rats placed in rotometers to count the rate of rotations. L-DOPA produced contralateral rotation within 15 minutes of administration, while D-DOPA produced contralateral rotational behavior after about 45 min. Once the rats began to rotate, the time course for both drugs was similar. The minimum dose of D-DOPA that induced rotational behavior was 30 mg/kg; 10 mg/kg had no effect. These results suggest that D-DOPA may be useful in the treatment of parkinsonism.

326.5 INBRED MOUSE STRAINS DIFFER IN SENSITIVITY TO STIMULANTS ACTING ON DIFFERENT DOPAMINE POOLS. R. Tintner and J. Proctor*. Dept. of Neurology, Southwestern Med. Sch., Univ. of Texas Health Sci. Ctr., Dallas, TX 75235-9036.

A number of diseases characterized by the presence of abnormal involuntary movements have a significant hereditary component. These movements are affected by drugs which alter dopamine (DA) action. Likewise, inbred strains of mice have been shown to have heritable differences in both spontaneous and drug-induced activity. Specifically, BALB/cj mice have been reported to have higher bseline activity, enhanced response to amphetamine, and higher numbers of substantia nigra DA neurons thans CBAs; however, BALB striatal DA content does not exceed that of the CBAs. We therefore examined the possibility that behavioral differences in two such strains reflect differences in compartmentation of DA, and not merely in numbers of DA neurons.

Locomotor activity (LA) and stereotypic activity (SA) were assessed in BALB/c and CBA mice after s.c. administration of either amphetamine (AMP), which releases DA from a cytoplasmic pool or amfonelic acid (AFA), which releases it from a storage (vesicularized) pool. BALBs had significantly greater baseline LA. AMP caused: 1) decreased BALB LA at low doses, but increased LA above 5 mg/Kg, 2) increased CBA LA at all doses, 3) greater SA in the BALBs than the CBAs. AFA increased LA at all doses tested in both strains. At AFA doses above 0.6 mg/Kg the CBAs had significantly greater LA than the BALBs; in addition, the CBAs had LA scores close to twice the maximum induced by AMP. This data is consistent with the hypothesis that a greater proportion of DA is stored in vesicular, as opposed to cytoplasmic, pools in the CBA mice. The role of different subcellular DAergic mechanisms in nonuniformity of drug action will be discussed.

326.6 OPPOSITE EFFECTS OF THE D1 AGONIST SK & F 38393 AND THE D2 AGONIST LY171555 ON ORAL MOVEMENTS IN RATS. Gaylord Ellison, Per Johansson*, Edward Levin, Lynn Furukawa*, and Lars Gunne* Dept of Psychology, UCLA and Psychiatric Research Center Uppsala Sweden

We report that D1 dopamine agonists have opposite effects on oral activity from D2 agonists, that these opposed effects can be dissociated from autoreceptor mechanisms, and further that chronic neuroleptic administration alters responsivity to these two drugs.

Oral movements (OMs) were monitored in rats administered one of three doses of either the D1 dopamine agonist SK&F 38393 or the D2 dopamine agonist LY171555. OMs were observed via closed-circuit television and simultaneously analyzed using a computerized recording of the distance between two fluorescent dots painted above and below the rat's mouth.

The human observer data indicated that the two drugs had distinctively different effects on oral movements. SK&F 38393 induced dose-dependent increases in tremorous movements of the masseter muscles, tongue protrusions, and, at the highest dose, an increase in repetitive chewing movements. Conversely, LY171555 produced an inhibition of oral activity at all dose levels. At the lowest dose tested this appeared to reflect an auto-receptor phenomenon, for all activity was decreased and when OMs occurred, they were sluggish in form. But higher doses of LY171555 induced hyperactivity and eventually stereotyped, repetitive headmovements wereas chewing movements, tremorous OM's, and tongue protrusions were decreased or completely absent. The computerized scoring system confirmed and extended these conclusions. Thus, D1 and D2 receptors appear to have opposite effects on OM's.

A second study attempted to determine how these findings might clarify the role of dopamine mechanisms in tardive dyskinesia. Using the same recording procedures, responsiveness to these two dopamine agonists was tested in rats which had been chronically pretreated with one of two classes of chronic neuroleptics (haloperidol vs. fluphenazine).

326.7 AUTORADIOGRAPHIC DEMONSTRATION OF BENZODIAZEPINE AND MUSCARINIC RECEPTORS IN TURTLE CORTEX. J.R. Schlegel*, S.J. Peroutka, and A.R. Kriegstein. (SPON: R. Cawthon). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

In vitro receptor autoradiography using radioligand binding techniques has yielded much information regarding the distribution of neurotransmitter receptors in mammalian brain. Using this method, we have investigated the distribution of benzodiazepine and muscarinic cholinergic receptors in turtle cortex. This relatively simple trilaminar structure consists of a pyramidal cell layer, an overlying molecular layer, and an underlying subcellular layer. Immunohistochemical and physiologic evidence suggests that intrinsic cortical stellate neurons mediate GABAergic inhibition of pyramidal cells. There is also evidence that neurons of the basal forebrain contribute a cholinergic innervation of the turtle cortex, and ACh produces predominantly excitation of cortical pyramidal cells (see Blanton et al., this meeting). We report here the results of autoradiographic studies demonstrating the cortical distribution of two important receptors associated with these transmitter systems.

Benzodiazepine binding sites were labelled with 1nM ^{3}H- Flunitrazipam (^{3}H-FLU). Specific binding was defined by the addition of 1 µm clonazepam. Autoradiograms were generated using tritium sensitive film, and the cortical distribution of binding sites was investigated further using the coverslip method developed by Kuhar and associates, allowing simultaneous observation of autoradiogram and cortical histology. ^{3}H-FLU binding was least dense in dorsomedial cortex, and was progressively more dense in ventromedial cortex and dorsal cortex. In all these cortical regions binding was greater over the outer half of cortex (outer molecular region) compared to the inner half (inner molecular region , pyramidal cell layer, and subcellular layer).

Muscarinic binding sites were demonstrated by 1nM ^{3}H-QNB, with specific binding defined by the addition of 1 µm atropine. ^{3}H-QNB binding was most dense in the rostral cortical region, and it was greatest in dorsal cortex compared to medial cortical regions. In contrast to ^{3}H-FLU binding, ^{3}H-QNB binding appeared more concentrated over the inner molecular layer compared to the outer molecular layer.

The differential regional and laminar distribution of benzodiazepine and muscarinic receptors in turtle cortex may be correlated with other neurochemical, anatomical, and physiologic data in this simple cortical model.

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326.8 FIGHTING STRESS INCREASES BENZODIAZEPINE RECEPTOR BINDING IN VIVO. L.G. Miller,* M.L. Thompson,* D.J. Greenblatt* (SPON: A. Tischler) Division of Clinical Pharmacology and Department of Biochemistry and Pharmacology, Tufts-New England Medical Center, Boston, MA 02111

We examined benzodiazepine receptor binding in response to a paradigm of stress in mice: attack by a resident mouse on an intruder. Adult male CFW mice were stressed by individual placement into home cages of resident CFW mice. After the intruder mouse had been attacked and bitten 100 times, mice were removed. Benzodiazepine receptor binding in vivo was determined by the method of Goeders and Kuhar (Life Sci., 1985, 37:345). Mice were injected with 5 uCi 3H-Ro15-1788 intravenously and then sacrificed and cortices removed, dissolved, and counted. Nonspecific binding was determined by pretreatment (30 min) with a saturating dose of clonazepam (CNZ, 5 mg/kg intraperitoneally)(i.p.). For affinity experiments, mice were treated with CNZ in varying doses (.02-2 mg/kg) i.p. after stress for 40 min and receptor occupancy was determined in half the cortex as above. CNZ concentrations were determined in the remaining cortex by electron-capture gas chromatography. In some experiments mice were pre-treated with lorazepam (LRZ .03-.3 mg/kg) prior to stress. Mean (±SD) receptor occupancy in intruder animals injected with radioligand immediately after stress (n=10) was 24.0±4.1 cpm/mg compared to that of unstressed controls (n=5, 14.5±4.2 cpm/mg) and 13.0±4.2, n=4 in resident CFW mice. Receptor occupancy remained elevated when radioligand was injected at 15 minutes after stress (156% of control, n=3) and at 30 minutes (141% of control, n=3) but at 45 and 60 minutes after stress occupancy had returned to control levels. Concentration vs occupancy for CNZ yielded IC_{50} 26 ng/g similar to those in unstressed animals (IC_{50} 21 ng/g). Pretreatment wth lorazepam (LRZ) for 1 hour at the IC_{50} dose (0.3 mg/kg) resulted in similar receptor occupancy in stressed animals (n=3, 2.89±0.3 cpm/mg) and controls (n=3, 2.24±2.07 cpm/mg). Pretreatment with LRZ at doses producing 20% and 5% receptor occupancy (n=3, .1 mg/kg and n=3, .03 mg/kg) resulted in increased receptor occupancy in stressed versus unstressed mice. Altered nonspecific uptake of Ro15-1788 did not explain occupancy changes, since cortical concentrations of unlabeled Ro15-1788 (5 mg/kg) were not changed by LRZ (4 mg/kg) administration. Thus fighting stress increases cortical benzodiazepine binding in vivo. Since receptor affinity for CNZ remains unchanged, the increase is most likely due to an increase in number of binding sites. Pretreatment with LRZ sufficient to produce 50% receptor occupancy abrogated the increase, but pretreatment with doses producing 25% occupancy or less did not.

326.9 ASSOCIATION OF BEHAVIORAL RESPONSES WITH ACTIVATION OF CENTRAL BETA ADRENERGIC SYSTEMS. James M. O'Donnell, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

The beta adrenergic receptor-adenylate cyclase system in the brain has been implicated in the actions of antidepressant drugs. It has been suggested that lipophilic beta adrenergic agonists may possess antidepressant activity. For this reason, it was of interest to begin to assess the neuropsychopharmacological effects of these compounds. Initial studies have involved the characterization of the effects of clenbuterol. Clenbuterol inhibited the binding of ^{125}I-iodopindolol to membranes prepared from rat cerebral cortex (mostly $beta_1$ subtype) and cerebellum (mostly $beta_2$ subtype), in vitro. In the absence of guanosine triphosphate (GTP), the apparent K_I value and Hill coefficient for clenbuterol in the cerebral cortex were 106±18 nM and 0.89±.02, respectively. 0.2 mM GTP increased the apparent K_I value to 124±18 and the Hill coefficient to 1.05±.02. In the cerebellum, the apparent K_I value was increased from 60±10 to 100±13 nM and the Hill coefficient was increased from 0.82±.04 to 1.05±.02 by 0.2 mM GTP. Nonlinear regression analysis of the competition curves for clenbuterol indicated that a lower percentage of the binding of clenbuterol was to a high affinity state of the receptor than was observed with the full agonist isoproterenol. Consistent with these findings, assessment of the ability of clenbuterol to increase levels of cyclic AMP in cerebral cortex or cerebellum showed it to be a partial agonist.

Since several lines of evidence indicate that clenbuterol is centrally active following peripheral administration, it was of interest to examine its effects on behavior. Clenbuterol (0.003 to 10 mg/kg, i.p., 30 minutes prior to testing) reduced locomotor activity in a dose-dependent manner with and EC_{50} value of approximately 0.03 mg/kg. Clenbuterol also reduced response rate under a multiple fixed-interval 5-minute, fixed-ratio 30-response schedule and reduced response rate and increased reinforcement rate under a differential-reinforcement-of-low-rate 72-second schedule. The effects of clenbuterol on locomotor activity and schedule-controlled behavior appeared to be mediated by beta adrenergic receptors since they were antagonized by the beta adrenergic antagonist propranolol in a dose-dependent manner. These behavioral effects of clenbuterol resemble those of tricyclic antidepressants and monoamine oxidase inhibitors. Studies are underway to determine if the behavioral effects of other centrally acting beta adrenergic agonists resemble those of clenbuterol. (Supported by AFOSR NL0054 and USPHS Grant MH40694.)

326.10 EFFECTS OF 5-HT DEPLETION AND OF 5-HT AND CATECHOLAMINE RECEPTOR ANTAGONISTS ON 8-OH-DPAT-INDUCED FEEDING IN THE RAT. C.T. Dourish[1], P.H. Hutson* and G. Curzon*. Dept. of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

In recent studies we have shown that the novel 5-HT agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) elicits feeding in satiated rats after both peripheral and intra-raphe injections. Since post-synaptic 5-HT receptor agonists generally decrease feeding we have proposed that 8-OH-DPAT-induced feeding may be due to activation of 5-HT autoreceptors which would decrease 5-HT synthesis and release. In the present study we examined the effects of 5-HT depletion and of various serotonergic, dopaminergic and adrenergic receptor antagonists on feeding elicited by peripheral injection of 8-OH-DPAT.

All feeding tests were conducted during the daytime in non-deprived male rats. To deplete brain 5-HT rats were injected with 150 mg/kg p-chlorophenylalanine daily for 3 days. This treatment produced a 91% depletion of whole brain 5-HT and 5-HIAA and abolished 8-OH-DPAT-induced feeding. In antagonist studies, drug or vehicle solutions were injected 30 min. prior to 1 mg/kg 8-OH-DPAT (sc) and food intake measured for 2 hrs. Feeding was unaffected by pretreatment with alpha-2-adrenoceptor antagonist idazoxan (3 mg/kg), the beta-adrenoceptor antagonist propranolol (8 mg/kg), the 5-HT2 antagonist ketanserin (2.5 mg/kg) or the non-selective 5-HT antagonist methysergide (10 mg/kg). In contrast, feeding was attenuated by (-)-pindolol (4 mg/kg) and by the neuroleptics spiperone (0.05 and 0.1 mg/kg) and haloperidol (0.1 and 0.2 mg/kg). These data show that 8-OH-DPAT-induced feeding does not depend on adrenoceptors or 5-HT2 receptors. Blockade of the response by the putative 5-HT1A antagonists (-)-pindolol and spiperone suggests mediation by the 5-HT1A receptor. This is consistent with evidence that 8-OH-DPAT has high affinity for this receptor and with the finding that 2 other putative 5-HT1A agonists buspirone and isapirone also elicit feeding. Further, the results indicate the involvement of dopamine receptors as the feeding response was blocked by haloperidol. The data as a whole suggest that 8-OH-DPAT-induced feeding involves the activation of 5-HT autoreceptors located on the cell bodies of neurones in the raphe nuclei. The resulting feeding response may depend on an indirect activation of dopaminergic receptor mechanisms caused by removal of tonic 5-HT inhibition.

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326.11 ACUTE AND CHRONIC BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF MPTP IN C57 BLACK MICE. W.C.KOLLER, G.HERBSTER*, N.UNG, and M.COLLINS. Department of Neurology and Biochemistry, Loyola Medical Center, Chicago, IL 60153

MPTP causes in humans and monkeys behavioral, biochemical, and pathological changes similar to Parkinson's disease. A rodent model of MPTP-induced parkinsonism would provide an inexpensive, manageable test for potential therapeutic agents. We have investigated acute (5 to 45 mg/kg) and chronic (30 to 45 mg/kg, 1 to 5 days) dosage paradigms in young (3-4 wks) and old (11-12 months) C57 black mice. Locomotion was measured by an activity monitor. Striatal dopamine and its metabolites were determined by HPLC. Dose-dependent hypokinesia (up to 90% reduction) was observed from 0.5 to 2.0 hrs. after single injections, which was more pronounced in older animals. Pretreatment with pargyline (MAO inhibitor), amfenolic acid (dopamine reuptake inhibitor) but not methylsergide (serotonin inhibitor) or ascorbic acid blocked MPTP's acute effect. The chronic injection of MPTP produced no behavioral changes or hypokinesia at 1 to 30 days after administration. Lethal effects, particularly in the older animals, were common. Striatal dopamine and its metabolites were diminished by MPTP treatment. Dopamine was decreased bygreater than 95% in older animals with chronic injections.

MPTP induces an acute hypokinesia that is related to dopamine dysfunction but no permanent behavioral syndrome was produced despite marked reduction of striatal dopamine. The MPTP mouse model of Parkinson's disease may not be adequate for the testing of putative antiparkinsonian drugs.

327.1 CHANNELS FROM ISOLATED GAP JUNCTIONS INCORPORATED INTO PLANAR LIPID BILAYERS FOLLOWING SELECTION BY A TRANSPORT-SPECIFIC DENSITY SHIFT. A. Walter*, J. Zimmerberg*, D. Paul* and A.L. Harris. LTB, NCI & LCB, NIADDK, NIH, Bethesda, MD, Dept. Anatomy, Harvard Med. Sch., Boston, MA and Dept. Biophys., Johns Hopkins Univ. Baltimore, MD.

Study of the junctional channel will be facilitated by its incorporation into planar bilayers. We have (1) incorporated material from isolated gap junctions into vesicles, (2) applied a density shift technique to select vesicles containing large open channels and (3) incorporated those channels into planar bilayers. We showed in preliminary studies (Lynch, Harris & Paul, Biophys. J. 45:61a) that channels from isolated gap junctions can be reconstituted into lipid vesicles and fused into planar bilayers. To improve the rate of success, we modified the permeability dependent density shift technique of Goldin and Rhoden (J. Biol. Chem. 253:2575) to select for open channels of large size. We used a well-characterized channel, voltage dependent anion channel (VDAC), to develop the technique. Lipids with or without VDAC were solubilized in octyglucoside and a low ionic strength buffer containing 459 mM urea and 10 mM calcein (a fluorescent dye; mw 622). Vesicles were formed on a gel-filtration column. The vesicles were monodisperse with a mean diameter of 730 A. +VDAC and -VDAC vesicles were spun on iso-osmolar urea to sucrose density gradients. In theory, vesicles with open channels will exchange urea for sucrose and become more dense. In fact, a significant fraction of the +VDAC vesicles shifted to a higher density. This shifted +VDAC peak contained 80% less calcein/lipid than did the lighter +VDAC peak or the -VDAC peak. Because calcein loss and density shift are correlated, we conclude that the shifted vesicles contained open channels permeable to calcein, urea and sucrose.

Material from isolated rat liver gap junctions (Fallon and Goodenough, J. Cell Biol. 90:521) was solubilized and incorporated into vesicles, which were separated by the protocol outlined above. Approximately one-half of the vesicles shifted to a higher density, indicating permeability to urea and sucrose. When aliquots of shifted vesicle fractions were added to a bilayer chamber under osmotic conditions known to promote fusion with planar bilayers, large (100-500 pS) irreversible step-wise increases in bilayer conductance were seen, corresponding to fusion into the bilayer of vesicles containing several channels. Subsequently, smaller conductance fluctuations of reproducible magnitudes were seen, typical of transitions between conductance states of channels. Predominantly three sizes of conductance changes were observed. In symmetric solutions containing 182 mM KCl they were 20-30, 60-70 and 110-130 pS, each having different degrees of voltage sensitivity, asymmetry and kinetics. In several cases, membranes containing several channels were anion selective. Because single fusion events often led to the appearance of small and large conductance transitions in the bilayer, we suspect that not all of the observed changes are due to channels permeable to sucrose, as is the junctional channel. We are presently pursuing steps to fractionate the channels further in order to isolate, identify and study the junctional channel. Supported by NIH GM18974-15 to DAG and GM36044-01 to ALH.

327.2 DEVELOPMENT OF A PHOTOAFFINITY LABEL FOR THE ISOLATION OF A Ca^{++} CHANNEL. David C. Wood, Behavioral Neuroscience Program, University of Pittsburgh, Pittsburgh, PA 15260.

An inward receptor current (I_R) carried largely by Ca^{++} ions is elicited in the protozoan, Stentor coeruleus, in response to mechanical stimulation. These mechanoreceptor currents are increased by depolarizing the membrane from the -50 mV resting potential to -25 mV though this procedure reduces the driving force. Conversely hyperpolarizing the membrane reduces I_R. Thus the channel carrying I_R is voltage dependent (12.6 mV per e-fold change in conductance). This voltage dependency can be explained by assuming the unstimulated channel can exist in 2 forms: a form which predominates at hyperpolarized potentials and which cannot be opened by a mechanical stimulus (the U form) and a form which predominates at depolarized potentials and which can be opened by a mechanical stimulus (the R form) (Wood, D.C., J. Comp. Physiol. 146:537, 1982).

Tubocurarine (TC) blocks I_R in a dose dependent fashion at concentrations of 1-30 μM (ED_{50} = 7.5 μM). This blockade is highly correlated with the binding of radioactively labelled TC (TC*) to living cells (K_D = 22 μM). Additional correlations based on incubation time and specific drug affinity support the concept that TC and other drugs of similar structure bind to and block the mechanoreceptor transduction mechanism. The binding of TC* is reduced when the incubation is carried out in depolarizing K^+ solutions as is the blockade of I_R when a depolarizing voltage step is applied to the cell. These data indicate that TC binds primarily to the U form of the mechanoreceptor channel (Wood, D.C., J. Exp. Biol., 117:215, 1985).

Since TC binding is of relatively low affinity and reversible, TC* cannot be used as a label for isolation of the Ca^{++} channel. I have therefore synthesized the photoaffinity label bis (3 azidopyridinium)-1, 10 decane perchlorate (DAPA) which contains the bisquaternary ammonium structure which characterizes TC. DAPA, like TC, blocks I_R in a dose dependent fashion and with a K_D somewhat smaller than TC. Action potentials and other electrophysiological parameters are not changed significantly at these concentrations of DAPA. Cells are incubated briefly in radioactively labelled DAPA (DAPA*) and then exposed to UV irradiation to covalently bind the DAPA*. This binding is irreversible since it is not removed from the cells by mild extraction with 1% SDS. The saturation curve for DAPA* binding (K_D = 10 μM) is highly correlated with the blockade of I_R by DAPA. DAPA* binding can also be largely inhibited by the presence of TC in the incubation medium. Like TC binding, DAPA* binding is inhibited by depolarizing the cells in K^+ solutions. It therefore appears that DAPA* binds irreversibly to the mechanoreceptor Ca^{++} channel and can be used to isolate this channel.

327.3 PERMEABILITY OF THE Ca CHANNEL OF RAT SKELETAL MUSCLE TO MONOVALENT CATIONS IN THE ABSENCE OF EXTERNAL Ca. I.D. Uribe and R.F. Valdiosera*. Dept. of Physiology and Biophysics. CINVESTAV-IPN. Apdo. Postal 14-740, México, D.F. 07000.

Recently we have recorded Ca currents from a fast twitch skeletal muscle of the rat (omohyoid) using the vaseline gap voltage clamp. (I. Uribe & R. Valdiosera, Soc. Neurosci., Abstr., Vol. 11, part 1, p. 520, 1985). We found that when Cs was the main internal cation and with 2 mM Ca in the external solution, Ca currents were followed by an outward current that was reversibly reduced or abolished when the external Ca concentration was raised to 10 mM. Both currents were blocked when 2 mM Cd were added to the bath. In a similar way when Na was the main cation in the external solution and in the presence of 2 mM Ca, the currents were followed by a second inward current that was reversibly reduced or abolished when the external Ca concentration was raised to 10 mM. Again both currents were blocked by 2 mM Cd. We suggested that these findings could be explained by Ca depletion in the tubular system, lowering the Ca concentration to values low enough to make the Ca channel permeable to monovalent cations as has been shown to occur in frog muscle. (Almers, W., E.W. McCleskey and D.T. Palade, J. Physiol. 353:565, 1984). Here we report the results of experiments carried out to explore this possibility. Recordings were made at room temperature (22°C) using the vaseline gap voltage clamp. External solutions contained in mM; $TMACH_3SO_3$149, MOPS 5, Glucose 12 and TTX 1 μM, fiber ends were cut in intracellular medium containing TMA Asp 125, $(TMA)_2$EGTA 20, TMA-Citrate 0.125 and MOPS 5, pH was adjusted to 7.35 in both solutions. Under this conditions no currents were recorded in response to membrane depolarizations indicating that TMA was not permeant through the Ca channel in the absence of extracellular Ca, however, when Na substituted TMA, a large inward current was recorded. This current started at -55 mV, reached a maximum peak value of 1 mA/cm^2 at -40 mV in 20 msec, varied with the external Na concentration and was blocked when 10 μM nifedipine was added to the bath. Similarly, substituting Cs for TMA in the internal solution an outward current was recorded. This current was also blocked in 10 μM nifedipine. Similar experiments using Li or Rb as the main extracellular cation were done. In this case the experiments were difficult, the fibers deteriorated rapidly, especially with Li, however some experiments were done in the presence of 2 mM Ca to see if we could detect a second peak of inward current. The results of these experiments suggest that these cations are not permeant in this condition. No current were recorded when K substituted TMA and 40 mM TEA was also present in the internal solution indicating that in contrast to frog muscle, K is not permeant through the Ca channel in the absence of Ca. In conclusion, the Ca channel of rat skeletal muscle in the absence of external Ca is permeable to Na and Cs, and very little if any to Li Rb and K.

327.4 **A GTP-BINDING PROTEIN MEDIATES INHIBITION OF CALCIUM CHANNELS IN AtT-20 CELLS.** Deborah L. Lewis and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852

Somatostatin inhibits the voltage-dependent Ca^{2+} current, I_{Ca}, measured by the whole-cell patch-clamp technique, in the neurosecretory pituitary cell line AtT-20/D16-16. We tested the involvement of guanine nucleotide-binding proteins, G proteins, in the signal transduction mechanism between the somatostatin receptor and the voltage-gated Ca^{2+} channel.

The patch-clamp method was used to record the Ca^{2+} current in the whole-cell voltage-clamp mode. The extracellular solution contained: 150mM TEA-Cl, 0.8mM $MgCl_2$, 5.4mM KCl, 10mM $CaCl_2$, 10mM HEPES/CsOH (pH 7.4), and 1μM tetrodotoxin with an osmolarity of 340 mosmol kg^{-1}. The intracellular patch pipette solution contained: 120mM CsCl, 11mM EGTA, 2mM TEA-Cl, 2mM $MgCl_2$, 10mM HEPES/CsOH (pH 7.4), 4mM MgATP, 20mM creatine phosphate, and 50 U/ml creatine kinase with an osmolarity of 318 mosmol kg^{-1}. The nonhydrolyzable GTP analog, guanosine 5'-(3-*O-thio*)triphosphate, GTPγS was added to the patch pipette solution at a concentration of 100μM. Some patch pipettes had an additional 100μM cAMP and 1mM IBMX. Cells were voltage clamped to a holding potential of -80mV and stepped to +10mV, the peak of the current-voltage relationship for I_{Ca}. All recording was at room temperature, 20-22°C. [D-Trp^8]-somatostatin was dissolved in the external solution from a stock solution of 100μM and applied to via a macropipette lowered into the recording bath near the surface of the cell under study. The macropipette was withdrawn from the bath after a 60s period of somatostatin application.

Pertussis toxin treatment (100ng/ml, overnight), which blocks the function of the GTP-binding proteins G_i and G_o, completely abolished the action of somatostatin on I_{Ca}. Intracellular application of 100μM GTPγS via the patch pipette renendered irreversible the somatostatin-induced inhibition of I_{Ca}. In addition, GTPγS alone caused a slow decline in the Ca^{2+} current amplitude over 650s. When I_{Ca} was inhibited by GTPγS (alone or with somatostatin), there was little or no response of I_{Ca} to subsequent application of somatostatin. The affect of somatostatin on I_{Ca} was not altered by intracellular application of cAMP (100μM) and IBMX (1mM) alone or in the presence of GTPγS. The results suggest that the somatostatin receptor is coupled to a GTP-binding protein which mediates inhibition of voltage-dependent Ca^{2+} channels in a cAMP-independent manner.

327.5 PROTEIN KINASE C MODULATES DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONIST BINDING IN PC12 CELLS. R.O.Messing, C.L.Carpenter* and D.A.Greenberg. Ernest Gallo Clinic and Research Center, Department of Neurology, University of California, San Francisco, CA 94110.

Agents that activate protein kinase C (PKC) modify voltage-dependent Ca^{2+} currents in neural cells. We have shown that tumor-promoting phorbol esters, which activate PKC, inhibit depolarization-stimulated ^{45}Ca uptake in the murine neural cell line PC12. To investigate the biochemical basis for this action, we examined the effect of phorbol esters on binding of the dihydropyridine Ca^{2+} channel antagonist [^{3}H](+)PN200-110 to intact PC12 cells and PC12 membranes. Phorbol 12-myristate 13-acetate (PMA) reduced specific (nifedipine-sensitive) binding to intact K^+-depolarized cells with IC_{50} = 16 ± 5 nM and 40 ± 5% maximal inhibition at 1 uM, whereas the inactive compounds 4-alpha-PMA and phorbol had no effect at 100 uM. In contrast, 1 uM PMA inhibited specific binding of [^{3}H](+)PN200-110 to PC12 membranes by only 14 ± 1%. To determine whether the diminished effect of PMA on [^{3}H](+)PN200-110 binding to membranes was due to depletion of PKC, we measured binding to membranes reconstituted with a cytosolic fraction enriched in PKC activity. Cells were homogenized and centrifuged at 100,000 x g, and the supernatent was subjected to DEAE-cellulose (DE52) column chromatography. PKC was eluted with a 0-300 mM continuous NaCl gradient and appeared as a single peak between 30 and 75 mM NaCl. Incubation of this fraction with [gamma-^{32}P]ATP, $MgCl_2$, $CaCl_2$, phosphatidylserine, and 1,2 diolein stimulated ^{32}P incorporation into histone type III-S five-fold over control samples incubated without lipids. Reconstitution of PC12 membranes with PKC-enriched cytosol, $CaCl_2$, ATP, phosphatidylserine, and 1,2 diolein, reproduced the maximal inhibitory effect of PMA on [^{3}H](+)PN200-110 binding (44 ± 3% inhibition). The extent of inhibition was reduced to 4-13% when ATP, lipids, or Ca^{2+} was omitted, but when 1 uM PMA was substituted for diolein as activator, inhibition was maintained (38 ± 3%).

These results demonstrate that tumor-promoting phorbol esters reduce Ca^{2+} channel antagonist binding in PC12 cells. This effect is Ca^{2+}- and phospholipid-dependent, and appears to be mediated by PKC. Modulation of the Ca^{2+} channel antagonist binding domain by PKC may therefore be involved in endogenous regulation of voltage-dependent Ca^{2+} channels.

327.6 ENHANCEMENT OF VOLTAGE-DEPENDENT CALCIUM FLUX IN CULTURED PC12 CELLS BY THE LECTIN, CONCANAVALIN A. D. A. Greenberg, C. L. Carpenter*, and R. O. Messing. Department of Neurology and Gallo Research Center, University of California, San Francisco, CA 94110.

Changes in surface membrane properties that alter cell adhesiveness are thought to influence neuronal migration, cell-cell recognition, and synaptogenesis. Adhesion-promoting lectins such as concanavalin A (Con A) modify membrane ion fluxes and intracellular ion concentrations in several experimental systems, suggesting that changes in the properties of membrane ion channels may contribute to the development of neuronal connections. Because Ca2+ in particular has been implicated in cell adhesion, in the formation, maintenance, and modification of synapses, and in the functional consequences of lectin stimulation, we examined the effect of Con A on 45Ca2+ influx through voltage-dependent Ca2+ channels in PC12, a clonal cell line of neural crest origin.

To measure 45Ca2+ influx, PC12 cells grown in the absence of nerve growth factor were incubated for 2.5 minutes at 25oC in non-depolarizing (5 mM K+) or depolarizing (50 mM K+) buffer containing 45CaC12. Depolarization increased 45Ca2+ uptake approximately ten-fold. Pre-incubation with 3-100 ug/ml Con A for up to 25 minutes had no effect on uptake under non-depolarizing conditions, but enhanced depolarization-induced uptake in a time- and concentration-dependent fashion. The effect of Con A was maximal (125 % of control) after 5-10 minutes of pre-incubation, and at a concentration of 30 ug/ml. No additional stimulation of uptake was observed when Con A was also added during depolarization. In both the absence and presence of Con A, 45Ca2+ uptake increased rapidly during the first 2.5 to 5 minutes of depolarization and reached a plateau between 10 and 20 minutes. Con A-stimulated 45Ca2+ uptake was abolished by the ionic Ca2+ channel blockers La3+ and Cd2+ (2 mM), and by the organic Ca2+ channel antagonists, verapamil (100 uM) and diltiazem (1 mM), indicating that the voltage-dependent Ca2+ channel was the route of 45Ca2+ entry.

These findings suggest that modification of cell surface glycoconjugates alters the functional properties of voltage-dependent Ca2+ channels. While the sites that mediate this effect are unknown, evidence exists for a glycoprotein component of voltage-dependent Ca2+ channels, which might interact directly with exogenous lectins or with endogenous cell adhesion factors.

327.7 DIFFERENTIAL ACTION OF PENTOBARBITAL ON ISOLATED Ca CURRENTS IN MOUSE SENSORY NEURONS. R.A.Gross and R.L.Macdonald, Department of Neurology, University of Michigan, Ann Arbor, MI, 48104, U.S.A.

Barbiturates have been shown to inhibit neurotransmitter release from both central and peripheral neurons; this is thought to be secondary to a block of Ca entry into presynaptic terminals. It has been shown that barbiturates shorten Ca-dependent action potentials and reduce Ca currents in dorsal root ganglion (DRG) neurons in culture (Werz and Macdonald: Mol. Pharmacol. 28:269, 1985.). Recently, Ca currents in DRG neurons have been studied in detail, and three distinct currents have been described (Nowycky, Fox and Tsien: Nature 316:440, 1985.). T currents are small transient currents that activate and inactivate near the resting membrane potential (RMP); N and L are large currents activated positive to the RMP, and unlike T, are sensitive to cadmium ions (Cd). Like T, N is transient. However, it is not known which calcium currents are affected by barbiturates and which, by inference, may be involved in transmitter release. We report here a differential action of pentobarbital (PB) on T, N, and L currents.

Intracellular recordings were obtained with micropipettes filled with 3 M CsCl (20-30 MΩ) from mouse DRG neurons in culture. Single electrode voltage clamp was used to isolate Ca currents (switching frequency 5-6 kHz; 30-70 duty cycle). Recording medium, pH 7.3-7.4, contained in mM: Tris 50; choline-Cl 142; KCl or CsCl 5; TEA, 25; $CaCl_2$ 2-5; glucose 5.6; $MgCl_2$ 0.8. Pentobarbital was applied by pressure ejection from blunt-tipped micropipettes (2-5 μm), and cadmium by diffusion from larger (10-20 μm) micropipettes. T currents were evoked from a holding potential of -100 - -90 mV with a depolarizing step to -60 - -50 mV; T was a small (0.5 - 1.0 nA), transient (~20 msec) current which inactivated at >-60 mV. With 20-40 mV steps from -60mV, a large (2-10 nA), partially inactivating (asymptote at 100-300 msec) current was evoked; this was presumed to be N and L. At -20 - -40 mV, depolarizing steps produced a moderate (1-3 nA) non-inactivating L current. T was insensitive to 20 μM Cd, while N and L were abolished.

PB, 500 μM, did not alter the amplitude or duration of T currents. In contrast, N was blocked 15-50% and L currents 30-80%. Since T was inactivated at a holding potential of -60 mV, and was insensitive to PB, while N and L were present at this potential and were sensitive to PB, it is likely that the effect of PB on Ca-dependent action potentials and on transmitter release are due to a selective effect of PB on the N and L Ca currents.

Supported by NIH NS01019 to RAG, and NIH NS19692 to RLM.

327.8 INTERACTIONS OF THE ANESTHETICS PENTOBARBITAL AND ALPHAXALONE WITH CALCIUM CONDUCTANCE MECHANISMS IN CULTURED RAT HIPPOCAMPAL (HPC) AND DORSAL ROOT GANGLION (DRG) NEURONS. D.E.R.Meyers[1], N.L.Harrison* and J.L.Barker. Laboratory of Neurophysiology and [1]Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20892.

Pentobarbital (PB) has several actions on neuronal membranes which may underlie its anesthetic activity, including potentiation and direct activation of GABA-activated Cl^- conductance (g_{Cl}) and a reduction of voltage-dependent calcium conductance (Werz and Macdonald, Mol. Pharmacol. 28: 269, 1985). The steroid anesthetic alphaxalone (AX) shares the actions of PB on GABA-activated g_{Cl} at anesthetic concentrations (1-10μM) in cultured CNS neurons. We have therefore studied the interactions of these two anesthetics with calcium conductance mechanisms in cultured neurons using the whole-cell recording technique. Patch pipettes contained (mM): 145 K gluconate or CsCl, 5 EGTA/0 Ca; the bathing medium contained 1uM TTX and 20mM TEA. In cultured rat HPC neurons, 1-10μM AX abolished Ca-dependent action potentials (Ca-APs) and produced a large increase in membrane conductance (g_M); these actions were reversed by addition of the $GABA_A$ antagonist bicuculline (100μM). In cultured rat DRG neurons, which lack functional $GABA_A$ receptors, AX was without effect on Ca-APs or Co^{2+}-sensitive inward currents recorded under voltage-clamp. In contrast, 200μM PB reduced the duration of Ca-APs in DRG cells and reduced the magnitude of the peak Ca current without increasing resting g_M. We conclude that the action of AX on Ca-APs in HPC neurons is secondary to an increase in g_{Cl}, and that the anesthetic action of this steroid primarily results from 'shunting' of neuronal membranes (both post- and presynaptic) which express functional $GABA_A$ receptors.

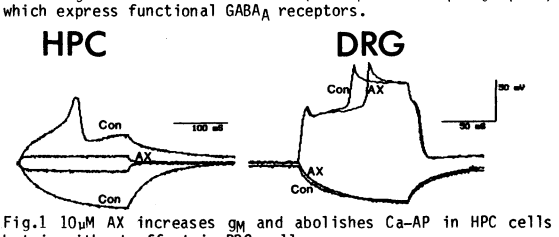

Fig.1 10μM AX increases g_M and abolishes Ca-AP in HPC cells but is without effect in DRG cells.

327.9 PHENYTOIN BLOCK OF LOW THRESHOLD CALCIUM CHANNELS IS VOLTAGE- AND FREQUENCY-DEPENDENT. D.A. Twombly* and T. Narahashi (SPON: D.L. Colbern). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The anticonvulsant properties of phenytoin (diphenylhydantoin, DPH) have long been suspected to derive, at least in part, from an inhibition of calcium flux through neuronal membranes. Recently, we reported that phenytoin suppresses low-threshold, inactivating ("type I") calcium channel currents in N1E-115 neuroblastoma; the non-inactivating ("type II") current component of these cells was insensitive to phenytoin concentrations within, and somewhat above the therapeutic range (Twombly & Narahashi, Soc. Neurosci. Abstr. 11, 1985). The objective of the present study was to determine whether phenytoin exerts its blocking action by modifying inactivation parameters of type I calcium channels.

Calcium channel recordings were obtained from cultured N1E-115 neuroblastoma cells with the whole-cell voltage clamp technique. Barium (50 mM) was included in the external medium to serve as charge carrier through calcium channels. Conditioning and test pulses were applied from a holding potential of -80 mV. Under control conditions (standard external solution, 22-23°C), type I currents were activated at potentials positive to -40 mV, with maximum amplitudes occurring at -20 mV. Phenytoin concentrations higher than 10^{-5} M significantly reduced the amplitudes of type I currents, without changing current-voltage relationships or the kinetics of activation and inactivation. To examine the effect of phenytoin on voltage dependent inactivation, -20 mV test pulses were preceded by five second conditioning pulses to various membrane potentials. In drug-free external media, type I current responses decreased in amplitude as the conditioning membrane potential became less negative. Phenytoin shifted the voltage dependence of this steady-state inactivation in the hyperpolarizing direction, allowing fewer channels to open during test pulses. Frequency dependence of type I currents was tested by applying a series of -20 mV pulses from a holding potential of -80 mV. At stimulation frequencies higher than 0.5 Hz, current amplitudes diminished with successive test pulses. This frequency-dependent reduction in current was even more pronounced in the presence of phenytoin. The "use-dependent block" by phenytoin was correlated with prolonged recovery from inactivation.

Voltage- and frequency-dependent blocking actions suggest that phenytoin can interfere with type I calcium currents by favoring the inactivated state of the channel. Block of these low threshold calcium channels by phenytoin could suppress a significant portion of the inward current underlying repetitive depolarization shifts. Such an effect may diminish opportunities for cellular synchronization, thereby limiting the spread of seizure activity. (Supported by the American Epilepsy Society and NIH grant NS14144)

327.10 EFFECT OF A CALCIUM SLOW CHANNEL INHIBITOR (NIMODIPINE) ON PICROTOXIN-INDUCED SEIZURES. R. L. Isaacson & J. Thomas (SPON: J. H. McLean). Dept. of Psychology, University Center at Binghamton, Binghamton, New York 13901.

Increases in the concentration of intracellular calcium have been implicated in the induction of seizures (Schartzkroin & Wyler, Ann. Neurol., 7:95, 1980). A clear understanding of the relationship between intracellular calcium levels and the induction of seizures is complicated, however, by the fact that in vitro studies have shown that some anticonvulsant as well as convulsant drugs inhibit calcium uptake (Pincus & Hsiao, Brain Res., 217:119, 1981). These findings indicate that there is a complex relationship between intra- and extracellular calcium levels, a general neuronal excitability, and the activity of specific brain regions that may mediate certain forms of seizures.

This study investigated the effects of the calcium slow channel inhibitor, nimodipine (NIM), on the pattern of seizures induced by picrotoxin in vivo. Four distinctive patterns of behavioral seizures were induced by s.c. administration of picrotoxin: myoclonic, "partial", "atonic", and generalized. It is likely that each reflects a different form of neurochemical mediation. Therefore changes in calcium influx could be involved in some types of seizures but not in others.

Male Long-Evans hooded rats were pretreated with 0, 5, 15, 25, or 50 mg/kg of NIM and 2 hr later given 4.0, 4.5, or 5.0 mg/kg picrotoxin and observed over a 60 minute period for behavioral signs of the various seizures.

None of the NIM doses had any effect on the incidence of myoclonic, "partial", or "atonic" seizures. The effect of NIM on the picrotoxin-induced generalized seizures varied as a function of the doses of picrotoxin and NIM used. In animals treated with 4 mg/kg of picrotoxin, pre-treatment with NIM increased the incidence of full seizures at the 5 mg/kg dose but reduced the incidence at doses of 15 mg/kg and higher. In animals treated with 4.5 mg/kg of picrotoxin, NIM had no effect on the incidence of full seizures at the 5 mg/kg dose but reduced their occurrence at the 15 and 25 mg/kg doses and increased their occurrence at the 50 mg/kg dose. Pre-treatment with NIM had no effect on the incidence of generalized seizures in animals treated with 5 mg/kg of picrotoxin.

These results suggest that there may be a biphasic relationship between inhibition of calcium entry and the antagonism of generalized seizures that is related to the level of neuronal activation.

327.11 CLONAZEPAM DIFFERS FROM DIAZEPAM AND NITRAZEPAM IN BLOCKING TWO TYPES OF CALCIUM CHANNELS. S. Watabe*, M. Yoshii*, N. Ogata* and T. Narahashi. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Recent action potential study suggests that benzodiazepines block calcium channels in neurons (Johansen et al., PNAS 82, 3935, 1985). However, the mechanism by which voltage-gated calcium channels are affected by benzodiazepines remains to be seen. We have examined the effects of benzodiazepines on calcium channels in mouse neuroblastoma (N1E-115) cells. Diazepam, nitrazepam and clonazepam were selected for the study: diazepam is a sedative as well as an antiepileptic; nitrazepam is a hypnotic; clonazepam is an antiepileptic. Calcium channel currents as carried by Ba^{2+} (50 mM) were recorded using a whole-cell variation of the patch-clamp technique.

Two types of inward Ba^{2+} current could be recorded from the same cell. A transient (type I) current was induced by a step depolarization to potentials more positive than -50 mV. A long-lasting (type II) current was induced at potentials more positive than -20 mV. The effects of diazepam, nitrazepam and clonazepam on the two channel types were compared. Diazepam at 100 μM reduced both types of current equally by about 30%. Nitrazepam showed a similar but slightly less potent blocking action on the two channel types. On the contrary, clonazepam (100 μM) reduced type I current by more than 50% without much affecting type II current. The suppression of type I current by diazepam, nitrazepam or clonazepam was accompanied by an acceleration of the falling phase of the current. Type II current in the presence of diazepam or nitrazepam showed a time-dependent decrease. The benzodiazepines caused no significant change in the voltage dependence of the steady-state inactivation of the type I channel. No use dependence was found in the type I channel block caused by diazepam (1 mM).

We conclude that benzodiazepines block voltage-gated calcium channels and that their blocking potency varies depending on the type of channels and the kind of drugs. Diazepam and nitrazepam do not exert differential actions on the two channel types, whereas clonazepam preferentially blocks type I channel. These differences in the action on the calcium channels might reflect different therapeutic profiles of these drugs. Supported by NIH grant NS14144.

327.12 BLOCKING ACTION OF CHLORPROMAZINE ON TWO TYPES OF CALCIUM CHANNELS IN NEUROBLASTOMA CELLS. N. Ogata*, M. Yoshii* and T. Narahashi (SPON: C.A. Berry). Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Biochemical studies have suggested that a certain class of neuroleptics affects calcium channels in nerve and muscle membranes. However, little is known as to how voltage-sensitive calcium channels are modified by the neuroleptics. We have investigated the effect of chlorpromazine (CPZ), a phenothiazine neuroleptic, on calcium channels in cultured mouse neuroblastoma (N1E-115) cells. Calcium channel currents as carried by Ba^{2+} (50 mM) were recorded using a whole-cell variation of the patch-clamp technique.

We have previously shown that neuroblastoma cells have two types of calcium channels. A transient (type I) current was induced at potentials positive to -50 mV, and a long-lasting (type II) current was induced at potentials positive to -20 mV. The amplitudes of the two types of current were equally reduced by 20% in the presence of 10 μM CPZ. Both currents were totally abolished by 0.5-1.0 mM CPZ, and recovered completely after washing out of the drug. There was no change in the time course or voltage dependence of either type of these currents. Apparent dissociation constants for CPZ block were 20-30 μM for both types of calcium channels. Type I currents recorded by a double pulse protocol in the presence of CPZ showed a significant delay in the time course of recovery from inactivation: the recovery time constant measured at -80 mV was increased approximately five times by 100 μM CPZ. CPZ (50 μM) also caused a shift in the potential dependence of the steady-state inactivation of type I channel in the negative direction by approximately 10 mV. These data indicate that CPZ reduces the backward rate constant of the inactivation process without affecting the forward rate constant. Since type II current lacked fast inactivation, such analyses on type II channels were not performed. No indication of the use dependence was obtained in either type of channels.

Our results suggest that CPZ blocks both types of calcium channels in their resting state. Interaction with the inactivation gate of the channel is also suggested. The CPZ-induced shift of the steady-state inactivation-voltage relationship in the hyperpolarizing direction would significantly modulate neuronal function, because a slight depolarization of the resting membrane would result in a sizable reduction in the calcium channel current. Supported by NIH grant NS14144.

327.13 SEROTONIN AND DOPAMINE REGULATE THE CALCIUM CURRENT OF THE IDENTIFIED APLYSIA NEURON R15. D.P. Lotshaw and I.B. Levitan. Dept. of Biochemistry, Brandeis Univ., Waltham, MA 02254

The identified Aplysia neuron R15 expresses an endogenous bursting pacemaker pattern of action potentials which is dependent on an oscillating calcium current. Synaptic stimulation modulates bursting activity in R15 and the neurotransmitters serotonin (5-HT) and dopamine mimic some of the effects of synaptic stimulation. We have used voltage clamp techniques to investigate effects of 5-HT and dopamine on the voltage dependent calcium current in isolated R15 neurons maintained in primary cell culture.

R15 was identified in the abdominal ganglion by its relative size, pigmentation and position. The somal diameter of cells successfully cultured (based on the ability to extend neurities) ranged from 75 to 150 microns. These cells extended neurites within 1 to 3 days after isolation but did not exhibit spontaneous bursting activity, although bursting activity could be induced by neurotransmitter treatment.

The voltage dependent calcium current was isolated by iontophoretically loading the cell with Cs^+, using Cs^+ acetate electrodes, substituting Tris for external Na^+ or adding 50 μM TTX to block Na^+ current, and adding 50 mM TEA and 5 mM 4-AP to the bath. Depolarizing 0.5 sec pulses from V_H=-60 mV induced an inward current proportional to the external calcium concentration. Peak inward current occurred between +10 and +20 mV membrane potential. 5 μM 5-HT reversibly increased the peak calcium current an average of 20% within 2 min after the onset of perfusion. This effect was mimicked by perfusion with 50 μM forskolin plus 15 μM Ro20-1724, an adenylate cyclase activator and phosphodiesterase inhibitor, respectively. These treatments primarily affected the inactivating component of the calcium current evoked at membrane potentials above -25 mV. The non-inactivating component present at membrane potentials less than -25 mV was also significantly increased. Treatment with 10 μM dopamine reversibly decreased peak calcium current approximately 20%, primarily affecting the inactivating component present at membrane potentials greater than -25 mV but also affecting calcium current present below -25 mV.

The calcium current regulates bursting activity in R15. Serotonin, previously shown to regulate an inwardly rectifying K^+ current, also stimulates the calcium current and spike activity in R15. Dopamine inhibits spiking and the calcium current in R15. Dopamine, like 5-HT, appears to affect other ionic currents as well. (This work supported by NSF grant BNS-8400875 to I.B. Levitan. D.P. Lotshaw was supported by a research fellowship from the American Heart Assoc., Massachusetts Affiliate, Inc.).

327.14 MODULATION OF APLYSIA BAG CELL NEURON EXCITABILITY BY SYNTHETIC DIACYLGLYCEROLS. K.R. Bley and L.K. Kaczmarek. Depts. Physiology and Pharmacology, Yale Medical School, New Haven, CT 06510.

In response to brief stimulation of an afferent pathway, the ordinarily silent bag cell neurons of Aplysia depolarize and fire repetitive action potentials for 20 to 60 minutes. The action potentials seen during this discharge have enhanced height and width relative to those which can be elicited in resting cells. Injection of purified protein kinase C (PKC) into isolated cultured bag cell neurons produces similar increases in action potential height. At concentrations which activate PKC in broken cell preparations, application of a phorbol ester (10-100 nM TPA) to these cells enhances voltage-sensitive Ca current. The basis of this enhancement is the unmasking of a previously covert species of voltage-activated Ca channel (J.A. Strong et al., Biophys. J. 49:430a).

We find that membrane-permeant synthetic diacylglycerols mimick the effect of TPA. At concentrations of 1 uM, 1,2-dioctanoylglycerol (DOG) and 1-myristeroyl-2-acetylglycerol (MAG) also enhance the amplitude of evoked action potentials. In contrast, 1-stearoyl-2-arachidonylglycerol (SAG), an impermeant product of inositol phospholipid hydrolysis, has no effect at this concentration. Large doses ($\leq$ 50 uM) of the inactive analogs 4-α-phorbol, 4-α-phorbol-12,13-didecanoate (4-α-PDD), as well as arachidonic, linolenic and linoleic acid, do not induce this effect.

We have also found that higher doses of TPA and membrane-permeant synthetic diacylglycerols have an additional effect that is not observed with the inactive analogs. At concentrations of TPA $\geq$ 1uM, or DOG and MAG $\geq$ 10uM, enhancement of depolarization-evoked Ca-dependent action potentials is followed by membrane potential oscillations and then by an extended period (20-70 min) of repetitive action potentials. The onset of repetitive firing caused by higher doses of TPA and DOG is associated with an increase in neuronal input resistance, not observed with 10-100 nM TPA. The time course of this increase parallels the latency of spike enhancement, yet occurs in both normal and calcium-free media. Thus it appears likely that additional currents in the bag cell neurons are affected by higher concentrations of PKC activators. Exogenous lipophilic compounds could produce several types of non-PKC specific effects. Our control experiments with inactive analogues suggest that a generalized lipid effect is unlikely. However, stimulation of other enzymes or a non-physiological hyperactivation of PKC are possibilities which require further analysis.

327.15 TRANSMITTER RELEASE FROM ELECTIC ORGAN NERVE TERMINALS: EFFECTS OF OMEGA CONUS TOXIN AND LEPTINOTARSIN. R.E. Yeager*, D. Yoshikami+, G.P. Miljanich* (SPON: L. Byerly). Biological Sciences, USC, Los Angeles, CA, 90089 and +Biology, U. of Utah, Salt Lake City, UT 84112.

Cholinergic synaptosomes from O. discopyge electroplax release ATP in a Ca^{2+}-dependent manner upon depolarization with high $[K^+]_o$. Others have shown that ATP and ACh are released from electroplax synaptosomes in the same molar ratio as in isolated synaptic vesicles from the same tissue, suggesting that ATP release reflects exocytosis of vesicle contents. Sr^{2+} and Ba^{2+} support depolarization-dependent ATP release when substituted for Ca^{2+}. Co^{2+} and Mn^{2+} block depolarization-dependent ATP release in the presence of Ca^{2+}. However, in the presence of the ionophore, A23187, 0.5 mM Mn^{2+} supports depolarization-independent ATP release to the same extent as 0.5 mM Ca^{2+}. While trifluoperazine (TFP) inhibited K^+-evoked ATP release by 65%, blockade of release by TFP was circumvented by A23187 in the absence of depolarization. Failure of TFP to block ATP release in the presence of A23187 suggests that the drug acts by blocking Ca^{2+} channels rather than by inhibiting a calmodulin-dependent process subsequent to divalent cation entry. Nitrendipine had no effect on ATP release.

The peptide shellfish toxins, omega Conus magus toxin (CmTX) and C. geographus toxin (CgTX), maximally inhibit K^+-evoked ATP release in the presence of Ca^{2+}, Sr^{2+}, or Ba^{2+} by 80%. Half-maximal inhibition of ATP release by CmTX and CgTX is reversible, is approximately first order, and is one-half complete in 40 min for CmTX and 15 min for CgTX. A23187 defeats blockade of Ca^{2+}-dependent ATP release by CmTX, consistent with direct blockade of Ca^{2+} channels by CmTX. Both spontaneous and K^+-evoked influx of $^{45}Ca^{2+}$ into the synaptosomes are inhibited by CmTX as well as Co^{2+}. We conclude that CmTX and CgTX inhibit ATP release by blocking Ca^{2+} flux through presynaptic, voltage-sensitive Ca^{2+} channels of the electric ray electroplax, and that these toxins are potentially useful for identifying and purifying Ca^{2+} channels from this tissue.

Leptinotarsin-d (LPTd), a putative Ca^{2+} channel agonist from potato beetles, causes Ca^{2+}-dependent ATP release from electroplax synaptosomes in the absence of depolarization. CmTX cannot block ATP release evoked by LPTd, suggesting that the two toxins act at distinct sites on one or more Ca^{2+} channels. ATP release by LPTd is saturable; maximal ATP released by saturating levels of partially purified LPTd was more than twice the amount of ATP released by depolarization with 140 mM K^+. TFP inhibited LPTd-evoked release in 1 mM Ca^{2+} by 85%. Mn^{2+} (5 mM) and Co^{2+} (5 mM) inhibited LPTd-evoked ATP release in 1 mM Ca^{2+} by 25% and 75%, respectively. Predepolarization of synaptosomes did not affect the extent of ATP release evoked by LPTd beyond the amount released by the K^+ itself. These data suggest that LPTd acts by opening presynaptic Ca^{2+} channels in nerve terminals from electric ray electric organ.

We thank T. Hsiao for leptinotarsin hemolymph and B. Olivera for Conus toxins.

327.16 STIMULATION OF CALCIUM CURRENTS AND ACTH SECRETION IN CLONAL PITUITARY CORTICOTROPHS BY BAY-K-8644 AND ITS ENANTIOMERS. S. Heisler*, Z. Miljkovic* and J.F. MacDonald (SPON: P. Pennefather). Bioregulation Cell. and Playfair Unit, Centre hospitalier de l'Universite Laval and Toronto Western Hosp., Sainte-Foy, PQ. G1V 4G2 and Toronto, Ont. M5T 2S8

The 1,4-dihydropyridine, BAY-K-8644, both stimulates and antagonizes the spontaneous secretion of immunoreactive ACTH from mouse clonal pituitary corticotrophs, AtT-20/D16-16 (AtT-20) Although BAY-K-8644 is known to facilitate inward calcium current in a number of different cell types, a similar effect these secretory cells has been assumed, but not demonstrated.

Whole cell voltage-clamp (discontinuous switching, 10 to 16 kHz) was performed upon cells at 60 to 70% confluency. Voltage steps (50 ms) from hyperpolarized holding potentials were used to evoke sustained inward calcium currents. BAY-K-8644 and its enantiomers BAY(+)R4407 and BAY(-)R5417 were applied by pressure to individual cells. BAY-K-8644 (<1 uM) reversibly potentiated calcium currents by shifting the current-voltage curves to the left and by increasing peak inward calcium currents. Higher concentrations (100 uM) depressed such currents. R4407 in in all concentrations tested caused a slight depression of peak calcium current while R5417 caused a potentiation similar to the racemer. Depression of potentiated currents (R5417) resulted from co-applications of both enantiomers. R5417 evoked a concentration-dependent increase in ACTH secretion. However, concentrations of 10 uM or more were less effective (a down turn in the concentration-response curve). In contrast, R4407 inhibited basal secretion of ACTH, regardless of concentration, and antagonized the stimulation of secretion by the other enantiomer.

The (-) enantiomer of BAY-K-8644 is responsible for its potentiation of calcium currents and stimulation of ACTH secretion in AtT-20 cells. On the other hand, a major portion of its antagonistic activity must orginate from the (+) enantiomer which appears to be an soley an antagonist. These results suggest that BAY-K-8644 actions on other secretory cells are also dependent upon the opposing effects of its enantiomers. (Support: Quebec Ministry of Education & the MRC)

327.17 PERTUSSIS TOXIN-SENSITIVE GTP-BINDING PROTEINS COUPLE ALPHA-2 AND GABA-B RECEPTORS TO INHIBITION OF NEUROSECRETION IN DORSAL ROOT GANGLION CELLS. G.G. Holz, K. Dunlap and R.M. Kream.* Depts. Physiol. and Anesthesia Res., Tufts Med. Sch. Boston, MA. 02111.

Norepinephrine (NE) and gamma aminobutyric acid (GABA) inhibit current flow through voltage-sensitive L-type Ca channels in chick dorsal root ganglion (DRG) neurons. This inhibition of Ca influx may explain how NE and GABA inhibit the release of substance P (SP) from these same cells (see abstract by Dunlap, Kream and Holz). To test this hypothesis, we investigated the involvement of GTP-binding proteins (G-proteins) in the action of the transmitters. It was previously reported that pertussis toxin (PTX, a toxin catalyzing ADP-ribosylation of G-proteins) blocks inhibition of DRG cell Ca channels by NE and GABA (Holz et al., Nature 319: 670). We now report that PTX also blocks the ability of NE and GABA to inhibit release of SP from DRG cells.

Primary cultures of embryonic chick DRG cells were grown on collagen-coated tissue culture dishes at a density of ca. 175,000 neurons/60 mm dish. After 9-16 days in culture, each dish contained 13,069 ± 653 pg (mean ± s.e.m., n=9) of substance P (as measured by RIA and authenticated by HPLC). Electrical field stimulation (1 Hz, 90 sec) of cultures bathed in saline containing (in mM) 132 NaCl, 2.5 KCl, 1.0 $BaCl_2$ and 2.0 $CaCl_2$ resulted in the Ca-dependent release of 451 ± 22 pg SP/dish (n=9). Each culture was stimulated 3 times: during S_1 and S_3 the saline contained no drugs, whereas during S_2 the cultures were exposed to either 50 uM d,l-arterenol (NE) or 10 uM GABA. By calculating the Release Ratio $S_2/[(S_1+S_3)/2]$ we compared SP release in different test solutions. Toxin-treated cultures were preincubated at 37 °C in 140 ng/ml PTX for 16-20 hr. Representative results are listed:

	Release Ratio (No PTX)	Release Ratio (PTX)
Control	1.06 ± 0.09 (n=3)	0.88 ± 0.06 (n=3)
50 uM NE	0.55 ± 0.06 (n=3)*	0.77 ± 0.11 (n=3)
10 uM GABA	0.64 ± 0.09 (n=3)*	0.92 ± 0.09 (n=3)

In cultures not treated with PTX, release was significantly inhibited by NE and GABA (* indicates the Ratio was significantly different from control, p 0.05), whereas in PTX-treated plates, no significant inhibition of release was observed. Although the identity of the G-proteins coupled to these NE and GABA receptors remains to be determined, the action of PTX was correlated with ADP-ribosylation of 39 and 41 kD substrates that co-migrate on polyacrylamide gels with alpha subunits of N_o and N_i G-proteins, respectively (the authors thank Eva Neer for her help). These findings suggest that NE and GABA inhibit exocytosis from DRG cells by modulating current flow through L-type Ca channels.

327.18 ALPHA-2 ADRENERGIC AND GABA-B RECEPTORS MEDIATE TRANSMITTER INHIBITION OF NEUROPEPTIDE SECRETION FROM DORSAL ROOT GANGLION CELLS. K. Dunlap, R.M. Kream* and G.G. Holz. Depts. Physiol. and Anesthesia Res., Tufts Med. Sch., Boston, MA 02111.

Norepinephrine (NE) and gamma aminobutyric acid (GABA) inhibit current flow through voltage-sensitive L-type Ca channels in cell bodies of chick dorsal root ganglion (DRG) neurons. This inhibition of depolarization-induced Ca influx results from the activation of alpha-2 adrenergic and GABA-B receptors. We now demonstrate that the Ca-dependent release of substance P (SP) from DRG cells is also inhibited by activation of alpha-2 and GABA-B receptors. These findings suggest that NE and GABA presynaptically inhibit primary afferent transmission by suppressing Ca influx through L-type Ca channels in sensory nerve terminals.

Chick DRG cell cultures were prepared for electrical field stimulation as described in the accompanying abstract (Holz, Kream and Dunlap). Each culture was stimulated 3 times: during S_1 and S_3 the bathing solution contained no drugs, whereas during S_2 drugs were added. By calculating the Release Ratio $S_2/[(S_1+S_3)/2]$ we compared SP release in different test solutions. Inhibition was calculated as: [1-(Test Ratio/Control Ratio)] x 100%. Drugs tested were (in uM): 50 d,l-arterenol (NE); 10 yohimbine (YOH, an alpha-2 antagonist); 10 prazosin (PRAZ, an alpha-1 antagonist); 10 propranolol (PROP, a beta blocker); 10 GABA; 10 baclofen (LIORESAL, a GABA-B agonist); 10 muscimol (MUSC, a GABA-A agonist); 50 bicuculline (BIC, a GABA-A antagonist). Representative results are summarized below. (* indicates the Ratio was significantly different from control; p 0.05; t-test).

Treatment	Release Ratio (+/- s.e.m)	% Inhibition
Control	0.97±0.11 (n=3)	---
NE	0.62±0.01 (n=3)*	36%
NE + YOH	0.90±0.03 (n=3)	7%
NE + PRAZ	0.63±0.02 (n=3)*	35%
NE + PROP	0.67±0.04 (n=3)*	31%
Control	0.93±0.02 (n=3)	---
GABA	0.65±0.03 (n=3)*	30%
LIORESAL	0.53±0.06 (n=3)*	43%
MUSC	0.84±0.10 (n=3)	10%
GABA + BIC	0.66±0.02 (n=3)*	29%

This analysis demonstrates that NE and GABA inhibit the release of SP by activating receptors similar, if not identical, to those responsible for inhibition of L-type Ca channels: i.e. both inhibitory processes are mediated by alpha-2 adrenergic and GABA-B receptors. Supported by NIH grant NS16483 to K.D.

327.19 REGULATION OF SUBSTANCE P RELEASE FROM CULTURED RAT SENSORY NEURONES. T.M. Perney*, D.A. Ewald and R.J. Miller. Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

We examined the regulation of substance P (SP) release from dorsal root ganglion (DRG) neurones taken from 2 day old rat pups and cultured for two weeks. Release of SP could be evoked by K^+-rich solutions, capsaicin, bradykinin (BK), Ba^{2+} and A23187. K^+ evoked release could be blocked by Cd^{2+} or in Ca^{2+} free solutions. GABA and norepinephrine also partially abolished release. SP release was extremely sensitive to inhibition by dihydropyridine (DHP) Ca^{2+} channel antagonists such as nitrendipine, being completely inhibited at 10^{-6}M. Ca^{2+} channel agonists enhanced the K^+ induced release of SP. In contrast, SP release induced by capsaicin was completely resistant to both Ca^{2+} channel agonists and antagonists. We performed studies on neurones that were cultured for various periods of time ranging from 1 to 60 days. Release of SP could be evoked by K^+ from neurones of all ages. Moreover, at all times evoked SP release was enhanced to approximately the same extent by BAY K8644 and completely blocked by nitrendipine (10^{-6}M). We also investigated the effect of culturing DRG cells with their target neurones. Co-cultures of DRG and spinal neurones were grown for a period of two weeks. However, even under these conditions the DHP sensitivity of SP release remained unaltered.

In order to further explore the properties of SP release from adult neurones, we have started to examined the K^+ evoked release of SP from slices of rat spinal cord. Perfusion of such slices in medium containing 70mM K^+ stimulated the release of SP greatly. Interestingly, preliminary studies using this system indicate that the K^+ induced release of SP is much less sensitive to DHP's than release evoked from cultured neurones. Whole cell voltage clamp studies on rat DRG neurones revealed that they appear to exhibit 3 components to their Ca^{2+} current. If cells are held at -80mV, small depolarizing voltage steps produce a transient Ca^{2+} current in some but not all cells. Larger depolarizing steps produce a second component of inactivating current as well as a large component which shows little inactivation. This pattern is virtually identical to that reported previously by Nowycky et al. (Nature, 316, 440, 1985) for chick DRG neurones. One difference however concerns the effect of the 27 amino acid peptide ω-conotoxin GIVA (ω-CGTx). This toxin blocks the two high threshold components of the Ca^{2+} current in chick DRG neurones (McCleskey et al., Biophys. J., 49, 431a, 1986). However in concentrations of up to 10^{-8}M the toxin had no inhibitory effect on K^+ evoked SP release from cultured rat DRG neurones. Preliminary studies also reveal no effect of toxin in concentrations up to 10^{-6}M on Ca^{2+} currents measured in these same cells. We have also observed that stimulated Ca^{2+} uptake into single DRG neurones can be measured using the Fura-2 ratio technique (Thayer et al., this volume. The properties of this influx are currently under investigation.

327.20 **DIHYDROPYRIDINE DRUGS AND cAMP APPEAR NOT TO AFFECT THE K^+ STIMULATED Ca^{++} INFLUX IN RAT SYNAPTOSOMES.** **M.E. O'Leary* and J.B. Suszkiw (SPON: S.H. Bryant). Dept. of Physiol. and Biophys., Univ. of Cinti., Coll. of Med., Cincinnati, OH 45267-0576**

The dihydropyridine-sensitive, voltage-dependent calcium channels in cardiac muscle appear to be modulated by cAMP dependent protein phosphorylation (Sperelakis, Membr. Biochem. 5, 1984). Recent evidence indicates that the nitrendipine receptor of calcium channels is a substrate for cAMP dependent protein kinase (Curtis and Catterall, PNAS 82, 1985). These results suggest that the regulation of the calcium channels by phosphorylation/dephosphorylation may be exerted via the dihydropyridine receptor of the channel. Both dihydropyridine sensitive and insensitive Ca^{++} channels have been detected in neurons (Nowycky et al., Nature, 316 1985), however it is not certain which type(s) of Ca^{++} channels are present in nerve terminals. The aim of this study was to determine if channel mediated Ca^{++} influx in synaptosomes is modified by dihydropyridines or cAMP. The Ca^{++} channel agonist, Bay K 8644 (0.01-10 μM) failed to enhance depolarization stimulated calcium uptake (20 mM K^+, 5 sec). The Ca^{++} channel antagonists, nifedipine and nitrendipine (0.1-10 μM), did not significantly reduce depolarization stimulated Ca^{++} uptake (52.5 mM K^+, 1 sec). Preincubation of synaptosomes with 1 mM 8-bromo cAMP or 50 μM forskolin did not significantly alter K^+-depolarization stimulated calcium uptake from control values (20 mM K^+, 5 sec). Under identical preincubation conditions forskolin, although not 8-Br-cAMP, caused detectable increases in ^{32}P incorporation into a number of synaptosomal proteins. These results suggest that synaptosomal calcium channels are not sensitive to dihydropyridine drugs and may not be regulated by cAMP dependent phosphorylation. Supported by NIH Grant NS 20786.

327.21 CHARACTERIZATION OF DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNELS IN STRIATAL SYNAPTOSOMES. J.J. Woodward* and S.W. Leslie (Spon: W.H. Riffee). Div. of Pharmacology and Toxicology, College of Pharmacy, Univ. of Texas at Austin, Austin, TX 78712.

Previous studies have demonstrated the existence of high-affinity binding sites for the dihydropyridine calcium channel antagonists in cardiovascular tissue, skeletal muscle, and brain. Brain calcium channels appear to be insensitive to the potent blocking action of the dihydropyridines observed in other tissues. However, it has recently been reported that the novel dihydropyridine, Bay K 8644, which possesses calcium agonist activity, can activate brain calcium channels under certain conditions (Woodward and Leslie, Brain Res. 370, 397, 1986). This activation was completely blocked by low nanomolar concentrations of the calcium antagonist nimodipine. The present study examined the sensitivity of brain calcium channels to dihydropyridine calcium agonists under various experimental conditions to more fully characterize the dihydropyridine sensitivity of these channels. Synaptosomes were isolated from the striata of male, Sprague-Dawley rats. Calcium uptake and endogenous dopamine release were measured as previously described (Leslie et al., Brain Res. 325, 99, 1985). Bay K 8644 (1 nM) potentiated synaptosomal calcium uptake and dopamine release only at 7.5 mM and 15 mM KCl concentrations. Calcium uptake and dopamine release were not altered by Bay K 8644 under resting conditions (5 mM KCl) or in response to KCl concentrations greater than 15 mM. Bay K 8644 (1 nM) also enhanced synaptosomal calcium uptake and dopamine release in a time dependent manner. Significant increases in both calcium uptake and dopamine release only occurred during the first three seconds of a 30 second potassium induced depolarization period. The greatest increase was observed during the first second of depolarization. The agonist activity of Bay K 8644 was attenuated by altering the calcium or magnesium ion concentrations in the incubation buffer. Bay K 8644 (1 nM) did not significantly increase KCl induced calcium uptake at magnesium concentrations of 0, 50, or 100 uM. Full agonist activity was restored when the magnesium concentration was increased to 500 or 1000 uM. Similarly, Bay K 8644 (1 nM) significantly enhanced KCl stimulated calcium entry at calcium concentrations of 100, 500, and 1000 uM (magnesium maintained at 1000 uM). Decreasing the calcium concentration to 25 or 50 uM abolished this effect. Finally, we examined the effects of the stereoisomers of Bay K 8644 and the dihydropyridine calcium agonist, 202-791. The (-) isomer of Bay K 8644 was approximately 1000 times more potent than the (+) isomer in enhancing synaptosomal calcium uptake and dopamine release. In contrast, the (+) isomer of 202-791 was roughly 100 times more potent than the (-) isomer of 202-791. Both active isomers of these compounds significantly increased calcium entry and dopamine release at a concentration of 1 nM. Neither of the less potent isomers possessed any calcium antagonist activity. (Supported in part by a grant from Miles Laboratories, NIAAA grant AA05809, a grant from the Texas Advanced Research Technology Program and a National Research Service Award to J.J.W.)

ACTION POTENTIALS AND ION CHANNELS VIII

328.1 CALCIUM TRANSIENTS RECORDED FROM MOUSE SPINAL CORD NEURONS UNDER VOLTAGE-CLAMP. M.L. Mayer, A.B. MacDermott, G.L. Westbrook, S.J. Smith and J.L. Barker. Labs Neurophysiology, NINCDS and Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Use of the calcium indicator dye arsenazo III in combination with voltage clamp techniques can provide useful information on changes in [Ca++]i in excitable cells. Traditionally such experiments were performed using large invertebrate neurons and muscle fibres; using whole cell patch clamp recording we have been able to measure optical transients reflecting calcium flux through both voltage-dependent and transmitter activated ion channels present in spinal cord neurons grown in tissue culture.

Patch recording was performed using K-gluconate, CsCl or $CsMeSO_3$ based solutions containing (mM) 1.1 EGTA, 10 HEPES and 0.8-1.5 arsenazo III; cultures were bathed in a balanced salt solution containing 2.5 mM calcium. Light passing through the cell was focussed onto a diffraction grating, and photodiodes used to measure the absorbance at 700, 660, 610 and 570 nM. [Ca++]i signals were recorded as 570-660 or 700-660 nM.

Depolarizing voltage steps from holding potentials of -50 to -60 mV evoked wavelength dependent increases in arsenazo III absorbance (660 > 610 >> 700 nM, with no change at 570 nM - the isobestic wavelength). Arsenazo III signals occurred with a threshold of close to -30 mV, peaked at +10 mV, and declined with further depolarization such that responses were too small to measure beyond +80 mV, consistent with a decrease in driving force for calcium entry during strong depolarizations. For voltage steps of < 500 ms duration the arsenazo III signal was a linear function of step duration, and under the conditions of our experiments (1 mM arsenazo III) intercepted the baseline at around 10-20 ms; with signal averaging it may be possible to detect Ca entry during single action potentials. For longer steps the arsenazo III signal rose exponentially to an asymptote with a time constant of close to 7 sec, suggesting that inactivation of Ca channels is slow and incomplete. Decay of the arsenazo signal following long steps has more complex kinetics and requires at least two exponentials, of time constants of around 10 to 100 sec, and probably reflects removal of intracellular calcium by extrusion, sequestration, and exchange with dye in the pipette. Similar responses have been recorded from molluscan neurons using microelectrode techniques (e.g. Gorman & Thomas, 1980).

Calcium signals were also recorded during depolarization evoked by excitatory amino acids. Arsenazo III signals to kainic acid were greatly reduced during voltage clamp; however arsenazo signals evoked by NMDA were less dependent on membrane potential depolarization, suggesting Ca entry through NMDA- but not kainate- or quisqualate-activated ion channels. Ca influx via NMDA-receptor channels may underlie long term regulation of excitability.

328.2 EFFECT OF PLATELET ACTIVATING FACTOR ON INTRACELLULAR FREE CALCIUM IN HUMAN PLATELETS: MEASUREMENT WITH FURA-2. M.A. Javors*, D.E. McGinnis* and C.L. Bowden. Depts. of Psychiatry and Pharmacology, The University of Texas Health Science Center, San Antonio, TX 78284.

Platelets have been used as a peripheral model for central nervous system nerve endings because they take up, store, and release serotonin, a neurotransmitter found in brain. The release of neurotransmitter in brain cells and platelets is regulated by intracellular free calcium concentration ($[Ca^{++}]_i$). The purpose of this study was to develop the platelet model for intracellular calcium function. The specific purpose of this study was to measure the effect of platelet activating factor on $[Ca^{++}]_i$ in human platelets in the absence and presence of extracellular calcium ($[Ca^{++}]_{ext}$). $[Ca^{++}]_i$ was measured with fura-2, a Ca^{++} -chelating fluorescent dye. Fura-2 was loaded into platelet cytosol where its fluorescence directly reflected $[Ca^{++}]_i$ according to Tsien et al (Cell Calcium 6:145, 1985). Platelet activating factor (PAF) is a lipid that binds to platelet membranes, raises $[Ca^{++}]_i$, activates platelets, and causes release of serotonin. We have shown in this study that $[Ca^{++}]_{ext}$ has an effect on the stimulation of $[Ca^{++}]_i$ by PAF. The Kd value for PAF for increasing $[Ca^{++}]_i$ does not differ in the absence vs. the presence of 1 mM $[Ca^{++}]_{ext}$, 7.07 ± 5.48 vs. 1.75 ± 1.15, respectively. For the experiments at zero $[Ca^{++}]_{ext}$, 50 μM EGTA was added. However, the maximal change in $[Ca^{++}]_i$ (delta $[Ca^{++}]_i$ max) differed in the absence vs. the presence of 1 mM $[Ca^{++}]_{ext}$, 45.0 ± 11 vs. 137 ± 26, respectively. These results suggest that calcium from an extracellular source enters the platelet when it is activated by PAF in the presence of 1 mM $[Ca^{++}]_{ext}$. They also suggest that Ca^{++} does not affect the binding of PAF to its receptor(s). Funded by a grant from the Meadows Foundation, Dallas, Texas.

328.3 IMMUNOLOCALIZATION AND CHARACTERIZATION OF THE CALMODULIN REGULATED Ca^{2+} PUMPING MEMBRANE ATPase IN NEURONAL TISSUES. M. Zurini*, B.F. Sisken and T.C. Vanaman* (SPON: R.L. Volle). Depts. of Biochem. and Anatomy, Univ. of Kentucky Coll. of Med., Lexington, KY 40536-0084

We report here recent studies on the localization and characterization of the 140 KDa calmodulin regulated Ca^{2+} pumping membrane ATPase in brain tissues. Antisera were prepared in rabbits by standard immunization procedures using the detergent solubilized Ca^{2+}-ATPase purified to homogeneity from human erythrocyte ghosts by calmodulin Sepharose affinity chromatography (Niggli et al., J. Biol. Chem. 254, 9955, 1979). A polyclonal IgG fraction, specific for the Ca^{2+}-ATPase, was purified from the resulting hyperimmune serum by affinity chromatography on a small column of immunobilized antigen. Monospecific and regiospecific anti Ca^{2+}-ATPase IgGs were isolated by preparative immunoabsorption on nitrocellulose transfers of SDS-PAGE resolved Ca^{2+}-ATPase and its limited trypsin digestion products (Zurini et al., J. Biol. Chem., 258, 618, 1984). Indirect immunofluorescence studies of paraformaldehyde fixed thin sections of rat brains, frozen immediately after sacrifice by decapitation, clearly demonstrate the presence of material which cross reacts with these monospecific immunoreagents, localized predominantly or exclusively in neurones throughout the brain. Intense immunoreactivity also was observed in RBCs present in vascular elements in these sections as would be expected. Preabsorption of the primary rabbit IgG with purified human erythrocyte Ca^{2+}-ATPase abolished all staining. Parallel immunoperoxidase localization studies confirmed the neuronal distribution of this enzyme and further suggested that it is present in neuronal processes as well as in the cell body. Immunoblot analyses of SDS-PAGE resolved membrane preparations, and enzyme assays demonstrated the presence of high levels of a 140 KDa polypeptide and calmodulin activated Ca^{2+}-ATPase activities in purified synaptosomal membranes from both human and rat brain tissues. Chemical and enzymological studies currently in progress should provide detailed information concerning the relationship between this synaptosomal membrane enzyme and the well studied Ca^{2+} pumping ATPase of human erythrocyte plasma membrane. The presence of this enzyme in high concentrations in the synaptosomal membrane provides an added dimension to the role of calmodulin in regulating key processes in the neurosecretory cycle. (Supported by NIH Grant NS 21868 and U.K. College of Medicine P.S.P. Funds.)

328.4 **Ca^{2+}/CALMODULIN DEPENDENCE OF REGULATION OF NEURON EXCITABILITY BY INTRACELLULAR pH.** R. Gillette & D.J. Green. Department of Physiology & Biophysics, Univ. of Illinois, Urbana, IL 61801. The activity and excitability of feeding command neurons in the buccal ganglion of the predatory mollusk Pleurobranchaea californica are sensitive to small changes in intracellular pH (0.05 unit) such as occur during normal activity. The sensitivity has been shown to arise from pH modulation of a slow Na^+ current co-activated by cyclic AMP and Ca^{2+}. The amplitude and duration of the current response are augmented by small changes in pHi, either acid or basic. This reflects a markedly steep pH/activity relation and indicates that the pH optimum for the enzyme normally lies at the resting pHi of the neuron.

At least two different mechanisms regulate sensitivity to pHi. Saline application of the calmodulin blocking agents W-7 and trifluoperazine (TFP) enhances the current response and also completely blocks augmentation of the response normally caused by intracellular alkalinization. However, the augmentation of the current response caused by intracellular acidification is not blocked by the calmodulin blockers. W-7 does not cause changes in pHi detectable with intracellular pH microelectrodes. Phorbol ester activators of Kinase C, a possible target of W-7 and TFP, have no effects on the cyclic AMP current response (N=6); this strengthens the likelihood that the actions of W-7 and TFP are effected through a Ca^{2+}/calmodulin pathway.

The pH sensitivity of the cyclic AMP stimulated Na^+ current appears to arise in large part from the sensitivity of the degradative enzyme for cyclic AMP, a Ca^{2+}/calmodulin dependent phosphodiesterase (PDE). PDE activity is reflected in vivo in the amplitude and duration of the Na+ current response to injected cyclic AMP (Soc. Neurosci. abs. 11, 238.7, 1985).

Our recent observations show that the pH optimum of the cyclic AMP current response can adapt to a new pHi (7.0 -7.6) maintained for 20-30 minutes. Such regulation of cell processes may relate to previous evidence that subcellular calmodulin distribution is highly sensitive to pH (Brain Res. 331,190, 1983) and the presence in CNS extracts of Ca^{2+}/calmodulin dependent phosphorylation also sensitive to pH (Lipeski, Gillette, & Gillette, this meeting). Supported by NSF BNS 83-08551.

328.5 NEURONAL LOCALIZATION OF A CYTOPLASMIC PROTEIN THAT REGULATES CALCIUM-DEPENDENT POTASSIUM TRANSPORT. Y. Tomozawa, L. S. Seinsoth*, P.H. White and G.A. Plishker*. Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

In the human red blood cell, Ca-dependent K transport is regulated by a 23,000 dalton cytoplasmic protein (calpromotin) that associates with the membrane in the presence of Ca. The amount associated with the membrane increases as Ca increases (10-100 μM). This effect is seen in intact cells and ghosts. The carboxymethylation of calpromotin correlates directly with the loss of Ca-dependent K transport in resealed ghosts (Am. J. Physiol. 248: C419-C424, 1985). Antibodies raised against calpromotin inhibit Ca-dependent K transport in resealed ghosts.

In one week cultures of dissociated rat embryonic cerebral cortex, calpromotin was localized in neurons and did not appear to be present in glia. Biotin-avidin immunostaining technique was used for detection. Preimmune serum did not show positive staining under the same conditions. Neuronal localization was established by double staining with antibodies against neuron specific enolase and calpromotin. Absence of this protein in glia was confirmed by double staining with antibodies against calpromotin and Glial Fibrillary Acidic Protein (GFAP). The calpromotin was stained by a biotin-avidin complex and astroglia marker was stained by immunofluorescence. There were a low number of oligodendroglia in these cultures.

Calpromotin was expressed in dissociated cultures prepared from 12-13 day old embryonic cerebral cortex. In cultures from 12 day embryonic cortex, 30% of the neurons stained positive for calpromotin and the subcellular localization of this protein was limited to the soma. Greater than 90% of the neurons from 14 day embryonic cortex stained positive for calpromotin. In cultures from 16 day embryos the calpromotin was found in both the soma and processes; with 19 day embryos its distribution extended into branched processes. Parallel quantitative studies of extracts from cerebral cortex established that calpromotin increased during embryonic development. Enzyme-linked immunoabsorbant assays (ELISA) showed that the amount of calpromotin doubled between 14 and 18 days of development. SDS polyacrylamide gels and immunoblots of extracts subjected to electrophoresis confirmed that the 23,000 dalton protein was increased.

This work was supported by National Institutes of Health Grants NS-11535 and GM-30594.

328.6 SINGLE CHANNEL CURRENTS RECORDED IN RAT PERITONEAL MAST CELLS. J.F. Fiekers. Dept. Anatomy and Neurobiology, Univ. Vermont College of Medicine, Burlington, VT. 05405.

Mast cells secrete a wide variety of chemical mediators which are thought to be involved in numerous allergic and inflammatory responses. Activation and degranulation of mast cells can result from diverse stimuli including specific antigens and substances like compound 48/80. The present study was designed to record and analyze the conductance changes in the mast cell membrane resulting from receptor-mediated mast cell activation. Mast cells were obtained by peritoneal lavage procedures and purified using density gradient centrifugation. Single channel currents were recorded from mast cells prior to and during mast cell activation using the patch clamp recording technique in both cell-attached and excised inside-out recording configurations. At least two types of single channel currents were recorded from mast cells in response to 48/80-induced activation.

48/80 when present in the patch pipette produced inward currents with brief (<1msec) and longer open times. Channels with longer open times frequently exhibited brief closing events within the open state. Hyperpolarization of the patch increased the current amplitude of both inward currents and prolonged the mean open times. Substitution of KCl for NaCl in the pipette or symmetrical solutions in excised patches did not alter the magnitude of the currents.

An additional outward current was recorded at depolarized clamp potentials. Both the frequency and duration of channel openings increased as the intracellular membrane surface was made more positive. Bursting behavior of this channel was also observed with channels open for seconds with an increase in open channel noise. Two exponentials were required to fit the observed channel open times. This channel was not present in on-cell patches without 48/80 in the pipette, mast cells previously treated with EGTA in the absence of 48/80 and on-cell patches with the pipette containing zero calcium with 48/80. After the establishment of an on-cell patch, superfusion of mast cells with either A23187 or 48/80 in the presence of calcium increased channel activity.

These results suggest that there are at least two channels present in the mast cell membrane- a nonspecific cation channel and a calcium-activated K channel. The cation channel requires external receptor activation while the Ca(K) channel is activated by a rise in cytosolic calcium and is influenced by calcium concentration and the clamp voltage. (supported by University of Vermont Biomedical Research Grant).

328.7 OSCILLATIONS OF Ca2+—ACTIVATED K+ CHANNELS AROUND VARIOUS CLOSED STATES IN BOVINE CHROMAFFIN CELLS. M.I. Glavinović (SPON: R. Chase). Depts. of Anaesthesia Research and Physiology and Biomedical Engineering Unit, McGill University, Montreal, Canada H3G 1Y6

Ca2+ activated K+ channels with large single channel conductance have been observed in a variety of cells. A model for gating of these channels has been suggested from studies in rat myotubes of (a) probability distribution of open and shut intervals and (b) bursting kinetics (Magleby & Pallotta, J. Physiol., 344:585-604, 1983; Magleby & Pallotta, J. Physiol. 344:605-623, 1983). Conditional probability analysis revealed however, that transitions to and from long shut states occur almost exclusively via short duration open state (Pallotta, J. Physiol., 363:501-516, 1985) and to and from short shut state through open states of long duration (McMannus et al., Nature, 317:625-627, 1985). This is an attempt to further examine the transitional pathways between various open and shut states of Ca2+ activated channels.

Experiments were done on excised inside out patches of bovine chromaffin cells. The pipettes were filled with Locke solution which consisted of (mM): 154 NaCl, 2.2 CaCl2, 1.2 MgCl2, 2.5 KCl, 2.15 K2HPO4; 0.85 KH2PO4 and 10 dextrose. The internal solution contained (mM): 160 KCl/KOH, 10 Hepes, 1 MgCl2, 0.5 EGTA and .279 CaCl2 at pH = 7.2, and nominally had 0.1 μM free Ca2+.

Our results confirm that long shut states are preceded and followed by short open states and short shut states by long open states. Oscillations around long shut state persist and as many as ten openings preceding and following it are shorter than average while the corresponding closings are above average. Oscillations around short shut state are also quite prolonged. No evidence of temporal asymmetry of opening and closing around either long or short state is observed. These findings suggest that a 'pair' of long shut and short open states is connected to another 'pair' of short shut and long open state through a shut state of intermediate duration - 'gateway state'. However, more than two 'pairs' of open-shut states may exist since the average open time per opening for each burst (excluding the first and last opening; burst is defined as in Magleby & Pallotta, J. Physiol. 344:605-623, 1983) is positively although weakly correlated with the number of openings per burst.

This study was supported by MRC and MDA (Canada).

328.8 MUSCARINIC RECEPTOR-LINKED INCREASE IN CALCIUM-ACTIVATED POTASSIUM CHANNEL ACTIVITY IN 1321N1 ASTROCYTOMA IS DEPENDENT ON EXTRACELLULAR CALCIUM. S.A. Oglesby & B.S. Pallotta, Curriculum in Neurobiology and Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27514

The plasma membranes of 1321N1 human astrocytoma cells contain a large conductance (290-320 pS) voltage- and Ca^{2+} -sensitive K^+ channel. Using conventional patch clamp techniques, we have previously shown that in excised membrane patches the channel is sensitive to sub-micromolar concentrations of Ca^{2+} (0.01 to 10 μM), and that the apparent sensitivity to Ca^{2+} is dependent upon the membrane voltage (-60 to +60 mV) such that membrane depolarization increases channel % open time (Pallotta & Oglesby, Biophys. J. (1986)49:171a). We now demonstrate that the channel is highly selective for K^+ over Na^+, and that activation of the channels in on-cell patches by bath-applied carbachol requires extracellular Ca^{2+} and is reversibly blocked by atropine.

Current-voltage relations constructed from single channel currents in inside-out excised patches were linear over the voltage range -50 to +50 mV in symmetric 110 mM KCl solutions. Replacing KCl with equimolar NaCl on the intracellular surface caused a pronounced curvature of the I-V relation such that no outward currents or reversal potential were observed at voltages as great as +100 mV. These results suggest that the channel is at least 50-fold more selective for K^+ than for Na^+. Current-voltage relations obtained from cells bathed in normal Na^+ (150 mM) suggested that normal cell resting potentials varied from -50 to -70 mV (41 cells) and that the normal intracellular K^+ concentration was ~110 mM.

Bath application of 1 μM carbachol+2.3 mM Ca^+ caused a rapid (sec) and large increase in channel open probability in 9 out of 21 polarized cells. In responsive cells, the % open time increased from a resting level of <1% to 38-61%. This effect did not occur when 1 μM atropine was present, although atropine would not decrease channel activity which had already been induced by carbachol. Application of carbachol in the absence of extracellular Ca^{2+}(0 added Ca^{2+}+1mM EGTA) did not increase channel activity in either polarized or depolarized (110 KCl) cells which responded in the presence of Ca^{2+}.

These results suggest that the observed carbachol-induced increases in channel activity required the stimulation of muscarinic receptors, and that at least one event linking channel activity with receptor activation required extracellular Ca^{2+}.

Supported by NIH grant GM32211.

328.9 CYCLIC AMP-DEPENDENT PROTEIN KINASE MODULATION OF SINGLE, CALCIUM-ACTIVATED POTASSIUM CHANNELS FROM RAT BRAIN IN PLANAR BILAYERS. D. K. Bartschat*, R. J. French*%, A. C. Nairn*#, P. Greengard#, and B. K. Krueger. (SPON: R. Gruener). Depts. of Physiology and Biophysics%, Univ. Maryland Sch. of Med., Baltimore, MD 21201 and Lab. of Molecular Cellular Neuroscience#, Rockefeller Univ., NY, NY 10021.

Calcium-activated potassium channels (CAKC's) from rat brain membranes were incorporated into planar lipid bilayers to determine whether the activity of these channels was susceptible to enzymatic modification by the purified catalytic subunit of cAMP-dependent protein kinase (CS). When brain membranes were added to one side of the bilayer, large, unitary, potassium-selective conductance events (250 pS in symmetrical 150 mM KCl) were observed under voltage clamp conditions. The probability of the channel being open (P_o) could be increased by depolarization or by increasing [Ca^{++}] on the normally intracellular side of the channel. Intracellular CS (1 - 10 μg/ml), in the presence of 2 mM Mg^{++}, 0.2 mM ATP and 25 μM Ca^{++}, caused the CAKC to enter a prolonged and completely nonconducting state; the latency from addition of enzyme to complete "closure" varied inversely with the CS concentration. At low CS (1 - 3 μg/ml), the CAKC frequently passed through a transient period of altered gating (stage 1) which invariably proceeded to complete quiescence (stage 2). Stage 1 was characterized by a low P_o, although depolarizing potentials, but not the addition of 200 μM $Ca^{++}{}_i$, increased P_o. During stage 2, neither high $[Ca^{++}]_i$ nor strong depolarizations elicited openings. Effects of CS were seen in about 80% of all experiments (N = 42). They were never observed with boiled CS, when MgATP was omitted, or when CS + MgATP was added to the extracellular side of the CAKC. In some preliminary experiments, protein phosphatases or CS + high ADP were able to restore channel activity resembling that of stage 1. These results indicate that CAKC's from mammalian brain can be modulated by phosphorylation of the channel or a tightly associated component by CS. Moreover, a channel may contain more than one regulatory site, each of which, when phosphorylated, alters channel gating and/or conduction. (Supported by NIH and the U. S. Army Medical Research and Development Command.)

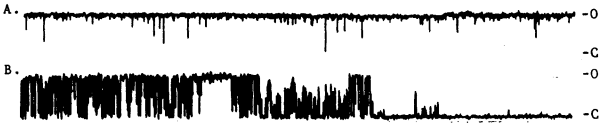

Figure: A. Control with intracellular MgATP. B. After addition of CS. Single channel current = 5 pA; each record is 50 sec long.

328.10 A TRANSIENT CALCIUM-DEPENDENT POTASSIUM CURRENT IN CULTURED RAT DORSAL ROOT GANGLION CELLS. K. Sugiyama* and M.L. Mayer. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

The characteristics of transient Ca-dependent potassium current (IKca) are not as well established compared to those of the sustained IKca (e.g. Adams et al, 1982). Calcium-dependent transient outward currents have been described in sympathetic and hippocampal pyramidal neurons (MacDermott & Weight, 1982; Zbicz and Weight, 1985). A recent study has shown a transient IK elicited in cultured rat dorsal root ganglion cells (DRGs, e.g. Kostyuk et al, 1981), however the involvement of Ca ions in the production of this transient IK was not examined. We have investigated the Ca-dependence of the transient IK in cultured rat DRGs, its kinetics and its sensitivity to potassium channel blockers, using whole cell patch recording.

Cultures prepared from neonatal rat DRGs were bathed in medium containing (mM) 2.5 Ca, 1.0 Mg, 142 Na and 2.5 K. The patch solution contained 140 $KMeSO_4$, 10 HEPES, 1.1 EGTA and 2 $MgCl_2$. Transient outward currents (TOC) were activated by membrane depolarization more positive than -60 mV after conditioning prehyperpolarization (-120 mV, 500 msec). Records with and without prepulses were subtracted to reveal the TOC which increased linearly in amplitude with increasing depolarization up to 0 mV. The time to peak of the TOC was 5-10 msec at -20 mV. Steady state inactivation for the TOC occurred between -120 and -60 mV with a half-inactivation at -84 +6 mV (n=5). Both the removal and the development of inactivation of the TOC followed an approximately single exponential time course with mean time constants of 30+3 msec at -120 mV and 82+28 msec at -50 mV (n=3). Mean reversal potential of the TOC was -74+4 mV (n=5) in media containing 2.5 mM K and the positive shift on raising the external K concentration 10-fold was 40+3 mV (n=5). The amplitude of the TOC decreased in low Ca media and was also depressed by Mn and Cd. The TOC was blocked by 4-aminopyridine (4-AP), barium and quinidine, but not by tetraethylammonium (TEA). Sustained outward currents which had a N-shaped current-voltage relation, could also be evoked by depolarizing voltage steps more positive than -30 mV from holding potentials of -50 to -60 mV, and were depressed by Mn and Cd. This sustained Ca-dependent outward current was also blocked by TEA, 4-AP and quinidine.

These results suggest that the transient outward current generated in cultured rat dorsal root ganglion cells is probably a Ca-dependent potassium current (IKca) with rapid voltage dependent activation and inactivation kinetics and that the transient IKca can be pharmacologically separated from the sustained IKca by TEA.

328.11 SENSORY NEURON ULTRASTRUCTURE FOLLOWING WHOLE CELL PATCH RECORDING. E.A. Neale, M.L. Mayer and L.M. Bowers*. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892

Following development of the improved patch-clamp technique by Neher, Sakmann and colleagues (Hamill, O.P., et al., 1981) whole cell recording has become widely used for voltage clamp experiments on vertebrate neurons. Whole cell recording has several well-known advantages over conventional recording techniques. However, the degree of cellular disorganization induced by perfusion of the cytoplasm with the pipette solution is not known.

We have examined, in the electron microscope, mouse dorsal root ganglion neurons in culture that were used for gigaseal whole cell recording. Pipettes were filled with KCl or K-aspartate (140 mM) solutions containing in addition (in mM) 1.1 EGTA, 10 HEPES and 2 $MgCl_2$ (pH, 7.2; ca. 315 mOsM). After establishing whole cell recording, the series resistance was monitored continuously for 20 min and kept between 5-10 MΩ; the pipette was withdrawn to form an outside-out patch, and within 10-20 min the cells were fixed for electron microscopy.

In some experiments, exchange of Lucifer Yellow from a patch pipette was followed using a fluorescence microscope; dye diffused into the neuronal soma and initial processes within seconds after breakthrough, suggesting rapid perfusion. With the recording solutions that we have tested, cellular ultrastructure showed some distortion, although overall, the organization of cellular contents was fairly well-maintained relative to 'control' neurons in the same field. The mitochondria of patched neurons were the most consistently altered organelles. In neurons patched with the KCl solution, mitochondria were smaller and more electron dense than those of control cells, and frequently were associated with myelin figures suggestive of degeneration. Although Golgi sacs were somewhat swollen and vacuolated, the rough endoplasmic reticulum and nuclear envelope did not exhibit signs of swelling. In K-aspartate patched neurons, mitochondria were also small and electron dense, and occasionally dumbbell- or cup-shaped. In these cells, there was less Golgi vacuolation. In order to compensate for possible loss of ATP during recording, 2 mM ATP was added to the K-aspartate recording solution. In two neurons patched with this solution, cisternae of the rough endoplasmic reticulum were somewhat swollen and some Golgi cisternae appeared vesiculated. However, mitochondria in these neurons were very similar to controls, suggesting that washout of ATP may be responsible for changes in mitochondrial morphology, perhaps reflecting transition to a state of high oxidative metabolism.

Our results suggest that whole cell patch recording need not produce grossly distorted ultrastructure. However, modifications of the recording solution (use of aspartate, addition of ATP) appear to minimize structural alterations in recorded cells.

328.12 PURIFICATION OF CHARYBDOTOXIN, A SPECIFIC INHIBITOR OF HIGH CONDUCTANCE CALCIUM ACTIVATED POTASSIUM CHANNELS. C.D. Smith*, M. Phillips* and C. Miller* (SPON: G. Yellen) Graduate Dept. of Biochemistry, Brandeis Univ., Waltham, MA 02254.

Charybdotoxin (CTX) is a high-affinity specific inhibitor of the large conductance Ca^{++} activated K^{+} (CaK) channel found in the plasma membrane of many vertebrate cell types. We have used CaK channels reconstituted into planar lipid bilayers to assay CTX channel blocking activity. Using this method, we have purified the toxin protein from the venom of the Israeli scorpion Leiurus quinquestriatus. Solubilized venom was fractionated on SP-Sephadex in a chromatofocusing mode to remove over 90% of the contaminating venom protein. The toxin is eluted from the column by a pH gradient of 9-12. All activity elutes in a sharp peak at pH 10.8-11.

The toxin is purified to homogeneity by C8 reverse phase HPLC. The mobile phase is a 0-50% methanol gradient in 50mM acetate pH4. The elution profile gives three peaks, the predominant one containing the CTX activity. We recover over 80% of the CTX activity and achieve a 300-fold purification over the crude venom by this protocol. The resulting protein shows a single band on SDS-urea polyacrylamide gels. This band elutes from nonreducing gels with full activity.

Amino terminal analysis of purified protein yields valine as the single amino group present in the protein sample. This result agrees with the N-terminus obtained in a partial protein sequence. Amino acid analysis further indicates that the protein is pure, has a molecular weight of 10kD, and contains a large proportion of basic residues. The toxin contains tyrosine and can be iodinated to a specific activity of $2x10^{8}$ cpm/nmole by the lactoperoxidase method with no loss of channel blocking activity. The protein also contains cysteines, at least some of which are present in disulfide linkages essential for channel blocking activity.

Under our standard assay conditions, the pure toxin inhibits CaK channels in a 1:1 stoichiometry with dissociation constant of 3.5nM. The protein is exceptionally stable, with activity being insensitive to boiling or exposure to organic solvents, urea or extreme ranges of pH and ionic strength. The toxin does lose all activity when cleaved with chymotrypsin or reacted with lysine acylating reagents such as citriconic anhydride.

We are currently characterizing ^{125}I-CTX binding to plasma membranes of bovine adrenal chromaffin cells. The high affinity and specificity of CTX for CaK channels make it a good candidate as a ligand to assay channels during purification.

328.13 LOW MICROMOLAR CONCENTRATIONS OF PHENOTHIAZINES AND HALOPERIDOL SELECTIVELY BLOCK CA-ACTIVATED K CHANNELS IN RAT BRAIN SYNAPTOSOMES. C.G. Benishin, B.K. Krueger, and M.P. Blaustein. Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

Micromolar concentrations of the phenothiazines, fluphenazine and trifluoperazine, selectively inhibited the Ca-dependent ^{86}Rb efflux from isolated nerve endings. ^{86}Rb efflux was stimulated with elevated extracellular K in the absence and presence of Ca. The Ca-dependent difference in flux represents flux through K channels activated by Ca and voltage (Bartschat and Blaustein, J. Physiol. 361: 419-440 and 441-457, 1985). Inhibition by the phenothiazines was dose dependent and revealed that the Ca-dependent K conductance consisted of two components with differing sensitivities to the phenothiazines (with IC_{50}'s of 20-30 nM and 1-3 μM). 10 nM to 30 μM phenothiazines minimally inhibited depolarization-induced ^{45}Ca uptake, ruling out the possibility that the phenothiazine effect on Ca-dependent K channels was due to an inhibition of Ca uptake. W-7, a non-phenothiazine calmodulin antagonist, was ineffective at inhibiting Ca-dependent K channels. However, haloperidol, another antipsychotic, but a weaker calmodulin antagonist, inhibited the Ca-dependent K channels with a single IC_{50} of 0.5 μM. Sulpiride, a potent antipsychotic with weak anti-calmodulin activity, was relatively ineffective at inhibiting Ca-dependent K channels, but a high concentration (10 μM) blocked the higher sensitivity component. These results indicate that several classes of antipsychotic drugs are potent and selective inhibitors of Ca-dependent K channels in nerve endings. This function does not appear to be mediated via calmodulin. One class of antipsychotics, the phenothiazines, distinguished two components of Ca-dependent K channels which differ in sensitivity to these drugs. This work was supported by an MDA Post-Doctoral Fellowship to C.G. Benishin, and NIH grants NS-16285 to B.K. Krueger and NS-16106 to M.P. Blaustein.

328.14 APAMIN DEPRESSES SELECTIVELY THE AFTER-HYPERPOLARIZATION OF CAT SPINAL MOTONEURONS. L. Zhang* and K. Krnjević. Departments of Anaesthesia Research and Physiology, McGill University, Montreal, Canada H3G 1Y6.

Apamin, a peptide component of bee venom, causes a block of Ca-activated K conductance ($G_{K(Ca)}$) in cells from a variety of tissues including liver, muscle and neuroblastoma (Haberman, 1984, Pharmac. Ther. 25, 255). According to Lazdunski (1983, Cell Calcium, 4, 421) only the slow $G_{K(Ca)}$ responsible for the late after-hyperpolarization (AHP) is sensitive to apamin, a suggestion confirmed by recent observations on bullfrog sympathetic ganglia (Pennefather et al., 1985, Proc. Nat. Acad. Sci. 82, 3040). So far a similar effect has not been seen in CNS neurons. In cats under pentobarbitone or chloralose anaesthesia, action potentials and AHP's were evoked in lumbosacral motoneurons by antidromic stimulation of ventral roots. A second, extracellular microelectrode, filled with 200 μM apamin (Sigma) in 0.15 M saline, was separated from the intracellular electrode by a distance of 30 μm. When released by a current of 80 nA for 3-5 min, apamin depressed the amplitude of AHP's and increased AHP resistance in 13 motoneurons, with only minor alterations in resting potential or resistance, and without affecting the size and time course of action potentials. The depressions of AHP amplitudes were by 22-74%; they were observed for as long as 20 minutes, with no sign of recovery. Motoneuronal firing, evoked by 500 ms depolarizing current pulses, was increased by 42-120% after iontophoresis of apamin, but postsynaptic potentials evoked by dorsal root stimulation were not obviously changed. These results confirm previous data (Krnjević et al., J. Physiol. 275: 199-223, 1978) indicating that the AHP of cat spinal motoneuron is mediated by a $G_{K(Ca)}$, which can modulate motoneuronal firing. They are in keeping with previous observations that apamin (given intrathecally) enhances spinal reflexes (Haberman, 1984).

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328.15 Ca^{++}-DEPENDENT OUTWARD CURRENTS ACTIVATED BY Ca^{++}-INJECTION IN VISCERAL SENSORY NEURONS. W.F. Wonderlin and D. Weinreich. Dept. Pharmacology & Exper. Therap. Univ. Maryland Sch. of Medicine, Baltimore, MD 21201.

Action potentials in C-fiber (unmyelinated) visceral sensory neurons of the rabbit nodose ganglion can activate two Ca^{++}-dependent spike afterhyperpolarizations (a.h.p.s): (1) a fast, TEA-insensitive a.h.p. lasting less than 500 msec, and (2) a slow a.h.p., lasting 1 to 30 sec. To characterize further the specific subcellular processes responsible for activation of these a.h.p.s, we have compared the Ca^{++}-dependent K^+-conductances activated during the fast and slow a.h.p.s with the outward currents that can be activated by intrasomatic ionophoretic Ca^{++} injection.

Acutely dissociated nodose neurons were impaled with two microelectrodes; one electrode was used to single-electrode voltage-clamp the membrane potential while Ca^{++} was ionophoretically injected from a second electrode that contained .2 M $CaCl_2$. Ionophoretic Ca^{++}-injection (3-7 nA, 500-1000 msec duration) activated two temporally and pharmacologically distinct outward currents in nodose neurons: (1) a fast, TEA-sensitive outward current ($I_{C,fast}$) that is strongly voltage-dependent with an e-fold increase in peak conductance for each ≈10 mV depolarization beyond -40 mV; and (2) a slow outward current ($I_{C,slow}$; τ_{on} ≈1200 msec, τ_{off} ≈1500 msec) which is TEA-insensitive, voltage-independent and exclusively associated with neurons that also exhibit a slow a.h.p. Forskolin (1 μM) completely inhibited $I_{C,slow}$ and the slow a.h.p. without effect on $I_{C,fast}$ or the fast a.h.p.

$I_{C,slow}$ probably represents the same K^+-conductance activated during the slow a.h.p., based on its slow time course, block by forskolin and insensitivity to TEA. With regard to the fast a.h.p., neurons that exhibit $I_{C,fast}$ also possess a Ca^{++}-dependent component of the fast a.h.p., as demonstrated by removal of external Ca^{++}. Inhibition of $I_{C,fast}$ by TEA also produces spike broadening in these neurons. However, we have not been able to activate with Ca^{++}-injection a TEA-insensitive outward current that corresponds to the TEA-insensitive, Ca^{++}-dependent component of the fast a.h.p. This work was supported by an NIH grant.

328.16 CALCIUM-ACTIVATED INWARD CURRENT IN APLYSIA NON-BURSTING NEURONS: EVIDENCE FOR A NONSELECTIVE CATIONIC CONDUCTANCE. Wilkie A. Wilson, Leslie S. Jones, Darrell Lewis, Depts. of Peds. (Neurol.) and Pharm., Duke Univ. Med. Center and the Veterans Administration Hospital, Durham, N.C. 27710

We have been studying the triphasic current response to Ca2+ iontophoresis into the silent, non-bursting neurons of Aplysia (Jones et al., Brn. Res., in press). This response consists of an early outward current, thought to be Ic, during the injection, followed by an inward current (phase II) that peaks 10-20 seconds after the end of the injection, then followed by a very slow outward current that develops over many seconds, peaking 50-100 seconds after the injection is terminated. We report here that the second phase, an inward current that occurs in response to the injected Ca2+, appears to be a nonselective cationic conductance carried by both Na+ and Ca2+.

Identified, non-bursting neurons were impaled with a recording electrode containing 1.5 M KCl and two electrodes for iontophoresis, one with .5 M KCl and the other with 50 mM CaCl2. The cells usually were voltage-clamped at -30mV, as the triphasic response is most apparent at slightly depolarized potentials, and Ca2+ was ejected for 20 seconds using currents of about 100 nA. To test the dependence of the inward phase II on external Na+, bathing solutions containing zero Na+ (Na+ replaced by sucrose or bis-tris-propane) were used. To test the dependence of phase II on external Ca2+, zero Ca2+ solutions were used (Ca2+ removed and 1 mM EGTA added), also Ca2+ channel blockers such as manganese (25 mM MnCl2) or lanthanum (3 mM LaCl3) were added to the bathing solution. Since none of these manipulations alone blocked the expression of phase II, in some experiments Ca2+ channel blockers were applied in conjunction with a zero Na+ bathing solution. Only under these conditions, where there was zero extracellular Na+ and a Ca2+ channel blocker was present (25mM MnCl2), was the appearance of phase II blocked. These results point to phase II being a current that can be carried either by Na+ or Ca2+, as we can block phase II only by preventing both cations from contributing to the current.

This work was supported by NSF grant BNS 850-7603, by a research grant from the Epilepsy Foundation of America, and by the Veterans Administration.

328.17 CALCIUM-ACTIVATED, INWARD CURRENT CHANNELS IN SNAIL NEURONS. L.D. Partridge, D. Swandulla* and H.D. Lux*. Max-Planck Institute for Psychiatry, D-8033 Planegg-Martinsried, F.R.G.

The depolarizing drive that maintains bursting in Helix pomatia neurons is carried by a long-lasting inward current. Pressure injection of Ca into many snail neurons activates a similar long-lasting inward current that is carried non-selectively by cations. This current has a reversal potential of approximately -20 mV and a decay half time of several hundred msec and it can be carried by ions as large as Tris (Swandulla and Lux, J. Neurophysiol. 54: 1430). Characterization of this current is hampered by the temporal overlap of several outward currents. Using cell-attached and inside-out patches, we have identified a population of channels that is probably responsible for this current. A similar Ca-activated, non-selective cation channel has been described in other tissues.

The right parietal fast burster and, occasionally, other large neurons of Helix pomatia were cleaned of glial covering with trypsin. They were voltage clamped with a standard two-electrode clamp, pressure injected with Ca from a third electrode, and patch clamped. Patches were excised directly into the stream flowing from a mini-perfusion pipette positioned about 100 u from the cell surface.

These patches exhibited from 1 to 6 unitary current levels, but only rarely were currents of different unitary amplitudes or both inward and outward current processes observed in the same patch. One population of unitary currents was inward at -50 mV and showed an increased probability of opening when Ca was injected or when excised patches were bathed in solutions with 10^{-7} M or more Ca. Cell-attached patches (patch electrodes with low Ca Ringer) had single channel conductances near 30 pS with reversal potentials near -20 mV; excised patches had similar conductances in symmetrical Na solutions and reversal potentials within a few mV of zero. Calculations assuming a simple spherical cell yield a channel density of only about 1/20 u^2. The increased channel opening probability characteristically persisted well beyond the duration of the transient whole-cell inward current. We conclude from this that the later phase of Ca-activated inward current is normally masked by an opposing outward current.

328.18 INTRACELLULAR CALCIUM ACTIVATES A NON-SELECTIVE CATION CHANNEL IN BC3H-1 CELLS. R. E. Sheridan. Dept. of Pharmacology, Schools of Med. and Dent., Georgetown Univ., Washington, DC 20007.

The BC3H-1 clonal cell line is generally considered to be a "muscle" cell line due to its expression of nicotinic receptors when the cells are either grown to confluency or grown in reduced concentrations of serum. However, the BC3H-1 clone line used in this laboratory can express both a cation current activated by extracellular cholinergic agonists and a separate non-selective cation current activated by intracellular Ca^{++}. These two channel types are distinguishable electrophysiologically with the patch clamp technique and are expressed under different tissue culture conditions.

The presence of micromolar concentrations of Ca^{++} on the cytoplasmic face of BC3H-1 cell membranes activates a cation conductance. In intact membrane patches, the intracellular Ca^{++} concentration is typically too low to activate these channels, although the nicotinic receptor channels can be readily activated by extracellular cholinergic agonists. Excised, inside-out patches of membrane with symmetric solutions of 155 mM NaCl inside and outside the patch exhibit Ca^{++}-dependent single channels with a conductance of 20 pS at 20°C and 34 pS at 30°C. By comparison, the nicotinic receptor channels in intact membrane patches have a conductance of 35 pS at 20°C. Like the nicotinic receptor channel, the Ca^{++}-activated channel shows little selectivity between Na^+ and K^+ but is highly selective for cations over Cl^-, as measured by shifts in the reversal potential following replacement of Na^+, K^+ and/or Cl^- on the extracellular face of the membrane patch. Kinetically, the Ca^{++}-activated channel has at least two mean open state lifetimes. At 20°C, one lifetime is in excess of several hundred msec while the other, shorter lifetime is less than 8 msec. The shorter lifetime is similar to the lifetime of the dominant open state in the nicotinic receptor channels under similar conditions.

Unlike nicotinic receptors, Ca^{++}-activated channels in BC3H-1 cells are present before the cells become confluent and when the serum levels are above 10-15%. Further, BC3H-1 cells grown in media supplemented with micromolar concentrations of polyunsaturated fatty acids do not have nicotinic receptors on their plasma membranes, although the Ca^{++}-activated channels are present and functional.

The Ca^{++}-activated cation channels may be coupled to alpha1-adrenergic receptors on BC3H-1 cells through mobilization of intracellular Ca^{++} (Brown et.al., J. Biol. Chem., 259:7554-7562, 1984) since exposure of intact cells to micromolar phenylephrine can stimulate the appearance of these channels in an otherwise quiescent intact patch of membrane.

328.19 ACTIVATION OF THE CALCIUM-DEPENDENT CHLORIDE CURRENT IN AtT-20 PITUITARY CELLS. Stephen J. Korn and Forrest F. Weight. Lab. of Preclinical Studies, NIAAA, Rockville, MD 20852.

A Ca^{++}-dependent Cl^- current has been observed in several mammalian and non-mammalian cell types, including both neurons and secretory cells. To understand the role of this current in the regulation of cell excitability, we are investigating the mechanisms that underly its activation and deactivation in AtT-20 mouse pituitary cells. The Ca^{++}-dependent Cl^- current can be activated in AtT-20 cells by step-depolarization into the range that activates the voltage-activated Ca^{++} current (Fed. Proc. 45, 1008, 1986). In the studies described below, we have used both the whole-cell and excised patch clamp technique to examine the roles of voltage and Ca^{++} in the activation of the Cl^- current.

Voltage-clamped cells were held at -80 mV and perfused internally with Cs^+ to eliminate K^+ currents. When the external solution contained Ca^{++}, voltage steps to between -30 mV and +60 mV activated the Cl^- current. With removal of external Ca^{++}, application of 0.4 mM Cd^{++}, internal perfusion of the cell with more than 1 mM EGTA or BAPTA, or replacement of external Ca^{++} with Ba^{++}, the Cl^- current was not activated. Cells that were bathed in an external solution not containing Ca^{++} and perfused internally with a solution containing 0.5 to 1 uM Ca^{++} displayed a steady leak current that was not observed when the internal Ca^{++} was buffered at 0.1 uM. This leak current was not diminished by hyperpolarization to -100 mV. Current-voltage plots demonstrated that the leak current rectified in the direction of the Cl^- concentration gradient. The direction of the rectification could be reversed upon reversal of the Cl^- concentration gradient. With Ca^{++}-free external solution and 1 uM internal Ca^{++}, a time-dependent current, similar in timecourse and direction to the Ca^{++}-dependent Cl^- current, was not observed until step depolarizations reached +40 mV. These data suggest that the Ca^{++}-dependent Cl^- current is activated primarily by Ca^{++}, and that voltage has little or no role in its activation.

Support for this hypothesis was obtained from single channel recordings made from excised membrane patches. The solution bathing the external membrane consisted primarily of N-methyl glucamine sulfate; that bathing the cytoplasmic surface consisted primarily of CsCl. No channel activity was observed when the cytoplasmic membrane was exposed to a Ca^{++} concentration buffered at 0.1 uM (Vm = -20 mV). Increasing the Ca^{++} concentration above 1 uM resulted in the occurrence of inward channel currents. These results suggest that this channel is the Ca^{++}-dependent Cl^- channel that underlies the current observed in whole-cell recordings.

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328.20 **A CALCIUM-DEPENDENT CHLORIDE-CONDUCTANCE IN AN IDENTIFIED LEECH NEURON.** J. Johansen and A.L. Kleinhaus. Dept. of Neurology, Yale Univ. School of Medicine, New Haven, CT 06510.

In this study we demonstrate the presence of a Ca-activated chloride-conductance in an identified leech neuron. Such a conductance has thus far been described in some algae and a few vertebrate preparations, but to our knowledge not in invertebrates.

Neurons from the glossiphoniid leech *Haementeria* were studied under two electrode voltage clamp with outward K-currents eliminated by replacement of intracellular K with Cs, and with Na-currents blocked by 1 μM TTX. Recording was done with 2.5 M CsCl containing electrodes. Due to leakage from the electrodes this typically resulted in an intracellular Cl-concentration of about 100 mM as estimated from analysis of tail-current reversal potentials. Among the leech neurons studied under these conditions, we found a specific chloride-conductance only in the identified AL1 cell (Kramer & Goldman, *J. Comp. Physiol.* **144** : 435; 1981) which is a putative motoneuron. The reversal potential of tail currents of this conductance varied with $[Cl]_o$ as predicted for the equilibrium potential of Cl by the Nernst equation and was unaffected by changes in the extracellular cation-concentration. The decay of the tail currents followed a process which could be decribed by the sum of two exponentials with time constants of about 100 msec and 750 msec, respectively. No voltage-dependence of the time constants was apparent.

All current flow was abolished in Mn-containing Ringer and the chloride-dependent current was absent when Ca was substituted with Ba. Furthermore, the activation and size of the chloride-current was directly related to the amount of Ca-influx, and no chloride-current flow could be induced at depolarizations at or beyond the apparent reversal potential for the Ca-current. These results strongly suggest that the chloride-conductance is dependent on Ca for its activation. However, the role of Ca could be substituted for by Sr which also was able to activate the Chloride-current.

Since only a few of the examined identified neurons in the leech possessed a Ca-activated chloride-conductance, it is conceiveable that this conductance may be directly involved in regulation of these cells' functional properties.

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EFFECTS OF CHRONIC DRUG ADMINISTRATION AND NEUROTOXICOLOGY II

329.1 EFFECTS OF PYRETHROIDS ON INTRACRANIAL SELF STIMULATION. E.A. Stein, C. Walczak* and A.S. Bloom[1]. Department of Biology, Marquette University, Milwaukee, WI 53233 and [1]Department of Pharmacology, Medical College of Wisconsin, Milwaukee, WI 53226.

The pyrethroid insecticides are known to have high insect: mammal lethality when used in acute, effective environmental doses. In addition, while high parental doses are known to result in profound behavioral syndromes often leading to death, understanding of the CNS effects of low doses in mammals is poorly understood. We have recently demonstrated that operant responding for food can be an effective indicator of the acute CNS toxicologic effects of subconvulsive doses of pyrethroids. However, it is possible that these agents acted on sites (perhaps in the periphery) to make food somewhat aversive and thus not act at neural sites responsible for this behavior. To test this possibility, intracranial self stimulation, a reinforcer that does not have to be consumed by the subject and is relatively non-satiable, was used to maintain operant responding. Monopolar stimulating electrodes were implanted into either the ventral tegmental area or medial forebrain bundle region of male, Holtzman rats. Following recovery, rats were tested for intracranial self stimulation behavior and trained to respond on a CRF schedule. Eight current intensities were presented for 2 min each in an ascending/descending manner. Technical Permethrin (0, 15, 30 or 60 ng/kg) or Deltamethrin (0, 1, 3 or 5 mg/kg) were given once/wk IP 20 min prior to testing. Preliminary evidence indicates minimal effects of either agent at any dose or current tested. There were also no effects of stimulation sites. Data are discussed in terms of the putative sites/mechanisms of action of the pyrethroids.

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329.2 EFFECT OF SOMAN ON BRAIN WEIGHT AND REACTIVITY TO AN ACOUSTIC STARTLE STIMULUS. H.E. Modrow*, B.L. Harding*, M.Z. Mays*, N.J. Jaax*, and J.A. Romano. U.S. Army Medical Research Institute of Chemical Defense, APG-EA, MD 21010-5425.

A single exposure to the organophosphonate soman may produce extensive neural damage, with lesions most severe in the hippocampus, septal nucleus and pyriform cortex. Since experimental lesions of either the septal nucleus or the hippocampus result in increased reactivity to various stimuli, this study examined whether rats exposed to convulsion-producing doses of soman would show similar changes in behavioral reactivity.

One week prior to soman exposure, baseline reactivity to an acoustic startle response was obtained for adult male rats (300 to 350 g), using a Columbus Instruments Responder IV apparatus. All rats were given 40 habituation trials, with each trial consisting of a 100-db 1000-Hz tone delivered for 500 msec. On the day of soman exposure, the rats were randomly divided into 3 groups and injected sc with saline or with 70 or 100 ug/kg of soman. Using the same test procedure as in the baseline phase, 10 rats from each group were tested at one of the following times: 3 hrs, 1 day, 3 days, 7 days, 1 month, 3 months or 6 months after exposure. After each post-exposure test, subjects were perfused with formalin, and the brains removed and weighed.

Exposure to 70 ug/kg soman resulted in approximately 30% lethality at 7 days, while administration of 100 ug/kg soman produced approximately 70% lethality. Among the surviving soman-exposed animals, significant changes in reactivity to the auditory stimulus were seen. Animals given either dose of soman were less reactive 1 and 3 days post-exposure, an effect probably due to general debilitation. But at 7 days post-exposure and at all later intervals, the reactivity of animals receiving 70 ug/kg soman was no different from controls. However, animals receiving 100 ug/kg soman were significantly more reactive to the stimulus at all test times after 3 days. These animals also did not habituate to the tone as rapidly as the controls.

Soman exposure also resulted in significant differences in brain weight. At 3 hrs post-exposure both soman-exposed groups displayed a nonsignificant tendency towards heavier brains than the controls. However, rats receiving 100 ug/kg soman and tested at 3 days post-exposure and later had significantly lighter brains than did the saline controls. These animals also displayed severe necrosis, mineralization and melacia throughout the limbic system and thalamus. Animals with severe pathology in the hippocampus and septal nucleus displayed greater levels of hyperreactivity. The correlation between the behavioral and pathological results confirm previous informal observations and demonstrate permanent irreversible deficits due to a single exposure to soman.

329.3 EFFECT OF GAMMA-HEXACHLOROCYCLOHEXANE (LINDANE) ON CEREBELLAR CYCLIC GMP. B.E. Fishman* and G. Gianutsos. Section of Pharmacol. & Toxicol. U. Connecticut, Storrs, CT 06268.

The active isomer of hexachlorocyclohexane (HCH), the gamma isomer commonly called lindane, has been used as a pesticide for a variety of purposes. Toxicity resulting from overexposure in mammals, including humans, is manifested by seizure activity. Other isomers (e.g., delta) are not useful as pesticides and are not convulsant agents. Recent work has attempted to define the mechanism of action of lindane. Lindane has been shown to inhibit the binding of radioligands for the GABA-mediated chloride channel in rat brain (Lawrence and Casida, Life Sci. 35:171, 1984). Other agents known to be antagonists at this site or at other sites of the GABA-receptor complex have been shown to increase cyclic GMP in the cerebellum (Mao et al., Arch. Pharmacol. 289: 369, 1975; Mattsson et al., Nature 268:52, 1977). In order to examine the effects of HCH isomers on cyclic GMP, mice were treated with gamma of delta HCH and sacrificed by microwave irradiation 1 hr after administration. Cyclic GMP was measured by radioimmunoassay using a commercially available kit. Lindane dose-dependently increased the accumulation of cyclic GMP in the cerebellum after IP injection of 40 - 120 mg/kg, while no increase in cyclic GMP was observed after a 400 mg/kg IP dose of delta HCH. The delta HCH also failed to induce seizure activity. In a related study, strychnine, a convulsant acting via glycine receptors, induced a significant increase in cyclic GMP at doses which induced seizures, but the magnitude of effect was smaller than that observed after lindane or picrotoxin, a convulsant drug believed to act at the GABA-mediated chloride channel. Since both lindane and picrotoxin (but not strychnine) inhibit the binding of 3H-TBOB, a ligand for this site, it is suggested that the elevation in cerebellar cyclic GMP observed after lindane may be due to an inhibitory effect at the GABA-mediated chloride channel. The dose-dependent increase in cerebellar cyclic GMP produced by lindane, combined with the lack of effect of larger doses of delta HCH suggests that this response is due to a specific (receptor) interaction and not to a general membrane effect. The results suggest that the increase in cerebellar cyclic GMP may play a role in the seizure activity manifested by lindane.

329.4 EFFECTS OF SOMAN OR PYRIDOSTIGMINE ON PRIMATE EQUILIBRIUM PERFORMANCE AND BLOOD CHOLINESTERASE. D. W. Blick*, M. R. Murphy*, G. C. Brown*, M. G. Yochmowitz*, and S. L. Hartgraves. Systems Research Laboratories, Inc. (DWB, MRM, & GCB) and Radiation Sciences Division, USAF School of Aerospace Medicine, Brooks AFB, San Antonio, TX 78235-5301.

Changes in serum ChE and in performance of a compensatory tracking task (Primate Equilibrium Platform, PEP) were measured following acute exposure of Rhesus macaques to an organophosphate (Soman) or a carbamate (Pyridostigmine) anticholinesterase administered by intramuscular injection. The PEP performance measure, the adjusted root-mean-square (i.e., σ) of platform position, was observed for five 5-min periods prior to and for fifteen 5-min periods after injection. The limits of normal performance were established through the use of baseline (placebo injection) runs and the Lieberman & Miller method of simultaneous tolerance limits ($P=.95$, $\alpha=.05$).

For Soman, the threshold dose producing performance decrements was determined by the up-and-down method. Eight monkeys were each exposed to a single dose (2.21 to 3.50 µg/kg). ED50 was 2.50 µg/kg (95% confidence limits: 2.30 to 2.72 µg/kg). The ED50 dose produced 69±3.5% inhibition of serum ChE measured 2 hr after exposure. Recovery of ChE activity was slow (half-time approximately 7 d), while performance generally recovered in 24 to 48 hr. The dose-effect function was very steep -- no performance decrement could be detected at 2.21 µg/kg, while 3 of 3 animals showed obvious decrements at 2.78 µg/kg.

For Pyridostigmine, eight monkeys each received four doses (.12, .24, .48 & .96 mg/kg) at one-week intervals. Peak serum ChE inhibition occurred about 30 min after exposure; recovery of ChE activity was essentially complete in 24 hr. The ED50 dose for performance decrement was estimated (method of splines) to be 0.66 mg/kg. This dose would produce approximately 77±3% inhibition of serum ChE.

Studies of the performance effects of drug combinations (soman + atropine and pyridostigmine pretreatment + soman + atropine) are currently in progress.

329.5 DISCRIMINATION-REVERSAL LEARNING IN OFFSPRING OF RHESUS MONKEYS (M. Mulatta) EXPOSED TO LOW LEVELS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN. S. L. Schantz*, R. E. Bowman, S. A. Ferguson*, H. C. Van Valkenberg* and K. M. Boehm* Harlow Primate Laboratory, University of Wisconsin, Madison, WI 53715

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic environmental contaminant which is lipophilic and poorly metabolized. Following maternal exposure, TCDD accumulates in adipose tissue and is mobilized and transferred to the developing organism during gestation and lactation. It is well documented that TCDD doses well below those toxic to the mother are fetotoxic and teratogenic to the offspring. However, the neurobehavioral effects of perinatal TCDD exposure have not been extensively studied.

We evaluated the long-term effects of perinatal TCDD exposure on cognitive behavior of rhesus monkeys. Adult female rhesus monkeys were exposed to 0, 5 or 25 parts per trillion (ppt) TCDD in their diets for approximately 4 years. The females were bred to unexposed males after 7 and 26 mo. of TCDD exposure (Cohort I and II offspring), and again 10 mo. after the end of TCDD exposure (Cohort III offspring). All infants were weaned at 4 mo. of age. The 25 ppt females gave birth to too few viable offspring to contribute to Cohorts I and II which included 0 and 5 ppt offspring only. Cohort III included 0, 5 and 25 ppt offspring.

Cohorts I and II were tested on 4 discrimination-reversal learning (DRL) problems in the Wisconsin General Test Apparatus at 14 mo. of age. Cohort III was tested on the same series at 16 mo. of age. Each problem consisted of an acquisition phase followed by a series of reversals. An initial spatial problem with no secondary stimuli and a spatial problem with color and shape as irrelevant stimulus dimensions were followed by two problems in which first color and then shape became the relevant stimulus dimension. The TCDD-exposed offspring of Cohorts I and II were not impaired in their ability to perform the spatial or color discriminations, but did show performance deficits on the shape discrimination. Cohort III is currently being tested on this problem series.

Perinatal exposure to polychlorinated biphenyls, which are structurally and toxicologically similar to TCDD, has been found to have very long-term effects on neurobehavioral function. Therefore, Cohort I was retested on another series of DRL problems at 64 mo. of age (young adulthood). The series included spatial, color, shape and size discriminations. No differences between the TCDD and control offspring were seen on any of these later discriminations. Cohort II will also be tested on this DRL series when they reach 64 mo. of age.

329.6 EFFECT OF NEUROLEPTIC MEDICATION ON THE REACTION TIME (RT) RESPONSE IN SCHIZOPRENICS. P.B. Vrtunski, D.M. Simpson#, K.M. Weiss#, and H.Y. Meltzer. Cleveland VA Medical Center, Brecksville OH 44141.

We have recently reported a difference in the motor component of a choice RT response between schizophrenic and nonschizophrenic subjects (Psychiatry Research, in press). Motor response trajectories in schizophrenics were longer and contained more motor discontinuities. Since most studied schizophrenics were receiving neuroleptics, the possibility that drug effects contributed to this difference could not be ruled out.

In the present follow-up study, the choice RT response was examined in three groups of inpatients: Nonpsychotic controls (N=20), drug-free schizophrenics (N=15) and neuroleptic-treated schizophrenics (N=16). Both pictures and spoken words were used as stimuli for the choice RT task. Three dependent variables were extracted from the analog record of force exerted on pressure transducers by the subjects' index fingers: premotor time (interval from stimulus onset until the initiation of response), motor time (from response initiation until the response criterion was attained), and number of motor trajectory discontinuities during the ascending portion of the motor response.

For all three dependent variables, the largest deficits were observed in medicated schizophrenics. For the three groups of controls, nonmedicated and medicated schizophrenics, the observed average values were premotor times: 771, 1019 and 1172 ms, respectively ($p<.0001$); motor times: 238, 318, and 420 ms, respectively ($p<.0001$); number of trajectory discontinuities: 2.9, 4.5 and 6.3 velocity changes per response, respectively ($p<.01$). The two stimulus conditions also were significanly different for all three dependent variables with words generating higher values than pictures. The two motor variables of motor time and number of trajectory discontinuities generated significant group x stimulus condition interactions, as well.

Post hoc analysis demonstrated that nonmedicated schizophrenics differed significantly from both control subjects (in all three dependent variables) and from medicated schizophrenics (in two motor variables). Therefore, we conclude that although neuroleptic medication appeared to have an effect on the motor component of the RT response, drug-free schizophrenics still exhibited abnormalities similar to those we have previously reported.

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329.7 CHARACTERISTICS OF ORAL DYSKINESIAS IN NEUROLEPTIC-TREATED RATS AS MEASURED BY TWO DIFFERENT ASSESSMENT TECHNIQUES. R.E.See, E.D.Levin, and G.D.Ellison. Dept.of Psychology, UCLA, Los Angeles, CA 90024.

Chronic treatment with neuroleptics has been shown to produce a syndrome in rodents characterized by an increase in spontaneous oral movement activity. It has been suggested that this may provide a valid animal model for tardive dyskinesia, however, it has been reported that these vacuous chewing movements develop too early following drug treatment to qualify as tardive and are normal in appearance and cannot be described as being dyskinetic in character.

We have developed a means of precisely measuring oral activity by computerized analysis of video images to achieve more objective recordings of neuroleptic-induced changes. The present study compared this technique (in which rats are partially restrained in a plexiglas tube) with the older technique of human observer recorded activity of the rats in a larger observation cage. Rats were administered chronic fluphenazine decanoate, haloperidol decanoate, or vehicle with injections occurring once every 3 weeks for four months. After a two month period of withdrawal, injections were reinstated. Oral movement activity was monitored both prior to and just after each drug injection.

Human observer scores showed a significant increase in vacuous oral movements after 4 months of treatment. The difference between controls and drug-treated animals was even greater at the end of the drug-free interval. Reinstatement of drug treatment caused a decrease in oral movement activity, but drug-treated animals remained significantly higher than controls. Initial computer scored results showed an acute sedation effect in both drug treated groups as measured by total number of oral movements. These animals also showed more aluggish openings and closings of the jaws as seen by the average slope of each individual movement. The computer records indicate that each animal has a characteristic type of oral movement and that changes in the form of these movements over time may indicate the development of a qualitatively dyskinetic syndrome.

These two assessment techniques provide different aspects of neuroleptic-induced oral dyskinesia in rats. The results obtained give additional support for the development of a tardive dyskinetic syndrome, which can be characterized by both quantitative increases in oral activity as well as more subtle changes in the type of oral movements seen in neuroleptic-treated animals.

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329.8 DYSKINETIC ORAL MOVEMENTS IN GUINEA PIGS FOLLOWING CHRONIC NEUROLEPTIC TREATMENT. D. L. Weinstein,* G. D. Ellison, E. D. Levin, and R. E. See. (SPON: D. Novin). Dept. of Psychology, UCLA, Los Angeles, CA 90024

The present study examined the effects of chronic fluphenazine decanoate on oral movement in guinea pigs. The guinea pig is ideal as an animal model of tardive dyskinesia (TD). It has an extremely well articulated oral region and develops a clear supersensitivity response to chronic neuroleptic treatment. We have studied in detail the changes in orofacial behavior in guinea pigs as a result of chronic fluphenazine decanoate treatment. TD-like oral movements ("vacuous" movements) were significantly elevated in the fluphenazine-treated animals following 4 months of treatment ($F=4.99$, $df=1,30$, $P<0.01$). The "low" and "high" groups received an injection of 0.07 mg/kg/day and 0.2 mg/kg/day fluphenazine decanoate respectively once every two weeks. Oral movement was recorded by both a computerized movement detection circuit and human observation.

Following 9 months of chronic treatment, the fluphenazine dose was increased to 1 mg/kg/day for both drug-treated groups and administered once a month. Oral movement was suppressed immediately following the dose increase. This effect mimics the human TD condition. However, a week later, vacuous movements did not appear as previously observed. Rather, small tick-like oral movements characteristic of twitching were elevated in the fluphenazine-treated animals (not significant). Human observation demonstrated a drug dependent effect on twitching behavior ($F=4.48$, $df=1,27$, $P<0.05$). In guinea pigs, chronic neuroleptic treatment appears to alter the form of TD-like oral activity over time, The initial occurrence of vacuous movements gradually declines and is eventually replaced by twitching movements. Twitching behavior is dyskinetic in nature which is consistent with the dyskinetic oral activity seen in human TD patients. In addition, twitching movement appears late in the course of treatment which is consistent with the time course of human TD. Additional testing following drug withdrawl will help to elucidate the various forms of oral activity in rodents which model human TD.

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329.9 BOTH NUCLEUS ACCUMBENS AND PERIAQUEDUCTAL GREY SITES PARTICIPATE IN MAINTENANCE OF OPIATE SELF-ADMINISTRATION. W.A. Corrigall* and F.J. Vaccarino (SPON: J.M. Khanna). Addiction Research Foundation of Ontario, Toronto, Canada M5S 2S1 and Department of Psychology, University of Toronto, Ontario, Canada M5S 1A1.

One of the first steps required in specifying brain substrates for opiate abuse is a mapping of the distribution of opiate receptor sites underlying venous self-administration. To this end different investigators have shown an involvement of receptors in the ventral tegmental area (VTA) and in the nucleus accumbens (n.ACC), using microinjections of opiate antagonists into these sites to block agonist binding locally. We are using a similar technique to determine the distribution of other brain sites involved in maintenance of low-dose i.v. heroin self-administration in rats. To this end we have examined two sites, the n.ACC in order to confirm an earlier report of its involvement, and the periaqueductal grey. The latter site was chosen because it has been implicated in both opiate reward and physical dependence.

Animals were prepared with chronic venous catheters and, following recovery from surgery, were allowed a period of 5-10 daily 180-minute sessions to acquire heroin self-administration at a dose of 0.06 mg/kg/infusion on an FR1 schedule. Animals were then prepared with brain cannulae directed at the target sites, and again allowed access to heroin in daily (Mon-Fri) sessions. On treatment days, animals received a bilateral infusion of the quaternary opiate antagonist methyl naltrexone, administered as a volume of 0.5 microl./side over a period of 2 minutes; doses of 0, 0.1, 0.3, 1.0 and 3.0 micrograms of antagonist were used.

In the n.ACC, these treatments caused dose-dependent increases in the number of heroin infusions taken by the animals, with prominent effects at the beginning of the session and, in addition, increases in the number of responses occurring during the time-out period following each drug delivery. This increase in response rate is interpreted as being a compensatory response of the animal to a decreased effectiveness of the reinforcer due to the action of the antagonist within the n.ACC.

In the PAG, antagonist pre-treatments also caused an increase in drug-appropriate responding with, however, a different time course of effect than in the n.ACC; in the PAG there was an initial suppression of responding followed by increases later in the session. These results are not interpreted as reflecting antagonist precipitated hyperactivity because there were no sustained trends on the activity control lever resulting from antagonist pretreatment. Rather, the data is interpreted to mean that both n.ACC and PAG sites participate in maintenance of opiate self-administration.

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329.10 NEONATAL CAPSAICIN MODIFIES MORPHINE WITHDRAWAL SIGNS IN THE RAT. L.G. Sharpe and J.H. Jaffe*. Addiction Res. Ctr., Nat. Inst. on Drug Abuse, Baltimore, MD 21224

Rats received injection of either capsaicin (50 mg/kg, s.c.) or the capsaicin vehicle at 2 days of age. When the animals were 90-120 days of age they were implanted with morphine or placebo pellets (s.c.) for 3 (1 pellet) or 6 (3 pellets) days. Naloxone (0.4 mg/kg, s.c.) produced no effects in the placebo pelleted groups but elicited salivation, lacrimation and rhinorrhea in the morphine-treated animals. These abstinence signs were less severe in the morphine pelleted rats (3- and 6-day groups) that were treated as neonates with capsaicin. However, the capsaicin treated animals that were implanted with morphine pellets exhibited more naloxone-precipitated wet-dog shakes than the vehicle controls; in the case of the 3-day but not the 6-day pelleted group the increase was statistically significant. Since capsaicin is known to induce long lasting depletion of substance P and other peptides in peripheral neurons, it was concluded that substance P may be involved in the expression of some signs of opioid withdrawal.

329.11 TOLERANCE AND SENSITIZATION TO MORPHINE IN RESTRAINED AND FREELY-MOVING RATS. K.S. Schwarz & C.L. Cunningham*. Dept. of Medical Psychology,Oregon Health Sciences Univ.,Portland,OR 97201.

Morphine causes an initial bradycardia followed by tachycardia when administered intravenously to rats. Restraint-stress augments the bradycardia and attenuates the tachycardia. The present study assessed tolerance and sensitization to the heart-rate effects of various doses of morphine in restraint-stressed and freely-moving rats.

Male albino rats were assigned to one of four dose groups: 0 (saline), 2, 4 or 8 mg morphine/kg body weight. Within each dose group there were both restrained (R) and unrestrained (U) rats matched in pairs by weight. The experiment consisted of two main phases: drug exposure and tolerance test. During drug exposure, all rats received one infusion daily for 12 days according to dose group assignment. During the tolerance test, rats were exposed to each dose on different days in a counterbalanced order. Magnitude of tolerance was assessed by comparing the responses of the saline groups with those of the drug groups. Heart rate was recorded for 1 hr before (baseline) and 2 hrs after infusion.

In general, heart rate was elevated initially due to handling and decreased over the pre-infusion baseline hour. Over the drug exposure phase, mean baseline heart-rate declined slightly in Dose Groups 0 and 2. In Dose Groups 4 and 8, mean baseline heart-rate increased and was significantly higher relative to Dose Groups 0 and 2. Upon morphine infusion, heart rate decreased, then increased in a dose-dependent manner. Over the drug exposure phase, a significant attenuation in the magnitude of bradycardia occurred in all morphine dose groups except Group R8. Group U8 also showed a significant attenuation in the magnitude of tachycardia; however, no significant change in tachycardia occurred in any other group.

During the tolerance test phase, separate comparisons among the R and U groups were made. Tolerance to the bradycardic effect was revealed in Groups R4, R8, U2 and U4, but not in Groups R2 and U8. Sensitization to the tachycardic effect was revealed in Groups U2, U4 and U8 but not in any of the R groups.

These results are consistent with Solomon and Corbit's (1974) opponent-process theory which states that for a given response (e.g., bradycardia) to a stimulus (morphine), there is an opposing response (tachycardia) generated which serves to decrease the magnitude of the first response. Chronic morphine exposure leads to strengthening of the tachycardia, reflected as a decrease in bradycardia (tolerance) and an increase in tachycardia (sensitization). In Dose Groups 4 and 8, expectation of drug effects may have resulted in the occurrence of tachycardia before morphine administration, implicating learning in the development of tolerance.(Funded by NHLBI #HL07332, N.L. Tartar Research Fund & NIDA #DA03608)

329.12 NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF CHRONIC NALTREXONE TREATMENT IN RATS. M. T. Bardo and J. L. Neisewander*. Dept. of Psychology, University of Kentucky, Lexington, KY 40506.

Adult male rats were implanted with a slow-release pellet of naltrexone that was removed 10 days later or were given sham surgeries. Twenty-four hours after pellet removal, specific binding of the mu-receptor radioligand ^{3}H-DAGO (4 nM) was assessed. The results showed that naltrexone pretreatment increased specific DAGO binding relative to sham-treated controls. Administration of morphine (15 mg/kg, s. c.) did not enhance specific DAGO binding in either naltrexone or sham-treated animals.

To assess the effect of chronic naltrexone treatment on dopaminergic activity, rats were administered morphine (15 mg/kg, s. c.) or saline on the day after pellet removal. Thirty min after this injection, animals were either sacrificed or injected with the tyrosine hydroxylase inhibitor alpha-methyltyrosine (250 mg/kg, i. p.) in order to assess functional dopamine turnover. Animals that were injected with alpha-methyltyrosine were sacrificed at either 60 or 120 min after alpha-methyltyrosine administration. Levels of dopamine and the metabolite dihydroxyphenylacetic acid (DOPAC) were measured in striatum using high-pressure liquid chromatography with an electrochemical detector. As expected, morphine increased striatal DOPAC levels. However, naltrexone pretreatment did not alter significantly this morphine-induced neurochemical effect. Further, naltrexone pretreatment did not alter significantly the level of dopamine in the striatum, indicating that functional turnover was not altered.

We also assessed the effect of chronic naltrexone treatment on the reinforcing efficacy of morphine using the single-trial place preference procedure. One day after pellet removal, half of the rats received morphine (5 or 8 mg/kg, i. v.) or saline and were placed in a white compartment for 30 min post-injection. The other half were placed in a black compartment for 30 min without drug. On the second day, the animals received the alternative treatment as that given on the first day. The third day, animals were given free-access to both environments simultaneously for 15 min. The results show that naltrexone-treated rats spent significantly more time in the morphine-associated compartment than sham-treated rats conditioned with morphine, although this effect did not reach statistical significance in a subsequent replication. Supersensitivity to the rewarding efficacy of morphine was apparent at 5 mg/kg, but not at 8 mg/kg morphine, following naltrexone pretreatment. (Supported by USPHS grant DA03460).

329.13 ROLE OF METABOLISM IN THE DEVELOPMENT OF TOLERANCE TO METHAMPHETAMINE (IN BIOCHEMICAL SYSTEMS), M. Alburges*, G. R. Hanson, J.W. Gibb, (SPON: J.W. Woodbury) Dept. Biochemical Pharmacology and Toxicology, College of Pharmacy and School of Medicine, University of Utah, Salt Lake City, UT 84112

We have previously observed (Schmidt et al., 1985) that pretreatment with gradually increasing doses of METH attenuates the effects of multiple doses of METH on the dopaminergic and serotonergic systems normally observed in naive rats. In order to understand the mechanism of this tolerance, untreated rats (naive) and rats pretreated with METH (2.5, 5.0, 7.5 and 10 mg/kg, s.c., 6-hr intervals with a 24-hr drug-free period between each increase in dose) were challenged with 5 doses of METH (15 mg/kg, s.c., 6-hr intervals). The metabolism of METH in brain, liver, and blood was studied by measuring the concentration of METH and its metabolites 2,4,6,8 and 10 hr after the last drug dose by gas chromatography/mass spectrometry techniques. Samples were homogenized in 0.1 M perchloric acid and extracted into n-butyl chloride. Pseudoephedrine and phentermine were used as internal standards; 3-fluoroacetylated derivatives were formed and injected onto a 12.5-meter cross-linked dimethylsilicone (0.2 mm) capillary column.

The forebrain concentrations of METH in the pretreated animals were significantly lower than those observed in the forebrain of naive animals. Liver concentrations of METH in the pretreated animals were not significantly changed with respect to the naive animals, but amphetamine and the p-hydroxylated metabolites, p-hydroxyamphetamine (p-OH-AMP) and p-hydroxymethamphetamine (p-OH-METH), were significantly greater than those observed in the naive group. Blood levels of METH and AMP were also significantly increased in the pretreated animals.
(Supported by USPHS grant DA 00869).

329.14 **CHRONIC AMPHETAMINE TREATMENT: EFFECTS ON BEHAVIOR AND LOCAL CEREBRAL GLUCOSE UTILIZATION.** **Michael R. Kozlowski, Lisa Condon*, Dori Fern*, Judy Houde*, Edward Martindale* and Robert Sklans*. Pfizer Central Research, Groton, CT 06340 and Dept. of Psychology, Connecticut College, New London, CT 06320.**

Chronic amphetamine ingestion in humans produces symptoms of endogenous psychosis, and therefore may represent a model of schizophrenia. The present study examined the effects of chronic amphetamine treatment on both behavior and local cerebral glucose utilization (LCGU) in the rat.

Fourteen male, Sprague-Dawley rats housed communally were observed for 2 hr daily, and rated in each of the following behavioral categories: withdrawal, social behaviors, startle, stereotypy and activity. After 1 week, the animals were divided into 2 groups and implanted with mini-osmotic pumps which delivered either 0.5 mg/kg/hr of amphetamine, or a similar volume of the vehicle (saline). The animals were rated for the next 3 weeks by observers who were blind to which treatment each group had received. LCGU was then measured in each animal by the technique of Sokoloff et al.(J. Neurochem. 28, 897, 1977).

During the first week of post-implantation observations, the amphetamine-treated rats showed a significant elevation of activity (t-test, $p < .05$), and an almost significant increase in stereotypy ($p = .05$) compared to the vehicle group. During the second and third weeks (combined data) the activity of the amphetamine-treated animals was still elevated relative to the vehicle group, but the stereotypy score was now at vehicle level. Other behaviors did not differ significantly at any time. When the LCGU values for the animals given amphetamine (n=4) or vehicle (n=5) were compared, 70% of the areas measured had elevated values (2-18%) in the amphetamine group. The increases in the olfactory tubercle, primary olfactory cortex, anterior nucleus accumbens septi, lateral prefrontal cortex and olivary nucleus were statistically significant (t-test, $p<.05$).

The persistence of hyperactivity but not of stereotypy and the pattern of changes in LCGU observed after 3 weeks of amphetamine treatment suggest a primary effect on limbic brain, which is consistent with the psychotomimetic effects of chronic amphetamine ingestion in man.

330.1 PHORBOL ESTER INDUCED CONRTACTURE OF THE MUSULATURE OF SCHISTOSOMA MANSONI. K.L.Blair, J.L.Bennett* and R.A.Pax. Zoology, Mich. St. Univ.,E.Lansing, MI 48824.

Experiments were conducted to assess the potential role of the protein kinase-C (PKC) in muscle conrtactility of the parasitic trematode Shistosoma mansoni. Various phorbol esters were used to stimulate PKC. β-12-O-tetradecanoyl-13-acetate (TPA), β-phorbol-12,13-dibutyrate, and β-phorbol-12,13-didecanoate produced dose dependent increases in muscle tension through the ranges tested (1 nM - 10 μM). This is in contrast to reports of no response or relaxation in vertabrate muscle preparations. Mezerin, another activator of PKC, also produced, at 10 μM, an increase in tension. α-Phorbol and α-phorbol-12,13-didecanoate were without effect on the musculature. The calcium ionophore, A-23187, produced a dose dependent increase in tension. Contractures induced by either TPA (100 nM) or A-23187 (10 μM) were reversed by carbachol (100 μM), but when both compounds were present, the increased tension was not reversed by the carbachol. Tamoxiphen, an antiestrogenic with anti-PKC activity, caused a relaxation of the musculature at 100 μM. 17-β-Estradiol(100 nM) was without effect on the musculature. DMSO (.1 - 1%), dibutyryl C-AMP (100 μM), and dibutyryl C-GMP (100 μM) were without effect on the contractility of the musculature. These results strongly indicate that PKC is involved in the regulation of muscle contractility and tension in this organism, but that the physiological role it plays may be different from that in vertabrate muscle.

330.2 CENTRAL CONTROL OF CILIARY ARREST IN THE ASCIDIAN CHELYOSOMA PRODUCTUM. S.A. Arkett*, (SPON: D. Paul). Dept. of Biology, Univ. of Victoria, Victoria, B.C. V8W 2Y2 Canada

Ascidians circulate water by ciliated stigmata of the branchial basket and feed on particulate matter suspended in these currents. Water enters the branchial siphon, passes through ciliated stigmatal openings, and exits through the atrial siphon. 'Foreign' material causes a basket-wide arrest of cilia beating. Electrical activity associated with ciliary arrests (ciliary arrest potentials, CAPs) has been recorded from Corella willmeriana (Mackie, G.O., et al. Proc. R. Soc. Lond. B., 187:1,1974). Ciliary arrest is often accompanied by rapid contractions of siphons, mantle, and branchial sac, sometimes resulting in a rapid ejection of water from the siphons (MacGinitie, G.E., Biol. Bull., 77:443, 1939). Coordination of ciliary arrest and siphon contraction is thought to be under CNS control, however, the lack of information on neuronal organization in ascidians has precluded any conclusions about the control of these effector systems. I describe a new preparation for physiological and morphological identification of neurons, which are involved in ciliary arrest and siphon muscle contractions, in the central ganglion of Chelyosoma productum.

The central ganglion, located between the two siphons and dorsal to the branchial basket, is exposed in a semi-intact animal and treated with collagenase to allow microelectrode penetration of individual cells for recording and Lucifer Yellow injection. Cells to date are putative motorneurons. One cell (RP -60mV) spikes 1:1 with CAPs recorded extracellularly from the branchial basket. This cell is rarely spontaneously active. Brief stimulation of one branchial medial root results in one to three action potentials (over 10s period) of 50mV amplitude and 40ms duration followed by a 5-10mV hyperpolarized phase lasting 1-3s. LY fills show a cell body (15-20 μm diameter) with a single, unbranched axon exiting the ganglion via one atrial medial root. Intracellular recordings from ciliated stigmatal cells (RP -40mV) show PSPs with a 20-35mV depolarized phase lasting ca. 400ms. PSPs are time-locked, 1:1 with CAPs. Branchial ciliary arrest is directly associated with these depolarizations. The depolarization in ciliated cells: 1) appears to be a post-synaptic event, produced by the identified central cell, 2) may spread either passively or actively through gap junctions connecting ciliated cells thereby causing ciliary arrest.

Supported by NSERC grant to G.O. Mackie.

330.3 NEURAL SUBSTRATES OF LOCOMOTION IN HERMISSENDA. E.M.Schuman, J.Farley, W.G.Richards. Prog. in Neurosci. & Behavior, Princeton University, Princeton, NJ 08544.

Locomotion in many molluscs is accomplished by means of either a pedal wave or by ciliary action. Previous studies of phototaxic behavior in Hermissenda have failed to establish which peripheral mechanism the animal is using, and instead have chosen to characterize the behavior in terms of its latency to commence and its velocity once initiated. We have analyzed high-resolution videotapes of magnified portions of the ventral surface of the animal's foot, while engaged in spontaneous and light-evoked locomotor behavior, and find that the characteristic mode of locomotion involves a pedal wave. During locomotion, longitudinal muscles which raise and move forward successive portions of the foot contract. These movements are accompanied by peristaltic-like contractions and expansions of the peripheral edges of the foot. In semi-intact, restrained animals, electrical stimulation of the two major pedal nerves (P1 & P2; Richards & Farley, Soc. Neurosci. Abstr., 1985) which innervate the pedal musculature revealed that stimulation of nerve P1 resulted in contractions of the longitudinal muscles of the posterior three-quarters of the foot, while stimulation of nerve P2 resulted in localized contractions of the anterior periphery of the foot.

Four new classes of putative motorneurons, with cell bodies in the pedal ganglia, have also been discovered and have been studied by simultaneous recordings of whole-nerve multiple unit activity (MUA) and intracellular recordings from putative MNs. Motorneuron 5 cells exhibit a transient light response but do not burst. Motorneuron 6 cells also display a transient light response but burst in phase with nerve P2. Motorneuron 7 cells undergo a marked increase in impulse activity during light and burst in phase with MUA activity in nerve P2. Motorneuron 8 cells are inhibited by light, burst in phase with P2 and exhibit a marked hyperpolarization between bursts. We have begun to relate the pattern of whole-nerve activity to the pedal wave with the aim of providing a more complete description of how locomotion is accomplished in this animal, and how associative training reduces phototaxis.

Supported by NSF BNS-8316707 to J.Farley.

330.4 NEUROMUSCULAR CONTROL OF UNSTEADY FLOWS BY DRAGONFLIES. M. Kliss* and M.W. Luttges. Aerospace Engineering Sciences, University of Colorado, Boulder, Colorado 80309.

The remarkable aerial maneuvers of dragonflies have been shown to rely on the utilization of unsteady separated flows. Such flows are both transient and spatially disparate. Specific constraints on flight rhythm are exacted by the use of unsteady flows. The present investigation focuses on the mechanisms through which the neuromuscular system can be coordinated to utilize wing interactions with these relatively complex flows. Understanding the operations of the centrally generated flight program is the goal of these studies. High speed 16 mm movies of restrained dragonfly wing kinematics demonstrate a spatial and temporal synchronization of both tandem wing sets with periodic vortical structures. Integrated force balance measurements correlated wing kinematics instances with high peaks of lift generation. Analysis of the wing box kinematics revealed that agonist and antagonist muscles act on opposite sides of a wing base pivot point to produce wing elevation and depression. Displacements and tilting of the scutum and sclerites are primarily responsible for changes in pronation and supination. Direct electrical stimulation of thoracic muscular systems show that gross wing kinematics are relatively robust, arising from a combination of peripheral musculature and mechanics. More subtle kinematics, such as phase angle between fore and aft wings, can be driven at 0° - 180° phase angles. Basic feedback controls also produce only minor modifications in wing kinematics. Removal of encephalization influences led to larger amplitude electrically-elicited wing responses. Flight episodes were of longer duration than those of an intact insect, but were more difficult to elicit. Histologic techniques were employed to determine populations, locations and functional connections of cell bodies for the main flight motor neurons. Graphic reconstruction provided a simplified wiring diagram for the cell bodies. Final motor path roles of the larger cells have been partially identified. Electrical stimulation of the meso and metathoracic ganglia elicited coordinated wing responses through a wide range of frequencies. High voltage stimulation levels produced complete flight episode kinematics that did not correspond to the stimulation frequency or phase relationship of the electrical stimuli. Initial extracellular recording studies reveal a relatively complex relationship between flight neuron firing patterns and the resultant driving of the flight musculature. These findings are compared to those known for flight control mechanisms of the locust. Unlike the locust, where details of the fluid-wing interactions are not well established, the results in the dragonfly can be related to the fluid dynamics that support the observed flight modes.

330.5 BREATHING IN FAT AND THIN LOCUSTS: ADAPTATIONS BY VENTILATION MOTOR PATTERN GENERATORS TO ALTERED MECHANICAL PROPERTIES OF THE ABDOMEN. S. F. Giszter* (SPON: E. Bizzi). Dept. Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

The principle breathing mechanism of resting locusts is a dorsoventral pumping of the tergum and sternum. This motion is produced by two antagonistic muscle groups in each segment. The motoneurons controlling these are driven by a master oscillator in the metathoracic ganglion by way of local segmental oscillators in abdominal ganglia. The local segmental oscillators are networked together as phaselocked loops with delay lines. The delay line arrangement forms a phaselocked loop able to closely follow the metathoracic command transitions of expiration to inspiration as frequency varies. In this manner, the abdominal segments pump in tight synchrony.

In young adult locusts, the duration of the expiratory portion of the pattern covaries with the duration of the total breathing cycle, while inspiration is relatively fixed. In contrast, older locusts show the same general pattern, but inspiration covaries with breath cycle and expiration is short and fixed.

Young adult locusts can be switched to the "older" pattern by appropriate loading of the abdomen, which increase the degree of expansion of tergum relative to sternum, and the extent of inspiratory motion. This switching pattern seems to be an adaptation to increase of fat supplies and egg loads in older animals.

The switching of duration control based on load experienced in the abdomen may be achieved in a number of ways. An adjustment might be made local to a segment based on its individual load, though this would interfere with segment synchronization. Alternatively, all segments could be switched, simultaneously, by alteration of signals from the metathoracic oscillator. In this latter case, the metathoracic ganglion could switch patterns based either on its own state alone, or on combined information from all segments to obtain some "consensus" decision as to which pattern to use.

Evidence for which of these switching mechanisms is used will be discussed.

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330.6 SEGREGATION OF VISUAL INTERNEURONS ONTO DENDRITIC DOMAINS OF BLOWFLY DESCENDING NEURONS IS REVEALED BY TRIPLE FLUORESCENT STAINING. N.J.Strausfeld and H.S.Seyan*, EMBL, 6900 Heidelberg, West Germany.

In insects, descending neurons (DNs) arise in the lateral deutocerebrum of the brain and send their axons to motor centers of thoracic and abdominal ganglia. DN activity is thought to play a crucial role in determining the release and maintainance of motor programs.

The dendritic trees of most DNs are complex. It is thought that each part of the tree receives a typical pattern of inputs; primary afferents from receptors and sensory interneurons originating in sensory neuropils of the brain and body-ganglia. Previous neuroanatomical studies demonstrating the convergence of sensory neurons at DNs relied on double staining - cobalt or HRP backfills followed by Golgi impregnation. So far, these somewhat capricious methods have provided descriptions of sensory convergence onto two DNs only,both postsynaptic to endings of giant motion-sensitive interneurons and to certain small visual elements (Strausfeld,N.J. and Bassemir,U.K., *Cell and Tiss.Res.*,240:617,1985 & 242:531,1985). However, the vast majority of small-field visual interneurons have proven relatively refractive to conventional neuroanatomical techniques.

We have now simplified the analysis significantly using two dyes, Texas Red (Sigma, as 4% solution) and Lucifer Yellow (Sigma, as 6% solution), applying them consecutively into either living or formalin-fixed tissue. Texas Red is introduced first, by diffusion into cut axons filling the cells completely after 18h. Lucifer Yellow is next applied iontophoretically (10-30 min) from an electrode which is inserted "blind" into a selected area. A tip diameter of 1-4 µm is sufficient to damage axons of up to a dozen neurons so that they too are filled in their entirety. This is an advantage over intraneuropil application of cobalt or HRP. After dye filling, tissue is fixed in buffered formalin (1h) and then postfixed in the same but containing 1% of the blue fluorescing nuclear stain, Hoechst bisbenzimide H33458.

Using appropriate excitation filters for viewing serial plastic sections, we make multiple exposures onto positive film from which we reconstruct complex cell relationships. Perikarya of dye-filled neurons can be directly related to the intensely blue-stained nuclei of the perikaryal rind. Typically, clusters of DNs arise from groups of cell bodies. Each DN cluster receives the endings of up to ten classes of small-field visual interneurons each of which projects to a characteristic part of the DN dendritic field. Thus, a single DN can receive up to 50000 visual interneurons, each class comprising about one ninth of the total number of ommatidia of the compound eye. The retinotopic organization of the small-field interneurons is usually lost during their passage from the lobula to the target DN where they segregate out into functional classes. We are now further developing the method for combined light and electronmicroscopy.

330.7 PROCESSING OF PROPRIOCEPTIVE SIGNALS BY SPIKING LOCAL INTERNEURONES IN THE LOCUST. M. Burrows. Department of Zoology, Downing Street, Cambridge CB2 3EJ, England.

Proprioceptors monitor the movement of joints and the prevailing status of the muscles to provide a means for rapid corrections of the motor outflow. Such corrections are initiated at several levels in the nervous system so that the central effects of proprioceptive afferents should be widespread. The connections made by afferents from a particular proprioceptor at the femoro-tibial joint in a hind leg of a locust, the femoral chordotonal organ, were therefore determined by making intracellular recordings from motor neurones and spiking local interneurones in the central nervous system and from afferent cell bodies in the periphery. This receptor was chosen because its apodeme can be moved without stimulating other receptors.

Staining the central projections of the afferent neurones with dye introduced into their axons at the receptor, and the intracellular injection of dye into motor neurones and interneurones shows that the branches of all three classes of neurone overlap in specific regions of neuropile.

Afferents excited by an extension of the femoro-tibial joint excite flexor tibiae motor neurones and some spiking local interneurones. Afferents excited by a flexion movement excite extensor tibiae motor neurones and a different set of spiking local interneurones. Spikes in afferents that excite flexor motor neurones evoke depolarizing potentials that follow each spike with a consistent central latency of some 1.5ms. The amplitude of these potentials is dependent upon the membrane potential of the motor neurone and each can evoke motor spikes. This evidence points to the connection being direct and the potentials being EPSPs. Simultaneous recordings from certain spiking local interneurones and flexor motor neurones show them to be driven in a parallel fashion by movements of the receptor apodeme. Spikes of some afferents evoke EPSPs in both neurones with the same consistency and with the same latency. An afferent can therefore synapse directly upon a motor neurone and a spiking local interneurone. Each afferent synapses on several motor neurones and several interneurones. In turn each motor neurone and each interneurone receives inputs from several afferents.

These pathways therefore underlie classical resistance reflexes whereby an imposed movement is resisted. The direct connections with motor neurones form the excitatory limbs of the reflex loops. The parallel connections with the spiking interneurones could provide for the inhibitory limbs by virtue of the inhibitory connections of these neuroens with the antagonistic motor neurones or with nonspiking interneurones.

This work was supported by NIH grant NS16058.

330.8 INTRACELLULAR ANALYSIS OF THE SYNAPTIC ACTIVITY IN THE POSTURAL MOTOR NEURONS EVOKED BY MECHANICAL STIMULATION OF THE SWIMMERET IN THE LOBSTER ABDOMEN. V.C. KOTAK* and C.H. PAGE. Rutgers University, Bureau of Biological Research, Piscataway, NJ 08854

Using extracellular recordings we have shown that strong stimulation of the swimmeret produces tonic extension in the abdomen of the lobster (J. Comp. Physiol., 158:225-233, 1986). The present investigation employed intracellular recording to examine the synaptic responses of the postural motoneurons that are evoked by the swimmeret mechanoreceptors. The preparation consisted of the second and third abdominal ganglia with attached second left swimmeret. Almost all of flexor excitor motoneurons (f1,f2,f3,f4 but not f6) as well as the flexor inhibitor (f5) were spontaneously active while the extensors were quiescent. Motoneuron potentials were recorded intrasomatically with 5% Lucifer yellow filled microelectrodes. A 5g, 100ms stimulus generated by a probe (diameter 0.5mm) fixed to a speaker was used to stimulate localized swimmeret mechanoreceptors to study evoked synaptic potentials and latencies in the postural motoneurons. Stronger responses were generated by manual stimulation with a fine brush.

Mechanosensory stimulation of the swimmeret surface produced the following motoneuron responses: The peripheral flexor inhibitor f5 was excited by epsps which led to an increase in its spontaneous firing while the extensor inhibitor e5 was hyperpolarized. Swimmeret stimulation also induced large ipsps in the flexor excitors f2 and f3 and epsps in unidentified extensor excitors which often led to their spiking.

The extent of synaptic excitation and inhibition in these abdominal positioning motoneurons was dependent upon the kind of receptors stimulated as well as the intensity, frequency and duration of the stimulus. Stimulation of the smooth hairs (female), feathered hairs (both sexes) and cuticular surface (both sexes) elicited extension responses whereas bristly spine (male) stimulation excited the flexor excitors. The rise time, shape and amplitude of the touch evoked epsps differed according to the kind of mechanoreceptors stimulated. The synaptic potentials produced in motoneurons following stimulation of the swimmeret were initiated after a minimal latency of 25 ms.

The results agree with our previous observation that tactile stimulation of a swimmeret usually favors abdominal extension. The variations in synaptic potentials and latencies arising by stimulation of different mechanoreceptors may indicate differences in the neuronal pathways that affect the motoneurons. The occurrence of excitatory and inhibitory postsynaptic potentials after a 'long' delay (>25 ms) suggests that these motor neuron responses are mediated by postural interneurons in the abdominal nerve cord.

330.9 INTERSEGMENTAL INHIBITION OF ABDOMINAL POSTURAL RESPONSES EVOKED BY MECHANOSENSORY STIMULATION OF THE SWIMMERET IN LOBSTER. F.A. ABENANTE*, V.C. KOTAK* and C.H. PAGE (SPON: J. INSERRA). Rutgers University, Bureau of Biological Research, Piscataway, NJ 08854.

Intersegmental modulation of postural activity produced by mechanostimulation of a swimmeret was investigated in a preparation that included the second swimmeret attached to an isolated abdominal nerve cord. The effects of three successive stages of isolation of the second ganglion were examined: (1) intact abdominal nerve cord; (2) after sectioning the posterior connective between ganglia 2 and 3; (3) after totally isolating ganglion 2 by sectioning the anterior connective between ganglia 1 and 2. The responses of the tonic flexor motor neurons (f1-f6) in ganglion 2 were analyzed based upon their relative spike amplitudes and firing patterns in extracellular recordings.

In the intact nerve cord, tactile stimulation of the feathered hairs, which line the rami, never produced a postural response. In contrast, after sectioning the posterior connective stimulation of the feathered hairs elicited a flexion inhibition response--an increase in firing of the flexor inhibitor (f5) and a decrease in firing of the flexor excitors. Total isolation of the second ganglion by sectioning the anterior connective further increased the response. In the intact nerve cord, threshold stimulation (2.5g) of the swimmeret surface produced weak flexion inhibition; which included excitation of the flexor inhibitor f5 and inhibition of the flexor excitors. When the posterior connective was sectioned, stimulation of the swimmeret surface (2.5g) produced stronger excitation of f5 and greater inhibition of the flexor excitors. Complete isolation of the second ganglion further increased the firing of f5 and inhibition of the flexor excitors. The response of the flexor inhibitor f5 to constant strength stimulation increased as the second ganglion was progressively isolated. The threshold for an f5 response decreased following each stage of second ganglion isolation, changing from a 2.5g tap in the intact preparation to a 0.5g tap in the isolated ganglion.

These results indicate that intersegmental interneurons can modulate the neuronal responses to swimmeret mechanostimulation. Isolating the ganglion by cutting the connectives enhanced the sensory responsiveness by disinhibiting neurons that mediate the flexor responses initiated by stimulation of the swimmeret mechanoreceptors. This disinhibition is particularly striking in the case of feathered hair stimulation which does not affect flexor motor activity unless the ganglion is isolated from adjacent abdominal ganglia.

330.10 ANATOMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE PERIPHERAL SEROTONERGIC NEURONS INNERVATING THE STOMATOGASTRIC GANGLION. P.S. Katz and R.M. Harris-Warrick. Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

The stomatogastric ganglion of decapod crustacea contains 2 central pattern generators for stomach muscle movements. These CPGs (pyloric and gastric) are modulated by inputs from other parts of the nervous system. Serotonin has been shown to be a neuromodulator of the pyloric rhythm in lobsters and crabs (Beltz, B., et al., J.Exp.Biol., 109:35-54, 1984). We have now identified and characterized the source of the serotonergic innervation in *Homarus americanus*, *Cancer borealis*, and *Procambarus clarkii*.

We have shown, using anti-serotonin antisera (Immunonuclear), that all three species have bipolar cells that are situated in peripheral nerves and send serotonergic processes centrally (as described by Beltz, et al., for *Cancer irroratus*.) We have found that the axonal projections, but not the somata and dendrites, of the bipolar serotonin cells in *Homarus* contain immunologically detectable levels of serotonin. However, preincubation of the tissue in 10^{-4}M serotonin allowed these cell bodies and their dendrites to be visualized using the antisera. There are no other cells in this region of the nervous system that take up serotonin.

The axons of the serotonin cells travel to the stomatogastric ganglion (STG) where they branch profusely. We found that they then exit the STG and continue through the stomatogastric nerve as a very tight bundle. Our electrophysiological and anatomical data show that each axon then bifurcates and sends one branch out each superior oesophogeal nerve, terminating in each commissural ganglion. There are no descending serotonergic axons projecting to the STG from central ganglia.

In *Homarus*, we have found that there are 8 bipolar serotonergic cells, 4 located in each peripheral lateral ventricular nerve (LVN). These cells send processes to the bilaterally symmetric gastric muscle gm9. They each send branches out small nerves and form arborizations and varicosities on the muscle as they run along it. In both *Cancer* and *Procambarus* there are 2 serotonergic neurons in each LVN. In *Cancer*, each of the cells projects to different bilaterally symmetric gastric muscles. One cell innervates gm9a, the other innervates gm8b. Both of these muscles, however, are driven by the same gastric motorneuron, the MG cell. The homologous gastric motorneuron innervates gm9 in *Homarus*.

The morphology of the bipolar cells is typical for arthropod sensory neurons. In *Homarus*, sensory action potentials were recorded in nerves closely associated with gm9 in response to muscle movement. Concurrently, a modulation of the pyloric pattern with some serotonin-like characteristics was observed. These data strongly suggest that the serotonergic bipolar cells may provide sensory feedback to the pyloric CPG.

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330.11 BACKWARD WALKING INHIBITS TAILFLIP IN CRAYFISH. N. S. Phillips and D. H. Edwards, Department of Biology, Georgia State Univ., Atlanta, GA 30303.

Animals must be able to discriminate between sensory input from external sources and that due to their own motor acts. One mechanism by which animals can reduce or eliminate behavioral responses to their own movement involves the production of a corollary discharge or efference copy. These serve to reduce or eliminate reafference that results from an animal's motor acts. As a result, inappropriate reflex pathways are not activated, and relevant environmental stimuli are not overlooked.

We have shown that stimulation of the command pathway for backward walking in crayfish inhibits elements of the lateral giant (LG) tailflip escape circuitry while it releases backwards walking. This centrally derived inhibition is supplemented by inhibition produced by leg proprioceptors that are active during walking (Fricke, R.A. et al., J. Comp. Physiol., 149(2):251, 1982). This inhibition is directed pre- and postsynaptically against mechanosensory interneurons that excite the LG command neuron for tailflip. Both types of inhibition would appear to protect the animal from reafferent stimulation of the escape system as it moves backwards into its burrow.

To determine the behavioral consequences of this inhibition, we have electrically stimulated freely behaving crayfish to tailflip when they were quiescent and while they were walking backwards. Electrical stimulation is provided by 1.5 ms voltage pulses across a pair of electrodes implanted around the fourth root of the terminal abdominal ganglion. This root contains sensory afferents to the escape system. Animals were stimulated over a range of voltages and the response (LG-mediated tailflip, as judged by latency and behavior) frequency was determined. We illuminate previously dark-adapted crayfish ventrally to cause them to walk backwards (Edwards, D.H. J. exp. Biol., 109:291, 1984) Illumination excites the caudal photoreceptor neurons which release backwards walking.

We found that a much larger voltage stimulus is necessary to elicit tailflip escape when the animal is walking backwards. The stimulus voltage necessary for a 50% response frequency increased from 0.9 V to 1.3 V, while the response frequency at 1.2 V decreased from 84% to 36%. There was no difference between tailflip responsiveness of control animals in the dark and those that were exposed to light but did not walk. This result is consistent with the observation that leg movement and/or backwards-walking command neuron activity is necessary to inhibit the escape circuitry. The relative contributions of leg proprioceptors and central walking command neurons to the behavioral inhibition are not yet known.

330.12 A MODEL DESCRIBING THE KINEMATIC PROPERTIES OF THE CRAYFISH CLAW DURING THE DEFENSE RESPONSE. T.M. Kelly, Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268.

In order to more fully describe the defense response in the crayfish, a kinematic model of the claw was developed. The model, based on experimentally observed parameters, produced simulated trajectories of the cheliped that exhibited close correspondence to actual trajectories.

The cheliped was represented as two segments. The proximal segment was a composite of the coxopodite, basipodite, ischiopodite and meropodite. In the crayfish, the basipodite and ischiopodite are fused and excursion at the ischiopodite-meropodite articulation is extremely limited. The distal segment corresponded to the carpopodite. The basal joints, the thoracico-coxopodite and the coxopodite-basipodite articulations, were modelled as a single joint with two degrees of freedom. This composite joint permitted movement in the elevation (pitch) and rotation (yaw) axes. The meropodite-carpopodite (MC) joint was modelled as a simple hinge joint with one degree of freedom. The axis of the MC joint was offset from the axis of the meropodite by a fixed angle that was determined for each animal.

The accuracy of the model was assessed by comparing the trajectories of the cheliped observed during a visually evoked defense response to those generated by the simulation. The recording of the meropodite, carpopodite and propopodite in three dimensional space by a computer controlled optical device described previously (Kelly,T.M., Soc. Neurosci. Abstr. 1985) was followed by the calculation of elevation, rotation and MC angles. When the Cartesian coordinates of the cheliped were regenerated from these joint angles and the measured segment lengths, raw and simulated trajectories were nearly superimposable.

The working model of the crayfish claw permits the assessment of the dynamics of cheliped motion during the defence response. This information, when combined with the EMG patterns recorded from relevant muscles, will be helpful in elucidating the mechanisms of control that are operative during this stereotyped movement.

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330.13 THE CAUDAL PHOTORECEPTORS: IDENTIFIED NEURONS THAT PROJECT THE LENGTH OF THE CRAYFISH CNS. Ted W. Simon and Donald H. Edwards, Department of Biology, Georgia State University, Atlanta, GA 30303.

The Caudal Photoreceptors (CPRs) of the crayfish are a pair of photosensitive neurons in the caudal abdominal ganglion which send their axons forward into the rostral CNS. Sudden bright illumination of the caudal ganglion will cause the CPRs to fire at well over 100Hz (L. Wilkens & J. L. Larimer, J. Comp. Physiol. 80:389-407, 1972) and to produce leg movements in an intact animal. Ventral illumination of intact freely moving animals will elicit backwards walking behavior. (D. H. Edwards, J. Exp. Biol. 109:291-306, 1984) We have previously shown that electrical stimulation of a single CPR at frequencies of greater than 60-70Hz can elicit a motor response identical to that produced by a backwards walking command system (T. W. Simon & D. H. Edwards, Neurosci. Abstr. 10:627, 1984). Cutting the nerve cord at the thoraco-abdominal junction will abolish the motor response. Thus, it is likely that the Caudal Photoreceptors excite this command system somewhere in the rostral nervous system.

To determine the locus of this excitation, we traced the projection of the CPRs into the rostral CNS. We wish to report that the Caudal Photoreceptors project all the way from the caudal ganglion to the supraesophageal ganglion or brain of the crayfish and have extensive terminals in both the brain and subesophageal ganglion.

The CPRs have a characteristic morphology in the caudal ganglion with several large branching dendrites and a contralateral soma. In all the connectives, the CPR axons maintain a characteristic ventromedial position. In the other abdominal ganglia and the thoracic ganglia, only small branches project laterally from the ventromedial location of the CPR axon.

In the subesophageal ganglion, the CPR axons ascend along the midline of the ganglion. Twelve evenly spaced branches project laterally. The four rostral branches in this ganglion are thicker and have a more extensive arborization than those located more caudally.

In the brain, the Caudal Photoreceptors give off terminal branches in both the tritocerebrum and deuterocerebrum. The caudal branches in the brain appear to project to the antennal-tegumentary neuropile and the rostral terminal branches project to the parolfactory or optic neuropile.

The Caudal Photoreceptors excite the backwards walking command system in the rostral portion of the CNS. Visual and antennal inputs can also evoke backwards walking, and therefore, the connection probably occurs in a region where these three inputs can converge -- either the brain or the subesophageal ganglion.

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330.14 SYNAPTIC INTEGRATION IN EXCITATORY AND INHIBITORY CRAYFISH MOTONEURONS. Donald H. Edwards, Department of Biology, Georgia State University, Atlanta, GA 30303.

The different shapes of neurons enable them to make correct connections and to integrate synaptic inputs in ways that produce appropriate responses. I have studied synaptic integration in two identifiable motoneurons in the abdominal ganglia of crayfish, a fast flexor excitor (FE) and a fast flexor inhibitor (FI) of the abdominal fast flexor muscle that produces tailflip.

I obtained measurements of each cell's membrane time constant (Tm), input resistance (Rn) and three-dimensional structure. For FE, Tm = 12 ms and Rn = 4.9 M; for FI, Tm = 36 ms and Rn = 2.5M. FI is considerably larger than FE, which accounts for its lower Rn. I used these measurments to construct multicompartment models of the passive electrical structures of both cells, and used these models to calculate responses to simulated synaptic inputs. Each compartment represented a small region of a cell, and was characterized by a lumped membrane resistance and capacitance in parallel to ground, as well as coupling resistances to neighboring compartments.

The responses of the models to simulated electrical synaptic input from giant neurons were quite similar to the responses of the two motoneurons to input from those cells: the FE response is larger, rises faster and decays more quickly than the FI response. This indicates that differences in the cells' passive integrative characteristics can largely account for their different responses to giant input. Those responses are then supplemented by input from corollary discharge interneurons (J. Wine & D. Mistick, J. Neurophys.,40:904, 1977), which together cause the FE to fire at 3 ms to produce a fast abdominal flexion, and cause the FI to fire at 15 ms to produce flexor muscle relaxation (D. Kennedy & K. Takeda, J. exp. Biol., 43:211, 1965).

The models were also used to study the integration of lateralized input to both neurons. While both cells have ipsilateral and contralateral dendritic branches, they are arranged differently. The major dendrites of FE all radiate from a small region (< 200 um) of the cell's integrating segment that remains unilateral, with a soma at one end and an axon at the other. The major dendrites of FI emerge along an integrating segment that projects across the ganglion (> 500 um) from a soma to a contralateral axon.

The response of the models to simulated lateral inputs indicated that the FE would give similar responses to equal inputs from left and right, while FI would respond asymmetrically, with inputs ipsilateral to the axon being more effective in firing the cell. These predictions were verified experimentally: left and right FE neurons produce very similar responses to unilateral stimulation of sensory afferents, while left and right FIs respond with spikes either alone or earlier to input that is ipsilateral to the cell's axon.

330.15 COORDINATION OF SWIMMERET RHYTHMS IN CRAYFISH. B. Mulloney Zoology Dept., Univ. of Calif., Davis, CA 95616

The coordinated movements of the abdominal limbs, or swimmerets, of crayfish show a characteristic phase-shift between limbs on adjacent segements of the body. Movement of each limb is driven by a central pattern-generating circuit in the abdominal ganglion that innervates it. To test the necessity of long-projection intersegmental connections to coordinate the swimmeret pattern-generators in different segments, we used proctolin to induce stable swimmeret rhythms in isolated ventral nerve cords and then surgically reduced the number of connected ganglia.

Perfusion with the peptide proctolin mimics stimulation of command interneurons that excite the swimmeret pattern-generating system in the crayfishes, Procambarus and Pacifasticus. The range of effective concentrations of proctolin is 10^{-7} to 10^{-5} M. Comparisons of the periods and intersegmental phases that occur in spontaneously active isolated nerve cords with those that occur in proctolin-perfused cords show that these parameters of the motor patterns are similar under these conditions. The durations of power-stroke bursts, however, are significantly different.

When the number of connected ganglia was reduced from six to two, neither the periods nor the phases of power-stroke bursts in neighboring ganglia changed. Two kinds of two-ganglion chains were tested -- G5-G4 chains and G4-G3 chains. Both kinds responded in the same way. Therefore, the information transmitted between adjacent ganglia during production of the swimmeret rhythm is sufficient to account for the metachronous phase-shift characteristic of the limb movements in the intact animal.

Swimmeret interneurons that project through one ganglionic tract have characteristics of coordinating interneurons. The roles of these interneurons in coordinating neighboring pattern-generating circuits will be described.

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331.1 ONTOGENETIC DEVELOPMENT IN THE NORMAL RAT, EXAMINING BEHAVIOR AND NEURAL RESPONSES DURING PREWEANING PERIOD IN CONNECTION WITH MATERNAL BEHAVIOR. Moto Sakuma and R. Petters. Dept. of Biol., Wayne State Univ., Detroit, MI 48202; Dept. of Biol. Wichita St., Univ. Wichita, KA 67208

Data from previous studies on the rat have demonstrated that the manipulation of pregnancy by diets of differing tryptophan contents can produce changes in offspring development (Sakuma et al., Fed. Proc. 622, 1984; Neuro Sci. 668, 1983). The objective of the present study is to characterize the normal development of rats fed Purina Lab Chow by a rating scale of behavior and neural reflex ontogenetically. Twenty six pups from three Purina Lab Chow dams (Sprague-Dawley) exhibited the stepwise development in the weekly base. The cry behavior and uprighting behavior from supinal position which are observed from birth. Three days after birth behaviors such as locomotion, abduction of limbs and pina detachment begin. On Day 5 climbing and running behaviors begin. On Day 7 body hair appears like frost, and coordinative behaviors such as resistance to gravity, head-lifting, face washing, and pivoting are started. Day 11 is observed by slit-like opening of one eye, attempts to grasp, attempts at hiding, and the positioning of the abdomen at a high level. On Day 14 eyes are completely opened; thus, the acquirement of vision proceeds to enlarge the action radius. Other sensory responses such as eye blink, movement of the ear, vision, grooming and sitting also occur on Day 15. However, response to sound and smell occurs at 17 days of age and on Day 21 the animals display rearing activity.

The nursing behavior of three dams are recorded by a rating scale. Dams display nursing activity in two manners designated as heavy nursing and light nursing. Until day 7 of lactation, dams show no locomotion, no rearing, no grooming, and no face washing activity. Rather, the remain the whole day in the nest caring for their pups. This heavy nursing gradually changes to light nursing which continues until weaning (23rd day of lactation). On Day 10 of lactation all dams stay outside the nest when resting, however, pup-cleaning, nest cleaning, and feeding activity are still carried out. Although pup-retrieval activity is not observed after day 14 of lactation, the nursing posture is continually observed until day 17 of lactation.

Thus, it is possible to affect the development of offspring described above by inappropriate nursing behavior, particularly during the heavy nursing period. Twenty six tested pups were found to have significantly heavier body weights at the 0.01 level (t-test) than 10 non-tested pups from birth to 23 days of age. All utilized animals were replaced to the other project and treated according to the NIH Guide for Care and the Use of Laboratory Animals (1985).

331.2 A STUDY OF THE TEMPORAL AND SPATIAL SEQUENCE OF THE SENSORY INNERVATION OF THE CHICK AUDITORY RECEPTOR. S.G. Hemond* and D.K. Morest. Univ. Conn. Health Ctr., Farmington, CT 06032.

The cochlear ganglion neuroblasts in the chick embryo separate from the otocyst by stage 20 (Hamburger-Hamilton). The peripheral processes of these neurons grow out during the next 3-4 d and penetrate the basal lamina underlying the epithelium of the developing basilar papilla (BP), which elongates considerably during this period. The purpose of this study was to determine if there is a temporal or spatial gradient of fiber ingrowth.

White Leghorn specimens, containing the otocyst with adjacent ganglia and medulla from stages 12-29, were prepared for light microscopy with 3-5 μm sections, toluidine blue staining, and mixed aldehyde fixation. Our findings apply to the first fiber bundles to reach each BP region but not isolated fibers or fibers of newly formed ganglion cells tracking earlier ones.

At stage 21, the BP anlage appears as a ventromedial evagination of the otocyst. The first few fiber bundles observed grow into the base of the evagination. By stage 22, the evagination elongates slightly to assume the tubular form of the BP; more fiber bundles appear at the base of the BP. By stage 24, the BP has lengthened by about 30% (all increases re: stage 22); while innervation still is seen at the base, a conspicuous bundle extends apically from the now elongating cochlear ganglion to a point about 3/4 of the distance to the apex. Although fibers clearly penetrate the basal lamina at that point, there is a mid-zone devoid of direct fiber ingrowth separating the two innervated areas. By stage 25, the BP has lengthened about 200%; the pattern of fiber ingrowth resembles that of the previous stage, but the mid-zone is now innervated. Fiber bundles are still not seen in the apical region. By stage 26, the BP has lengthened 300%. The basal region of the BP is innervated, but the extent of this region is relatively reduced compared to the present length of the BP. The apical region is innervated practically to the end. Notably lacking in direct fiber ingrowth is the mid-basal region, which appears to be newly formed. Underlying the epithelium in this region are mesenchymal cells, which separate the ganglion from the receptor epithelium. By stage 29, the BP has lengthened about 350%, and a similar innervation pattern appears. The uninnervated zone in the mid-basal region has become relatively reduced and no doubt is subsequently innervated.

We conclude that there is a distinct temporo-spatial pattern of the ingrowth of fiber bundles to the developing BP. The primary pattern is not described as any simple linear gradient. Fiber ingrowth begins basally, shifts to an apical and then to a mid-basal region. This pattern may reflect not only the sequence of fiber ingrowth but also the passive displacement of fibers by new cells in the elongating BP. Supported by NIH grant NS14354.

331.3 THE PROJECTION OF SPINOCEREBELLAR FIBERS IN THE CHICK FOLLOWING TARGET REMOVAL. S. Furber* and N. Okado. Dept. of Anat., Univ. of Tsukuba, Inst. Basic Med. Sci., Ibaraki 305, Japan.

The present study was carried out to determine the mechanisms involved with pathway formation. We injected lectin horseradish peroxidase (HRP) in the lumbar spinal cord of the chick embryo and anterogradely labelled the terminals of the mossy fibers as well as the fibers from the cells of origin up to the target. On embryonic day (E) 7 a small number of fibers are seen penetrating the cerebellar plate. On E8 a large number of fibers are found in the cerebellar plate, decussating at the dorsal part of the cerebellar plate; growth cones are also labelled with HRP. Also on E8 no major errors in the projection pattern to the cerebellar plate were observed. On E10 cerebellar lobules can be distinguished and mossy fiber terminals appear in the internal granular layer. By E14 the terminals form longitudinal strips in the cerebellum. The topographical organisation of this pathway in the chick embryo makes this an extremely useful model for studying pathway formation.

We intend to clarify whether the target is necessary for formation of the correct pathway. Removal of the cerebellar plate was carried out on E4 1/2 - E5 (stages 24-26) using electrocautery. The brain and spinal cord were processed and examined to determine the formation of the projection pattern and to confirm the success of the cerebellar plate removal. In both normal and experimental cases the spinocerebellar fibers appear in the dorsal part of the lateral funiculus of the spinal cord, the ventral part of the medulla and the cerebellar peduncle. Also in cases with partial removal of the cerebellar plate, fibers were found in the remaining portion of the cerebellar plate and growth cones were found on the tips of the axons. In a case with almost total removal of the cerebellar plate, fibers were found in the rostral part of the cerebellar peduncle and the axon terminals were projecting in many directions as if searching for their target. Our present findings indicate that the spinocerebellar fibers choose the correct pathway even if no target is present.

331.4 PATTERNS OF NEURAL CREST MIGRATION IN THE CHICK EMBRYO. J.F. Loring* and C.A. Erickson* (SPON: H. Anderson). Dept. of Zoology, Univ. of Calif., Davis, CA 95616.

Neural crest cells migrate during embryogenesis to give rise to segmented structures of the vertebrate peripheral nervous system: dorsal root ganglia and the sympathetic chain. However, neural crest cells arise from the dorsal neural tube where they are apparently unsegmented. It is generally agreed that the segmentally arranged somites impose segmentation on migrating crest cells, but there is disagreement about two basic questions: (1) exactly what pathways do neural crest cells use to move through or around somites, and (2) do neural crest cells actively migrate or are they passively dispersed by movement of somite cells? The answers to both questions are critically important to any further understanding of the mechanisms underlying the precise distribution of the neural crest cells that develop into ganglia.

We have done an thorough study of the locations of neural crest cells in chick embryos during early stages of their movement, using antibodies to neural crest cells (HNK-1), growing nerve processes (E/C8, a gift from G. Ciment), and extracellular matrix molecules. Our results show that: (1) Some neural crest cells invade the extracellular space between adjacent somites, but the majority move into the somites themselves along the border between dermomyotome (DM) and sclerotome. (2) Neural crest cells remain associated with the anterior half of the DM of developing somites as they travel, suggesting that the DM may be used as a migratory substratum. Supporting this idea is our observation that the development of the DM basal lamina coincides in time and location with the onset of crest migration through the somite. (3) Neural crest cells advance through the somite while the length of the DM pathway remains constant, suggesting active locomotion at least in this early phase of development. (4) Neural crest cells leave the DM at a later stage of development, to associate with the dorsal aorta where sympathetic ganglia form, and to associate with newly emerging fibers of the ventral root nerve, where they persumably give rise to neuronal supportive cells. Thus we propose that the establishment of the segmental pattern of the peripheral ganglia and nerves depends on the timely development of appropriate substrata to guide and distribute migrating neural crest cells during the early stages of embryogenesis.

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331.5 **DEFECTIVE INNERVATION OF CHICK LIMBS IN THE ABSENCE OF PRESUMPTIVE SCHWANN CELLS.** Ellen M. Carpenter and Margaret Hollyday. Committee on Neurobiology, University of Chicago, Chicago, IL 60637.

We have examined a possible role for Schwann cells in the development of limb muscle innervation in chick embryos. We first studied the relationship of presumptive Schwann cells to outgrowing axons in normal embryos using immunohistological methods. Staining was carried out with antibodies to NgCAM (kindly provided by G. Edelman), and HNK-1, a specific marker for neural crest cells (Becton Dickinson). Some sections were counterstained with Hoechst 33258 (a fluorescent nuclear dye). Examination of the normal embryos at stages 25-26 (during the period of initial fiber outgrowth) revealed that outgrowing fibers are associated with a distinctive population of cells having elongate nuclei. These cells have been tentatively identified as Schwann cells (cf., Harrison, JCN 37:123-205 (1924)). Elongate nuclei were seen along the entire length of the peripheral nerve pathway, from the ventral root exit zone to the distal growing axon tips in the limb bud, with a higher concentration of cells in the distal portions of the pathway.

Schwann cell precursors were destroyed by electrocauterization of the neural crest in spinal cord segments known to supply either wing or hindlimb muscles. Crest lesions of brachial cord segments were done in embryos having 15-18 somite pairs; operations meant to affect lumbar segments were performed in embryos having 22-24 somite pairs. The embryos were allowed to survive until they reached stages 25 or 26 (wing) or stage 30 (hindlimb). They were then fixed and processed for immunohistochemical or conventional Nissl staining techniques. After radical neural crest removals, the presumptive Schwann cell population was largely absent as judged by a lack of elongate nuclei present in fiber pathways, and as confirmed in some cases by lack of staining with antibodies to HNK-1. In addition, all DRGs and some sympathetic ganglia were also missing in embryos in which the neural crest removal proved extensive enough to ablate Schwann cells. In all embryos lacking Schwann cells, axonal trajectories as reconstructed from sections stained with NgCAM were normal proximal to either the brachial or lumbar plexus and in the ventral axial nerve branches. However, in the absence of Schwann cells, motor axons failed to penetrate limb tissue. Fibers converged at the base of the limb, medial to the limb girdle, in the region of the plexus. A division of fibers into dorsal and ventral populations was observed, but no further distal growth could be seen unless fibers encountered blood vessels. No significant differences were seen between experimental wings and hindlimbs. In some of our cases, the crest removals were extensive enough to ablate the DRGs, but did not fully remove the Schwann cells. Axonal outgrowth and motor innervation were not disrupted in these embryos.

We conclude from these observations that Schwann cells may be involved in promoting motor axon growth beyond the plexus region.

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331.6 **PATHWAY SELECTION IN MUSCLELESS CHICK LIMBS.** K. Phelan* and M. Hollyday. Committee on Neurobiology, Univ. of Chicago, Chicago, IL 60637.

We have examined axonal guidance in chick limbs which are devoid of skeletal muscle fibers, in order to determine whether myocytes are required for pathway selection by motoneurons during development. Chick/quail chimera studies by others have shown that the limb musculature is exclusively derived from somitic mesoderm, with connective tissue and cartilagenous elements arising from lateral plate mesoderm (Christ et al., Anat. Embryol. 150:171-186, 1977). By surgically removing those somites unilaterally which normally contribute to the wing musculature before their cells have begun lateral migration, we produced embryos lacking muscle in one limb. Our primary criteria for lack of muscle in experimental limbs were: 1) absence of motility in ovo, 2) complete unilateral absence of the myotomes adjacent to the spinal cord at wing levels, as assessed histologically, 3) absence of myosin immunoreactivity, as visualized with mAb 31A directed against embryonic chick myosin (Sweeney et al., PNAS 81:797-800, 1984). The contralateral limb was used as an internal control in all embryos.

The chicken spinal cord is organized such that motoneurons which lie medially in the lateral motor column (LMC) normally innervate muscles derived from the ventral muscle mass, while those located laterally innervate muscles derived from dorsal muscle mass. Axons from segments 13-16 converge at the brachial plexus and subsequently diverge along two major pathways, one leading to ventral muscle mass and the other to dorsal muscle mass. Injections of WGA-HRP were made into peripheral nerves (Brachialis inferior, Pectoralis) which diverge from the ventral nerve trunk just distal to the dorsal-ventral branch point. When these nerves were injected in normal embryos, only medially positioned motoneurons were labelled in the LMC. WGA-HRP was also transported anterogradely, which allowed us to assess the specificity of the peripheral nerve injection. We tested the pathfinding abilities of motor axons in stage 28 embryos. At this stage muscle fibers in the wing normally express the myosin subtype to which our antibody binds and the specific motor projection pattern is established in normal embryos.

In those embryos which were muscleless according to the above criteria, and were specifically injected in the periphery, we detected no aberrantly positioned motoneurons in the spinal cord. In no case did we obtain labelled cells in the lateral portion of the LMC after a specific injection into a nerve projecting to ventral mass. Labelled cells were also appropriately positioned along the rostro-caudal axis of the spinal cord, as determined from spinal cord reconstructions and comparison to control motor pool maps. From these experiments we conclude that the presence of muscle is not required for pathway selection at the dorsal-ventral branch point in the chick wing. It is likely that lateral plate mesodermal (somatopleural) derivatives are sufficient for axon guidance at this choice point.

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331.7 **PROXIMAL TISSUES AND PATTERNED NEURITE OUTGROWTH INTO THE CHICK HINDLIMB.** K. W. Tosney. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

Tissues between the spinal cord and the base of the hindlimb in the chick embryo have been suggested to provide pathways and specific cues that guide motoneuron growth cones (Tosney & Landmesser 1985, Develop. Biol. 109: 193). I have examined the role of the somite and its individual components, the myodermatome (the dorsal portion of a somite, which gives rise to axial muscles and dermis) and the sclerotome (the ventral portion of a somite, which gives rise to cartilage). I removed these tissues before or during early neurite outgrowth (stages14-18) and assessed both the development of the gross anatomical nerve pattern and the precision with which motoneurons select the dorsal and ventral nerve trunk pathways in the limb.

When one or more myodermatomes were ablated the gross anatomical nerve pattern developed normally and motoneurons projected correctly to dorsal and ventral targets. This shows that the myodermatome is not an obligatory substratum for growth cones and it does not have to be present for outgrowth to become segmented into discrete spinal nerves, for the myotomal nerve (which normally innervates the myodermatome) to form, or for axons to deploy into the proper nerve trunk pathway.

In contrast, the sclerotome appears to be required for spinal nerve segmentation. When an entire somite (myodermatome and 80-100% of the sclerotome) was removed, axons from the deprived segment joined adjacent spinal nerves. When less sclerotome was removed spinal nerves were small or abnormal. These results suggest that the sclerotomal mesenchyme that lies in the path of outgrowing axons is of critical importance to spinal nerve formation.

Removal of several adjacent lumbosacral somites at stages 15-17 often resulted in smaller limb muscles and altered spinal nerve segmentation. Despite this, neurites continued to project correctly to dorsal and ventral targets. This suggests that, even though muscle nerves do not form in the absence of muscle (Lewis et al. 1981 J. Embryol. Exp. Morphol. 64: 211), the specificity of motoneuron projection is to a large extent independent of the migration of muscle precursor cells from the somite.

A few embryos seemed to show a shift in the spatial relationships among the notochord, spinal cord and limb following removal of several somites. In these cases some axons made -- and corrected -- projection errors. Axons were observed to course into the dorsal nerve trunk pathway, make a U-turn in the limb, return to the plexus region and project correctly along the ventral nerve trunk pathway. This reduction in the precision with which growth cones select the proper dorsal or ventral pathway is being analyzed further.

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331.8 DISTRIBUTION OF LAMININ AND COLLAGEN TYPE IV DURING EARLY AXONAL GROWTH IN THE MOUSE CNS. A.Madsen*, S.Palm*, L.Furcht, and P.Letourneau. Depts. of Cell Biology and Neuroanatomy, and Laboratory Medicine and Pathology, Univ. of Minnesota, Minneapolis, MN. 55455.

Little is known about the morphogenesis of the highly ordered axonal circuitry of the mammalian CNS. However, it is known that growth cones must adhere to surfaces to be able to advance. Previous in vitro demonstrations of neurite outgrowth on laminin (Lm)- and collagen-treated surfaces have led us to study the distribution of these extracellular molecules relative to the formation of axonal pathways in the mouse CNS.

Mice from embryonic day 8 to newborn were dissected free from membranes following removal from the uterus. They were immersed in 4% phosphate-buffered paraformaldehyde, then tranferred to 5% sucrose in phosphate-buffered saline. Frozen 10-15 μm sections were cut in a cryostat and incubated overnight in a combination of primary antibodies. Alternate sections were reacted with polyclonal antibodies to Lm or collagen type IV in conjunction with a mouse monoclonal antibody to the 200kD neurofilament protein (RT97; gift of Dr. John N. Wood). Rhodamine- and fluorescein-labeled secondary antibodies were used.

Immunoreactivity for Lm and collagen type IV were present at day 8 in the external limiting membrane (ELM) of the brain and spinal cord, as well as in somites, skin and blood vessels. Neurofilament immunoreactivity was present at day 9 and later. In addition to intense staining of the ELM, concentrated punctate staining for Lm was seen in the lateral margins of the neural tube in close association with early developing tracts of axons, proximal to the ELM. The floor plate of the nerve tube did not show the same punctate staining, and axons crossing in this region were close to the Lm-containing ELM. This staining for Lm seemed to be on the basolateral surfaces of neuroepithelial cells constituting the wall of the neural tube. In older embryos with multilayered tracts of axons, the concentrated punctate immuoreactivity for Lm was absent. At no time during CNS development was comparable punctate immunoreactivity for collagen type IV seen.

These results suggest that Lm is located within the developing CNS, and may be accessible to elongating CNS growth cones. Growth cones may encounter Lm not only in the ELM, but also on the surfaces of the primitive glia or neuroepithelium of the neural tube. (Supported by NIH grants CA21463, CA29995, AM32660, HD19950 and a grant from the Spinal Cord Society.)

331.9 LAMININ IS TRANSIENTLY EXPRESSED IN THE DEVELOPING RAT OPTIC NERVE DURING THE TIME RETINAL AXONS ARE GROWING. S.C. McLoon, L.K. McLoon, S.L. Palm* and L.T. Furcht. Depts. of Cell Biology & Neuroanatomy, Ophthalmology and Laboratory Medicine & Pathology, University of Minnesota, Minneapolis, MN 55455.

Axons in the developing central nervous system grow along unique and highly stereotyped courses often through a complex cellular terrain. One possible explanation for the unique course taken by different axon populations is that there are a variety of axon substrate molecules, each with a unique distribution in the developing brain. Growing axons require a substrate to which they can adhere in order to extend. However, no axon-substrate molecules have been definitively identified in the developing central nervous system. Evidence obtained in tissue culture suggests that laminin is a suitable substrate molecule for the growth of retinal axons. We have screened the optic stalk and nerve of developing rats before, during and after the period in which retinal axons are growing for the presence of laminin. Rats were fixed at two day intervals from embryonic day 14 (E14) to postnatal day 14 (P14). The optic stalk or nerve of these animals was processed for immunohistochemistry and reacted with an affinity purified antibody to laminin. On E14 laminin immunoreactivity was present in the ventral portion of the optic stalk, the same region in which the first retinal axons grow. Over subsequent days as retinal axons grew into the stalk the optic nerve was formed. Between E16 and P10, cells positive for laminin were distributed throughout the cross-sectional area of the nerve. The laminin positive cells in the E20 to P10 nerve were also reactive for glial fibrillary acidic protein (GFAP) which indicates they are astrocytes. By P12 the only laminin reactivity which remained within the optic nerve surrounded the vasculature. At this age and all older ages the staining for laminin on astrocytes was no longer visible. P12 is after the last retinal axons have grown through the optic nerve. The presence and distribution of laminin in the optic stalk and nerve is consistent with the hypothesis that laminin is an adhesive substrate for growing retinal axons and that laminin may in part regulate the path taken be growing retinal axons.

In order to determine the cellular basis for the loss of laminin immunoreactivity in perinatal optic nerve, we examined the type of astrocyte which is laminin positive. Monolayer cell cultures were prepared from perinatal rat optic nerves and stained immunohistochemically for GFAP, A2B5 and laminin. Type-1 astrocytes which compose the immature nerve are $GFAP^+$, $A2B5^-$ and $laminin^+$. Type-2 astrocytes which compose the mature nerve are $GFAP^+$, $A2B5^+$ and $laminin^-$. Thus, the loss of laminin immunoreactivity appears to correlate with the maturation of the astrocyte population of the nerve.

(Supported by NIH grant EY05371.)

331.10 AXONS ADDED TO THE OPTIC PATHWAY SUBSEQUENT TO ITS REGENERATION REESTABLISH THE NORMAL FIBER TOPOGRAPHY LOST DURING REGENERATION. R. Bernhardt, S.S. Easter, Jr., P. Raymond, and P. Rivlin*. The University of Michigan, Ann Arbor, MI 48109.

We have previously shown that axon topography in the goldfish optic pathway reflects the retinal position of the parent somata (Bernhardt and Easter, Soc. Neurosci. Abstr. 11:174, 1985). We have now examined the axon topography in the optic pathway of fish which had survived for more than one year after a nerve crush. Because ganglion cells continue to be added at the retinal margin (Johns, J. Comp. Neurol. 176:343, 1977) two groups of axons were present: those from ganglion cells in central retina, born before the time of nerve crush, had regenerated simultaneously; and those from peripheral cells, born post-crush, had been added to the optic pathway one after another over the entire post-crush period. Axons of these two groups were labeled selectively on the tectum and their paths and retinal origins were studied. To distinguish between retina already present pre-crush and retina added only post-crush, ganglion cells formed at the time of nerve crush were labeled with 3H-thymidine, for later identification in autoradiographs of retinal wholemounts.

Labeling fascicles on central dorsal tectum backfilled ganglion cells that were scattered over central retina rather than confined to a ventral annulus as in normals (Stuermer and Easter, J. Neurosci. 4:1045, 1984). These somata were confined within the ring of 3H-thymidine labeled cells. They had therefore been present before the time of nerve crush. Labeling a fascicle at the tectal periphery backfilled cells occupying a partial annulus which, as expected from normal animals, extended from the nasal pole into the ventrotemporal retinal quadrant. These cells were always peripheral to the ring of radioactive cells and thus must have been added to the retina subsequent to the nerve crush.

Axons labeled from central tectum were scattered throughout the tract rather than clustered as in normals. Axons labeled at the tectal periphery were clustered in a narrow band near the pial surface, the normal location.

Axons added to a regenerated pathway in a normal developmental sequence reestablish a normal fiber topography even though this has been lost by the regenerated axons. Therefore, growing optic axons do not navigate by tracking the axons of neighboring, slightly older ganglion cells. (Supported by EY-00168 to SSE and a grant from the Janggen-Poehn Foundation to RB.)

331.11 SOME CALLOSAL NEURONS OF THE FETAL MONKEY FRONTAL CORTEX HAVE AXONS IN THE CONTRALATERAL HEMISPHERE PRIOR TO THE COMPLETION OF MIGRATION. M.L. Schwartz and P.S. Goldman-Rakic, Sec. Neuroanat., Yale University School of Medicine, New Haven, CT 06510.

Developmental studies of the cerebral cortex have revealed that many migrating cells of the subplate and intermediate zones have axonal processes, although little information is available on the targets or extent of elaboration of these processes. In the present report we present evidence that in the macaque frontal cortex, some callosal projection neurons may have axons within the contralateral hemisphere prior to the completion of their migration into the cortical plate. We addressed this issue using retrograde tracing methodologies and ^{3}H-thymidine autoradiography in monkeys ranging in age from embryonic day 82 (E82) to birth.

Between E82 and E104 large injections of HRP or retrograde fluorescent tracers into the cortical plate and prospective white matter (the intermediate and subplate zones at this stage of development) of one hemisphere resulted in the following pattern of label in the opposite hemisphere: 1) a large number of labeled neurons in the lower half of the cortical plate (CP; prospective layers V and VI); and 2) a moderate number of labeled neurons in both the subplate zone(SPZ) and intermediate zone(IZ). Comparable injections at or after E104 resulted in a different pattern in which: 1) few if any cells were labeled within the cortical white matter; 2) the density of labeled neurons within infragranular layers V and VI was reduced; and 3) labeled neurons were found in layer III, which emerges as a distinctive stratum at this age.

To determine whether any of the callosally labeled neurons in the IZ, SPZ and infragranular CP prior to E104 might be prospective layer III callosal neurons in the process of migrating to more superficial positions in the CP, we examined sections from monkeys prepared for ^{3}H-thymidine autoradiography that had received a pulse injection of ^{3}H-thymidine between E70 and E80, the period of generation for layer III neurons (Rakic and Wikmark, personal communication) and which were sacrificed between E80 and E91. As late as 14 days after their last cell division, many layer III cells are still migrating to positions in the cortical plate and can be found in the superficial IZ, SPZ and at all depths in the CP. These results indicate that prospective layer III neurons are still migrating through the IZ, SPZ and lower CP at the ages in which we found retrogradely labeled callosal neurons in these same zones. These data strengthen the possibility that some of the HRP labeled neurons in these regions at midgestation might be migratory neurons. To obtain more definitive evidence for this possibility we have injected ^{3}H-thymidine into a fetal monkey at E75 and then subsequently injected HRP into one hemisphere on E84 to label callosal neurons in the opposite hemisphere. The analysis of this latter double-label experiment has yet to completed; however, on the basis of the single-label results described above we predict that a population of cells in the IZ, SP and deep half of the CP will be double labeled with both HRP and ^{3}H-thymidine.

In summary, we have found ectopic callosal neurons in zones of the fetal monkey cerebrum at ages when these zones contain high numbers of migrating neurons which are destined for supragranular cortical layers. These findings are compatible with the hypothesis that some cortico-cortical neurons of the primate neocortex have axons which approach distant targets of the contralateral hemisphere well in advance of reaching their final position within the cortical plate. Supported by NS 22807 and MH 38546.

331.12 DEVELOPMENT OF ACETYLCHOLINESTERASE ACTIVITY IN DORSAL THALAMIC NUCLEI: TRANSIENT AND PERMANENT PATTERNS OF ACTIVITY. Richard T. Robertson, Sayed J. Mirrafati* and Jen Yu. Departments of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

We have demonstrated recently that the primary sensory areas of cerebral cortex in the rat display patterns of acetylcholinesterase (AChE) activity transiently during the first three weeks of post-natal life. The areal and laminar patterns of the AChE correspond to the thalamic recipient zones of these cortical areas and suggest that the AChE may be a marker for zones of thalamocortical terminations. The present study was undertaken to determine whether thalamocortical neurons display AChE.

Subjects were laboratory born Long-Evans or Sprague-Dawley rats of 0-30 post-natal days (DPN) of age. Animals were sacrificed by vascular perfusion with 10% formalin. Some animals received systemic injections of 1.5 mg/kg DFP 3-5 hr prior to sacrifice. AChE activity was detected histochemically in series of 40-64 um frozen sections by a modified version of the method of Koelle. Non-specific cholinesterase activity was inhibited by 10^{-4}M iso-OMPA. Adjacent sections were processed for a Nissl stain.

AChE positive thalamic nuclear groups could be divided into two groups. Group 1, which included the anterior dorsal nucleus, the ventral nucleus of the lateral geniculate body and the parafascicular nucleus, displayed AChE staining of neuronal somata that first appeared at 5-8 DPN and remained prominent through adulthood. Group 2 included the ventrobasal complex (VB), the dorsal nucleus of the lateral geniculate body (dLGN) and the ventral nucleus of the medial geniculate body (vMG). These nuclei display intense, but transient, patterns of AChE during the first two postnatal weeks. AChE staining of neurons is present in VB at birth, reaches peak intensity at about 5-7 DPN and then disappears by about 14 DPN. AChE staining of dLGN appears at about 4-5 DPN, reaches peak intensity at about 7-9 DPN, and then is reduced to adult levels by about 20 DPN. The vMG shows onset of AChE activity at 3-4 DPN, peak activity at 7-10 DPN and is eliminated by about 16 DPN. Experiments with DFP administration demonstrated that AChE activity in each of these three thalamic nuclear groups was present in neuronal somata.

These data demonstrate that thalamocortical neurons in the three primary sensory relay nuclei display transient AChE activity during the first two postnatal weeks of life. Taken together with the findings that transient patterns of AChE are also seen in somatosensory, visual and auditory areas of the cerebral cortex, these data suggest that transient patterns of AChE are characteristic of thalamocortical axon terminals at the time when these axons are invading the cerebral cortex and establishing synaptic connections with cortical neurons.

331.13 REPAIR OF NERVE DAMAGE IN FLATWORMS OCCURS BY AXONAL FUSION. M.A. Holman* and H. Koopowitz (SPON: R. Hammerschlag). Developmental and Cell Biology, University of California, Irvine, CA 92717

The objective of this study was to examine at the cellular level the extent of specificity of individual axons during repair of cut nerves and to correlate the time course of repair with behavioral recovery.

The animal used was Notoplana acticola, a free-living marine flatworm that has a single discrete bilobed ganglion and an intricate nerve plexus anastomosing into six paired ventral nerve cords that enter the brain. The rate of repair of these nerve cords and return of behavioral function is extremely rapid--between 24 and 48 hours after injury. Three possible mechanisms for this recovery are the use of alternate existing pathways in the nerve plexus, very rapid regeneration to target tissues, or rejoining of cut nerve ends. We are reporting on the third mechanism. Histological studies indicate that the cut ends of major nerves realign during healing, but the specificity of individual axonal realignment has not been examined.

In order to test for axonal fusion, animals had their major longitudinal nerve cut on one side of the body at t=0. At various times after this, animals were prepared for intracellular dye injection by clearing the epithelium from over the brain and partially digesting the brain sheath with collagenase. The brains were pinned with cactus spines to immobilize them. Cells were filled iontophoretically with Lucifer Yellow and examined under a fluorescence microscope. Axonal sprouting was evident by 8 hours, and by 23 hours, numerous elongated filipodia could be seen in the area of the cut. Between 24 and 27 hours, a filipodium made contact with a distal axon segment and the two ends fused, resulting in the distal end filling with Lucifer Yellow. Axonal fusion in this brief time frame was seen in over twenty animals and in a variety of different cells. This mechanism differs from other invertebrate preparations where cutting axons results in new axonal growth to target tissues.

Concanavalin A delayed behavioral recovery for 24 hours when it was applied to the cut ends at t=0, and its effects on axonal fusion and specificity have been investigated. Evidence for specificity was seen in a small number of animals where two filipodia from one axon made contact with a single distal axon segment. Two filipodia were never seen fusing with two distal segments, indicating a high degree of specificity in axonal fusion.

331.14 VARIANTS OF ASCARIS WITH MIRROR IMAGE NEURONAL MORPHOLOGIES
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The nervous system of Ascaris contains 298 neurons; each neuron is an identified cell whose morphological features are reproducible from animal to animal. The neurons have been divided into 123 morphological classes; the morphology of neurons in 25 of the classes is asymmetric in the right/left plane. The largest classes of asymmetric cells are motor neurons whose cell bodies are located in the ventral nerve cord. Each motor neuron belonging to classes DE1, DE2, DE3, DI or VI (Stretton, A.O.W., et al., PNAS 75: 3497, 1978) has a single process, refered to as a commissure, which exits from the ventral cord and runs circumferentially around the body to the dorsal nerve cord. Although in general the commissure of each neuron is reproducibly to the right or to the left, we have found rare individuals in which the handedness of specific commissures is reversed.

In some animals only one or two commissures are reversed; these reversals are not randomly distributed but occur at specific sites in the animal.

We also find two types of variant animals in which entire classes of motor neurons have reversed commissures. In one type, all DE2, DE3 and DI commissures are reversed whereas DE1 and VI commissures are normal. In the other type of variant, all five classes of motor neurons have reversed commissures. Since in C. elegans, neurons analogous to the DE2, DE3 and DI neurons are generated during embryogenesis whereas the DE1 and VI neurons are from post-embryonic lineages (Sulston, J. E., Phil. Trans. Roy. Soc. Lond. B275:287, 1976), we refer to these two types of variant animals as MIR-EMB and MIR-ALL respectively. Analysis of additional asymmetric neurons indicates that other embryonic neurons have mirror image morphologies in both MIR-EMB and MIR-ALL animals whereas other post-embryonic neurons have normal shapes in MIR-EMB animals but are reversed in MIR-ALL variants. In contrast to these changes in process morphology, we find that the locations of asymmetrically positioned neuronal cell bodies are not reversed in either of the variants. Thus it appears that MIR animals are not complete mirror images of the normal animals; rather the alterations observed are restricted to process morphology. Somewhat surprisingly, neither MIR-EMB nor MIR-ALL animals have any readily apparent behavioral alteration; all of the animals found were fully grown adults. These variants may reflect (genetic?) alterations of the mechanism responsible for specifying the right/left axis in normal animals.

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331.15 unc-5 & unc-6 MUTANTS DISRUPT DORSAL/VENTRAL GUIDANCE CUES FOR THE OUTGROWTH OF BOTH AXONS AND OTHER TISSUES. D.H. Hall, E.M. Hedgecock* and J.G. Culotti*. Dept. Neurosci., AECOM, Bronx, NY 10461; Roche Inst. Mol. Biol., Nutley, NJ 07110; and Dept. Biol. Sci., Northwestern U., Evanston, IL 60201.

Axonal guidance is believed to involve adhesive molecules on the substratum which are recognized by the growth cone. Different neurons may be guided by overlapping subsets of guidance molecules in order to reach different targets in 3D space. In the nematode, Caenorhabditis elegans, we have studied a series of mutations affecting the development of many different cells. Dorsal axonal migrations are disrupted in the unc-5 mutants, causing a complete absence of the dorsal and dorsal sublateral nerve cords. Both dorsal and ventral axonal migrations are disrupted in unc-6 mutants. Serial thin section studies of two unc-5 mutants revealed that in the absence of dorsal cues, some motor axons adopt a new set of characteristic lateral positions and form NMJs onto both appropriate and inappropriate muscle cells. Non-neuronal cell migrations along the dorsal/ventral axis can also be affected, including those of the excretory canals and of the distal tip cells (DTC) of the gonad. Some of these phenotypes were scored by Nomarski optics or FITC staining of living animals, revealing substantial variations within and between several mutant alleles of each gene. In some alleles, the dorsal/ventral guidance cues are more disrupted for cell migrations topologically external to the epidermal basement membrane, while in other alleles the reverse is true. This suggests the unc-5 and unc-6 gene products may normally be localized on both the basement membrane and the epidermal plasma membrane, and that some mutations alter the pattern of localization rather than the adhesiveness of the guidance cue. Serial reconstruction of three DTCs during migration showed that this cell lacks leading filipodia or growth cones, but that its blunt leading edge adheres closely to the basement membrane of preferred cell types along its path. The trailing edge of the DTC adheres to previous guide cells; the rest of the gonad is not bound to its neighbors. The DTC also forms its own thick basement membrane and is filled with perinuclear cisternae containing floccular material resembling the basement membrane. Smaller numbers of cisternae are located at the trailing edge of the DTC. The contents of the cisternae may be related to the cell's adhesivity, since their distribution matches the stickiest portions of its perimeter. Thus the DTC may migrate by a different mechanism from axonal outgrowth, yet shares some of the same guidance cues. In all alleles, migration of the DTC is more often normal in the anterior than in the posterior, suggesting that some other dorsal/ventral cues are retained near the nerve ring, and that these guidance cues may be diffusable.

331.16 EFFECTS OF ALTERED MEMBRANE RECYCLING PROCESS ON NEURONAL DEVELOPMENT: ANALYSIS OF CULTURED NEURONS OF THE DROSOPHILA MUTANT, SHIBIRE^{ts1}. Y.-T. Kim* and C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, Iowa 52242.

The membrane recycling process in the temperature-sensitive mutant of Drosophila, shibire^{ts1} (shi^{ts1}), has been shown to be reversibly blocked at restrictive temperature (> 29° C) because of enhanced vesicle fusion and disrupted membrane pinch-off (Poodry and Edgar, J. Cell Biol., 81:520, 1979; Kosaka and Ikeda, J. Neurobio., 14:207, 1983 and J. Cell Biol., 97:499, 1983). We examined the role of membrane recycling in neuronal development at the single-cell level by comparing neuronal cultures dissociated from shi^{ts1} and normal larval CNS.

At 30° C, adhesivity of shi^{ts1} neurons was reduced, as indicated by a decreased number (30% of that at 22° C) of cells attached to substratum during plating. In contrast, the number of attached cells in normal cultures at 30° C was increased to 130% of that at 22° C. The compounding effect of membrane recycling block and reduced adhesivity resulted in poor growth cone formation. After 4-8 hours exposure to 30° C, fewer growth cones could be detected in shi^{ts1} cultures. SEM samples of the heat-treated shi^{ts1} neurons showed no lamellipodia, but only filopodia, at the tips of neurites.

Time-lapse studies demonstrated that, at 30° C, neurite initiation and elongation in shi^{ts1} cultures were suppressed. However, following the down-shifts of temperature, the rates of neurite initiation and elongation showed a rebound and were even accelerated. Even well differentiated shi^{ts1} neurons were affected, exhibiting retraction or complete reabsorption of neurites after long-term (24 hours) incubation at 30° C.

Decrease in neurite branching in shi^{ts1} neurons became evident after only 4 hours exposure to 30° C. At restrictive temperature, shi^{ts1} neurons exhibited mainly primary neurites and no tertiary branch could be detected, whereas the secondary and tertiary branches were abundant in normal neurons at the same temperature.

Our results provide a new line of evidence that membrane recycling mechanisms take active parts in regulation of neuronal membrane adhesion and growth cone activity, which in turn determine neurite initiation, elongation and branching.

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331.17 **ANDROGEN PREVENTS SYNAPSE ELIMINATION IN A HORMONE-SENSITIVE MUSCLE OF THE RAT.** C.L. Jordan, M.S. Letinsky and A.P. Arnold. Departments of Psychology and Physiology. University of California, Los Angeles, CA 90024.

Mammalian muscle fibers are multiply innervated at birth. In rats, the adult pattern of single innervation is generally established by the end of the second postnatal week. The levator ani (LA), an androgen-sensitive perineal muscle of the rat, is unusual in that synapse elimination occurs later (3-4 weeks after birth), and some adult fibers remain multiply innervated (Jordan et al., Neurosc. Abstr., 11:31.1, 1985). Furthermore, androgens delay the normal elimination of multiple inputs during postnatal development (Jordan et al., NMD VI, 1986). We now report that androgens given postnatally result in a dramatic increase in the amount of multiple innervation present in the adult LA, long after cessation of androgen treatment.

Male rat pups were assigned to one of 3 groups (n=4-5 per group): 1) gonadectomy at postnatal day 7 (P7) plus testosterone propionate (100 ug/50g BW), 2) gonadectomy plus the vehicle oil, or 3) sham gonadectomy plus oil. Treatments were given P7 to P34. Four weeks from the end of treatment (P63-66), the LA was dissected fresh and stained with tetranitroblue tetrazolium. We estimated the number of preterminal axons synapsing onto single muscle fibers, the number of separate terminal sites per muscle fiber and the size of muscle fibers for all surface muscle fibers having fully visible synapses ($\geq$ 35 muscle fibers per muscle sampled).

Androgens had three effects: 1) Multiple innervation was markedly increased in androgen-treated muscles (71$\pm$2% vs 27$\pm$4% in sham operated controls, and 17$\pm$1% in oil treated castrates). 2) Androgen-treated muscles were also unusual in that substantial numbers of muscle fibers had multiple terminal sites (24$\pm$5% vs. 4$\pm$1% in oil treated shams and 0.6$\pm$0.4 in oil treated castrates). 3) Androgen-treated muscles had larger muscle fibers (33$\pm$2 um) than oil treated castrates (26$\pm$0.4 um), and oil treated shams had the largest LA fibers (37$\pm$0.4 um).

Androgens during development are critical in determining the innervation pattern of the adult LA. During postnatal development, androgens can regulate two parameters of muscle fiber innervation, promoting both multiple innervation as well as multi-site innervation. Because androgen treatment overlaps with the period of normal synapse elimination in the LA, these results suggest that androgens prevent synapse elimination in the LA, causing multiple innervation to persist, perhaps permanently, into adulthood.

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331.18 **DEVELOPMENT OF AXONAL CONNECTIVITY IN RELATION TO A LEARNED BEHAVIOR.** S.W. Bottjer, S.A. Brown* & E.A. Miesner*. Department of Psychology, UCLA, Los Angeles, CA 90024

Male zebra finches learn a specific vocal pattern during a "critical" period of development. We have shown that the forebrain nucleus MAN (magnocellular nucleus of the anterior neostriatum) is uniquely involved in vocal learning during a restricted period of development: Lesion of MAN in juvenile males during early vocal development (25-50 days) produces profound and permanent impairment of song behavior, whereas the same lesion in older birds has no effect (Bottjer et al, 1984). There is a massive loss of neurons from MAN during the time that lesions are effective (Bottjer et al, 1985), and this loss appears to be due to a period of cell death that peaks around 30 to 35 days. Thus, MAN has many neurons when birds are first learning to produce song (25 days), but has only half as many neurons by the time lesions are becoming ineffective (55-60 days). During the period of neuronal loss, the absolute number of androgen target cells in MAN remains constant, although the ability of individual MAN cells to accumulate androgenic hormones is much greater in older (60-day) birds (Bottjer & Arnold, 1985).

We now report that the only known afferent input to MAN (from the medial nucleus of the dorsolateral thalamus: DLM) is established very late in development. Injection of horseradish peroxidase (HRP) into DLM of adult birds (>90 days) results in heavy anterograde label over MAN. The same technique applied to younger birds yields the following pattern: DLM axons have grown up to MAN by 25 days, but they remain clustered around the mediodorsal border until at least 40 days; by 45 days there are some thick labeled processes throughout MAN, but the fine-grained label characteristic of the adult pattern only appears some time between 45 and 60 days. In contrast, the only efferent projection from MAN (to RA: robust nucleus of the archistriatum) is established by 25 days. RA in turn projects directly onto the vocal motor neurons.

The overall pattern of results suggests the following: MAN may subserve some function that is critical for vocal learning, and may be directly involved in vocal production by juvenile birds (although it is clearly not on the main motor pathway in adulthood). The loss of neurons from MAN may be importantly related to vocal development, but does not correlate directly with the apparent change in function of MAN. The formation of axonal connections from DLM to MAN does seem to correlate with the decreased effectiveness of MAN lesions. One hypothesis we are currently investigating is that DLM input increases the ability of MAN cells to concentrate androgen, which could in turn promote permanent biochemical or morphological changes in RA that are necessary for vocal learning.

ENDOCRINE CONTROL OF DEVELOPMENT I

332.1 DYE COUPLING AND PHYSIOLOGY ARE SEX SPECIFIC PROPERTIES OF LARYNGEAL MUSCLE FIBERS IN THE FROG, *XENOPUS LAEVIS*. M.L. Tobias and D.B. Kelley, Dept. Biol. Sci., Columbia Univ., New York, N.Y. 10027

Xenopus laevis uses sexually dimorphic vocalizations as part of its reproductive repertoire. We have previously shown that male laryngeal muscle is capable of contracting at rates required to produce the male specific mate call. Female laryngeal muscle cannot contract as rapidly, and is thus incapable of producing the mate call (*Soc. Neurosci. Abs.* 11:152.4, 1985). In a further effort to characterize the basis of sexual dimorphism in this system we have examined the physiological and morphological properties of individual fibers in male and female laryngeal muscle. Our results indicate that even the physiological characteristics of individual muscle fibers are sex-typical. Further, labelling with Lucifer Yellow has revealed that only females display dye coupling between fibers.

Male laryngeal muscle contains two predominant fiber types. Type 1 fibers respond to single shocks of the laryngeal nerve in a voltage dependent manner: a low voltage stimulus results in an EPSP and a higher voltage stimulus results in an action potential. Stimulus trains at the subthreshold voltage result in an initial EPSP followed by action potentials which follow stimulus pulses 1:1 for the duration of the train. Type 2 fibers require repetitive stimulation to produce an action potential. Single stimuli never result in an action potential regardless of stimulus strength. Stimulus trains result in EPSPs which increase in amplitude and eventually give rise to an action potential. Type 2 fibers can be further subdivided. In some cells (Type 2a) action potentials are produced after a specific number of subthreshold events, regardless of stimulus frequency. In the other type (2b), the number of action potentials produced is frequency dependent; the first action potential is generated earlier in the train as the frequency increases. Lucifer Yellow injection into either Type 1 or Type 2 fibers results in labelling of only one fiber.

Female laryngeal muscle contains one predominant fiber type, whose physiological characteristics are distinct from male fibers. Female fibers respond in an all or none fashion to single stimuli. Subthreshold voltages result in no response, suprathreshold voltages result in an action potential. Stimulus trains result in action potentials which follow stimulus pulses 1:1. Lucifer Yellow injection into 3/5 female fibers results in multiple labelled cells; as many as twelve fibers are labelled per injection.

In either sex, physiological fiber types typical of the other sex are rarely encountered. We have recorded 4/35 male type fibers in females, and 3/36 female type fibers in males.

In males, repetitive firing of laryngeal axons should first recruit Type 1 and then Type 2 fibers; thereby increasing the number of fibers contracting as the stimulus proceeds. This pattern of recruitment is consistent with the increase in amplitude of individual clicks within a male mate call trill. In females, coupling of fibers may insure an effective contraction in response to the low stimulus rates used to produce the female-typical vocalization, ticking.

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332.2 ORGANIZATION AND ACTIVATION OF SEXUALLY DIMORPHIC VOCALIZATIONS; ANDROGEN LEVELS IN DEVELOPING AND ADULT *XENOPUS LAEVIS*. L.T. Lambdin* and D.B. Kelley, Dept. Biol. Sci., Columbia Univ., New York, N.Y. 10027

Mate calling in adult male South African clawed frog *Xenopus laevis* is regulated by gonadal androgens. Gonadotropin induces and gonadectomy eliminates mate calling; calls can be reinstated by exogenous androgen (Wetzel and Kelley, *Hormones and Behavior*, 1983). Females do not mate call even when androgen treated (Hannigan and Kelley, *J. Comp. Physiol.*, 1986). The neuroeffector organ for vocal behaviors, the larynx, undergoes dramatic physiological and morphological changes in response to androgen during early post-metamorphic (pm) development (Sassoon *et. al., Dev. Biol.*, 1986). Androgen induces myogenesis and chondrogenesis resulting in masculinization of the phenotypically female, juvenile larynx. Both adult and developing larynx and laryngeal motor neurons express high levels of androgen receptor (see Kelley, *J. Neurobiol.*, 1986 for review). The larynx of adult females hypertrophies in response to androgen. Thus, endogenous levels of androgen are critical to the normal development of the larynx and also to the ultimate expression of vocal behavior. In this study we measured circulating androgen levels in males and females at various stages of development and in adulthood.

Blood was collected and total androgen titers measured by radioimmunoassay from five developmental stages: 1) 0 months pm; 2) 2-3 months pm; 3) 6 months pm; 4) 10 months pm; 5) > 24 months pm (adults). Androgen levels at 0 months pm were sexually monomorphic: 34 pg/ml. Male levels had risen to 446 pg/ml by 2-3 months pm whereas female levels were 115 pg/ml. Six month pm males had levels of 862 pg/ml, comparable to adult male levels of androgen. Female 6 month values were 138 pg/ml. Male values at 10 month pm were 900 pg/ml, while 10 month female values were 221 pg/ml. Circulating adult values were sexually dimorphic: male, 955 pg/ml and female, 369 pg/ml. Thus, androgen levels were sexually dimorphic throughout post metamorphic development. Injection of human chorionic gonadotropin (HCG) into adults increased androgen levels in both sexes. Peak values 6 hours after injection were approximately 8 times basal levels. Androgen levels in HCG-injected females were approximately twice basal values in uninjected males.

The failure of HCG and of exogenous androgen to induce male mate calling in females suggests that adult females are behaviorally insensitive to androgen. Developing males exhibit high levels of androgen by 6 months pm, whereas females undergo a gradual increase in androgen levels throughout development. The period from metamorphosis to 6 months is a critical one for the establishment of sex differences in the larynx. We suggest that male-specific levels of androgen secretion during this period are responsible for laryngeal masculinization and the ultimate expression of a masculine vocal repertoire.

Supported by NS 23684

332.3 PROGRESSIVE SEXUAL DIFFERENTIATION OF BRAIN AROMATASE AND REPRODUCTIVE BEHAVIOR IN THE JAPANESE QUAIL.

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The brain metabolism of testosterone (T) determines the sensitivity to the circulating hormone. In the hypothalamus, T is converted into behaviorally active metabolites such as estradiol (E2) and 5a-dihydrotestosterone (5a-DHT) and into inactive 5b-reduced metabolites. In quail like in many other vertebrates, aromatization of T into E2 is important for the activation by T of male sexual behavior.

Sexual differences in brain sensitivity to behavioral effects of T have been observed and have been related to differences in T metabolism. In most mammals and birds, females are less sensitive than males to the activating effects of T. In quail, T induces copulatory behavior in castrated males but not in ovariectomized females. T also induces less efficiently the preoptic aromatase activity in gonadectomized females than males. This represents a precise molecular correlate for the behavioral dimorphism and many experiments suggest that it could have a causal value. We are currently investigating the precise neuroanatomical distribution of the sexually dimorphic aromatase using the Palkovits punch technique.

It is generally accepted that the behavioral dimorphism in adults is due to the permanent organizing action of steroids on the brain during a limited period of early development. We have shown however that in quail, the sexual differentiation is not restricted to a short embryonic period but only ends several weeks post hatching. Similarly, the T-inducible aromatase is not sexually differentiated during the first week post-hatch as it is in adults. There is a developmental decline in sensitivity of the female brain to the inductive effects of T on aromatase which correlates with the postnatal loss of sensitivity to the behavioral effects of T. Additional experiments relating the preoptic aromatase to the differentiation and activation of copulatory behavior in males and females during development are now in progress and support the notion that preoptic aromatase plays a causal role in the control of behavior.

332.4 SEX DIFFERENCES IN THE SONGBIRD BRAIN INVOLVE NEURONS BORN DURING ADOLESCENCE.

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In adult zebra finches, several neural regions controlling song behavior contain more neurons in males (who sing) than in females (who do not sing). Regional differences in the ontogeny of these sex differences suggest several mechanisms through which neuron number may be regulated. For instance, in nucleus robustus archistriatum (RA) and magnocellular nucleus of the anterior neostriatum (MAN), males retain neurons that are lost in females during adolescence. In contrast, males (but not females) add neurons to hyperstriatum ventrale pars caudale (HVc) during this same juvenile period. In the present study, we determined whether these apparent differences in the regulation of neuron number correspond to regional differences in the incorporation of neurons born during adolescence.

Beginning at 20 days of age, 3 zebra finches of each sex received injections of 3H-thymidine (2.5 uCi/gm) every 12 hours for 10 days. At 55 days of age, birds were killed and coronal sections (10um) were processed for autoradiography. In HVc and RA, the incidence of labeled neurons was determined for both sexes. The incidence of labeled neurons in MAN and Area X was only determined in males, since these regions are not readily identifiable in females. Cells with a large nucleolus and a distinct nucleus surrounded by darkly staining cytoplasm were classified as neurons.

In RA, virtually no thymidine-labeled neurons were present in either sex (<2%). Likewise, in male MAN few labeled neurons were present (2%). The paucity of labeled neurons in these two regions is consistent with there being no addition of RA or MAN neurons in either sex after 20 days of age (i.e., the first day of 3H-thymidine injections).

In contrast, our data indicate many HVc and Area X neurons are born during adolescence. In male Area X, 14% of the neurons were labeled by 3H-thymidine, suggesting that sexual differentiation of this area may involve neurons born after 20 days of age. Likewise, a large number of thymidine-labeled neurons were present in HVc. More importantly, the proportion of labeled HVc neurons in males (13%) was higher than in females (6%). Since the total number of HVc neurons is much greater in males than in females at 55 days of age, the above data reflect a robust sex difference in the absolute number of surviving HVc neurons born during adolescence. Moreover, since these newly generated neurons are born after the time during which hormone exposure can induce masculinization of song control nuclei, our data suggest that hormones may enhance neuron proliferation, as well as retention, to create sex differences in neuron number.

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332.5 THE DEVELOPMENT OF SEXUAL DIMORPHISM IN AREA X OF THE ZEBRA FINCH SONG SYSTEM

J. R. Kirn and T. J. DeVoogd, Department of Psychology, Cornell University, Ithaca, NY, 14853

Area X exhibits the largest sexual dimorphism of any nucleus in the Zebra Finch song system. This nucleus is prominent in adult males but absent in females (Nottebohm & Arnold, 1976; Ryan & Arnold, 1979). Ontogenetically, X may also be unique among song nuclei. HVc (Hyperstriatum ventrale pars caudale) and RA (Robustus Archistriatalis) are less dimorphic during development than in adulthood (Bottjer, et al., 1985; Konishi & Akutagawa, 1985), whereas female X is not visible with Nissl stain at any age. Differential cell death is involved in the establishment of sex differences in HVc and RA cell number (Kirn & DeVoogd, 1985). This report describes cell death in Area X and surrounding Lobus Parolfactorius (LPO).

In 10-20μ cresylecht violet stained coronal brain sections from 10 (10-11), 15 (15-17) and 20 (19-25) day old finches, the position of X in LPO was determined in males and was used to define putative X in same-age females (n = 2-3 birds/sex/age). In 5 sections, the entire LPO including X was scanned for degenerating cells, and the number of degenerating cells per $1000\mu^2$ was calculated.

Collapsing across ages, no overall sex difference in LPO cell death was observed ($\bar{x} \pm SD = 4.93 \pm 3.13$ degenerating cells (dc) in males; 5.24 ± 1.73 dc in females), or on day 20 (males = 3.87 ± 0.97; females= 3.76 ± 0.75). In contrast large sex differences were observed on days 10 and 15. (Day 10 males = 9.38 ± 0.98 dc; females = 5.36 ± 1.83 dc; day 15 males = $2.74 \pm .001$ dc; females = 5.95 ± 2.16 dc).

The occurance of greater cell death in males than in females at 10 days appears paradoxical: a simple process of sculpting a male-female dimorphism by differential cell death appears unlikely. However, perhaps cells destined for X are born and die in a restricted time interval after day 10. Preliminary ^3H thymidine data indicate that neurons destined for X in males may be generated as late as 4 weeks after hatching. Further thymidine and degeneration analyses are in progress to resolve this issue.

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332.6 DENDRITIC RETRACTION AND SPINE ELIMINATION IN FEMALES CONTRIBUTE TO SEX DIMORPHISMS IN THE AVIAN SONG SYSTEM

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The avian song control network has proven to be a highly productive model system in which to study neural sex differences in relation to a dimorphic behavior. Typically, male songbirds sing and females do not; the brain regions involved in song production are substantially larger in males than in females.

We have begun to study the development of dimorphic anatomy in song system nucleus RA in zebra finches. It has been shown that this nucleus is 5.5 times larger in mature males than mature females (Nottebohm and Arnold, 1976), and that divergence in nucleus volume in the early weeks of life results both from growth in the male and from condensation in the female (Konishi and Akutagawa, 1985). We have shown that during this time interval, cell death is occuring in females at 2-4 times the rate seen in males, suggesting that divergent cell death contributes to the sex difference in RA size (Kirn and DeVoogd, 1985). We now assess the morphology of dendritic fields and spines during the time in early life of morphological divergence between the sexes.

Male and female zebra finches were killed at weekly intervals from 2 to 8 weeks after hatching. The brains were stained with a Golgi-Cox procedure (Van der Loos, 1956), sectioned at 90-120μm and counterstained with thionin and methylene blue. Well-stained type IV neurons (DeVoogd and Nottebohm, 1981) in RA were quantified in three dimensions using a semi-automated microscope system, in order to obtain total dendritic field size, and spine density at 20 μm intervals from soma to dendritic tips.

We find similar dendritic fields in males and females at 2 weeks (927 and 871μm respectively). Female fields remain constant to 4 weeks and decrease thereafter such that at 8 weeks, a 23% decrease in field size has occured.

Spine density is also similar in males and females at 2 weeks, then rises in males at 3 weeks leading to a density 20-25% higher in males than in females through 8 weeks. We then multiplied mean spine density by mean dendritic field size to get an estimate of spines/neuron at each age. Spine number follows the same pattern: similar between the sexes at 2 weeks (M = 582, F = 659), substantially different at 3 weeks because of a male rise (M = 1425, F = 789), a difference that persists in spite of subsequent drops in both sexes (M = 1092, F = 542 at 8 weeks).

We believe the male hypertophy in these measures at three and four weeks of age and the female atrophy somewhat later may result from the afferentation from HVc which is then occuring in males but not females (Konishi and Akutagawa, 1985). Thus the growth and regression seen in the nucleus as a whole may result in part from changes occuring at the dendritic and synaptic level.

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332.7 EFFECT OF BIRTH ON PLASMA STEROIDS AND ON HYPOTHALAMIC ACTIVITY OF AROMATASE AND CONCENTRATIONS OF ESTRADIOL IN THE FERRET. M.S. Erskine, S.A. Tobet and M.J. Baum, Dept. of Biology, Boston University, Boston, Ma. 02215.

Circulating and hypothalamic concentrations of testosterone (T) and estradiol (E_2) increase dramatically in male rats within two h. after birth (Rhoda et al., Endocrinol., 114:1754, 1984). Since masculinization of sexual behavior in male ferrets may be promoted by postnatal secretion of testicular steroids (Erskine & Baum, Endocrinol. 114:2440, 1984), we examined the patterns of T and E_2 secretion immediately after birth in this species. Initially, ferret kits were delivered by caesarian section on gestational days 40 or 41 (normal gestation = 42 days). Plasma and preoptic area (POA) + mediobasal hypothalamus (MBH) were taken either immediately (0 h) or 2 h after delivery, and were pooled prior to steroid RIA. Plasma T concentrations were significantly higher at 2 h than at 0 h in both sexes and were significantly higher in males than in females at both times (means for males: 0 h, 3.3 ng/ml, 2 h, 9.5 ng/ml; for females: 0 h, 1.2 ng/ml, 2 h, 3.4 ng/ml). Plasma E_2 levels were below the assay detection limit in 17/22 samples. The levels of E_2 measured in the POA + MBH were variable, but tended to be higher 2 h after delivery, regardless of sex. Estimates of aromatase activity in the POA and in the MBH were equivalent at 0 h and at 2 h in both sexes. Thus any rise in POA + MBH concentrations of E_2 after birth probably results from increased availability of T substrate and not from activation of aromatase. In a subsequent study ferret kits were removed from their mothers immediately after natural delivery, and plasma was taken at 0 h and at 2 h. Again, in both sexes significant increments in circulating T occurred within 2 h, and at both 0 h and 2 h T values were higher in males than in females (means for males: 0 h, 6.4 ng/ml, 2 h, 22.0 ng/ml; for females: 0 h, 2.8 ng/ml, 2 h, 6.6 ng/ml). Plasma E_2 levels were equivalent in males and females, with significant elevations being detected 2 h after natural delivery (means of combined sexes: 0 h, 66 pg/ml, 2 h, 292 pg/ml). Absolute plasma concentrations of T and E_2 were at least 2 times higher in kits delivered naturally as opposed to by caesarian section. Within 2 h of natural delivery plasma T and E_2 concentrations reached or exceeded the levels of these steroids normally seen in adult male and female ferrets during the breeding season.

These results corroborate previous findings in the rat, and raise the possibility that in ferrets, as in rats, large postnatal increments in sex steroid availability may influence brain and behavioral sexual differentiation.

(supported by HD 21094 and MH 00392)

332.8 GONADAL-INDEPENDENT ACTION OF MELATONIN ON PULSATILE LH RELEASE IN OVARIECTOMIZED PREPUBERTAL RATS. H.F. Urbanski* and S.R. Ojeda* (SPON: S.M. McCann). Department of Physiology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235-9040.

In female rats a diurnal pattern of LH secretion becomes established during the juvenile-peripubertal period, with LH discharges being more prominent in the afternoon than in the morning. We have recently found that this activation of LH release originates centrally as it can be demonstrated even in the absence of the ovaries, using a short-term ovariectomy (OVX) paradigm. In the present study we investigated the possibility that the well-known delaying effect of melatonin on the timing of puberty may be related to an alteration of this centrally-driven diurnal pattern of LH release.

Immature female rats were maintained under a 14L:10D photoperiod (lights on 5.00-19.00 h) and given daily s.c. injections of melatonin (100 µg in saline) at 16.00 h (from day 20 until the time of vaginal opening). Melatonin significantly ($P<0.05$) delayed the age at vaginal opening (41.6 ± 1.2 days, n=22, melatonin-treated animals vs 37.8 ± 0.8 days, n=21, saline-treated controls). This same regimen was then used to examine the effect of melatonin on LH release in short-term OVX, 36-day-old rats. The animals were treated daily with melatonin (from 20-35 days of age). They were OVX at 32 days and 4 days later blood samples (150 µl) were collected every 5 min (from 13.00-18.00 h) using a previously described automated blood sampling technique. The plasma was assayed for LH by RIA. Individual plasma LH profiles were analyzed using the PULSAR algorithm and compared to those obtained from untreated OVX animals of the same age, either in the morning (from 8.00-13.00 h) or in the afternoon (from 13.00-18.00 h). As recently shown (Proc. Soc. Study of Fertility, 1986), the total LH secretory output in untreated OVX rats was greater in the afternoon than in the morning. Melatonin significantly ($P<0.05$) reduced the total afternoon LH secretory output so that it became indistinguishable from that observed in the morning. Inhibition of enhanced afternoon LH secretion by melatonin was further indicated by a significant ($P<0.05$) suppression of the nadir plasma LH levels. On the other hand, no differences were found between LH pulse amplitude, interpulse interval or peak plasma LH levels of the various groups. These findings suggest that melatonin may exert its delaying effect on the timing of female puberty by inhibiting the centrally-driven afternoon activation of LH release that occurs during the peripubertal period of development. (Supported by NIH Grant HD-09988, IV)

332.9 TREATMENT OF NEONATAL RATS WITH GONADAL STEROIDS AFFECTS IMMUNOREACTIVE BETA-ENDORPHIN LEVELS IN THE ADULT. L. Forman, and S. Estilow*, Department of Medicine, UMDNJ-School of Osteopathic Medicine, Camden, NJ 08103.

Administration of testosterone to neonatal male rats or estrogen to neonatal female rats, has a profound effect on the function of the hypothalamic-pituitary unit in the adult animal. Considering that gonadal steroids modulate hypothalamic and pituitary levels of beta-endorphin (BE) in adult male and female rats, the effects of neonatal gonadal steroid treatment on BE levels in the adult animal were investigated. Neonatal Sprague-Dawley (S-D) male rats were administered testosterone proprionate (500 ug/rat/day, sc in corn oil) at 2,4,6 and 8 days of age. Neonatal S-D female rats received estradiol benzoate (50 ug/rat/day, sc in corn oil) at 2-10 days of age. Matched control littermates were administered corn oil alone. All animals were sacrificed at 90 days of age and immunoreactive beta-endorphin (IR-BE) was measured by radioimmunoassay and column chromatography. Neonatal gonadal steroid treatment did not affect the level of IR-BE in the anterior pituitary (AP) of male rats, but did result in a significant increase in IR-BE in the AP of female rats. Neonatal administration of gonadal steroids produced a significant increase in IR-BE in the neurointermediate lobe of the pituitary (NIL) of both male and female rats, with the magnitude of the increase being greater in the NIL of the female rats. IR-BE levels in the hypothalamus of the male or female rats were not altered by the treatments. Column chromatography indicated that the increase in IR-BE in the AP of female rats represented a proportional increase in BE and beta-lipotropin, whereas the reduction in IR-BE in the NIL of the treated rats represented a reduction in BE. These findings suggest that gonadal steroids may influence a) the development of the neurotransmitter systems which regulate BE levels in the adult pituitary, b) the development of the biosynthetic mechanisms of the adult pituitary, c) or both. Furthermore, the developmental effects of gonadal steroids appear to be more pronounced in female than in male rats.

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332.10 SPECIFIC PROTEINS OF THE ARCUATE-MEDIAN EMINENCE AFFECTED BY ESTRADIOL IN PREPUBERAL FEMALE RATS. J.F. Rodriguez-Sierra, W.E. Heydorn, G.J. Creed* and D.M. Jacobowitz. Dept. Anatomy, Univ. Nebraska Med. Ctr., Omaha, NE 68105 and Lab. Clinical Science, NIH-NIMH, Bethesda, MD 20892.

The arcuate nucleus of the hypothalamus (AH) undergoes major structural changes during normal puberty or if exposed to a pulse of estradiol in the prepuberal period. Those changes are expressed by increased synaptogenesis and by a drastic alteration in the feedback control of anterior pituitary gland hormone release. Two studies were conducted to determine whether the effects of estradiol affected the concentration or formation of proteins in the AH. In the first experiment, 25-day-old female rats were administered estradiol benzoate (EB, 10 ug, sc in oil) or vehicle. Forty-eight hours after treatments, animals were decapitated their brains removed, and sectioned. The AH-median eminence region was dissected out and processed for isoelectric focusing followed by slab gel electrophoresis. The resulting 2-dimensional electrophoretic gels were further analyzed to quantitate the specific proteins resolved using a scanning microdensitometric method. Out of 235 proteins measured, 8 proteins were found to be significantly increased and 4 were decreased by EB treatment. The proteins that were increased in concentration ranged in molecular weight (MW) from 14,000 to 43,000 Daltons and isoelectric points (pI) of 4.9 to 7.0 The four proteins that were decreased by EB treatment were 43,000, 67,000, 70,000 and 75,000 Daltons in MW and their pI's ranged from 6.3 to 7.2 In the second experiment, 25-day-old female rats were administered EB (10 ug, sc in oil) or oil vehicle. The animals were decapitated at either 17 or 42 hours after EB treatment. The brains were removed, blocked and serially sectioned with a Vibratome (300 um). The AH-median eminence area was dissected out and incubated for 6 hours in a medium containing ^{35}S-methionine and ^{35}S-cysteine. Proteins were separated by two-dimensional gel electrophoresis, and the resulting autofluorographs were analyzed by scanning densitometry. Our results show that incorporation of labelled amino acids was increased in 10 proteins of rats killed at 17 hours after EB, while 3 proteins decreased. At 42 hours after EB, 6 proteins showed an increased incorporation of labelled amino acids and two proteins showed a decrease. Our results point to the possibility that one or more of the affected proteins is involved in the neuroendocrine and morphological changes produced by estradiol during the puberal period of development in the female rat and to the isolation of a synaptic growth factor induced by estradiol. (This work was partially supported by a Faculty Development Fellowship from the Univ. Nebraska Medical Center).

332.11 ONTOGENY AND STEROID DEPENDENCY OF A SEXUALLY DIMORPHIC OPIATE SYSTEM IN THE PREOPTIC AREA OF THE RAT. R.E. Watson, Jr., G.E. Hoffman, S.J. Wiegand, K.A. Allan*, and T.S. Peppy*. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

A profound sexual dimorphism in the density of methionine enkephalin (m-ENK) immunoreactive (ir) fibers exists in the periventricular preoptic area (pePOA), a cytoarchitectonically dimorphic region, in the rat. A dense plexus of m-ENK ir fibers, approximately 100 µm wide, is present in the subependymal zone in females, but not in males. We have observed that expression of the female-typical system can be altered in both an organizational and activational manner by the gonadal steroids. Exposure of the female neonate to aromatizable androgen blocks expression of the m-ENK system at adulthood. Additionally, ovariectomy of the adult female causes loss of expression of the system, whereas estrogen replacement reverses it. Preliminary experiments also showed that the female-typical system was not expressed in the juvenile pre-pubertal female. Therefore, we undertook a more complete study examining the temporal relationship of expression of the pePOA m-ENK system with pubertal onset.

Juvenile rats were perfused prepubertally (days 10-30), or peri-pubertally (days 32-40), and their brains processed for the immunocytochemical localization of m-ENK using the biotin-avidin technique. The results showed that the female-typical dense m-ENK fiber plexus was not expressed until the peri-pubertal period and during this time there was considerable variation in the density of m-ENK ir present in the pePOA.

The effects of exogenous estrogen exposure were also examined in the prepubertal female. On postnatal days 15, 20, or 25, rats were injected with 10 µg of estradiol benzoate, and on the following day were implanted sc. with a Silastic capsule (10 mm. in length) containing estradiol. Control animals received an oil injection and were implanted with an empty Silastic capsule. Following steroid exposure for a total of 3 or 7 days, the animals were perfused, and m-ENK immunocytochemistry performed. At all time points, exposure to estrogen for 3 days resulted in detectable expression of the m-ENK ir fiber plexus, whereas exposure for 7 days brought about full expression of system, such that it was indistinguishable from that of normal adult females. Control animals had no enhanced m-ENK ir in the pePOA.

These results further demonstrate the steroid dependence of this opiate system within a sexually dimorphic component of the preoptic area. These data, together with our recent finding with immunoelectron microscopy of enkephalinergic terminals in the pePOA, suggests that this region is a critical site at which opiate effects are exerted upon sexually differentiated functions.

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332.12 SEXUAL DIMORPHISM OF VASOPRESSIN INNERVATION OF THE PREOPTIC AREA OF THE HAMSTER BRAIN. G.E. Hoffman, R.E. Watson, Jr., and S.J. Wiegand. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, N.Y., 14642.

The preoptic area (POA) is host to a variety of sexually dimorphic reproductive functions including the regulation of hormone cyclicity and appropriate sexual behavior. Cytoarchitectonic sex differences in POA nuclear structures (Bleier, et al., JCN 212: 118, 1982; Gorski, et al., JCN 193: 529, 1980) as well as differences in the innervation of the POA (Simerly, et al., Br. Res. 330: 55, 1985; Watson, et al., Soc. Neurosci. Abstr. 11: 160, 1985) may underlie these functional differences. But thus far, generally, a direct link between the regulated functions and structural dimorphisms is lacking.

In the hamster, infusion of the neuropeptide vasopressin (VP) into the POA of either males or females elicits flank-rubbing behavior, generally typical of the male (Ferris, et al., Sci 224: 521, 1984). This suggests that the target neurons of the POA that are sensitive to VP are present in both sexes, but that the VP signal may differ in males and females. Thus, in the present study VP innervation of the POA was examined in 6 male and 6 female (in proestrus) adult Syrian hamsters. The animals were anesthesized and perfused transaortically with saline followed by Zamboni's fixative. Each brain was removed, sectioned on a vibrating microtome and processed using the avidin-biotin technique for the immunocytochemical localization of specific neurophysins. Antiserum to rat neurophysin (RNP) 2, the binding protein specific for vasopressin, was purchased from Cal. Med., while anti-RNP 4, specific for both oxytocin and vasopressin, was generously provided by Dr. Alan Robinson.

The results showed virtually no difference in the patterns of labeling in the POA between brains stained for RNP 2 and RNP 4, indicating that the innervation is principally vasopressinergic. Comparison of tissue from males and females revealed a clear sexual dimorphism in a component of the POA. Specifically, in males, the anteriorly situated medial preoptic nucleus (Bleier, et al., JCN 212: 118, 1982) and its immediately surrounding lateral zone were consistently more heavily innervated. This study demonstrates that the hamster, like the rat (Watson, et al., Soc. Neurosci. Abstr. 10: 436, 1984), possesses a striking sexual dimorphism of VP innervation in the POA, and suggests that differences in the VP input to the POA dictate certain sexually dimorphic behaviors.

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332.13 PRENATAL MANIPULATION OF SEROTONIN AND DEVELOPMENT OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. M. Hines and R.A. Gorski. Dept. of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA Sch. of Med., Univ. of California, Los Angeles, CA. 90024.

The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) is several times greater in adult male than in adult female rats, and is approximately 2 times greater in male than in female neonates. Several studies indicate that the size of the SDN-POA is related closely to the gonadal hormone environment during early life. Recently, we have been interested in the possibility that serotonin (5HT) also may influence SDN-POA development. Neurons in the SDN-POA appear to be innervated by 5HT fibers (Simerly et al., J. Comp. Neurol. 225: 151-166, 1984; Larriva-Sahd et al., Soc. Neurosci. Abstr. 11: 1229, 1985), and neural development in other brain regions that receive 5HT innervation is influenced by prenatal manipulation of 5HT (e.g., Lauder, Psychoneuroendo. 8: 121-155, 1983). In a prior study we treated pregnant rats with an inhibitor of 5HT synthesis, parachlorophenylalanine (PCPA), or with saline, and assessed the volume of the SDN-POA in male and female offspring on the day of birth (Handa et al., submitted). The PCPA treatment eliminated the sex difference in the SDN-POA by enhancing the volume of the nucleus in female rats. This influence appeared to be independent of steroid hormone secretion. Plasma testosterone and estradiol levels were unchanged in PCPA-treated rats. The present study was intended to determine if the effect of prenatal exposure to PCPA on the SDN-POA persists into adulthood. Prenatal treatments were identical to those used in the previous study. On day 8 of gestation, pregnant rats were implanted subcutaneously with osmotic minipumps (ALZET CORP.) containing either 400 mg PCPA in 2 ml saline, or 2 ml saline alone. These pumps released approximately 0.14 ml solution per day until parturition. Offspring of PCPA- and saline-treated pregnancies were sacrificed in adulthood and SDN-POA volumes were assessed as described in previous papers. Results suggested that prenatal exposure to PCPA had no influence on adult SDN-POA volumes. There were comparable (approximately 5-fold) sex differences in saline- and PCPA-treated groups. Also, both PCPA-treated male and female rats had similar SDN-POA volumes to those of saline-treated animals. One possible explanation of these data is that inhibition of 5HT synthesis delayed synaptic activity and functional innervation of SDN-POA neurons, and thus postponed their death in the female rat. If so, mechanisms underlying neuronal death in the SDN-POA may resemble those observed for motor neurons in the chick spinal cord, where blockade of neuromuscular transmission delays cell death until the blocking agent is removed (e.g., Pittman and Oppenheim, J. Comp. Neurol. 187: 425-446, 1979). Supported by USPHS Grants #NS 20687 to MH and #HD01182 to RAG.

332.14 LONG TERM EFFECTS OF NEONATAL SEX HORMONE MANIPULATION ON MPOA OPIATE RECEPTORS. M. Hijazi* and R.P. Hammer, Jr. (SPON: H. Gillary). Depts. of Physiology and Anatomy & Reprod. Biol., Univ. of Hawaii School of Medicine, Honolulu, HI 96822.

In the adult rat the opiate receptor distribution in the medial preoptic area (MPOA) of the hypothalamus has been shown to be sexually dimorphic (Hammer, Brain Res. 308:172). Normal adult females in estrus exhibit a region of high opiate receptor density within the MPOA, whereas males show a less dense and more uniform MPOA opiate receptor distribution. The sex difference in MPOA opiate receptors is apparent by postnatal day 6. Moreover, this early sex-specific opiate receptor distribution can be altered by neonatal sex hormone manipulation (Hammer, Brain Res. 360:65); neonatally-castrated male pups develop the typical female MPOA receptor concentration by day 6, while female pups treated with testosterone from birth to postnatal day 5 develop the male pattern of opiate receptor distribution. The purpose of this study is to determine whether perinatal sex hormone manipulation can permanently alter MPOA opiate receptor distribution. One group of male pups were castrated at birth (day 1). Another group of female pups were injected with testosterone propionate (TP-250ug/day) subcutaneously for 5 consecutive days starting on day 1 and ending on postnatal day 5. Control groups include sham-operated males and untreated females. These animals were decapitated at 60 days of age. Brains were rapidly removed, frozen in -30°C isopentane, and sectioned in a cryostat at -14°C. Tissue sections were incubated in 2.5 nM [^{3}H]naloxone at 0°C for 60 min, rinsed, dried, fixed, defatted, and exposed to tritium-sensitive film. Film autoradiographs were quantitatively analyzed using a computer-assisted densitometry system. Preliminary results indicate the presence of an apparent opiate receptor density in normal females and neonatally-castrated males. Sham-operated males and TP-treated females, on the other hand, did not exhibit an MPOA opiate receptor concentration. Thus, the sex hormone-induced alteration of MPOA opiate receptor content endures throughout development and into adulthood. This long-term effect of perinatal sex hormone manipulation on MPOA opiate systems could prove to be the basis for some behavior differences in these animals. (Supported by PHS Grant HD 19951)

332.15 MODULATION OF AROMATASE ACTIVITY BY TESTOSTERONE IN TRANSPLANTS OF FETAL RAT HYPOTHALAMUS-PREOPTIC AREA. C.M. Paden and C.E. Roselli. Dept. of Biology, Montana State Univ., Bozeman, MT 59717 and Dept. of Physiology, Oregon Health Sciences Univ., Portland, OR 97201.

Conversion of androgens to estrogens by neural aromatase appears to be a prerequisite for a variety of effects of androgens on brain function, including sexual differentiation. Activity of aromatase is modulated by its substrate testosterone (T) in adult hypothalamus-preoptic area (HPOA), resulting in significantly higher levels in the male (Roselli et al.,1985, Endocrinol. 114:192). Perinatal sex differences in activity have also been observed in hypothalamus, POA and/or amygdala (Reddy et al.,1974, Endocrinol. 94:117; MacLusky et al., 1985, Psychoneuroendocrinol. 10:355; Tobet et al., 1985, Dev. Brain Res. 23:171). It is not known if higher levels in the perinatal male occur in response to circulating androgens.

In order to investigate the influence of early steroid exposure on the development of neural aromatase activity, E17 fetal HPOA was transplanted onto the choroidal pia of adult ovariectomized-adrenalectomized (OVX-ADX) Holtzman female hosts as previously described (Stenevi et al., 1976, Brain Res. 114: 1). In the first experiment, the effect of androgen exposure on aromatase activity in mature HPOA transplants was determined. Hosts received T silastics sc or sham surgery 7 weeks after transplantation and were sacrificed 7 days later. Aromatase activity was determined in vitro using the stereospecific production of ^3H_2O from [1β-^{3}H]androstenedione as an index of estrogen formation (Roselli et al., ibid.). Aromatase activity was significantly greater in T-treated HPOA versus controls (44.18 vs 10.81 femtomoles/hr mg protein; $F=10.54$, $p<.005$, $df=1/26$). Activity was not affected by the sex of the donor fetus. In the second experiment, the effect of androgen exposure during the first 6 days following transplantation of E17 HPOA (corresponding to the last gestational week) was determined. OVX-ADX hosts were either sham operated or implanted sc with T silastics. Aromatase activity was much higher than in 8 week transplants, and T treatment again resulted in significantly enhanced levels versus controls (324.9 vs 118.7 fmoles/hr mg; $F=9.97$, $p<.01$, $df = 1/11$). There was no effect of the sex of the fetal donor.

These results indicate that both basal levels and the androgen sensitivity of aromatase will develop in the rat HPOA of both sexes without exposure to gonadal steroids after E17, and without the influence of neural inputs to the HPOA. In addition, they establish that the mechanism of androgenic modulation of HPOA aromatase is functional in the late embryonic period. However, further studies are needed to determine if normally occurring levels of circulating T are sufficient to cause the reported perinatal sex differences in rat brain aromatase.

This research was supported by NIH grants NS17974 and HD18196.

332.16 AROMATASE ACTIVITY IN EXPLANT CULTURES OF THE DEVELOPING MOUSE BRAIN: IS THE RODENT CEREBRAL CORTEX A TARGET FOR LOCALLY-SYNTHESIZED ESTROGEN? N.J. MacLusky*, A.S. Clark and C.D. Toran-Allerand, Dept of OB/GYN, Yale University Medical School, New Haven, CT.06510 and Center for Reproductive Sciences, College of Physicians and Surgeons, Columbia University, New York, NY.10032

Estrogen receptors are present in the rodent cerebral cortex during perinatal and early postnatal life (MacLusky et al, Brain Res. 178:129, 1979; Gerlach et al, Dev. Brain Res. 11:7, 1983). Estrogen has been reported to exert maturational effects on the rat cerebral cortex and to enhance neurite outgrowth from mouse cingulate cortex in organotypic culture (Toran-Allerand, Prog. Brain Res. 61:63, 1984). The physiologic role of the cortical estrogen receptors, however, remains uncertain. Unlike other estrogen-sensitive regions of the developing rodent brain, the cortex has not been shown to contain the aromatase enzyme complex necessary for estrogen biosynthesis from circulating androgens. In the present study, we have examined the ability of organotypic cultures prepared from regions of the newborn mouse brain to aromatize 19-hydroxy androstenedione (19OHA)$_2$. Tissues were cultured for three days in the presence of [6,7-^{3}H] 19OHA (8.7 pmol/culture). Unlabeled estrone (250 pg) and estradiol (2.5 ng) were added to each culture to limit further metabolism of the estrogen products. Radioactive metabolites in the cultures were characterized by thin layer chromatography, acetylation, reverse isotope dilution and crystallization to constant specific activity. Highest rates of estrogen biosynthesis were observed in cultures from the septum, anterior hypothalamus and preoptic area (270-470 fmol product/μg tissue DNA/day). Aromatization was not demonstrable in cultures from the pons, medulla, cerebellum or spinal cord (<2 fmol/μg DNA/day). Low levels of estrogen biosynthesis were observed in cultures from the midbrain central grey (19.6 +/- 3.1 fmol/μg DNA/day; mean +/- SEM, N=3), hippocampus (13.1 fmol/μg DNA/day; N=2) and cingulate cortex (13.4 +/- 2.8 fmol/μg DNA/day; N=5). These results demonstrate that several areas of the newborn mouse brain are capable of synthesizing estrogen under in vitro organotypic culture conditions. They also suggest that the estrogen-mediated developmental effects of aromatizable androgens may not be restricted to the hypothalamus, preoptic area and amygdala, but may also include other areas in the midbrain and limbic system. The high estrogen receptor levels observed in the cingulate cortex during early life may thus be involved in responses to locally-synthesized estrogen, as well as to the circulating hormone (Supported by NIH grants NS07724, HD08364 and MH00192).

332.17 STEROID HORMONE BINDING AND METABOLISM IN CEREBRAL CORTEX AND LIMBIC STRUCTURES OF THE DEVELOPING RHESUS MONKEY. A.S. Clark, N.J. MacLusky* and P.S. Goldman-Rakic. Sect. Neuroanatomy and Dept. OB/GYN, Yale Univ. Sch. Med., New Haven, CT 06510.

In many mammalian species sexual differentiation of the brain reflects early exposure to sex-specific patterns of gonadal steroid secretion. Many studies have examined the actions of steroid hormones on neuroendocrine tissues, particularly the hypothalamus. These actions are known to influence reproductive endpoints including gonadotropin secretion and sexual behavior. The possibility exists that maturation of the cerebral cortex may also be regulated by gonadal hormones. Our laboratory is investigating the role of gonadal steroids in the development and organization of the primate neocortex. This work has implications for understanding the impact of exposure to gonadal steroids during early life on the development of cognitive function.

Androgen binding and metabolism were assessed in brain regions from individual fetal (E127 days onward) and postnatal (birth-8 months) monkeys. Most commonly, one hemisphere was taken for steroid binding experiments while the other was processed for metabolism studies. Tissues taken for analysis included: hypothalamus-preoptic area, amygdala, hippocampus, dorsolateral prefrontal cortex, orbital prefrontal cortex, anterior cingulate cortex, parietal, visual, somatosensory and motor cortices. Androgen binding was determined in the cytosol fraction using {^{3}H}methyltrienolone (R1881) as the ligand. The metabolism of 4-{^{3}H}androstene-3,17-dione in tissue homogenates was studied using standard product isolation techniques.

Specific high-affinity androgen binding sites (K_d for {^{3}H}R1881 0.04-0.3 nM) were observed in many areas of the early postnatal monkey brain. These areas included (but were not limited to) the hypothalamus, orbital prefrontal cortex, visual and somatosensory cortices. The highest level of binding was observed in the hypothalamus (8-11 fm/mg protein). Cortical samples had consistently lower binding levels (1-5 fm/mg protein).

Androgen metabolism via aromatization and 5α-reduction pathways displayed a distinct regional pattern and an interesting developmental profile. Overall production of estrogens and 5α-reduced androgens declined substantially from late fetal to early postnatal life. Fetal monkeys had measurable aromatase complex activity in limbic areas and in every cortical region studied. Early postnatal animals had measurable aromatase activity in limbic tissues and in the association cortices; however, activity in the primary cortical areas was dramatically reduced. 5α-Reductase activity appeared to be evenly distributed in the brain regions studied, and this distribution did not change appreciably during development.

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332.18 ULTRASTRUCTURAL CHANGES IN CEREBRAL AND CEREBELLAR CORTICES OF DEVELOPING CRETINOID RATS Max Hamburgh*,Ilya I.Glezer,Charles Gison*,Ebba K.Ebba*,Mark Levie*,Calvert Warren*,Gerard Lois-Jacques*,Will Lee* and Astrid Mendoza*. The City College of New York and CUNY Medical School,New York,NY 10031

Our research was concentrated on finding permanent morphological changes of cortical neurons in developing rats subjected to chronic treatment with a goiterogen. Hypothyroidism was induced by offering goiterogenic diet,containing 0.2% propylthioyracil (PTU) starting 15 -th day prenatally and up to 60-th day postnatally. Neurons of both cerebral and cerebellar cortices are characterized by a transitory reduction in free polysomes, retarded development of the endoplasmic reticulum, mitochondria and components of the synapses(synaptic vesicles, pre and post-synaptic membranes and their densities). However, mentioned ultrastructural changes have disappeared with progress of the brain development. In cerebral cortex they disappeared by day 20 of postnatal age, in cerebellum by day 25. The most consistent change in both cerebral and cerebellar cortical neurons is associated with the plasma membrane of neuronal perikaryon, that was interrupted by osmiophobic gaps in cretinoid animals. This feature was visible at birth and persisted till day 60 when experiment was terminated. In cerebellar neurons(granular cells and Purkinje cells) the damage of the plasma membrane was less severe than in neurons of the frontal and occipital cortices. Probably different regions of the brain respond differently to the lack of thyroid hormone during maturation.It is tempting to speculate that the permanent damage to the plasma membrane induced in hypothyroid animals may be instrumental in bringing about the lasting mental and motor disabilities which are associated with cretinoid syndrome. (Supported in part by NIH grant RR 08168, the MBRS Program and CRS Program of the City College of New York).

332.19 DEVELOPMENTAL EXPRESSION OF THE SOMATOSTATIN GENE IN RAT BRAIN IS REGION SPECIFIC.

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The polypeptide hormone somatostatin (SRIF) has been demonstrated in multiple regions of the brain by radioimmunoassay and immunocytochemistry. Except for the role of hypothalamic SRIF in the regulation of growth hormone and TSH secretion, the exact function of SRIF in the brain remains undefined. To examine the possible role of SRIF in Sprague-Dawley rat brain development, the levels of SRIF RNA in olfactory bulb (OB), hypothalamus (HTH), brainstem (BST), cortex (COR) and cerebellum (CER) were determined during 8 stages of development, from 16-day embryos (16E) to 82 days postnatal (82P). Hybridizations were performed using a rat SRIF cDNA probe which included the entire coding region as well as short portions of the 3'- and 5'-untranslated regions of SRIF mRNA. Hybridization of the ^{32}P-labelled probe to Northern blots of total RNA under stringent conditions demonstrated the presence of SRIF RNA in all brain regions studied; this RNA consisted of a single hybridizing band $\sim$ 670 base pairs in length. Quantitation of SRIF RNA levels was performed by densitometric scanning of slot blots of the same RNA samples hybridized with the SRIF cDNA probe.

	16E	21E	1P	3P	8P	13P	22P	82P
CER	3.0	3.7	4.3	3.6	3.0	3.1	1.4	0.3
COR	1.1	2.3	2.4	4.3	6.6	8.1	5.8	5.5
BST	7.5	12.1	9.9	10.9	13.9	12.9	5.8	4.9
HTH	1.2	1.7	2.0	3.0	3.6	3.4	3.3	3.2
OB	-	0.2	0.4	0.3	0.4	0.6	0.8	2.3

SRIF RNA levels expressed as arbitrary densitometric units/µg total RNA.

Different developmental patterns of SRIF gene expression were seen. SRIF RNA levels were high in BST and CER early in development with a marked decrease late in development, whereas the opposite pattern was seen in COR and HTH.

We conclude that transcription and presumably translation of SRIF RNA occurs in multiple regions of rat brain and that the pattern of apparent SRIF gene expression differs in these various brain regions during development. These changes in SRIF gene expression during development suggest that SRIF may influence brain development.

332.20 SEX AND ENVIRONMENTAL EFFECTS ON THE ULTRASTRUCTURE OF THE RAT CORPUS CALLOSUM. J. R. Kopcik*, J. M. Juraska and D. L. Washburne*. Dept. of Psychology and Neural and Behavioral Biology Program, Univ. of Illinois, Champaign, IL 61820.

Juraska and Meyer (Neurosci. Abst., 11:528,1985) found that there were no sex differences in the gross mid-sagittal size of the corpus callosum in rats at 55 days of age. However, the rearing environment (complex vs. isolated) did affect the size of the posterior two-thirds of the corpus callosum (by 15-17%). In the present study we examined the splenium of the corpus callosum with electron microscopy in hooded rats of both sexes raised for one month post-weaning in either a relatively complex (with objects and other rats) or an isolated environment. Eight micrographs per animal were examined in five litters (20 animals).

The ultrastructural analysis revealed differences that were not apparent in the gross size measures. Unmyelinated axons were the most numerous (90%), and females had significantly more (10%) unmyelinated axons per micrograph ($p<.004$), so that even though there were no overall sex differences in the gross size of the area, females had more total unmyelinated axons than males. In addition, rats of both sexes raised in the complex environment had 9% fewer unmyelinated axons per micrograph ($p<.007$) than isolated rats. However, the 17% greater area of the corpus callosum in complex environment rats indicates that rats from this environment still had a somewhat greater total number of axons. There was a significant sex by environment interaction ($p<.01$) in the number of myelinated axons per micrograph such that females showed no differences between the environments while in males, there was a higher number of myelinated axons per micrograph in isolated rats (17.5%). This indicates that the sexes differ in what underlies their equivalent environmental effect (complex>isolated) on the gross size of the corpus callosum. Females from the complex environment would have more myelinated axons than isolated females while in males from the complex environment, the lower number of myelinated axons would be offset by the larger overall area and axon number would not change between groups. Measurements of axon size are currently being made. The sex differences in unmyelinated axon numbers and the sex differences in reaction to the environments in myelinated axon numbers indicate that many differences can be concealed within unchanging overall size.

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ENDOCRINE CONTROL OF DEVELOPMENT II

333.1 MORPHOLOGY OF RAT SPINAL MOTONEURONS WITH NORMAL AND HORMONALLY ALTERED SPECIFICITY. E.M. Kurz, D.R. Sengelaub, and A.P. Arnold. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The rat lumbar spinal cord contains two sexually dimorphic motor nuclei, the spinal nucleus of the bulbocavernosus (SNB) and the dorsolateral nucleus (DLN). In males, these nuclei project to the bulbocavernosus/levator ani (BC/LA) and ischiocavernosus (IC) muscles respectively. Normal adult females lack these muscles; however, females treated prenatally with the androgen dihydrotestosterone propionate (DHTP) retain the BC/LA muscles, which are anomalously innervated by motoneurons in the DLN (Breedlove, '85). The morphology of SNB and DLN motoneurons normally differs significantly; to investigate the factors which determine neuronal morphology, we compared the structure of SNB and DLN motoneurons having either normal or hormonally altered specificity.

Timed pregnant rats (Sprague-Dawley) received DHTP (2 mg/day in oil, SC) from embryonic (E) days 17 to 22; at birth (E23) pups were cross-fostered to other lactating females. At 60 days of age DHTP-females were given testosterone implants to increase muscle mass for reliable identification. Motoneuron somas and dendritic arbors were visualized by injecting 0.5-1.0 ul of cholera toxin-HRP (CT-HRP, .2%) unilaterally into either the BC/LA (n=5) or the IC (n=5) of males, or the BC/LA (n=6) of DHTP-females, at 83-85 days of age. After 48 hours, cords were processed with TMB and the number, location, soma size, number and orientation of primary processes, and distribution and amount of dendritic arbor of HRP-labeled cells were recorded.

Comparable numbers of motoneurons were labeled across groups ($\bar{x}$=41-51 per animal); in males, 96% of the cells labeled after BC/LA injections were in the SNB, while after IC injections 95% were in the DLN. In contrast, only 35% of the cells labeled after BC/LA injections in DHTP-females were located in the SNB, and 65% in the DLN. In males, SNB motoneurons had significantly more primary processes (6.9 vs. 5.4) and more arbor per cell (4280 vs. 2390 um) than motoneurons in the DLN, although soma area did not differ (1185 vs. 1066 um^2). The orientation of primary processes also differed significantly, as did the distribution of dendritic arbor, being bilateral in the SNB and unilateral in the DLN.

SNB motoneurons in DHTP-females were identical in all respects to those of males. However, while process orientation and arbor distribution of motoneurons in the DLN of DHTP-females were like those of males, soma area (568 um^2), number of primary processes (4.7), and amount of arbor per cell (1375 um) were significantly lower. These results suggest that motoneuronal morphology is not determined by the peripheral target, and only partially influenced by anatomical location.

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333.2 DEVELOPMENT OF NEURON NUMBER IN A SEXUALLY DIMORPHIC SPINAL NUCLEUS IN THE ANDROGEN INSENSITIVE (Tfm) RAT. D.R. Sengelaub, C.L. Jordan, E.M. Kurz, and A.P. Arnold. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) contains many more motoneurons in male rats than in females. Androgens produce this sex difference by regulating normally occurring cell death, and perinatal androgen treatment attenuates this death in females. Male rats with the testicular feminization mutation (Tfm) have reduced androgen receptors, and their SNB has a feminine number of motoneurons in adulthood. Given the importance of androgens for the development of a masculine SNB, we compared the development of the SNB in Tfm males to that of normal males and females to determine how their insensitivity to androgens affects the ontogeny of motoneuron number and extent of cell death.

Normal males, females, and Tfm males were obtained from timed pregnant females (King-Holtzman) at embryonic (E) day 22, and postnatal (P) days 1, 4, and 10 (P1= day of birth); these days encompass the major period for the development of sex differences in SNB motoneuron number. The lumbar spinal cords were removed, embedded in paraffin, sectioned, and stained with cresylecht violet. Counts of SNB motoneurons were made in alternate sections and corrected for sampling frequency and split nucleoli. Counts of degenerating cells in the SNB were also made at all ages.

At E22 SNB motoneuron number is substantially elevated over later levels, and normal males and Tfm males have significantly more motoneurons than females (375 and 284 vs. 188). Between E22 and P1, motoneuron number declines precipitously and significantly in all groups, and at P1 Tfm males no longer differ from females (135 vs. 111), and both have significantly fewer motoneurons than normal males (289). Motoneuron number declines to adult ranges in all groups by P10, with normal males having significantly more motoneurons (237) than females or Tfm males which do not differ (88 vs. 90). During this period of motoneuron loss, significantly more degenerating cells can be seen in the SNB of females and Tfm males than in normal males, and by P10 the frequency of degenerating cells is near zero.

Our results suggest that the development of SNB motoneuron number in Tfm males and females is similar. Just as androgen treatment attenuates cell death in the SNB of females, resulting in masculine motoneuron numbers, the insensitivity to androgens of the Tfm male results in a female-typical cell death, producing a feminine SNB motoneuron number.

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333.3 EFFECTS OF TESTOSTERONE ON REINNERVATION OF BULBOCAVERNOSUS MUSCLES IN ADULT MALE RATS. Mark N. Rand & S. Marc Breedlove, Dept. of Psychology, University of California, Berkeley, CA 94720.

In rats, the spinal nucleus of the bulbocavernosus (SNB) and its perineal target muscles, bulbocavernosus (BC) and levator ani (LA) are sensitive to androgen exposure during development and in adulthood. We decided to measure reinnervation of SNB motoneurons following a nerve crush procedure in adult male rats to see whether testosterone accelerates speed of axonal regeneration of these motoneurons, as it does hypoglossal motoneurons (Yu and Yu, Exp. Neurol. 80:349, 1983). Data from the present study do not indicate that testosterone has a facilitative effect upon speed of reinnervation in this hormone-sensitive system.

The branch of the pudendal nerve innervating the BC muscle was unilaterally crushed in either gonadally-intact or gonadectomized male rats. Side of crush was randomly selected. Gonadectomized animals were implanted with 30 mm silastic capsules containing testosterone or blank silastic capsules at the time of nerve crush. Gonadectomized rats given blank capsules were also given a 5 mg injection of Flutamide (Schering Corp.). The crush procedure was verified in pilot animals by injecting horseradish peroxidase (HRP) into the BC muscles the day after the crush and seeing no HRP in the SNB nucleus ipsilateral to the crush. Six days after crush all animals had 18 µL of HRP bilaterally injected into their BC muscles (six 3-µL injections, 3 injections/side). Animals were sacrificed 20 hours later, and the SNB area of the spinal cord was cut and processed for HRP reaction product. SNB cell bodies with discernable nuclei were counted, and the ratio of labelled cells on the crushed side:intact side was calculated. The mean proportions ± SEM (N) were:

T	Blank	Intact
0.832 ± 0.10(6)	0.852 ± 0.11(8)	0.898 ± 0.10(7)

There were no significant differences between the three groups. While it may be that shorter survival times and/or more severe crush procedures might reveal an androgen effect, there was no evidence for androgen acceleration of SNB regeneration in this experiment.

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333.4 POSSIBLE BRAIN SITES INNERVATING SPINAL NUCLEUS OF THE BULBOCAVERNOSUS. Edward P. Monaghan & S.M. Breedlove. Dept.of Psych., Univ. California, Berkeley, CA.

The spinal nucleus of the bulbocavernosus (SNB) innervates the bulbocavernosus and levator ani muscles which are present in male but not in female rats. The motoneurons in the SNB region of males are significantly larger and more numerous than those in the same region of female spinal cords. The dimorphic nature and hormone sensitivity of this neuromuscular system makes it useful in studying the development of the nervous system. The purpose of the present work is to identify supraspinal areas which might innervate the SNB, thus taking the first step in defining a sexually dimorphic system from peripheral muscles to brain nuclei.

One of two retrograde tracers, horseradish peroxidase (HRP) or wheat germ agglutinin conjugated HRP (WGA-HRP), was injected (1 µl) into the SNB region of adult male rats. After 48 hours, the animals were sacrificed and perfused. Their brains and spinal cords were cut into 50 µm sections, and labelled cells were visualized by a tetramethyl-benzidine procedure. The spinal cord was examined to verify the accuracy of the injection site. The brain sites which were labelled in both HRP and WGA-HRP injected animals were considered as possible areas innervating the SNB. These sites included reticular nuclei of medulla and pons, raphe nuclei, vestibular nuclei, red nuclei, nucleus of solitarius, locus ceruleus, and the hypothalamus, specifically the paraventricular, periventricular, dorsomedial, and lateral nuclei. If these candidate sites are confirmed to be afferents to the SNB, then the sex difference in SNB numbers may result in sexual dimorphism in the supraspinal afferents as well.

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333.5 RADIAL GLIA IN THE FETAL RAT SPINAL CORD MAY GUIDE THE MIGRATION OF SEXUALLY DIMORPHIC MOTONEURONS. Renata B. Fishman & S.M.Breedlove, Dept. Psych., Univ. Cal., Berkeley, CA 94720.

In the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB), motoneurons are located in the dorsomedial portion of the ventral horn of the spinal cord spanning L5 and L6. Embryonic SNB cells may undergo a secondary migration from the lateral dorsolateral nucleus (DLN) to the more medial SNB region. Horseradish peroxidase (HRP) injected into the target bulbocavernosus (BC) muscle at embryonic day 18 (E18), postnatal day 1, and E19-21 labelled cells in the DLN, SNB, and intermediate positions respectively; those intermediate motoneurons showed morphological characteristics of migrating cells (Sengelaub and Arnold, Soc. Neurosci. Abstr., 10: 453, '84). Furthermore, androgens may affect the specificity of motoneuron to BC connectivity by altering SNB cell migration. HRP injected into the BC muscles of prenatally dihydrotestosterone propionate (DHTP) treated females labelled motoneurons residing in the DLN (Breedlove, Science, 227: 1357, '85). It is possible that DHTP prevents SNB cell lateral to medial migration.

In the present study we examined whether or not SNB cells normally undergo a secondary migration from the DLN to the SNB. In the CNS, radial glia appear to guide migrating neurons to their appropriate destination. Using rabbit antibodies to glial fibrillary acidic protein (GFAP; courtesy of L. Eng) and monoclonal antibodies to vimentin (BioGenex) we stained for the presence of radial glia. Sprague-Dawley rat fetuses aged E20 and E22 were perfused with saline and cold para-formaldehyde solution, their spinal cords removed, and segments L5 and L6 were parraffin embedded and sectioned at 6 microns. Sections were stained for GFAP or vimentin by incubation with anti-GFAP or anti-vimentin, and PAP or ABC immunoperoxidase. Sections were counterstained with hematoxylin to visualize motoneurons.

Antisera to both GFAP and vimentin stained radial glia in the lumbar spinal cord at the level of the SNB at E20 and E22. At both ages, radial glia traverse the pathway between the DLN and SNB, and motoneurons can occasionally be seen in association with them. The presence of radial glia in the lumbar cord at E20 and E22 suggest that SNB cell migration may occur at these times and that radial glia could provide the necessary pathway.

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333.6 STRAIN DIFFERENCES IN MORPHOLOGY OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS (SNB) IN MICE. B. E. F. Wee and L. G. Clemens. Neuroscience Program and Zoology Dept., Michigan State University, East Lansing, MI 48824

The Spinal Nucleus of the Bulbocavernosus (SNB) of the spinal cord exhibits a sexual dimorphism in the rat (Breedlove, S. M., Prog. Br. Res., 61:147-170, 1984). SNB neurons are larger and more abundant in males than in females, and both the neurons and their target musculature bind androgen. In rats, castration reduced the size of SNB neurons, but had no effect on cell number.

The purpose of the present study was to examine the effects of castration and genotype on SNB morphology in mice. Adult male mice (Mus musculus) from three inbred strains were used : DBA/2J, C57Bl/6J, and the B6D2F1 hybrid (resulting from the cross between a C57Bl/6J female and a DBA/2J male). All mice were tested for masculine sexual behavior before and after castration or sham operation (Wee et al., Neurosci Abs., 11:738, 1985). At the end of the sex behavior tests (approx. 7 months after surgery), males were sacrificed, and spinal cords were removed and processed for histology following procedures described by Breedlove and Arnold, Br. Res., 225:297-307, 1981), except that 60 um thick sections were used. Data were analysed with Kruskall-Wallis and Mann Whitney U nonparametric tests, with significance levels of $p < .05$.

Among the gonadally intact males, the DBAs had significantly fewer SNB neurons than either the C57s or the F1s, which did not differ significantly from each other. In contrast, genotype did not influence neuronal area in the intact animals. No significant differences were observed between the castrates of the three strains for either neuronal size or number.

Castration significantly reduced neuronal area in the F1 hybrid and DBA (the paternal strain), but not in the C57. Further, castration significantly reduced neuronal number in the F1 and C57 (the maternal strain), but not in the DBA.

In contrast to the rat, castration influenced both neuronal number and neuronal size in B6D2F1 mice. The effects of castration on SNB morphology observed in the hybrid occured in one, but not both of the parental strains. Therefore, the effect of castration on neuronal size appears to be inherited independent of that for neuronal area. The observation of strain differences in SNB cell number further demonstrates the influence of genotype on SNB morphology.

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333.7 ABSENCE OF ANDROGEN ACCUMULATION BY MOTONEURONS OF NEONATAL RATS. S.M.Breedlove, Psych. Dept., U.C. Berkeley, CA.

Perinatal androgen spares motoneurons in the spinal nucleus of the bulbocavernosus (SNB) and their bulbocavernosus (BC) target muscles from dying in male rats. The absence of androgenic hormones in developing females results in the loss of both BC and SNB cells, and consequently, a very sexually dimorphic SNB system. In adult males, both SNB cells and BC muscles have androgen receptors, which suggests two hypotheses of androgen action: that hormone first spares the muscles and thereby indirectly spares SNB cells, or that androgen spares the motoneurons and thereby indirectly spares the BC. We have previously shown that SNB cells which are themselves androgen insensitive can be spared by androgen, and that the BC muscles of newborn females can be maintained by androgen in the absence of innervation from the SNB. These findings are compatible only with the first hypothesis. We now report repeated failure of newborn rat motoneurons to exhibit androgen accumulation in autoradiography, further suggesting that the motoneurons themselves are not the primary target for androgenic masculinization of the SNB.

Male rat pups were castrated (N=6) or castrated and adrenalectomized (N=2) on the day of birth and injected (either s.c. or i.p.) on the second day of life (N=6) or the third day of life (N=2) with one of 3 tritiated steroids (87-152 Ci/mmol; New England Nuclear): testosterone (N=1 @10μCi, 1 @100μCi), dihydrotestosterone (N=1 @10μCi, 1 @100μCi) or the synthetic androgen methyltrienolone (R1881: N=2 @9μCi, 2 @25μCi). In addition, two female pups were injected with 8 μCi of R1881 on day 2 after day 1 ovariectomy, and a male and female pup were each injected with 10 μCi R1881 on day 2 without previous gonadectomy. Pups were sacrificed by decapitation 53-122 min after injection. The spinal cords were rapidly removed, frozen and sectioned 5-6 μm thick in a darkroom. Sections were applied to slides previously dipped in nuclear track emulsion and stored at 4°C in light tight boxes for 29-624 days. The slides were then photographically developed, stained with thionin and examined with light microscopy. At least 25 SNB cells and 25 other motoneurons were examined from each animal.

Despite the heterogeneity of treatments, the resulting autoradiograms from these 12 rats were very similar: there were no convincing signs of steroid accumulation over the nuclei of motoneurons in either the SNB or other lumbar motor groups. The density of silver grains over these cell nuclei was always less than 3 times background density, although other cells in the brain or spinal cord of each animal did reach this criterion, and in adulthood the majority of motoneurons exceed the criterion. These findings do not rule out a steroid action independent of nuclear accumulation and, in theory, another technique might reveal accumulation. Still, it seems unlikely that motoneurons are androgen targets in newborn rats.

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333.8 THE MAJOR PELVIC GANGLION: ANDROGEN CONTROL OF POSTNATAL MATURATION. R.W. Hamill and J.E. Melvin. Dept. of Neurology, Neurobiology and Anatomy, Univ of Roch Med Ctr and Monroe Community Hospital.

Previously we reported the effects of postnatal castration on the postorganizational development of the sympathetic hypogastric ganglion (Hamill & Guernsey '83; Melvin & Hamill '86). We now report results which suggest that the classically defined parasympathetic major pelvic ganglion (PG) is similarly regulated by testosterone during development. Choline acetyltransferase (CAT) and tyrosine hydroxylase (TOH) activities were utilized to examine normal PG ontogeny. During normal development, CAT and TOH activities increase 50 and 110 fold respectively and these two neurotransmitter synthesizing enzymes exhibited different developmental profiles: CAT increase displayed a consistent slope reaching a plateau between 60-90 days whereas the increase in TOH activity accelerated periodically and failed to reach a plateau.

Postnatal castration at 10-11 days of age completely prevented the postorganizational developmental increase of TOH activity. At 12 postoperative weeks TOH activity in castrates was approximately 6% of the control animals (control 2880±127 pmoles/ganglion.hr, castrated 161±16 pmoles/ganglion.hr; $p<0.001$). In fact, by only one postoperative week the maturation of TOH activity was already significantly delayed in castrated animals (control 480±69 pmoles/ganglion.hr, castrated 179±6 pmoles/ganglion.hr; $p<0.001$). CAT activity and total ganglion protein were also significantly reduced by one postoperative week. In contrast to TOH activity, however, these indices continued to develop at diminished rates. By 12 postoperative weeks CAT activity and total ganglion protein in castrates were 30% and 50% of control values, respectively. Additionally, enzyme specific activity for both CAT and TOH were significantly altered at all time points following castration. Testosterone replacement reversed the castration induced developmental deficits of TOH and CAT activities.

These results suggest that hormonal mechanisms regulate the postorganization development of the parasympathetic major pelvic ganglion. Viewed with previous studies of the sympathetic nervous system, these investigation suggest that hormonal factors regulate presynaptic and postsynaptic elements of the parasympathetic as well as sympathetic components of the peripheral autonomic nervous system.

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333.9 ONTOGENY OF SEX DIFFERENCES IN NUMBER OF TRIGEMINAL MOTONEURONS IN GUINEA PIGS. Kate Townsend-Merino & S.M. Breedlove. Dept. Psych., Univ. of California, Berkeley, CA 94720.

The jaw closing muscles of guinea pigs are very responsive to adult androgen manipulation, and we previously reported a sex difference in the motoneuronal pool innervating these muscles. Specifically, male guinea pigs have more neurons in the trigeminal motor nucleus (MoV) than do females. This sex difference cannot be accounted for by differences in body weight alone, and there is no sex difference in MoV of rats (Neurosci. Abstr. 11, 530, 1985). In preparation for studies of the ontogeny of this sexual dimorphism in guinea pigs, we now report that the number of MoV neurons has reached adult values in newborn males, but are greater in newborn females than adult female guinea pigs. These results suggest that motoneuronal death in the MoV is complete at birth in male but not female guinea pigs. We also counted neurons in the mesencephalic trigeminal nucleus (MesV), since a portion of these cells provide afferent information from jaw closing muscles.

Three male and 5 female guinea pigs were overdosed with pentobarbital on the day of birth and perfused with saline and buffered formalin. The brains were removed, postfixed in formalin for at least 2 weeks, paraffin embedded, sectioned at 15 μm and stained with thionin. Bilateral counts of MoV and MesV cells were made in every fifth section. Cell nuclear cross-sections were measured and cell counts corrected for split nuclei error.

There were 2475.2 ±181.8(SEM) MoV neurons in male newborn guinea pigs, which is not significantly different from the number in adulthood (2260.9 ±108.1). However, newborn females had 2265.7 ±164.0 MoV neurons, which is significantly more than in adulthood (1706.4 ±70.0). There was no sex difference in the cross-sectional area of MoV neuronal nuclei at either age, nor was there a sex difference in the number of adult MesV cells.

The finding that the adult sex difference in the number of MoV is not yet present at birth suggests that the period of ontogenetic cell death which has been demonstrated for several motoneuronal groups is not yet complete in the newborn female guinea pig's MoV. The MoV of newborn males, on the other hand, is at or near the adult number, suggesting that MoV cell death is complete in that sex. If the eventual sex difference in MoV is the result of androgen action in males, then either there is a neonatal sex difference in androgen levels, or else prenatal androgen programs a postnatal sex difference in the death of MoV neurons. The MesV contains afferents from the jaw closing muscles and from the periodontium. The lack of a sex difference in the adult number of MesV neurons suggests that either there is no sexual dimorphism in the afferents to the jaw closer muscles, or only a small proportion of MesV neurons serve this role.

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333.10 AXONAL MATURATION IN THE RAT BRAIN: EFFECTS OF EARLY HYPO- AND HYPERTHYROIDISM. R. Hawkes*, R. Sasseville*, and C. Gravel (SPON: L. Poirier) Laboratory of Neurobiology, Laval University, Quebec, Canada, G1J 1Z4.

In hypothyroid rats there is an almost complete supression of a neurofilament-associated epitope (monoclonal antibody mabN210) in the basket cell axons of the cerebellar cortex and in the axons of the corpus callosum while other cerebellar and cortical axons were lightly or not affected. It appears that axons already in place before the onset of thyroid function (around E18 in rats) are insensitive to thyroid suppression, and that axons growing afterwards are dependent upon thyroid hormone levels. To explore this further, pregnant rats and their litters were treated with n-propyl-thiouracil from E16-17 to P25 or P45. At birth, some pups received daily thyroxine injections, scheduled and dosed so as to fulfilled minimal thyroxine requirements, either from P0-P25, P14-P25, P34-P45 or only at P14 and P15. Other groups received no thyroxine and finally some groups received excess thyroxine from P0 to P25 dosed to produce either light or severe hyperthyroidisms. Animals were sacrificed by intracardiac perfusion of fixative at various ages and the cerebella were sectioned and processed for mabN210 immunocytochemistry. The corpus callosum from P25 control and hypothyroid animals was Epon-embedded, cut a 1um and stained with methylene blue to count myelinated axonal profiles. Ultrathin sections were also cut for electron microscopy.

The results were as follows:

The deficit in mabN210 immunoreactivity seen at the level of basket cell axons in the cerebellum of hypothyroid animals can be almost completely restored by thyroxine injections starting from P0 or P14, but not from P34. Thus, the disturbance in axonal maturation can be cured past the normal birthdate of basket cells, but the period of plasticity is limited.

- Excess thyroxine administered to hypothyroid animals from P0 led to a precocious maturation of mabN210 immunoreactivity and resulted at P25 in a dose-dependent supra-normal production of basket cell axons.
- The callosal deficit in mabN210 immunoreactivity observed in P25 hypothyroid animals correlated with a deficit in the number of myelinated axons. By contrast, electron microscopy revealed that the total number of callosal axons was much higher in hypothyroids than in normals. Thus, the absence of mabN210-immunoreactivity from the corpus callosum reflects the immaturity of the projection rather than the lack of axons.

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333.11 SYMPATHETIC NEURON DEVELOPMENT IS ALTERED IN THE PRESENCE OF ABNORMAL LEVELS OF CIRCULATING THYROID HORMONES. P. Beaston-Wimmer*, J. Franzer* and A.J. Smolen (SPON: W.H. Goldman). Dept. of Anatomy, The Medical College of Pa., Philadelphia, PA 19129.

Abnormal levels of circulating thyroid hormone are known to result in dramatic abnormalities in the development of certain regions of the central nervous system. The present series of experiments was undertaken in order to examine the role that thyroid state plays in the development of sympathetic neurons. All experiments were performed using Sprague-Dawley rats. One group of rats was made hyperthyroid by daily subcutaneous administration of triiodothyronine (T3; 0.1 mg/kg) for the first two weeks of life. Littermates were treated daily with vehicle alone. A second group was made hypothyroid by daily subcutaneous administration of propylthiouracil (PTU; 50 ul of a 0.2% solution) for the first two weeks of life. Some animals were examined during the treatment (postnatal day 7), some immediately following the treatment (day 14), and others after a period of recovery (90 days). The number of neurons in the superior cervical sympathetic ganglion (SCG) were counted, and the content of norepinephrine (NE), the neurotransmitter of these neurons, was measured. In the hyperthyroid animals, no significant alterations were observed on postnatal day 7. By day 14, however, the protein content of the SCG of treated rats was less than in controls. NE content was even more greatly reduced, resulting in a reduction of transmitter per mg protein. This deficit persisted at 90 days. There was no alteration in the number of sympathetic neurons in the hyperthyroid rats, so that the amount of NE content per sympathetic neuron was decreased. This decrease may represent increased activity and release of transmitter from cells in the hyperthyroid animals. In the hypothyroid rats, NE content was slightly lowered at 14 days. There were significantly more cells in the SCG of hypothyroid rats at 14 days, due to a reduction in the extent of naturally occurring neuron death. By 90 days, the animals which were hypothyroid had significantly fewer neurons in the SCG. Thus the reduction in naturally occurring neuron death was only temporary, and neuron survival was ultimately not as good in the hypothyroid animals. NE content at 90 days was slightly reduced, but there was no difference in NE content expressed per mg protein or per sympathetic neuron. Thus either hyper- or hypothyroidism during the neonatal period results in dramatic deficits in the development of sympathetic neurons.
(Supported by NINCDS Grant NS15952 to A.J.S.)

333.12 ULTRASTRUCTURAL DISTURBANCES OF DEVELOPING INTRAOCULAR LOCUS COERULEUS NEURONS INDUCED BY THYROID HORMONE DEFICIENCY.
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The thyroid hormone dependency of the developing central nervous system has long been known. A number of light microscopical studies have shown morphological disturbances of brain maturation. However, very few ultrastructural studies have been undertaken. Central monoamine neurons have recently been shown to be markedly dependent on normal levels of thyroid hormones for organotypical maturation upon transplantation to the anterior chamber of the eye. Reduced fascicle formation and numerous accumulations along axon terminals were found by monoamine histofluorescence. The aim of the present study was to ultrastructurally scrutinize the abnormal development of locus coeruleus-derived, noradrenaline-containing neurites in the hypothyroid state of intraocular brain stem grafts. The abundant peripheral accumulations of fluorescent material, only found in the thyroidectomized group, corresponded ultrastructurally to axonal swellings, usually exceeding five microns in width and filled with dense-cored vesicles, mitochondria or smooth endoplasmic reticulum. No microtubules were observed. Locus coeruleus axons with considerably thinner diameters but with similar contents were numerous. Axonal swellings in the host irides were found all over the outgrowth derived from the locus coeruleus, although their numbers were higher close to and underneath the graft. The swellings also appeared in neuropil of the brain stem graft. Outgrowing monoaminergic fibers in the thyroidectomized group usually lacked accompanying glial cell processes, while controls contained fiber bundles ensheathed by glial cells. Membrane specializations were never observed on axonal swellings containing dense-cored vesicles or mitochondria and only rarely on those containing smooth endoplasmic reticulum. Injections of thyroid hormone given to thyroidectomized hosts resulted in fiber outgrowth and axonal ultrastructure similar to that of the controls. This strongly indicates a role for thyroxin in the process of nerve fiber formation from grafted locus coeruleus neurons. Since thyroxin has been shown to play an important role for microtubule assembly, it is possible that the morphological disturbances reported here, indicating a hampered axonal transport of organelles and a lack of microtubules, are caused by the hypothyroid effects on tubulin assembly in the immature locus coeruleus neurons.

333.13 CELLULAR DISTRIBUTION OF THYROID HORMONE BINDING PROTEINS (THBP) IN THE CEREBELLAR CORTEX: IMMUNOFLUORESCENT DEMONSTRATION. L.L. Hall* and K.R. Latham* (SPON: G.J. Bennett). Depts. of Physiology and Medicine, USUHS, Bethesda, MD 20814-4799.

The arborization of cerebellar Purkinje cells during development is one example of the profound dependence of the central nervous system (CNS) on thyroid hormones but detailed information on which cells in the CNS are thyroid hormone targets has been limited. In the present studies, we have utilized the specificity of monoclonal antibodies (MAB) directed against THBP to identify cells in sections of cerebellar cortex that contain receptors.

A library of MAB was previously developed against THBP purified from bovine liver (Sellitti et al., Exp. Cell Res., 158:223,1985). Previous light microscopic immunofluorescent studies of benzpyrene-transformed bovine endothelial cells (BEC) identified two high affinity antibodies with different specificities: MAB 1-11 demonstrated extensive cellular staining in a pattern resembling the distribution of the intermediate filament (IF) vimentin while MAB 5-6 showed extensive nuclear staining.

In the present study, 5 µm frozen sections of adult bovine and rat cerebellar cortex were stained with MAB to mouse vimentin MAB 1-11, and MAB 5-6. Following incubation in primary MAB (1:1000 in PBS with 1% normal goat serum) or control serum at 4°C overnight and several washes (PBS with 1%BSA), the sections were labeled with FITC-conjugated goat-antimouse IgG (1:20, Cappell) for 30 minutes. Washed sections were mounted and fluorescent photomicrographs prepared. Parallel tissue sections were stained with Cresyl violet for classical identification of cell types.

Antivimentin MAB positively stained Bergmann fibers from Golgi Epithelial cells and a few astrocytes in the white matter of both the bovine and rat cortex. Bergmann fibers displayed a familiar parallel arrangement to the longitudinal plane of the folium in the molecular layer. In contrast to BEC staining, MAB 1-11 did not show a vimentin staining pattern and did not bind to any other cellular component in the rat or bovine cerebellum. However, MAB 5-6 showed a strong nuclear staining in granule cells in both bovine and rat cerebellum .

The present data suggest a heterogeneity of THBP distribution in different TH target cells; while MAB 1-11 showed intense BEC staining, no reactivity was demonstrated in the cerebellar cortex. Glia and all cells in the molecular layer are not TH targets by our criteria since receptor staining was conspicuously absent in these populations. The absence of Purkinje cell staining suggests that this cell population may be a TH target only during development; alternatively, Purkinje cells may respond to products from other TH target cells in the cerebellum. The strong positive staining of granule cells by MAB 5-6 is evidence in support of this neural subpopulation as a thyroid hormone target.

333.14 CORTICOSTERONE EFFECTS ON IN VITRO DEVELOPMENT OF CHICK EMBRYO AND HUMAN FOETAL BRAIN.
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Tissue cultures provide a very useful tool to study the effects of endogenous and exogenous substances on neuronal differentiation and maturation. In previous work we have shown that corticosterone affects neuronal sprouting in vitro. In vivo, it induces the appearance of a new muscarinic cholinergic receptor subclass which is not present in the controls. Also adrenergic receptor maturation is affected by hormone treatment.

Human brains, obtained from either spontaneous or from medically induced abortions, or 8-day old chick embryo retinas were freshly dissected and incubated with 0.2% of sterile trypsin. Cells were mechanically dissociated, diluted with medium (DMEM) + 10% fetal calf serum and plated on polylysine-treated dishes. After 24-48 hours in vitro, cells attached to the substratum and started developing processes. At different intervals, cells were fixed with cold methanol for immunocytochemistry. Monoclonal antibodies against choline acetyltransferase (ChAT), glial fibrillar acid protein (GFA-P), vimentin (VT), desmin (DM) and neurofilament subunits (NF68K, NF160K, NF200K) were used. Results showed that the distribution of different antigens varied with development. In particular, NF200K were prevalently localized inside the cell bodies, whereas NF160K were prevalent in the processes and inside the nucleus. GFA-P never stained retina cells. On the contrary, in human cultures some cells with the typical morphological features of astrocytes appeared to be GFA-P positive. Very seldom, cells positive for ChAT were present in both culture types. VT stained both neuronal and non-neuronal immature cells.

When cultures were treated with corticosterone (10^{-7}M) the distribution of neurofilament subunits was affected. The nuclear staining disappeared in treated cells. The other antigens were not affected. These findings could be correlated with the capability of the hormone to bind to nuclear acids and consequently affect cell maturation.
(Supported by M.P.I. Grant 1985 to F. G.)

333.15 THE RELATIONSHIP BETWEEN ENRICHMENT- AND ADRENALECTOMY-INDUCED CORTICAL GROWTH. L.Devenport, S. Dallas*, C. Carpenter*, and M. Renner. Department of Psychology, University of Oklahoma, Norman, OK 73019, and University of Wisconsin at Oshkosh, Oshkosh, WI 54901.

Enviromental enrichment conditions (EC) have long been known to alter brain development. The most striking effect of EC is an increase in brain weight that is chiefly attributable to the thickening of neocortical tissue layers (1). Adrenalectomy also increases brain growth (2) owing to the removal of glucocorticoids (3), and it too thickens neocortex (4). This being the case, we wondered if the EC effect might not be mediated via the adrenal cortex.

If true, EC effects would depend upon variable adrenocortical output and should be abolished by adrenalectomy with constant glucocorticoid replacement. Sprague-Dawley rats (Charles River, Male, 30 days old) were adrenalectomized (ADX) or sham-operated. Half the animals from each surgical condition were maintained for 30 days in enriched enviroments (social housing with changing physical items) or in the standard isolated laboratory condition (IC). ADXs were implanted with continuous infusion devices (Alza) that delivered corticosterone at a dose of about 2.0 mg/kg body weight. Shams were implanted with blank pumps. At the end of the environmental treatment, the animals were sacrificed, their brains fixed in buffered formalin, and coded for blind analysis. Frozen sections 40u were taken at various planes, and the dimensions of 17 structures and areas were determined by projection microscope.

Significant EC-induced increases in cortical depth in frontal, parietal and occipital regions were observed, and the EC effect was as evident in ADXs as in Shams. No subcortical structures or fiber tracts were changed by EC. The replacement dose of corticosterone was not high enough to completely suppress ADX effects, and a variety of areas were increased in size owing to surgical condition. In nearly all cases, these were the same regions that were sensitive to EC ($r = .88$). This frequently resulted in the doubling of the EC effect. Thus, while EC- and ADX-induced growth were completely independent of each other, they operated on the same large contiguous patch of "plastic" neocortical tissue.

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2. Devenport. (1979). Behav. Neur. Biol., 27, 218.
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333.16 DEVELOPMENT OF SEX DIFFERENCES IN NEURON NUMBERS IN THE RAT SUPERIOR CERVICAL GANGLION: EFFECTS OF DEAFFERENTATION. R. Calegas* and L.L. Wright (SPON: D. Lake). Boston University School of Medicine. Boston. MA 02115.

The normal sex difference in neuron numbers in the superior cervical sympathetic ganglion (SCG) develops over the first two postnatal weeks. During this time, 30-40% of the principal neurons die, and synaptogenesis within the ganglion and innervation of the targets is progressing. Neonatal deafferentation of the SCG prevents the normal development of tyrosine hydroxylase within the ganglion and the normal innervation of its target tissue (Black & Mytilineou, 76). The present study was undertaken to determine whether the presence of afferent input to the SCG was required for the development of this sex difference.

Male and female Sprague Dawley rats received bilateral transection of the cervical sympathetic trunk (CSTX) on the day of birth, when numbers of neurons in the superior cervical ganglion (SCG) are equivalent in males and females. On postnatal day 15, after normal development of the SCG sex difference, the animals were killed, and their superior cervical ganglia were removed and embedded in paraffin. Counts of neuronal nuclei were taken from sections throughout the ganglia, and were corrected by the Abercrombie (46) method.

Our results confirmed our previous finding of more SCG neurons in males than in females. In addition, the results showed a difference in response to the CSTX in males and females. Males with CSTX had significantly fewer neurons (26%) in the SCG than did sham operated littermates. Females with CSTX showed only 14% fewer SCG neurons than female sham operates, which was not statistically significant. Because of the disparate effects of CSTX on SCGs of males and females, the sex difference was reduced from 28% more neurons in males than females in the control group to 11% more neurons in males than females after CSTX.

These results suggest that the development of the normal sex difference in numbers of SCG neurons requires an intact afferent input.

Numbers of SCG neurons at 15 days
(n per group, standard error)

	Males	Females
sham controls	26500 (19,2379)	20661 (16,1108)
CSTX	19683 (19,1003)	17751 (17,1257)

This work has been supported in part by NIH grant NS21577 to LLW.

333.17 LESION-INDUCED PLASTICITY: GENETIC OR HORMONAL CONTROL? J.K.Morse, S.T. DeKosky, and S.W. Scheff. Depts. of Anatomy and Neurology, VA and Univ. of Kentucky Medical Center, and Sanders-Brown Research Center on Aging, Lexington KY 40536

We have been investigating the role of sex steroids in lesion-induced circuity changes in the CNS. Untreated male and female rats demonstrate equivalent plasticity in the hippocampus following removal of the entorhinal cortex (ERC). However, gonadectomized (GDX) animals display a sexually dimorphic sprouting response, with only the ovarectomized females showing a significant reduction from control levels. It appeared that estrogen, the female gonadal hormone, was necessary, while the male gonadal steroid testosterone was not. Could this dimorphic sprouting reaction be due to the genetic makeup of the animal or do circulating neonatal sex steroids play a key role, as they do in the differentiation of the brain?

Litters of newborn Sprague Dawley pups were separated by sex and culled to 10 pups each. Male pups were feminized by castration on postnatal day 5 (grp 1). This creates a genetic male animal with a female brain. Female rat pups were masculanized by injection of testosterone on day 4, thus creating a genetic female rat with a male brain. The females were divided into 2 groups (grp 2 & 3). On day 35 the group 2 females were ovarectomized. At 60 days of age all animals were subjected to unilateral removal of the ERC and subsequently killed 15 days later. Changes in the hippocampal commissural-asociational fiber plexus were quantified using light microscopic techniques.

Genetic males pups with feminized brains decreased their sprouting response, equal to that of adult ovarectomized females. Masculinized, ovarectomized, genetic females responded with an outgrowth equal to adult controls and adult castrated males. However, the masculinized genetic females who were not ovarectomized, decreased their sprouting response significantly from both the controls and adult ovarectomized females. These results indicate that the sprouting mechanism is indeed influenced by the perinatal hormone environment, and infact overrides the genetic makeup of the animal.

(Supported by NIH grant NS21541 and the VA Medical Research Service)

333.18 GLUCOCORTICOIDS INCREASE THE LEVEL OF INTRACELLULAR DOPAMINE IN FETAL RAT BRAIN NEURONS CULTURED UNDER SERUM-FREE CONDITIONS. A. Al-Hader*, T. Schmidt* and R.E. Fellows. Dept. of Physiology, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Elevated glucocorticoids promote behavioral and aging-associated disorders in adult brain. However, relatively little is known about the effects of glucocorticoids on CNS neurons during fetal development. Cytoplasmic glucocorticoid receptors have been found in regions of fetal brain from several mammalian species. While glucocorticoids are known to induce cholinergic systems during CNS development, their effects on monoaminergic neurons in developing brain are less clear.

In the present study, we have determined the effects of glucocorticoids on the intracellular levels of dopamine (DA) in neuron-enriched cultures of fetal rat brain grown in serum-free defined medium. Intracellular levels of DA were measured by HPLC with electrochemical detection in 0.1 M PCA extracts of neuronal cultures from 18 day fetal rat diencephalon-mesencephalon. Cells were prepared by mechanical and enzymatic dissociation, plated at a density of 8000 cells/mm^2 and maintained in serum-free defined medium. Basal levels of intracellular DA increased from 126.5±9.19 pg/mg protein at the time of plating to 571.0±2.8 pg/mg protein after 12 days in culture, with no significant increase in cell number. Neurons were maintained in medium alone for 3 days before exposure to steroids. To determine its effects on DA concentrations as a function of time, 250 nM triamcinolone acetonide (TA) was added every 3 days. Maximum increases in DA levels were observed after 6-7 days of treatment. To determine concentration dependence, medium was replaced with defined medium containing 1.0-1000 nM TA or corticosterone (CS) after 3 and 6 days and cells were harvested at 9 days. Significant increases ($p<0.05$) in DA levels were observed at 25 nM and maximum increases were obtained at 250 nM CS or TA. At that concentration, CS and TA increased DA levels 104% and 117% over control, respectively. At 250 nM, 17β-estradiol and diethylstibestrol also increased DA levels, by 72% and 63% respectively. The effects of 250 nM 17 β-estradiol and 250 nM TA were additive, increasing DA levels 185%. Progesterone, aldosterone or 5α-dihydrotestosterone at 250 nM concentrations had no significant effect on DA levels.

These data demonstrate that both natural and synthetic glucocorticoids modulate levels of intracellular dopamine in cultured fetal rat brain neurons and suggest that in this culture system glucocorticoids, as well as estrogens, may be important in the expression of the dopaminergic phenotypes.

333.19 GLUCOCORTICOID RECEPTORS IN FETAL RHESUS MONKEY BRAIN.
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Glucocorticoids can influence neurological development of the fetus. Presumably, these effects are mediated through the interaction of glucocorticoids with high affinity intracellular receptors located in the brain. In the present study we sought to demonstrate and characterize glucocorticoid-binding activities in fetal rhesus monkey brain.

Cytosolic extracts of brain from 135 day postconception male and female fetuses were prepared using a buffer containing 10 mM Tris-HCl, 1.5 mM EDTA, 10% (v/v) glycerol, 1 mM mercaptoethanol, and 25 mM sodium molybdate (pH 7.4). Cytosolic extracts from cerebral cortex were incubated for 16 hr at 0-4° C with 0.4-28 nM ^{3}H-dexamethasone (^{3}H-dex) in the presence or absence of 200-fold excess unlabeled cortisol. Macromolecular bound radioactivity in duplicate 200-µl samples of the incubates was determined by Sephadex LH-20 gel filtration. Scatchard analysis revealed high affinity saturable binding with an apparent Kd=1.94 nM and Bmax=56.3 fmol/mg protein. The steroid specificity of the binding reactions was studied by incubating cytosolic fractions with 13 nM ^{3}H-dex and various unlabeled steroids at 10-, 100-, and 200-fold excess concentrations. Cortisol was the most vigorous competitor followed by progesterone, while estradiol and dihydrotestosterone were ineffective competitors. Single point analyses of binding activity in discrete brain regions using 13 nM ^{3}H-dex revealed a widespread distribution of binding which included frontal, parietal, temporal, and occipital lobes of cerebral cortex, cingulate gyrus, amygdala, hypothalamus-preoptic area, and hippocampus (range 15.3-35.6 fmol/mg protein). Evidence of dex-induced depletion of cytosolic receptor binding was obtained from an experiment showing that administration of unlabeled dex to the pregnant mother (2.5 mg/kg) every 12 hours beginning 72 hr and ending 36 hr prior to delivery of the fetus resulted in a 58-92% decrease in ^{3}H-dex-binding activity depending on the brain region being sampled. In contrast, administration of a single 15 mg/kg injection of dex 72 hr before delivery did not result in any change in binding activity.

These findings verify the existence in the brain of fetal rhesus monkeys of a specific glucocorticoid receptor which may be important in mediating influences of glucocorticoids on neurological development.

(Supported by NIH grants HC27358 and RR00167)

333.20

WITHDRAWN

333.21 ONTOGENY OF MESENCEPHALIC TYROSINE HYDROXYLASE IMMUNOREACTIVE NEURONS IN THE BRAIN OF THE FARM PIG
T.B. Freeman, L. Brandeis*, J. Pearson, R.A. Noonan* and J-P. Michel* NYU Medical Center, Depts. of Neurosurgery, Pathology, New York, Ny 10016; Hann Biologics, Inc., Berkeley, CA 94710

The ontogeny of tyrosine hydroxylase was studied immunocytochemically in the brain of the farm pig.

At embryonic day (ED) 22 (CRL = 11-12 mm), labelled cell bodies with short processes are first apparent in the mantle layer of the caudal mesencephalic flexure just ventral to the ependymal zone. They are restricted to the midline.

By ED 24 (CRL = 15-16 mm) the number of labelled cells in the mesencephalic flexure has increased. There is lateral and rostral migration of some cell bodies to the region of the mid mesencephalic flexure. Cell processes have sparsely penetrated the newly developing striatum.

At a later stage of development ED 26 (CRL = 19-20 mm) the lateral and rostral migration of cell bodies has continued, and the most laterally placed cells are found at the rostral mesencephalic flexure. Cell extensions have begun to form a dense arborization within the striatum.

The cells that form the presumptive nigrostriatal pathway in pig therefore become differentiated, migrate and begin extending axons from ED 22-24. The terminal fields are first detected from ED 24-26 and have reached their target before lateral migration of their cell bodies is completed.

334.1 TOPOGRAPHY OF MOTOR FUNCTIONS IN THE RAT'S STRIATUM: FORELIMB FIXATION, BITING AND LOCOMOTION. M. Pisa. Depts. of Neurosciences and Psychiatry, McMaster University, Hamilton, Ont., Canada L8N 3Z5.

The aim of this study was to examine the effects of regional neurodegenerative lesions of the rostral striatum on postural fixation of the forelimbs, biting and locomotion. After training to run for food reward on an elevated wooden beam 5 cm wide, male Wistar rats were anesthetized with Equithesin (3.5 cc/kg ip) and assigned to groups (N=8 to 10) for bilateral stereotaxic injections of either ibotenic acid (7.5 ug in .5 ul of PBS vehicle solution) or an equal volume of vehicle solution into either of these regions of the rostral striatum: dorsomedial (DMS); dorsolateral (DLS); ventrolateral (VLS); dorsomedial and ventromedial (MS); dorsolateral and ventrolateral (LS). Both preoperatively and on postoperative Days 2, 15 and 30, the rats were videotaped during 5-min sessions of manipulation and eating of relatively large (5 g) food pellets, and during trials of food reinforced locomotion across the beam. In the locomotor task, the number of footslips and falls and the time to locomote across the beam were recorded. In the manipulatory task, feeding efficiency (food intake/feeding time), duration of the food bites, and number of stable and unstable postural fixations of the forelimbs during pellet holding and biting were recorded. At the end of testing, the sites of axon-sparing, somatodendritic lesions were verified histologically. Enduring impairments produced by the lesions were as follows: 1) In the manipulatory task, the LS lesions produced the most severe impairments of feeding efficiency, biting speed and forelimb fixation; the VLS lesions produced qualitatively similar, but less severe impairments of all these behaviors; the DLS lesions reliably impaired feeding efficiency and forelimb fixation, but not biting speed; neither the MS lesions nor the DMS lesions reliably altered any of the measured behaviors. 2) In the locomotor task, none of the lesion treatments reliably increased the foot slips or the falls off the beam, and only the LS lesions produced a chronic increase of locomotion time.

Consistently with previous findings (Pisa, Soc. Neurosci. Abstr., 1985, 11:685), the present data indicate a topographically organized role of the rat's rostrolateral striatum in orofacial and forelimb motor control, with the dorsolateral region apparently facilitating postural fixation of the forelimbs, and the ventrolateral region facilitating both forelimb fixation and biting speed. From the present findings it appears, however, that the rostrolateral striatum does not play an important role in limb control during locomotion, and that the rostromedial striatum does not importantly influence any physical parameters of movement (supported by the MRC of Canada. M.P. is an OMHF Scholar).

334.2 CORTICAL-STRIATAL RELATIONSHIPS: SENSORIMOTOR AND VISUAL FUNCTIONS IN THE RAT. D.B.Neill* and K.E.Sabol* (SPON: K. Wallen). Dept. of Psychology, Emory University, Atlanta, GA 30322.

Subregions within striatum receive anatomical projections from cortex in a topographical manner. Divac (J.of Comp.and Physiol.Psych.,63:184-190,1967) suggested that functions of striatal subregions are related to the functions of cortical regions based on the pattern of cortical innervation. The purpose of these experiments was to study this hypothesis in regard to 2 functional systems: sensorimotor and visual systems. Based on anatomical evidence available, the cortical projection theory predicts a functional relationship between sensorimotor cortex and lateral striatum; and between visual cortex and posterior striatum.

Expt.1: 42 rats were given bilateral aspiration lesions of sensorimotor cortex (SMC), occipital cortex (OC) or were sham operated (Sh). One week after surgery the rats were tested on a forelimb task. They were required to reach into narrow slots for small 45 mg food pellets. After 3 weeks they reached for larger, 190 mg pellets. SMC lesioned rats were severely impaired on both small and large pellet testing, while the OC and Sh rats were unimpaired.

Expt.2: 68 rats received bilateral chemical lesions of striatum. The neurotoxin quinolinic acid (6.25 ug) or vehicle (PBS,pH=7.0) was injected into one of 4 sites: medial anterior (MAS) A 8.6, L 2.0, H 2.0 (reference to the interaural line and nose bar set at 5.0); lateral anterior (LAS) A 8.6, L 3.5, H 0.5; lateral central (LCS) A 7.2, L 4.0, H 0.5; posterior (PS) A 5.8, L 4.5, H 0.5. The MAS and LCS rats were unimpaired on the reach task. PS rats were impaired on small pellet testing and the LAS group was impaired only on the large pellet test.

Expt.3: The cortically lesioned animals described above were tested on the acquisition of 2 visual tasks: horizontal-vertical (H-V) and brightness discriminations. The rats were given 50 trials/day in a comupter controlled apparatus with food reward. SMC lesioned rats did not differ fron controls in either discrimination. Eleven of 12 OC animals failed to acquire the H-V task after 2500 trials. This group was also impaired on brightness.

Expt.4: The striatal lesioned animals were tested in the two visual tasks. Only the PS group was impaired on the H-V discrimination. None of the striatal groups showed a deficit on the brightness discrimination.

Overall, these results support the cortical projection theory of striatal function. However, other interpretations of the data are discussed.

334.3 SOME EFFECTS OF CAUDATE NUCLEUS LESIONS IN RATS DURING A FIXED-RATIO TASK ON RESPONSE INITIATION AND FORCE EMISSION. J.Y. Harrell and W.T. Hayes*. Dept. ofPsychology, Hampden-Sydney Col., Hampden-Sydney, VA 23943.

We have examined the effects of caudate nucleus lesions in rats on ability to perform ballistic movements required in an FR-5 operant task. The task required the rats to press with its forepaw upon an isometric, force transducer with a force above 20 g in order to advance the fixed-ratio counter. Five rats received bilateral caudate nucleus (CN) lesions, 2 rats received bilateral parietal cortex (PC) lesions, and three rats were unoperated. The PC and unoperated rats served as controls.

Subjects were trained to reach through a small opening in Skinner box and press upon a disc attached to the transducer in order to receive water reinforcers. This arrange-ment assured a uniform topography and eliminated extraneous forms of responding. Response parameters studied were peak force of responding, response duration, and response effort. In addition, strip chart recordings of selected sessions were made. All programming contingencies, data collection, and data storage were accomplished by computer in conjunction with standard reed relay and solid state buffer devices..

After shaping and stabilization on the operant task, a baseline of 20 days data was collected. The following day, under pentobarbital anesthesia, lesions were performed using a monopolar electrode, insulated to within 0.5 mm of the tip. Lesioning parameters were 2 ma of current for 20 sec for both CN and PC animals. The day after surgery animals testing resumed under preoperative conditions and ran continuously for 24 days. Thereafter they were tested twice more at three week intervals for three consecutive days. Thus the final postoperative session occurred 61 days following surgery. All lesioned animals were then perfused and histologies performed to verify lesion placement.

Primary lesion effects in the CN animals were 1) disruption of ability to initiate responses and 2) loss of ability to make sustained bursts of responses, primarily within the first 10 postoperative sessions. With the exception of one subject which never responded postoperatively, all CN subjects were responding independently (without prompting) by the eighth postoperative session and by the second postoperative week were able to make sustained bursts of responses. Control subjects showed no response dysfunction at any point postoperatively.

While all CN subjects received substantial damage to the caudate nucleus subjects with the greatest task-related deficits also received damage to the globus pallidus (GP). The one non-responding CN received, in particular, received substantial bilateral GP damage. The last CN subject to recover independent, postoperative responding also received significant GP damage, although limited more to one hemisphere. In general, observed deficits appeared to be correlated to lesion placement. Overall, lesion size was consistent across subjects.

These data are in marked contrast to those from a previous study using a similar apparatus but requiring sustained, precision force output for reinforcement. The previous data suggested that damage to fairly disparate regions of the caudate nucleus significantly and permanently disrupted ability to perform sustained responses. Results from these studies support views of the basal ganglia in rats as serving ramp-generating functions and governing sustained more than ballistic type responses.

334.4 CHARACTERIZATION OF A DIETARY HALOPERIDOL REGIMEN TO INDUCE CHANGES IN DOPAMINE AND GABA RECEPTOR BINDING WITHIN RAT BASAL GANGLIA, J.M. Frey, W.W. Morgan, M.K. Ticku and R.D. Huffman, Dept. of Pharmacology and Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284.

Several biochemical and behavioral measures were employed to assess the effects of chronic haloperidol [HAL] administration on the dopamine [DA] and GABA systems within the rat basal ganglia [BG]. For these experiments, HAL was administered in the diet for 30 days in three increasing concentrations [7-15 mg/kg/day] (Huffman and Ticku, Pharmacol Biochem Behav, 19:199,1983). Since other studies of the effects of chronic HAL on GABAergic binding within the BG have been conducted with rats that had received HAL by parenteral modes of administration (Ferkany et al., J Neurochem, 34:247,1980; Gale,K., Nature, 283:569,1980), we also decided to compare the results of our feeding model [CHAL] with those obtained from one of the parenteral models [0.7 mg/kg, once daily, SC;SCHAL]. As indices of the "sensitivity" of the DA system within the BG, striatal 3H-spiroperidol binding, apomorphine [APO]-induced stereotyped behavior [SB] and spontaneous locomotor activity [SLA] were measured. In addition, DA binding was examined within the globus pallidus [GP] and the substantia nigra, pars reticulata [SNR], and GABA binding was measured within the GP, SNR and the pedunculopontine nucleus [PPN]. Scatchard analysis of 3H-spiroperidol binding isotherms revealed significant increases [39-60%] in the Bmax of high affinity binding sites [KD≈55pM] within the striatum of both SCHAL- and CHAL-treated rats following cessation of HAL treatments [Control: 319 ± 12(SE),N=4; CHAL:509 ± 26,N=4; SCHAL:444 ± 34fmol/mg prot,N=3]. A nonsignificant trend toward a higher Bmax was observed within the striatum of CHAL-treated rats. Within the SCHAL-treated group, a second, lower affinity site was resolved which was not detected within the striatal homogenates of CHAL-treated or control animals [KD=336 ± 74pM; Bmax=786 ± 139fmol/mg prot]. At 48 hrs posttreatment: a) SLA and APO-induced SB were increased in both groups of HAL-treated rats [p<.05]; APO-induced SB was significantly greater in CHAL- vs SCHAL-treated rats at 8 days after cessation of the chronic HAL treatments; b) Specific 3H-spiroperidol [0.1nM] binding was increased by more than 200% within the GP of CHAL-treated rats vs control animals [p<0.001,N=3]; c) GABAA receptor [3H-muscimol] binding was enhanced 30-50% within the GP and the SNR of CHAL-treated rats [p<.05,N=3], whereas preliminary binding experiments indicated that GABAB [3H-baclofen] binding was greatly reduced within these regions as well as within the striatum [≈80%,N=1]; d) Within the PPN of CHAL-treated rats, a second, lower affinity 3H-GABA binding site was detected which was not present in control animals [KD=226 ± 56nM; Bmax=353 ± 46 fmol/mg prot, N=4].

334.5 INTRASTRIATAL ESTRADIOL ENHANCES HALOPERIDOL-INDUCED CATALEPSY. C. Van Hartesveldt, A. Kalehua* and B. Boeve*. Psychology Department, University of Florida, Gainesville, FL 32611.

Previous research has shown that exogenously administered estradiol enhances haloperidol-induced catalepsy. Since haloperidol induces catalepsy by blocking striatal dopamine receptors, it is possible that estradiol also acts on the striatum to enhance catalepsy. However, 1) there are no classical estrogen receptors in the striatum, and 2) peripherally administered estrogens might change the rate of metabolism of haloperidol. The present study was conducted to determine whether estradiol acts directly on the striatum to enhance haloperidol-induced catalepsy.

Male Long-Evans hooded rats were anesthetized and bilaterally implanted with permanent 21 GA stainless steel guide cannulae positioned 1 mm above target sites in the anterior dorsal striatum, ventral striatum, or cerebral cortex. After a 1 week recovery period, 27 GA cannulae filled with either 17α-estradiol or 17β-estradiol were inserted through the guide cannulae. Half of the animals received 17α-estradiol and the other half, 17β-estradiol. Forty-five minutes after the hormone treatment the rats were given 0.0625 mg/kg haloperidol S.C. in the flank. After 15 min rats were tested for bar clinging every 15 min for the next 2 hr. After 1 week, rats received the alternate form of estradiol and were tested in the same way. Separate groups of rats received the same hormone treatments but were administered the vehicle solution.

Implants of 17β-estradiol in the dorsal striatum significantly enhanced haloperidol-induced bar clinging in comparison with intrastriatal implants of 17α-estradiol. Implants in the ventral striatum and cerebral cortex were without effect. Neither hormone affected bar clinging in any group of rats administered the vehicle solution.

Unilateral intrastriatal implants of 17β-estradiol were previously shown to induce ipsilateral postural deviation (Joyce and Van Hartesveldt, Pharmacol. Biochem. Behav., 20:575-581, 1984). The present results lend further weight to the idea that estradiol acts directly on the striatum to affect dopamine-mediated behaviors.

334.6 **CORRESPONDING REGIONAL INFLUENCES OF THE WEAVER MUTATION ON STRIATAL DOPAMINERGIC INNERVATION AND RECEPTOR DISTRIBUTION.** **J.M. Pullara*, D.W. Sapp*, J.N. Joyce, and J.F. Marshall.** **Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.**

In addition to their cerebellar defects, mice homozygous for the autosomal recessive gene weaver (wv) exhibit a region-specific depletion of forebrain dopamine (DA). This has been demonstrated biochemically and visualized anatomically with catecholamine histofluorescence. DA is reduced approximately 70% in the dorsal striatum of homozygotes (wv/wv) relative to heterozygous (+/wv) controls while DA content in ventral striatum (nucleus accumbens and ventral caudate-putamen) is unchanged (Schmidt et al, J. Neurosci. 2, 376-380, 1982; Roffler-Tarlov and Graybiel Nature 307, 62-66,1984).

The goal of the present study was to determine if the loss of forebrain DA in wv/wv mice can be accounted for by abnormalties in midbrain DA-containing cell bodies and whether this loss is accompanied by changes in striatal D_2 receptors. We examined catecholamine histofluorescence in the ventral midbrain of wv/wv mice and +/wv littermates. Compared to +/wv controls, wv/wv mice had far fewer flourescent cell bodies in the substantia nigra (SN) and a less pronounced, more diffuse reduction of ventral tegmental area (VTA) fluorescent somata. We used quantitative autoradiography to determine the density of [^{3}H]spiroperidol binding sites in the striatum of wv/wv mice and +/wv littermates. Brain sections were incubated in 0.7 nM [^{3}H]spiroperidol (89 Ci/mmol) in the presence of 40 nM ketanserin with or without 1 μM (+)-butaclamol. Afterward, tissue sections were dried completely, fixed using paraformaldehyde vapors, and the white matter removed by chloroform extraction (Sapp et al, this meeting). Tissue sections and standards were apposed to tritium sensitive film for 17 days. Autoradiographs were digitized, linearized, and the density of specific [^{3}H]spiroperidol binding in regions of striatum determined by computerized image analysis. Compared to +/wv littermates, wv/wv mice exhibited higher D_2 binding in the striatum. The most pronounced and consistent increase in D_2 binding was found in dorsomedial caudate-putamen.

These results suggest that the regional effects of the weaver mutation on striatal DA content result from a differential loss of DA neurons in SN and VTA. The correspondence between the regional loss of striatal DA and the regional increases in [^{3}H]spiroperidol binding shown in the present study suggest that a regional reduction in DA results in a localized increase in D_2 receptor density and/or affinity. Thus, the weaver mouse appears to provide a model for studying regional receptor regulation under conditions of naturally occuring neurotransmitter deficiency. Studies now underway will examine the distribution of D_1 receptors and DA uptake sites in the striatum of these mice.

334.7 HEREDITARY EFFECTS ON MESOTELENCEPHALIC TYROSINE HYDROXYLASE ACTIVITY AND COMPONENTS OF OPEN-FIELD BEHAVIOR. C. Vadasz*, G. Kobor*, I. Sziraki*, A. Lajtha (SPON: D. Ford). Neurochemistry Division, Nathan S. Kline Institute, Orangeburg, N.Y. 10962.

Phenotypic consequences of genotypic differences can be expressed in different functions and forms of behavior, and in the central nervous system. Usually, when the genotypes differ at several genetic loci, it becomes very difficult to disentangle the pleiotropic effects of the individual genes. As part of our work to construct animal model lines that differ at only one or a few loci affecting central dopamine systems, male mice of (C57BL/6ByJXBALB/cJ)F_2 (Type"C"), and (C57BL/6ByJXCXBI/ByJ)F_2 (Type "I") segregating generations were rank ordered according to their mesencephalic TH activity and animals with extreme high or low values were back-crossed to C57BL/6ByJ females. Resemblances between the male offspring and their F_2 fathers were investigated for neurochemical and behavioral traits, including mesencephalic and striatal TH activity (TH/SN and TH/CS respectively), distance covered in an open-field test, time spent not moving, speed of locomotion, agoraphilia, frequency of exploratory rearing on the hind legs, touching the wall with forepaws, repetitive facial grooming bouts, and overhead stroke grooming patterns. Genetic correlation matrices, based on offspring-parent cross-covariances of non-transformed data of Type "C" and Type "I" populations were further analyzed by multivariate methods.

Factor analysis yielded different loading patterns for Type "C" and Type "I" correlation matrices, reflecting differences in the genetic architecture of the two populations. In Type "C" high factor loadings (>0.5) were found for speed of movement and TH/SN on Factor 1, for rearing and agorophilia on Factor 2, for defecation on Factor 3, and for overhead stroke and TH/CS on Factor 4. In Type "I" high factor loadings (>0.5) were found for speed, distance covered, rearing, and agoraphilia on Factor 1, for TH/SN on Factor 2, for repetitive facial grooming, defecation and rearing on Factor 3, and for overhead stroke and TH/CS on Factor 4. Since factors formed from genetic correlation presumably represent groups of characters affected by the same gene(s), results of genetic factor analysis may allow insight into the architecture of brain-behavior relationships.

334.8 MOTOR EFFECTS OF RIMORPHIN IN THE RAT SUBSTANTIA NIGRA. A.M.Lohof*, R.R.Matsumoto, R.L.Patrick, and J.M.Walker. Department of Psychology and Section of Neurobiology, Brown University, Providence, RI 02912.

Interactions between the striatum and the substantia nigra have been shown to be important in the production of movement. A striatonigral tract which releases products of the opiate precursor prodynorphin into the substantia nigra pars reticulata has been identified. Other investigations suggested that neural activity in the pars reticulata affects the dopaminergic nigrostriatal projection. The prodynorphin derived opioid peptides could therefore have a function in motor behavior.

Unilateral administration of rimorphin in the substantia nigra pars reticulata of the rat (doses: 2.67, 5.33, and 10.65 nmol) resulted in significant contralateral rotation, indicating motor activation on the side of injection. The effect was antagonized by systemic administration of naloxone, indicating that rimorphin actions are mediated by opiate receptors.

The hypothesis that the dopaminergic striatonigral tract mediates the motor effects was tested in two ways. The concentrations of dopamine and the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were measured in the left and right striata 20 minutes after the unilateral intranigral infusion of rimorphin. Catecholamine levels were quantified using high pressure liquid chromatography and electrochemical detection. In every animal tested, concentrations of both dopamine and DOPAC were higher in the striatum ipsilateral to the side of injection. Additionally, the percentage elevation in DOPAC concentration in the injected hemisphere was significantly correlated with the numbers of contralateral rotations that the animals performed.

The involvement of dopamine was also suggested by experiments in which selective lesions to the pars compacta dopamine neurons were made using 6-hydroxydopamine (6OHDA). The motor effects normally produced by rimorphin were significantly reduced in rats treated with 6OHDA. These results suggest that rimorphin, released from the striatonigral tract, exerts its motor effects via the pars compacta dopamine neurons.

334.9 ROTATIONAL BEHAVIOR FOLLOWING INTRANIGRAL MICROINJECTION OF SPECIFIC MU, KAPPA, AND DELTA OPIATE AGONISTS. R.R.Matsumoto, K.H.Brinsfield*, D.L.Cherlin* and J.M.Walker. Department of Psychology, Brown University, Providence, RI 02912.

Opiates induce contralateral circling when unilaterally injected into the substantia nigra pars reticulata (SNR). Circling produced by drugs such as morphine, dynorphin, and Met-enkephalin may result from interactions with various opiate receptor types. This possibility is supported by receptor autoradiographic studies which show populations of mu, delta, and kappa opiate receptors in the substantia nigra. However, past studies have been unable to elucidate the extent of involvement of each opiate receptor type in circling behavior since highly selective ligands have been developed only recently. The purpose of this study, therefore, was to use these highly selective ligands to assess the involvement of the mu, delta, and kappa opiate receptor types in eliciting rotational behavior in the rat.

The following ligands were chosen for their 200-fold or greater affinities for select opiate receptor types: [D-Pen2,5]-enkephalin (DPEN, delta ligand), [D-Ala2,MePhe4,Gly-ol5]-enkephalin (DAGO, mu ligand), and U-50,488H (kappa ligand). Three equimolar doses of each drug, adjusted for peptide content, were infused into the SNR through a 31-gauge microinjection needle in a 0.5 ul saline vehicle. A rotometer was used to count the total number of contralateral and ipsilateral rotations and these numbers were recorded every minute for 30 minutes. The results show that all three ligands are capable of producing significant contralateral rotations in a dose-dependent manner. Preliminary indications suggest that these effects are naloxone reversible. The peak effect of each drug occurs during different intervals following administration. At the 5 nmol dose, the effects of U-50,488H peak after 5 minutes; the effects of DAGO level off after 20 minutes; and the effects of DPEN are still increasing after 30 minutes.

The ability of all three receptor specific ligands to induce rotational behavior suggests that all three opiate receptor types are involved in eliciting circling in rats. A major input of opiates to the substantia nigra has been attributed to the dynorphinergic pathway from the caudate. The potent effects of DPEN on rotational behavior in this study, however, suggests a significant enkephalinergic impact via its input to the pars compacta (SNC). The origin of the SNC enkephalinergic cells are as yet unknown, but warrant further investigation. Also, interactions of opiate receptors with dopaminergic and GABAergic systems have yet to be investigated.

334.10 INTRANIGRAL INFUSIONS OF GABA ANTAGONISTS ELICIT VACUOUS CHEWING BEHAVIOR. Susan E. Bachus, Karen Gale & Lars M. Gunne*, Dept. Pharmacol., Georgetown Univ. Sch. Med. & Dent., Wash. D.C., 20007; and *Uppsala Psychiatric Res. Ctr., Sweden.

Chronic neuroleptic treatment has been found to cause a significant depletion of nigral GABA in those animals exhibiting persistent dyskinetic symptoms (Gunne & Häggström, Psychopharm. 81:191, 1983; Gunne et al., Nature 309:347, 1984). We were therefore interested in examining the ability of acute interference with nigral GABA transmission to induce dyskinetic behaviors.

Bilateral intranigral infusion of the GABA receptor antagonist bicuculline methiodide (BIC) (98 pmoles/.5µl/4 min, given simultaneously on each side) through chronically implanted cannulae produced an increased rate of vacuous chewing movements (VCM) in rats. The chewing rate increased from a preinjection level of 3.7 VCM/min to a maximum of 18.0 VCM/min at 5 min after the end of infusion. By 15 min after infusion, the VCM incidence was nearly back to pre-drug baseline level. In addition, isonicotinic acid hydrazide (INH), an inhibitor of GABA synthesis, was infused bilaterally into the substantia nigra in a concentration (.51 µmoles/.5 µl/4 min) that causes depletion of nigral GABA by at least 50%. INH also induced VCM which reached a maximum of 23.6/min at 20-30 min after infusion. During the peak effect the vacuous chewing behavior was occasionally replaced by object-directed biting, such as that reported by Arnt & Scheel-Krüger to be evoked by BIC (Eur. J. Pharmacol. 62:51, 1980).

After intranigral injection, INH depletes GABA after a 20 min latency (Iadarola & Gale, Brain Res. 183:217, 1980); our behavioral observations were consistent with this time course. The fact that the onset of action of the direct receptor antagonist BIC was very rapid (within min) whereas the effect of the GABA depleting agent INH occurred only after a significant latency, strongly implicates reduction of nigral GABA transmission as the mechanism responsible for inducing VCM.

We have previously implicated the GABAergic nigrotegmental projection in mediation of dyskinetic behaviors (Childs & Gale, Life Sci. 33:1007, 1983). We also examined whether the VCM elicited by intranigral INH could be modified by intervention at the nigrotegmental target region. Muscimol infusions into this region completely blocked VCM evoked by intranigral INH. Control tegmental saline infusions did not diminish peak INH-evoked VCM.

The observations reported here may link a reduced nigral GABAergic tone to an increase in involuntary oral activity, in support of the GABA hypothesis for tardive dyskinesia (Gunne et al., Nature 309:347, 1984).

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334.11 CONTRALATERAL ROTATION TO AMPHETAMINE FOLLOWING UNILATERAL LESIONS OF OLFACTORY TUBERCLE DOPAMINE. J.DeVeaugh-Geiss*,M.R. Lynch and R.J.Carey, Dept. Psychiatry,S.U.N.Y. Health Sci.Ctr. and V.A. Med. Ctr., Syracuse, NY 13210

We have previously reported that rats with severe bilateral (>40% loss) depletion of DA in the olfactory tubercle region are hypersensitive to the locomotor stimulatory effects of d-amphetamine. Thus, whereas DA depletion of the nucleus accumbens abolishes amphetamine-induced behavioral stimulation (indicating the importance of this limbic structure in facilitating behavioral output), other terminal projection areas of the mesolimbic DA system may serve an antagonistic function, i.e., behavioral inhibition (Cools & Van Rossum, Life Sci.,27:1237-1253,1980). More recent findings from our laboratory suggest that this hypersensitivity to d-amphetamine is manifest in behaviors typically observed only with higher doses of this drug (>1.0 mg/kg). That is, rats with severe bilateral (<22%) DA depletions of the olfactory tubercles and concomitant asymmetrical striatal DA concentrations show vigorous turning in response to 1.0 mg/kg d-amphetamine. This is in contrast to animals with selective unilateral depletion of caudate DA, who typically fail to turn in response to doses below 2.0 mg/kg. Also, amphetamine-induced turning in these tubercle-lesioned rats is contralateral to the side of the most severe DA depletion (versus ipsilateral in caudate-depleted animals). We infused 5 rats unilaterally into the olfactory tubercle (A+1.6,L+2.5,D-8.1) with 2 µl of 3 µg/µl 6-OHDA (as base) following DMI pretreatment. As we have previously found with bilateral tubercle lesions, these rats were severely hypoactive for 5 days post-surgery. They also rotated spontaneously ($\bar{X}$=4.6 turns/10 min on day 2 and 5.2 on day three) in a direction ipsiversive to the lesioned hemisphere. Treatment with 1.0 mg/kg d-amphetamine (1/2 hr before testing) on day 4, however, produced vigorous contraversive rotation ($\bar{X}$=85 turns/10 min). Four animals unilaterally lesioned with 6-OHDA in the caudate also turned spontaneously, ipsiversive to the lesioned hemisphere on days 2 and 3 post-surgery but made few turns in response to d-amphetamine on day 4 ($\bar{X}$=2.4). (Those turns that were recorded were ipsilateral to the lesioned hemisphere.) Also, these caudate-lesioned animals showed stimulation of locomotor activity in an open field, paralleling that observed for vehicle-infused control rats treated with amphetamine. Lastly, turning responses were at no time observed in 5 vehicle-infused control animals. In conclusion, hyperreactivity to the behavioral stimulatory effects of d-amphetamine observed in this and our previous studies, in olfactory tubercle depleted rats, indicates a disinhibition of the dopaminergic areas that regulate this behavioral output.

334.12 ULTRASTRUCTURAL AND BEHAVIORAL EFFECTS OF MPTP IN MICE. J.C. Linder, H. Klemfuss, and P.M. Groves, Dept. Psychiatry, M-003, Univ. California, San Diego, La Jolla, CA 92093.

Chronic exposure to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes parkinsonian symptoms in primates and long-term loss of dopaminergic cells in the pars compacta of the substantia nigra of primates, mice and other animals. These dopaminergic cells have their terminals in the caudate nucleus where acute systemic administration of MPTP alters dopamine metabolism and 2-deoxyglucose uptake, but where acute ultrastructural actions of MPTP have not been previously reported.

We examined the behavioral and ultrastructural effects of subcutaneous injections of 20-40 mg/kg MPTP or saline in 35 adult C57 Black mice. The presence or absence of unusual behaviors was noted for four 15-min intervals after each injection, and at 30-min intervals until the behavior returned to normal or the animals were killed. After survival times of 1.5 to 75 hr, mice were perfused and fixed for electron microscopy using standard procedures.

MPTP produced a variety of behaviors in the mouse, but almost all animals showed piloerection, salivation and reduced locomotion beginning within 30 min of injection. After 30 or 40 mg/kg doses, these symptoms were often followed by 0.5-1.5 hr of dramatically increased locomotion and hyperreactivity, usually followed by catalepsy for 1-3 hr. Some mice also had periods of gnawing, teeth chatter, Straub tail, and clonic jerks. After 40 mg/kg MPTP, over half of the mice had convulsions and died. Most of the others appeared to return to normal within 4 hr.

Electron microscopy revealed a large amount of dark degeneration in the caudate nuclei of MPTP-treated mice compared to control animals. Degeneration reached a maximum at 6-10 hr and then declined, although some was present as early as 2 hr after a single 30 mg/kg injection. Areas of degeneration could contain as many as 0.8 dark processes/100 µm^2 of tissue, but not all areas of the caudate contained degeneration. Most degenerating synapses made symmetric contacts with dendrites or dendritic spines, but some asymmetric synapses onto spine heads were also present. Dark postsynaptic elements were seen in a minority of animals. Serial section analysis revealed some very black, highly branched structures whose form resembled that of glial processes. In several mice, mitochondria showed signs of alteration.

This study has clearly shown that MPTP can produce degeneration in the caudate nucleus of mice within a few hours of injection. We may be recording the beginning of the process that leads to the dopaminergic cell death in the substantia nigra.

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334.13 EARLY MORPHOLOGICAL CHANGES IN SUBSTANTIA NIGRA OF CAT AFTER MPTP ADMINISTRATION. I.J. Bak, J.S. Schneider and C.H. Markham. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024

Our experiments were designed to study early MPTP effects on ultrastructural level in the cat substantia nigra where dopaminergic neurons are profuse. After intraperitoneal injections of 10 mg/kg per day for two days, animals were perfused with paraformaldehyde and glutaraldehyde fixative. Brains were removed and substantia nigra and putamen punched out. These small tissue blocks were further postfixed in cacodylate buffered osmium tetroxide fixative for 2 hrs and dehydrated and embedded in araldite and polymerized.

There were 3 distinct early morphological effects in substantia nigra. First, in cross section, loosely bundled thinly myelinated axons, 0.8 μ to 5 μ in diameter, often showed myelinated membrane inside the axoplasm. Some were clear invaginations, continuous with the outside membrane. Others showed herniated myelinated membrane outside the axon. Cross-sectioned axons showed normal tubules and mitochondria. Possibly the membrane changes interfere with axonal transport.

Second, glial cells contained multilamilated membrane structures. These glial cells made extensive surface contact with nigral neurons. Such contacts were not observed in normal controls.

Third, in the neuropil, unusual electron-dense particles were observed in glial processes and also in the dendrites of nigral neurons as well as in some boutons. These structures, measuring 600-700 A° in diameter, resembled glycogen particles.

Conclusion: It is clear that major changes occur in the substantia nigra soon after MPTP administration. It is not certain whether neural or glial changes are first.

334.14 MPTP INDUCES SELECTIVE TERMINAL DEGENERATION IN THE MOTOR REGION OF THE STRIATUM IN DOG. G. D. Morrow*, J.S. Wilson and B.H. Turner. Department of Anatomy, Howard Univ., Washington, D.C. 20059.

The neurotoxin MPTP induces a Parkinsonian-like syndrome in dogs and monkeys that is associated with neural degeneration of the pars compacta of the substantia nigra and loss of striatal dopamine. We have made intracellular recordings in the caudate (Cd) nucleus of MPTP-treated dogs showing this syndrome and found the greatest physiological abnormalities in neurons recorded dorsolaterally. These data suggest that MPTP may be selectively toxic to dopamine terminals in the dorsolateral Cd nucleus; however, there is no anatomical data to support this in dog. Therefore, the purpose of this study was to map anatomically the pattern of terminal degeneration produced by MPTP. A Parkinsonian-like syndrome was induced in beagles by making a single injection of MPTP (3.0 mg/kg; i.v.). We have found that this protocol results in extensive neural degeneration of the pars compacta. One and two dogs were sacrificed 2 and 4 days after the injection, respectively. Coronal sections of the brains were cut and degenerating axoplasm was stained by the Fink-Heimer and cupric silver methods. Two days after the injection of MPTP, we found little indication of cellular, axonal or terminal degeneration. Four days after the injection, intense terminal degeneration was found throughout the putamen. Moderate terminal degeneration was also found in the dorsolateral body and head of the Cd nucleus at the level of the anterior commissure. Little or no terminal degeneration was observed rostral to the anterior commissure or within the medial or ventral regions of the Cd nucleus. Although the observed degeneration was very evident at 4 days, the cells of the pars compacta appeared normal.

This pattern of terminal degeneration is very similar to the corticostriate projections which originate from motor areas 4 & 6 (Tanaka et al., Brain Res., 172:150, 1979). These data indicate that the nigral projections to the motor areas of the striatum are most vulnerable to MPTP. We also suggest that the cells of the nigrostriatal pathway later die retrogradely after MPTP destroys their axon terminals. (Supported by MBRS S06-RR-08016).

334.15 IMMUNOCYTOCHEMICAL ALTERATIONS INDUCED BY 1-METHYL-4-PHENYL-1,2,3,6 TETRAHYDROPYRIDINE (MPTP). J.L.Carlson, M.E. Melnick and L.L. Vacca-Galloway. Departments of Anatomy and Physical Therapy Education, University of Kansas Medical Center, Kansas City, Kansas 66103.

The C_{57} Black mouse demonstrates severe loss or damage to nerve cells in the zona compacta of the substantia nigra (SN) when subjected to MPTP. It was also demonstrated that MPTP induces neurotoxic action on both dopamine and noradrenaline terminals in the mouse brain (Hillman et al. 1984). This condition mimics the human pathology induced by MPTP which produces symptoms of Parkinson's disease. Our study investigated long-term immunocytochemical changes in two neuromodulators (Substance P,SP; and Leucine Enkephalin, LE) and one dopamine transmitter enzyme (Tyrosine Hydroxylase, TH) after MPTP treatment of C_{57} Black Mice. The mice were treated for 3 days, 5 days, or 10 days with either MPTP (30 mg/kg/day, i.p.) or the solvent solution. The mice were perfused at 30 and 60 days after initial injection. Immunocytochemical alterations were then examined for each immunoreactive (IR) antigen in the 30 day animals. It was concluded that MPTP effected an increase of IR-SP in the striato-nigral neurons of the caudate nucleus and at their terminals in substantia nigra. Additionally, MPTP decreased IR-TH at the terminal fields of the dopaminergic nigro-striatal neurons in caudate nucleus. IR-LE demonstrated a small increase within the intrensic neurons of caudate nucleus after MPTP treatment. Animals examined 60 days after initial injection are currently being analyzed qualitatively for alterations in their immunoreactivity.

334.16 DESTRUCTION OF NORADRENERGIC AND DOPAMINERGIC NEURONS IN THE MURINE BRAINSTEM BY 1-Methyl-4-Phenyl-1,2,3,6-tetrahydropyridine (MPTP). N.A. Seniuk* and W.G. Tatton. Playfair Neuroscience Unit, Toronto Western Hospital, University of Toronto, Toronto, Canada, M5T 2S8.

The meperidine analogue, MPTP produces a Parkinsonian-like syndrome in humans and non-human primates. It reportedly causes selective destruction of dopaminergic neurons in the substantia nigra compacta (SNc) in primates and rodents while the numbers of aminergic neurons in other CNS structures such as the locus coeruleus (LC), ventral tegmental area (VTA) and hypothalamus are unchanged. The apparent discrepancy between the restricted cell death in the MPTP syndrome and the widespread histopathology of idiopathic Parkinson's disease (IPD) prompted the reexamination of the presumed selective cytotoxic action of MPTP. The effect of varying total dosages of MPTP (37.5-300 mg/kg) was examined in the C57BL/6J mouse using quantitative morphological techniques. Catecholaminergic neurons were immunocytochemically defined using a tyrosine hydroxylase (TH) specific antiserum. Serial, 20 μm thick, coronal sections were cut on a cryotome and thaw-mounted on slides. One series of alternate sections was incubated with a 1:500 dilution of antiserum and processed using an immunoperoxidase method with DAB as the chromogen. The second series was Nissl stained. Immunoreactive cells were localized in discrete, defined CNS regions. Individual cell counts were made in the SNc, LC, VTA and in A13 of the hypothalamus. Only immunoreactive cells containing a nucleus were counted. Individual section cell counts were normalised against an average control value. Computer-reconstructed serial sections of the brainstem volumes containing TH+ neurons, made from camera lucida drawings, illustrated the extent of cell loss among MPTP-treated animals as compared to normal controls. Histograms of the TH+ somal locations for the LC, SNc, VTA and A13 revealed a similar distribution of neurons among the control populations within each structure. There was a detectable cell loss with the lowest cumulative dose of 37.5 mg/kg of MPTP. There was a monotonic cell loss for each dosage schedule among the specific neural populations and there was a progressive loss of TH+ neurons as the cumulative dose of MPTP increased. Normalised total cell counts taken from all catecholaminergic nuclei examined were pooled and the mean values plotted for each dosage of MPTP. The "cumulative dose-cell number" curves reveal a strikingly similar and progressive loss of catecholaminergic neurons in all nuclei with increasing amounts of MPTP. These results establish differential cytotoxicity over selected dosage ranges for specific catecholaminergic neural populations and appear to support homology between cell loss in IPD and MPTP-parkinsonism in the murine model. Supported by MRC grant MT5218 and the Parkinson Foundation of Canada.

334.17 INTRACAROTID AND INTRAVERTERBRAL INFUSIONS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) IN PRIMATES AND CANINES. R.S. Burns*, L.E. Baker*, R.W. Kessler*, D.E. Schmidt and M.H. Ebert*. Dept. of Psychiatry, Vanderbilt Univ., Nashville, TN 37232.

Unilateral vs bilateral representation of extrapyramidal motor function in the striatum was studied using selective delivery of MPTP into the internal carotid artery (ICA) in primates and canines. In a pilot experiment, 2 rhesus monkeys received MPTP by ICA infusion (0.5 mg/kg in 12 min). Motor changes included a Glabellar reflex, marked hypokinesia and flexed posture of the arm, and mild hypokinesia of the leg contralaterally. These findings suggested that in primates the head and the arm are predominantly unilaterally represented in the striatum and the leg is represented bilaterally.

A preliminary study was carried out in the dog to determine the experimental parameters (dose, duration of infusion, brain:body weight ratio, blood flow rate) to destroy nigrostriatal dopamine neurons on one side of the brain with minimal damage to the other side. Dogs are highly sensitive to cell destruction by MPTP (Burns, R.S. et al., Soc. Neurosci. Abs., 1984). The greater (4-5X) body:brain weight ratio of the dog than the monkey is a theoretical advantage in reducing the toxic effects on brain of MPTP which 'spills over' into the general circulation after ICA infusion. Decreasing the cerebral blood flow rate uniformly by raising the intracranial pressure was used to increase the residual time of MPTP in brain. To investigate whether MPTP 'acts' at the cell body or terminal region of the cell, MPTP was also infused slowly (0.75 ml/min) into one vertebral artery (VA) and, assuming laminar flow in the basilar artery, the results compared with ICA infusion. Twelve dogs (16-25 Kg) received MPTP by infusion into the ICA (1 mg in 33 min; 2.5 mg in 33 min; 2.5 mg in 98 min) or into the VA (2.5 mg in 98 min) and were killed at 30 days. Two dogs given 2.5 mg into the ICA developed ipsilateral circling, and a flexed posture of the forelimb and hypokinesia and tremor of both limbs contralaterally. Nerve cell loss in the substantia nigra compacta (SNC) on one side of the brain vs the other was found after ICA infusion. Dopamine levels were reduced by up to 70% and HVA levels by up to 90% in the ipsilateral caudate nucleus after infusion of MPTP into the ICA.

334.18 ALTERATIONS OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN A PRIMATE MODEL OF HEMIPARKINSONISM. L.J.Porrino, E.Palombo*, V.W.Ho*, A.M.Crane*, K.Bankiewicz*, I.J.Kopin, L.Sokoloff. ARC,NIDA,Baltimore, MD 21224/ NIMH/NINCDS,Bethesda, MD 20892

Parkinson's disease in its initial stages is often characterized by symptoms limited to one side of the body. An animal model of hemiparkinsonism is provided by the unilateral intracarotid injection of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin known to destroy dopaminergic cells of the substantia nigra pars compacta (SNpc) in rhesus monkeys. These monkeys display unilateral bradykinesia and tremor, and in some cases spontaneous turning behavior towards the MPTP-treated side. We have used the quantitative autoradiographic 2-[^{14}C]deoxyglucose method (Sokoloff L et al, J.Neurochem.,28:897,1977) to define the patterns of local cerebral glucose utilization (LCGU) associated with this hemiparkinsonian syndrome.

LCGU was measured in 3 monkeys (Macaca mulatta) at least 3 months following intracarotid injection of MPTP. Decreases in LCGU were evident on MPTP- treated side in the SNpc, subthalamic nucleus, motor and premotor cortex, as well as in basolateral nucleus of amygdala. A significant increase in glucose use was seen in the external segment of the globus pallidus on the treated side. No side-to-side differences were seen in the striatum or in structures of the mesocorticolimbic system. The pattern of unilateral changes in energy metabolism found here is similar to the pattern of bilateral LCGU alterations associated with Parkinsonism (Porrino LJ et al, J.Cereb.Blood.Flow and Metab.,5:S167,1985), resulting from systemic administration of MPTP to rhesus monkeys.

L-DOPA treatment reverses the symptoms of Parkinsonism in the unilaterally treated monkeys and causes turning away from the side of MPTP treatment. Rates of LCGU were measured in 3 monkeys with unilateral SNpc damage following 4 days of L-DOPA (200 mg/day) and carbidopa (20 mg/day) therapy. Animals were studied 30 minutes after the last dose of L-DOPA. In these animals no evidence of asymmetry in glucose use was found. In those areas in which side-to-side differences had been found prior to L-DOPA therapy, L-DOPA restored LCGU on the MPTP-treated side of the brain to normal levels. This normalization is in sharp contrast to the effects of L-DOPA treatment in monkeys with MPTP-induced bilateral Parkinsonism, in which large increases in LCGU above normal control rates were seen throughout the brain. The extent of damage to the SNpc then, appears to be an important factor in determining the effects of L-DOPA on local cerebral energy metabolism.

334.19 ACUTE MPTP TREATMENT INCREASES GLUCOSE UTILIZATION IN SUBSTANTIA NIGRA PARS COMPACTA. E.Palombo*, L.J.Porrino, A.M.Crane*, V.W.Ho*, K.Bankiewicz, I.J.Kopin, L.Sokoloff (SPON:T.Jay). NIMH, NIDA and NINCDS, Bethesda, MD, 20892.

Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in humans and monkeys is known to produce a chronic syndrome closely resembling idiopathic Parkinson's disease. These symptoms develop over weeks following MPTP exposure (Davis GC et al, Psych.Res., 1:249, 1979; Burns SR et al, Proc.Nat.Acad.Sci., 80:4546, 1983). In contrast to the long term effects, acute administration of MPTP results in immediate symptoms consisting of sudden involuntary jerks, salivation and eyelid closure, followed by extension of the head and eye-widening. These behavioral changes have been attributed to transient activation of both serotoninergic and opioid systems (Langston WJ, Life Science, 36:201, 1985). In order to elucidate the anatomical and functional basis of the acute pharmacological effects of MPTP, the 2-[^{14}C]deoxyglucose (DG) method (Sokoloff L et al, J.Neurochem., 28:897, 1977) was used to measure rates of local cerebral glucose utilization (LCGU) in 4 rhesus monkeys (Macaca mulatta). Two received a single I.V. injection (4 mg/kg of MPTP dissolved in saline) 15 min. prior to initiation of the DG procedure; throughout the experiment they displayed behavioral changes consisting mostly of head extension, eye-widening, grimacing and struggling. Two monkeys received vehicle alone. All physiological parameters remained normal.

LCGU was measured in 73 brain areas. Most of the regions showed a decrease in energy metabolism. The most prominent decreases (-30 to -45%) were found in neocortical, hippocampal, thalamic and cerebellar regions. Smaller decreases (-25 to -35%) were also found in the extrapyramidal system and in the mesolimbic structures. In contrast, striking increases of LCGU were observed in substantia nigra pars compacta (+70%) as well as in the ventral lamella of inferior olive (+43%). Our study demonstrates that the acute pharmacological effects of MPTP are widespread in their distribution. Moreover, substantia nigra pars compacta is an immediate target of this compound, and is affected in an unique way as compared to other structures. The present data show a clear difference in the pattern of metabolic changes compared to the results obtained in chronic MPTP-treated Parkinsonian monkeys (Porrino LJ, J.Cereb.Blood Flow and Metab., 5:S167,1985), where the effects occur in areas specifically related to motor control.

334.20 MPTP-INDUCED PARKINSONIAN SYNDROME ASSOCIATED WITH ALTERATIONS OF GLUCOSE UTILIZATION IN THE PRIMATE OCULOMOTOR SYSTEM. V.W.Ho*, L.J.Porrino, E.Palombo*, A.Crane*, K.Bankiewicz*, I.J.Kopin, L.Sokoloff (SPON:C.Kennedy).Howard Hughes Medical Institute/NIDA/ NIMH/NINCDS, Bethesda, MD 20892

Chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment in primates causes symptoms of akinesia, rigidity, tremor and freezing, which closely resemble idiopathic Parkinson's disease (Burns RS et al, Proc.Nat.Acad.Sci., 80:4546,1983). In addition, deficiency in eye movement production, another feature of parkinsonism, was observed in MPTP-treated monkeys. In order to delineate those circuits controlling eye movements affected by MPTP treatment, the quantitative autoradiographic 2-[^{14}C]deoxyglucose (DG) method (Sokoloff L et al, J.Neurochem., 28:897,1977) was employed.

Rates of glucose utilization (LCGU) were measured in 11 monkeys (Macaca mulatta) divided into four groups: normal, normal with chronic L-DOPA administration, MPTP-treated, and MPTP-treated with chronic L-DOPA administration. Each MPTP-treated monkey received a total of 4 mg/kg MPTP i.v. divided over a five day period. At least three months elapsed between MPTP treatment and the DG studies. LCGU was determined in 20 structures known to be involved in the production of eye movements. Decreases in LCGU in MPTP-treated monkeys as compared to normal controls were observed in the paralamellar portion of the mediodorsal nucleus of the thalamus and its cortical projection in the frontal eye field. In contrast, no differences between normal and MPTP-treated groups were found in any portion of the superior colliculus, the accessory oculomotor nuclei, or brainstem nuclei innervating the extraocular muscles. MPTP treatment, then, tended to alter LCGU in those portions of the oculomotor system involved in the initiation of voluntary eye movements, the most prominent oculomotor deficits of Parkinsonian patients. Furthermore, whereas L-DOPA administration produced no significant changes of LCGU in normal monkeys, it increased LCGU above control values in all structures of the oculomotor system of MPTP-treated monkeys.

LCGU was also measured in 6 monkeys with unilateral Parkinsonism induced by a unilateral intracarotid injection of MPTP (2.5 mg/kg) at least three months earlier. These animals showed behavioral asymmetries characterized by rotation and gaze towards the treated side. LCGU was decreased in the trochlear and the oculomotor nuclei but increased in the abducens nucleus of the treated side compared to the normal side. L-DOPA treatment reversed the rotational behaviors and the predominant gaze direction as well as the pattern of LCGU in the brainstem nuclei innervating the extraocular muscles.

335.1 Ca^{++} CHANNELS AND DEVELOPMENT: ω-CONOTOXIN BINDING IN CHICK BRAIN. M.J. Litzinger*, A. Azimi, L.J. Cruz and B.M. Olivera. Depts. of Pediatric Neurology and Biology, University of Utah, Salt Lake City, UT 84112 and Dept. of Biochemistry, University of the Philippines, Manila.

The development of the voltage sensitive calcium channel (VSCC) has been studied in vertebrate tissue culutre (Litzinger and Brenneman, BBRC, 127:112,1985) and membrane preparations (Marangos et al., J. Neurochem., 42:1338, 1984) using [^{3}H]-Nitrendipine (NTP), a calcium channel ligand. Recent electrophysiologic evidence shows 3 types of calcium channel (N, T, L). NTP is believed to act at the L channel (Nowychy et al., Nature, 316:440, 1985). ω-Conotoxin (ωCgTx) a peptide neurotoxin from the Conus snail, has been shown to act on the N and some L channels (McCleskey et al., submitted, 1986). Binding experiments show that NTP binds at sites different from the ω-CgTx (Cruz and Olivera, J. Biol. Chem., in press, 1986). We have measured ω-CgTx binding in chick whole brain preparations as a function of development to compare to already published data for NTP binding in chick whole brain preparations (Marangos et al., 1984).

Membranes were prepared from chick brain essentially as described by Catterall et al. (J. Biol. Chem., 254:11379, 1979). To measure nonspecific binding, 0.25 μM of cold ω-CgTx was preincubated for 30 min with this membrane preparation before ^{125}I-ω CgTx was added. The mixture was then incubated at room temperature for 30 min, filtered, washed and the filters counted as described previously (Cruz and Olivera, 1986).

The developmental curve for ω-CgTx binding ranged from day 10 of incubation to day 4 after hatching. The ω-CgTx binding remained stable and low until days 18-20 of incubation; the period during which the chick is hatching. Preliminary data show that ω-CgTx binding levels off after the chick has hatched. This pattern is markedly different than that seen with NTP which increases gradually throughout development.

We interpret these data as showing developmentally that the ω-CgTx binding site is different from the NTP binding site. More importantly, the number of ω-CgTx sites show a sudden increase associated specifically with the chick's hatching. Supported by GM22737.

335.2 CALCIUM CHANNEL BINDING SITE DISTRIBUTION IN CAT AND KITTEN VISUAL CORTEX. C. Shaw[1], M. Wilkinson[2] and M. Cynader[1,2]. [1]Depts. of Psychology and [2]Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada.

In a recent series of publications we have described the characteristics and laminar distributions of various neurotransmitter (NT)/neuromodulator (NM) receptors in cat visual cortex during post-natal development. The function of NT/NM receptors may involve coactivation of voltage dependent calcium (Ca^{++}) channels whose presence has been related to the density of synaptic connections. We have characterized Ca^{++} channel binding sites in cat visual cortex and examined their laminar distributions during post-natal development using [^{3}H] PN-200-110 ([^{3}H] PN; Amersham, 82 Ci/mmol), a 1.4 dihydropyridine Ca^{++} channel antagonist.

Sixteen micron thick sections of cat visual cortex were cut within the same 24 hr. period and thaw-mounted onto subbed glass slides. The sections were pre-incubated for 10 min. in cold Tris HCl (170 mM, pH 7.7) with the addition of 0.2% formaldehyde. Two additional 5 min. rinses in Tris HCl alone completed the pre-incubation. In initial characterization studies the sections were incubated in 0.1-1.0 nM [^{3}H] PN in Tris HCl. Equilibrium binding was reached in 30 min. Rinses in cold Tris HCl (with 0.25 mg/ml fatty acid free BSA) reduced specific binding to 50% at 18 min. [^{3}H] PN binding was saturable and reversible. Non-specific binding (0.1 nM [^{3}H] PN) in the presence of 10^{-5}M nifendipine was about 10% of total binding. Eadie-Hofstee analyses of binding data in adult cats showed a K_D of 0.05 nM.

Sections from the visual cortex of animals of different ages were incubated with 0.1 nM [^{3}H] PN as described above and exposed to LKB ultrofilm. The autoradiograms revealed a laminar specific pattern of binding at each age examined: in young kittens (<20 days of age) binding was densest in layer 4; after 30 days post-natal, laminae 1-3 and to a lesser extent, lamina 6, showed the densest binding.

Similar redistributions of receptor binding during post-natal development have been shown to be a characteristic of a number of NT/NM receptors in cat visual cortex (eg. muscarinic ACh, cholecystokinin). In young kittens, most receptor populations are concentrated in layer 4 as are Ca^{++} channel binding sites. The Ca^{++} channel binding pattern in adult cat visual cortex resembles that of muscarinic ACh, cholecystokinin, adenosine and β-adrenergic receptors, as well as opiate and glutamate-related binding sites.

The present data suggest the possibility of a functional correlation, at certain ages, of several different NT/NM receptors with Ca^{++} channels.

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335.3 ONTOGENY OF CRF RECEPTORS IN RAT BRAIN AND PITUITARY. T.R. Insel, D. Fairbanks* and E.B. De Souza, Lab. of Clinical Science, NIMH, Poolesville, MD 20837 and Neuroscience Branch, ARC, NIDA, Baltimore, MD 21224.

Receptors for the 41 amino acid peptide, corticotropin releasing factor (CRF), have been previously identified, characterized, and localized in rat brain and pituitary using both homogenate binding and in vitro autoradiographic techniques (De Souza, E.B. et al., J. Neurosci., 5:3189-3203, 1985). Although CRF receptors in brain and pituitary show similar kinetic properties, pituitary receptors appear to be regulated differently than those in the brain (De Souza, E.B. et al., Neurosci. Letters, 56:121-128, 1985). To further compare CRF receptors in brain and pituitary, we investigated the ontogeny of the receptor in both sites.

Fetuses of timed pregnant S-D rats were delivered by C-section every other day from day 15 through day 21 of gestation. In addition, rat pups at post-natal days 2, 8, 14, 21, and 28 were used. Brains (and post-natal pituitaries) were removed, dropped into 10% sucrose and saline at 4° for 5 minutes, and then frozen at -70° C. Because of the difficulty of obtaining intact pituitaries from fetal rats, in an additional series of animals, the entire skull was frozen without dissection. CRF receptors in brain were measured using both whole brain homogenates and in vitro autoradiography. Both methods employed a 120 minute incubation with 0.1 nM ^{125}I-tyr-O-rCRF with non-specific binding determined using unlabeled r-CRF (1 uM). Pituitary CRF binding was measured using in vitro autoradiography alone.

^{125}I-CRF binding in whole brain homogenates appeared by day 17 of gestation (B_{max} = 20.6 ± 1.3 fmol/mg protein) increasing steadily to a peak at post natal day 8 (B_{max} = 150.1 ± 7.5 fmol/mg protein), then decreasing to lower values at days 21 and 28 (55.7 ± 3.4 and 48.1 ± 3.5 fmol/mg protein). This pattern did not reflect changes in protein content. Autoradiographic images revealed a similar time course with dense labeling in the developing cortex, as well as in several other regions that appear less conspicuously labeled in the adult. Pituitary CRF receptors followed a similar time course with specific binding present by day 19 gestation and marked binding evident from post-natal day 2 onwards.

Recently, physiologic studies have demonstrated that CRF receptors in pituitary are functionally active early in development (Walker, et al., Endocrinology, 118:1445-1451, 1986). The ontogeny of brain receptors for CRF appears to parallel this development in pituitary, although the function of these receptors in the developing brain remains to be elucidated.

335.4 D-1 AND D-2 DOPAMINE RECEPTOR DISTRIBUTIONS IN KITTEN AND MONKEY STRIATUM. E.K. Richfield, A.B. Young and J.B. Penney. Dept. of Neurology, University of Michigan, Ann Arbor, MI, 48104.

D-1 and D-2 dopamine receptors were studied in two neonatal kittens (P2 and P7) and four adult monkeys using quantitative autoradiography. Adjacent unfixed cryostat tissue sections were processed for D-1 and D-2 dopamine receptors and acetylcholinesterase (AChE) staining. D-1 receptors were labeled using [3H]-Schering 23390 and D-2 receptors were labeled using [3H]-spiroperidol. Both ligands were incubated at concentrations equal to their respective K_Ds and at one-half K_D to allow direct comparison of the number of D-1 and D-2 receptors. Specific binding was determined using 500 nM cis-flupenthixol as a blank for D-1 binding and 10 μM dopamine for D-2 binding. AChE staining was performed using a modified method of Geneser-Jensen and Blackstad (Z. Zelliorsch., 114:460, 1971). AChE staining durations were varied for kittens and monkeys to reveal maximal contrast between areas of high and low activity. Areas of increased staining (patches) were easily seen in kittens, while areas of decreased staining (striosomes) were easily seen in monkeys. Both patches and striosomes had appearances and distributions consistent with other reports.

D-1 receptors were heterogeneous in kitten striatum with a moderate amount in matrix and denser areas of increased binding that were often in register with AChE patches. D-2 receptors in kittens were more homogeneous, but with small areas of decreased binding that corresponded to areas of low AChE staining. D-1 receptors were much greater than D-2 receptors at both ages. In the P2 kitten, D-1 and D-2 receptors were present in the matrix with a decreasing gradient from dorsal to ventral. In the P7 kitten, these gradients were not present. The P7 kitten demonstrated increased binding of both D-1 and D-2 receptors in the matrix compared to that at P2, which tended to mask the heterogeneities present. The AChE stain demonstrated a decreasing gradient from lateral to medial, but not dorsal to ventral at both ages.

In the adult monkeys, D-1 receptors were also much greater than D-2 receptors. Both D-1 and D-2 receptors appeared quite homogeneous when compared to the AChE heterogeneity. There was a 5 to 15% variation in binding in some areas, but these areas did not correspond to striosomes seen with AChE staining.

Dopamine D-1 and D-2 receptors are heterogeneous in developing kitten striatum. The development of D-1 and D-2 receptors are distinct and differ from each other. The ontogeny of the dopamine receptors parallels, but differs from AChE staining in their distributions. Adult dopamine receptors are more homogeneous and do not match the distinct heterogeneity of AChE staining.

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335.5 ONTOGENY OF B_1 AND B_2 ADRENERGIC RECEPTORS IN THE BABOON: AN IN VITRO AUTORADIOGRAPHIC STUDY. P.A. Slesinger, P.R. Lowenstein, L.C. Walker, M.F. Casanova*, D.L. Price, J.T. Coyle and H.S. Singer*. Depts. of Neuroscience, Pathology and Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Norepinephrine (NE) appears early in development and has been shown to be involved in synaptogenesis and neuronal plasticity. Adrenergic receptors, which mediate the action of NE, are divided into two classes, Alpha 1 and 2 and Beta 1 and 2. Since NE is implicated in development, a detailed description of the ontogeny of Beta (B) receptors might prove useful in elucidating how and where NE acts. We report on the ontogeny and developmental pattern of B_1 and B_2 receptors in baboon, using in vitro autoradiography.

Brains from term baboons (approx. 180 days) and juvenile baboons (3 years old) were processed for Beta receptor autoradiography with ^{125}I-iodocyanopindolol, using standard autoradiographic techniques. Beta receptor subtypes were distinguished with the selective antagonists for B_1 (ICI 89,406) and for B_2 (ICI 118,551) receptors.

In the term baboon, areas of high binding to B_1 receptors include the caudate and putamen (striatum), globus pallidus, thalamus, lateral geniculate body, cerebral cortex (layers I,II,III and VI) and hippocampus. B_2 receptors are seen only in the dentate gyrus. Distribution of B_1 and B_2 receptors is similar in the juvenile baboon. In contrast, the pattern of binding in the cortex and striatum is strikingly different between the term and juvenile baboons. Patches of B_1 receptors and a pronounced medial (high binding) to lateral (low binding) gradient are observed in the striatum of the term baboon, whereas the striatum of the juvenile baboon is denser and more uniform. Additionally, only the term baboon has B_1 receptors in layer VI of the cortex.

The primary projection of NE fibers to cortex is from the locus coeruleus. The B_1 receptors found in the cortex of both term and juvenile baboons may mediate the effects of this NE input. The loss of B_1 receptors in cortical layer VI of the juvenile baboon indicates some reorganization of these receptors during development. In contrast, the striatum receives little NE innervation, yet B_1 receptors are present in high concentrations. In addition, we have found that B_1 receptors are localized into discrete patches in the striatum, and this pattern of binding becomes more uniform during development. Other investigators have shown a similar developmental pattern with acetylcholinesterase staining, dopamine histochemistry, opiate receptors, and Hemicholinium-3 binding sites. This study provides evidence for a dynamic, organized pattern of Beta receptors during development of the primate striatum and cortex.

335.6 DEVELOPMENT OF CHOLINERGIC AND DOPAMINERGIC PRE- AND POSTSYNAPTIC MARKERS IN THE BABOON AND HUMAN STRIATUM. P.R. Lowenstein, P.A. Slesinger, H.S. Singer*, L.C. Walker, M.F. Casanova*, D.L. Price and J.T.Coyle. Depts. of Neuroscience, Pathology and Neurology, The Johns Hopkins Univ. School of Medicine, Balto., MD 21205.

The caudate and putamen, which participate in the processing of motor and cognitive information, are part of parallel basal ganglia-thalamocortical circuits. Rather than being a homogeneous structure, these nuclei are divisible into distinct domains, based on anatomical, biochemical and electrophysiological characteristics. To understand the ontogeny and developmental organization of these circuits, we studied the maturation of two neurotransmitter systems within baboon and human striatum. Markers for pre- and postsynaptic cholinergic (striatal interneurons) and dopaminergic (nigral afferents) systems were investigated using autoradiographic and histochemical techniques.

Cerebral hemispheres from term baboons, human abortuses (approx. 18 weeks) and 3 year old baboons were rapidly dissected, frozen on dry ice and processed for autoradiography. Serial sections were obtained through the entire rostro-caudal extent of the corpus striatum. [^{3}H]Hemicholinium-3 ([^{3}H]HCh-3), which binds to high affinity choline uptake, was used as a presynaptic cholinergic marker. [^{3}H]Mazindol, which binds to dopamine uptake in the striatum, was used as a presynaptic dopaminergic marker. M1 muscarinic receptors were measured using [^{3}H]Pirenzepine. AChE histochemistry was performed on frozen and fixed tissue.

In the term baboon, [^{3}H]HCh-3 autoradiography demonstrated patches of high activity in a lateral (high) to medial (low) density gradient. This distribution of binding sites is paralleled by the AChE stain. A similar [^{3}H]HCh-3 and AChE pattern is also evident in an 18 week human fetus. In contrast, [^{3}H]HCh-3 binding in the 3 year old baboon striatum showed a lateral to medial gradient, with no high density patches apparent. [^{3}H]Mazindol autoradiography in the term baboon also showed patches, with non-patch areas having homogeneous binding, whereas in the adult, these dopaminergic terminals displayed a fairly homogeneous pattern. [^{3}H]Pirenzepine labelled muscarinic M1 receptors in both the term baboon and fetal human striatum showing patches in the caudate and putamen. In the 3 year old baboon, M1 receptors showed a lateral to medial binding gradient to the caudate and putamen.

These results emphasize the existence of an important reorganization within the postnatal caudate and putamen. This reorganization occurs after the generation of striatal neurons and after cortical and nigral innervation of the striatum have been achieved. Further studies are required to determine whether these patterns are modulated by intrinsic or afferent systems.

335.7 POSTNATAL DEVELOPMENT OF GP-50, A MAJOR GLYCOPROTEIN OF ISOLATED RAT BRAIN SYNAPTIC MEMBRANES, STUDIED WITH A MONOCLONAL ANTIBODY

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We have described previously a monoclonal antibody, mabSMgp50, that recognizes a neuron-specific, concanavalin A-binding glycoprotein that is enriched in isolated synaptic membranes (Beesley et al.; Biochem. Soc. Trans., in press). Immunocytochemical staining of adult rat cerebellar cortex suggests that the epitope is concentrated in the granule cells. This mab has now been used in biochemical and immunocytochemical experiments to study the developmental profile of the epitope in rat forebrain and cerebellum. Solid phase radioimmunoassay and Western blots of forebrain and cerebellar homogenates show that gp50 is present only at low levels (10% or less of adult) during the early stages of postnatal development. In forebrain the gp50 antigen increases to 80% of its adult level between postnatal days P16 and P33. The developmental profile shows a 2 day lag in cerebellum. Specific granule cell immunoreactiyity is first identified at P12-P15 and increases thereafter. At P25 the intensity of staining is still well below that of adult cerebellar cortex. Analysis of a range of forebrain and cerebellar subcellular fractions, including synaptic membranes, microsomes and the P2 pellet, shows that the developmental profiles mimic that for the parent homogenate with no redistribution of gp50 between fractions. However, in synaptic membrane the major increase in gp50 is delayed with respect to the homogenates, occurring between P24 and P33. Gp50 is present in cerebellar granule cell cultures but not in cultures enriched in astrocytes. It is first detected in granule cell cultures after 4 days in vitro and continues to increase in concentration throughout the culture period (maximum 12 days in vitro).

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335.8 **Na^+,K^+-ATPase Activity in the Developing Brain of Fetal Guinea Pigs During Normoxia and Hypoxia.** O. P. Mishra and M. Delivoria-Papadopoulos,* Dept. of Physiology, University of Pennsylvania, Philadelphia, PA. 19104

The guinea pig is noted for its high degree of neurological maturity at birth. In this species, the rate of brain growth in terms of increase in weight is maximum at 35 days of gestation. However, maximum increase in terms of DNA and cholesterol content occurs during the last week of gestation. The present study aims to examine the developmental pattern of Na^+,K^+-ATPase activity, an index of membrane functional maturation, in guinea pig fetal brain during the period of gestation and determine the effect of prenatal hypoxia on the activity of this enzyme.

Guinea pig fetuses of different days of gestation (35, 40, 45, 50, and 60 days) were obtained from mothers. Following decapitation, fetal brains were quickly removed and frozen in liquid nitrogen. The brain tissue was homogenized in 50 mM Tris-HCl buffer, pH 7.4, to produce a 2.5% W/V homogenate. ATPase activity was determined in 1 ml assay medium containing 100 mM NaCl, 20 mM KCl, 6 mM Na_2 ATP, 6 mM $MgCl_2$, 50 mM Tris-HCl buffer, pH 7.4, and 0.1 ml tissue homogenate. The reaction was carried out in both the presence and absence of 1 mM ouabain at 37°C for 5 min. Ouabain-sensitive activity was taken as a measure of Mg^{++}-ATPase. Protein was determined by the method of Lowry et al. Enzyme activity was expressed as umoles of Pi/mg protein/hour.

Na^+,K^+-ATPase, Mg^{++}-ATPase, and total ATPase activities remained unchanged during the early periods (35 - 45 days) of gestation. However, these activities increased significantly during the last 10 - 12 days of gestation. The activity of these enzymes at 35, 40, 45, 50, and 60 days of gestation are as follows: Total ATPase (5.89±0.86, 6.56±0.97, 6.79±1.21, 9.67±0.62, and 16.75±1.10 umoles of Pi/mg protein/hour); Na^+,K^+-ATPase (4.71±0.28, 4.33±0.58, 4.17±0.75, 5.89±0.51, and 8.29±0.93 umoles of Pi/mg protein/hour); Mg^{++}-ATPase (1.57±0.40, 2.24±0.46, 2.61±0.60, 3.78±0.36, and 8.45±0.81 umoles of Pi/mg protein/hour). Na^+,K^+-ATPase activity in the 60 day fetus was sharply reduced (50%) by maternal hypoxia (F_iO_2 7% for 40 min), whereas the 50 day fetal brain Na^+,K^+-ATPase was unaffected. Results show that a rapid increase in the Na^+,K^+-ATPase activity occurs during the last week of gestation and the activity is highly sensitive to hypoxia during this period. On the basis of these results, it is proposed that the fetal brain cell membrane functions are more susceptible to hypoxia at term than in preterm periods.

335.9 TRANSFERRIN BINDING IN CULTURED EMBRYONIC CHICK RETINAL NEURONS. G. D. Zeevalk & A. G. Hyndman Rutgers University, Dept. Biol. Sci., Piscataway, N.J. 08854

As previously reported (Neurosci. Abs., '85), developing chick retinal neurons bind human transferrin in vitro. Evidence in the literature (Kimura, et al., Develop. Growth & Differ., 24:369, '82 and others), however, suggests species differences among transferrins with regard to binding and functional properties. We therefore, investigated chick transferrin (conalbumin) binding in our cultured retinal neurons. Since the major role for transferrin is the transport of iron into the cell, we also investigated human and chick transferrin mediated iron uptake by our cultured neurons. Transferrin binding was examined by fluorescence immunocytochemistry using a rabbit polyclonal antibody against chick transferrin (generous gift of Dr. George Markelonis) or a goat polyclonal antibody against human transferrin (Cappel Lab.). Iron uptake was examined using radioactively labeled ^{59}Fe. At 72h in vitro, neurons derived from 8 day embryonic retina and grown in a transferrin-free defined medium, bound both human and chick transferrin on their surface when assayed at 4°C. When incubated with human or chick transferrin at 37°, then fixed and solubilized with cold 95% ethanol:2 parts glacial acetic acid, binding was found to consist of brightly fluorescent granules within the soma suggesting internalization of the transferrin. While both human and chick transferrin showed binding and internalization, the amount of fluorescent label associated with the neurons was always much greater with chick transferrin. Iron uptake by cultures grown in the absence of transferrin was 43±1 fmoles/10^3 cells after 20min of incubation. The addition of human or chick transferrin (62.5nM final concentration) enhanced iron transport into the neurons, however, the amount of iron taken up was much greater in the presence of chick transferrin (60±1.3 fmoles/10^3 cells for human transferrin compared with 107±1.2 fmoles/10^3 cell for chick transferrin.).

These results show that while cultured chick neurons can bind and utilize human transferrin, they do so with less efficiency than their own species of transferrin. This may explain, in part, why many defined media that contain human transferrin use unphysiologically high concentrations.

335.10 VITAMIN D DEPENDENT CALCIUM BINDING PROTEINS IN THE CEREBELLUM OF RODENTS. J. Mariani, C.O. Parkes*, M. Thomasset*, J.L. Guenet* and N. Delhaye-Bouchaud*. UA 1199 CNRS and Lab. Neurophysiologie Ontogénétique, Univ. P. et M. Curie, Paris, and Institut Pasteur, Paris, France.

Among the large family of intracellular calcium binding proteins, some are ubiquitous (eg calmodulin) whereas others show a specific tissue distribution. In the latter group are the cholecalcins or cholecalciferol induced calcium binding proteins (CaBPs). In mammals a small 9K cholecalcin is found in the intestine and a 28K cholecalcin is found mainly in the kidney and in the central nervous system, where the largest concentration is by far observed in the cerebellum. Moreover recent histochemical studies (Jande et al., 1981; Baimbridge and Miller, 1982; Legrand et al., 1983) have clearly shown that cerebellar CaBP is restricted to the Purkinje cells, in both chickens and rats. The physiological function of this protein and its role in the regulation of the internal concentration of free Ca^{++} in the cell remains however largely unknown.

In the first part of the present suty the CaBP content was measured with a specific radioimmunoassay (RIA) in the cerebellum of different mutant mice; in three of them, ie Purkinje cell degeneration (pcd), nervous (rd) and staggerer (sg)whose cerebellum is almost lacking of Purkinje cells, the CaBP was almost absent in the cerebellum.

By contrast the CaBP level was almost normal in the cerebellum of reeler and weaver mice which are deprived of granule cells. These results give quantitative support to the idea that the entire pool of cerebellar CaBP is associated with the Purkinje cell (P.C.) population.

In the second part of this study we have tried to manipulate the intracerebellar level of 28K CaBP without changing the size of the PC population. Preliminary results indicate that the cerebellar level of CaBP is severely depleted in two new mutants, hyperspiny Purkinje cell (hpc) and nodding (nd), without no gross change in PC's number. Despite the defect in CaBP content disclosed by radioimmunoassay, immunocytochemistry revealed that all the PCs present in these mutants were uniformely stained with an anti CaBP antibody. Since the Purkinje cells in these mutants exhibit morphological and electrophysiological abnormalities, especially of their dendrites, they could be useful models to study the role of 28K CaBP in the development and the function of PCs.

335.11 CYTOCHALASIN B BINDING SITES IN ADULT AND NEONATAL RAT BRAIN. A.M. Morin, B.E. Dwyer*, D.G. Fujikawa and C.G. Wasterlain*. Neurology and Epilepsy Res. Labs, V.A. Med. Ctr., Sepulveda, CA 91343 & Dept. Neurology & Brain Research Inst., U.C.L.A. School of Medicine.

During seizures or hypoxia, brain glucose is profoundly depleted in normoglycemic neonatal animals. This inability of glucose supply to keep up with glucose utilization has been suggested as a cause of selective neuronal damage suffered during prolonged seizures. One possible explanation for brain glucose depletion is that vulnerable areas in neonates contain fewer glucose transport sites and thus have decreased access to glucose at a time when it is most needed. The object of this study was to measure the binding of 3H-cytochalasin B to the glucose transporter in adult and 4 day old rat pup brains and to correlate regional differences in transporter with areas of increased vulnerability. The binding of 3H-cytochalasin B, a potent inhibitor of glucose transport, is used as a measure of transporter (Dick et al., Proc. Natl. Acad. Sci., 81:7233, 1984). Specific binding is the difference in binding in the presence of D-glucose (500mM) or L-glucose (500mM). The results of binding studies in whole homogenates (washed twice with 0.05M Tris-HCl, pH 7.4) indicate that in cortex, there is significantly less transporter in neonate than adult (neonate, 1.98±1.13 pmol/mg prot; adult, 8.78±0.92 pmol/mg prot, P<0.025). Scatchard analysis of 3H-cytochalasin B binding in cortex indicated adult cortex has a higher Bmax (46.8pmol/mg prot) than neonatal cortex (6.8 pmol/mg prot) while the binding affinities are relatively close (adult Kd0.20uM; neonate Kd=0.23uM). In contrast, binding in brain stem and cerebellum were not significantly different when neonates were compared with adults. The results indicate that of the three brain areas examined, transporter concentration increases greater than 5 fold as the cortex matures while brainstem and cerebellum transporter concentrations remain constant. The limited transport capacity of neonatal cortex may explain its selective vulnerability and the ictal development of cortical glucose depletion in the face of normoglycemia.

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335.12 DEVELOPMENTAL CHANGES IN THE NEURONAL CYTOSKELETON OF PYRAMIDAL TRACT NEURONS IN GOLDEN HAMSTERS: BIOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDIES. J. Pickett* and M.M. Oblinger (SPON: C.M. Combs), Dept. Biol. Chem. & Struc., Chicago Med. Sch, N. Chicago, IL 60064

The biochemical composition of the neuronal cytoskeleton determines many of its structural and functional properties. During development, the axonal cytoskeleton undergoes marked morphological changes and certain functional properties of axons, such as plasticity, also change. At present, little molecular information is available on changes in the composition of the cytoskeleton during development of CNS neurons. To address this, we examined the expression and transport of cytoskeletal and associated proteins in developing corticospinal neurons. Since hamster pyramidal tract axons grow postnatally, this system was used to assess changes which occur during the period of initial axon growth and maturation.

Cytoskeletal proteins were labeled during synthesis by injections of 35S-methionine into the motor cortex of golden hamsters aged 1-28 d; animals were sacrificed 1hr-7d later. To examine axonally transported proteins, pyramidal tract axons were dissected from 0.5-1 mm frozen serial sections of brains and spinal cords and subjected to SDS-PAGE/fluorography. In another experimental series, samples from motor cortex and pyramidal decussation were dissected from 1-28d old pups and equal amounts of protein were run on SDS-PAGE and electrophoretically transferred to nitrocellulose. The blots were probed with monoclonal antibodies to phosphorylated or nonphosphorylated neurofilament (NF) proteins, antibodies to tubulin (T) and a monoclonal antibody to the tau class of microtubule associated proteins. For histological studies, brains were fixed in 4% paraformaldehyde and sections were cut either without embedding at 50 um using a Vibratome or at 10 um after parafin embedding. Histological material was stained using the PAP procedure with various cytoskeletal antibodies.

Gel analysis of axonally transported proteins revealed a number of significant changes. These included markedly faster rates of transport of major cytoskeletal proteins in younger compared to older postnatal or adult axons and major increases in the proportion of labeled NF protein to tubulin between 3 and 28 days. Immunoblots indicated that the first significant expression of the 200 kd NF subunit in the motor cortex occurred on day 3. Additionally, the expression of a group of 3 tau proteins (60-65 kd) began on day 9 and increased through day 28; between days 1-9 the expression of a 57 kd tau decreased to zero. In the caudal pyramidal tract, a 110 kd tau protein was first observed on day 9. Examination of histological material provided additional detail on rostro-caudal gradients in the molecular development of the cytoskeleton in corticospinal neurons.

335.13 **THE EFFECTS OF PERINATAL PHENYTOIN ADMINISTRATION ON CEREBRAL GLUCOSE METABOLISM IN THE ADULT RAT.** J.W. Melisi*, D.L. Dow-Edwards, and T.H. Milhorat.(SPON: A.I. Kobrine) Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Health Science Center at Brooklyn, Brooklyn, N.Y. 11203.

Phenytoin administration during pregnancy may have adverse morphologic, behavioral and physiologic effects in both human and animal offspring. In order to identify the neuronal structures which might be responsible for these changes, we administered the drug during the postnatal period when the brain is rapidly developing. After reaching maturity, we employed the quantitative [^{14}C]deoxyglucose autoradiographic method to measure the rate of in vivo glucose utilization in the rat brain (Sokoloff et al, J Neurochemistry 28:897-916, 1977).

Newborn male rat pups were randomly assigned to either experimental or control groups. The experimental group received one subcutaneous injection of 0.25mg Dilantin (phenytoin sodium inj 250mg/50ml, Parke-Davis) in a total volume of 5ul on postnatal days 1, 3,and 5 and 0.5mg in 10ul on days 7 and 9 (approximately 23mg/kg). The control group received equivalent volumes of vehicle kindly supplied by Parke-Davis. Each dam nursed a total of eight pups. Animals were housed under standard laboratory conditions with 12 hour light-dark cycles and food and water ad lib. All animals were weaned at 21 days. No differences in body weight gain were observed between the groups.

At three months of age, cerebral glucose metabolic activity was assessed. Structures examined included those from the forebrain, midbrain and hindbrain. All data was subjected to statistical analysis using Student's T-test. Only very subtle changes in local cerebral metabolism were noted in the drug-treated group compared to control animals. This minor effect on glucose metabolism will be discussed in light of our present understanding of the fetal hydantoin syndrome.

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335.14 THE DEVELOPMENTAL SEQUENCE OF LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU). E.L. McCann, H.W. Holloway, D.M. Larson and S.I. Rapoport* (SPON: W.B. Marks).Laboratory of Neurosciences, National Institute of Aging, National Institutes of Health, Bethesda, MD 20892.

Past developmental investigators have found generalized increases in the cerebral metabolic rate for glucose (CMRglu) between 30 and 90 days in developing rats (London, et al., 1981). However regional determination of CMRglu in a number of age groups during development have not been performed. Therefore, Fischer-344 rats between the ages of 7 and 90 days received [14-C]-2 deoxyglucose with subsequent determinations of local cerebral glucose utilization (LCGU) according to the Sokoloff method (Sokoloff, et al., 1977).

As illustrated in Table 1 (below), LCGU increased between 7 and 90 days of age with a significant peak ($p < 0.05$) at 45 days in 40 % of 106 brain regions examined. Between 45 and 90 days the increase of cerebral metabolism was of the order of 15 -35%. A phylogenetic sequence of development was found with the caudal brain areas (myelencephalon) having a higher LCGU than the rostral brain sections (neocortex) prior to 20 days. A reversal occurred after 20 days in the neocortex/myelencephalic ratio. Plasma ketone bodies were elevated throughout the suckling period (less than 20 days) with a significant peak ($p < 0.05$) at 15 days.

In conclusion, there is a gradual increase in LCGU from the postnatal period through adulthood with a peak at 45 days. Regional analysis of this increasing LCGU demonstrates adherence to a phylogenetic sequence (Leon and Clark, 1984). The 45 day peak for glucose utilization is in contrast to the 20 day peak of plasma palmitate incorporation, which corresponds to the maximal rate of brain lipid synthesis (Tabata, et al, 1985). The changes occur in the presence of high plasma concentration of ketone bodies and enzymes for their utilization, functioning at 4 times their adult capacity (Yeh, et al, 1977 and Booth, et al, 1980). Glucose utilization reflects the developmental time course of brain oxidative metabolism, as opposed to plasma palmitate incorporation, which reflects the rate of brain lipid synthesis during develpment.

Table 1: Cerebral glucose utilization and neocortex/myelencephalic ratio in rats at different ages (6 animals for each age).

DAYS	7	30	45	90
LCGU, AVERAGE	10.8+2.0	77.0+2.0	104.4+3.0	86.1+3.0
NEOCORTEX	9.2 + 0.1	98.2 + 5.5	130.9 + 4.0	107.6 + 3.0
NEO/MYEL LCGU	65.7 %	120.2 %	115.8 %	125.2 %

Values are mean + SEM.

335.15 LOCAL RATES OF CEREBRAL GLUCOSE UTILIZATION IN ADULT MALE RATS FOLLOWING 30 DAYS OF AN "ENRICHED ENVIRONMENT". M.C. Diamond[1], C.B. Smith, A. York[2], and E.R. Greer[1]*. Depts. Physiology/Anatomy[1] and Zoology[2], Univ. CA., Berkeley, CA. 94720 and Lab. Cereb. Metabolism, NIMH, Bethesda, MD. 20892

The purpose of these experiments was to examine the effects of 30 days of an "enriched environment" on local rates of cerebral glucose utilization in adult male Long-Evans rats. "Enriched conditions" consisted of housing 12 rats in a large cage with novel objects which were changed twice weekly. "Standard conditions" consisted of rats housed 3/small cage without novel objects. It had been shown previously (Diamond et al., J. Neurobiol. 7: 75-85, 1976) that adult rats maintained for 30 days under these "enriched conditions" have significant increases in cortical thickness in occipital, frontal and parietal cortex as compared with age-matched animals maintained under "standard conditions". In these present studies the quantitative autoradiographic deoxyglucose method (Sokoloff et al., J. Neurochem. 28: 897-916, 1977) was used to measure local rates of glucose utilization in 30 brain regions. Results from 4 "enriched" rats were compared with results from 6 "standard" rats. Because this was a pilot study statistical analyses were carried out with an analysis of variance and a Student's t-test. No correction was made for multiple comparisons. Brain regions examined included 8 cortical areas, 9 limbic areas, 10 other subcortical areas, 2 regions of white matter, and the reticular formation. Of these regions only frontal and parietal cortex were significantly changed ($P<.05$). In both of these areas rates of glucose utilization were 13% lower in the "enriched" rats as compared with the "standard" controls. Whether or not these effects are random events or are actually due to the experimental conditions can only be determined by a second series of experiments. It is of interest that these changes occurred only in brain regions in which changes in cortical thickness had been found previously. That the "enriched" animals had decreased rather than increased rates of glucose utilization was somewhat unexpected. It is conceivable that differences in adaptability between the "enriched" and "standard" animals to the experimental environment during the measurement of cerebral glucose utilization may have contributed to these results.

335.16 LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE (LCMRglc) DURING HUMAN BRAIN DEVELOPMENT STUDIED WITH 2-DEOXY-2[18F]FLUORO-D-GLUCOSE (FDG) POSITRON EMISSION TOMOGRAPHY (PET). Harry T. Chugani*, Michael E. Phelps and John C. Mazziotta (SPON: Eduardo H. Rubinstein). Departments of Neurology and Pediatrics, and the Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024, USA.

The delineation of maturational changes occurring in different brain regions during development would provide a means whereby various behavioral phenomena can be related to specific brain structures, and enhance our understanding of cerebral structure-functional relationships. We have measured LCMRglc using FDG and PET in over 100 children with various neurological disorders in order to determine functional brain disturbances characterizing these disorders. From this larger group of children, we retrospectively selected out a subgroup of children who had suffered transient neurological events that did not significantly alter neurological development, and therefore, are reasonably representative of normal children. Using a similar strategy in 9 children, we have previously described (Science 231:840-843, 1986) changes in the anatomical distribution of LCMRglc during the first year of life. We now report absolute (as opposed to relative) values of LCMRglc in 23 such children (age 5 days - 12 years). These values were compared to those of 7 young healthy adults (age 19-30 years) studied under similar conditions. Our results show that LCMRglc are low at birth (2-4 mg/min/100gm), rapidly rise to reach adult values (4-5 mg/min/100gm) by 2 to 3 years but then continue to rise until, by 4 to 5 years, LCMRglc in most regions reach values of 8-11 mg/min/100gm. These high rates are maintained throughout the remainder of childhood when, at about 10 years, they begin to decline and reach adult rates again by about 12 to 14 years. LCMRglc for thalamus, cerebellum and brainstem at birth are closer to their adult rates compared to most other structures, and this is consistent with the relatively early functional maturation of these phylogenetically older structures. The high energy demand of the developing brain between 3 and 11 years of age can possibly be accounted for by the high energy expenditure required for maintenance of membrane potentials as a result of excessive production of neurons, synapses and dendritic spines during brain development. The subsequent selective elimination of excessive neurons and other cellular elements during their 'functional stabilization' leads to a lower cerebral energy demand during adolescence. Other aspects of cerebral maturation, such as myelination, may also contribute to the high energy expenditure of the developing brain. The mapping of functional changes in the normally developing brain is a prerequisite to the study of central nervous system plasticity in different disease states.

335.17 CYTOCHROME b561 IS ASSOCIATED WITH ASCORBIC ACID-REQUIRING ENZYMES IN MANY NEUROENDOCRINE SECRETORY VESICLES. Rebecca M. Pruss* and Emily A. Shepard*(SPON: R.Cook-Deegan). Lab of Cell Biology, NIMH, Bethesda, MD 20892.

Cytochrome b561 (cyt b561) is a major chromaffin granule protein. It represents between 10-20% of the granule membrane protein and about 3% of the total protein of the adrenal medulla. It is the only heme- or iron-containing protein in the granule membrane, and thus the only candidate for an electron carrier in these granules. Dopamine β-hydroxylase (DBH) is a mixed function oxygenase which requires copper, molecular oxygen, and ascorbic acid for converting dopamine to norepinephrine within chromaffin granules and adrenergic or noradrenergic synaptic vesicles. Ascorbic acid donates an electron during this reaction and cyt b561 is proposed as the electron carrier for regenerating reduced ascorbate in chromaffin granules. We have a monoclonal antibody which is specific for cyt b561. This antibody was generated by immunizing mice with chromaffin granule membranes. Although it detects cyt b561 in homogenates of adrenal medulla, this monoclonal antibody has not detected cyt b561 in homogenates of other tissues. Immunocytochemistry allows one to localize an antigen which is not abundant if it is concentrated in discrete subcellular regions. We have used our monoclonal antibody to study the distribution of cyt b561 in bovine tissues fixed by immersion in 4% paraformaldehyde. Since the function of cyt b561 as an electron carrier has not been absolutely established, we compared its distribution with that of catecholamine synthetic enzymes, DBH and PNMT, chromogranin A (another major chromaffin granule protein), and a number of neuropeptides and peptide hormones (VIP, enkephalin, NPY, galanin, and insulin). Cyt b561 was found in both the neural and endocrine lobes of the pituitary, the inner nuclear layer of the retina, gastrointestinal nerve fibers and plexuses, intestinal crypt cells (including typical enteric endocrine cells), heart muscle fibers, and all adrenal medullary chromaffin cells. Its distribution was best correlated with that of DBH or amidated neuropeptides. Many neuropeptides share the feature that their C-terminal amino acid is amidated. This modification is the result of a mixed function oxygenase found in peptidergic secretory vesicles. This peptide α-amidating enzyme, like DBH, requires copper, molecular oxygen, and ascorbic acid. Since the peptide-containing vesicles of the gut, pituitary, and retina do not contain DBH, it appears that cyt b561 may be required for ascorbic acid regeneration in peptidergic ascorbic acid-containing vesicles as well as DBH-containing granules. Cyt b561 may, therefore, be a useful and interesting marker for studying the development or pathology of peptidergic and DBH-containing neural and endocrine tissues.

SEROTONIN III

336.1 A PHARMACOLOGIC PROFILE OF MDA AND MDMA ON BRAIN RECEPTORS AND UPTAKE SITES. Theresa Kopajtic*, George Battaglia, and Errol B. De Souza (SPON: W. Kinnier). Neuroscience Branch, Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224.

MDA and MDMA, ring substituted derivatives of amphetamine and methamphetamine, respectively, retain the stimulant properties of their parent compounds but additionally exhibit psychotomimetic properties. Although these compounds are reported to exhibit similar pharmacologic profiles in various species including man, recent behavioral data from stimulus generalization studies suggest that the effects of MDA and MDMA might differ markedly in some respects. For example, (+)MDA exhibits complete stimulus generalization to (+)MDMA, amphetamine, cocaine and hallucinogens (eg. LSD, (+)DOM) but (+)MDMA does not stimulus generalize to the hallucinogens. Additionally, in man, R(-)MDA is considered the more active isomer while for MDMA it is the S(+) isomer which exhibits greater activity. These data suggest that while some of the effects of MDA and MDMA might be mediated through similar neuronal systems their effects might be quite different on others. These studies were designed to delineate an in vitro pharmacologic profile for MDA and MDMA at various brain recognition sites using radioligand binding techniques. Our data indicate that MDA and MDMA exhibit similar and preferential high affinities at 5HT and NE uptake sites (1-2uM) as well $5HT_2$ receptors (4-5uM) with significantly lower affinities at DA uptake sites (45-70uM) and $alpha_1$ adrenergic receptors (25-50uM). In contrast to their affinities at $alpha_1$ adrenergic receptors, MDA and MDMA exhibit substanitally higher affinities for $alpha_2$ sites (2-6uM). Of interest, (+)MDMA has a high affinity (2.5 uM) for sigma receptors which is nearly 5-fold greater than that of (+)MDA (12uM), suggesting that perhaps some of the psychotomimetic effects of MDMA may be sigma receptor-mediated as are those of PCP, SKF 10,047 and other sigma agonists. Affinities in excess of 100 uM were observed at mu and delta opiate, benzodiazepine and corticotropin-releasing factor receptors and calcium channels. The effects of in vivo administration of MDA and MDMA on the regulation of the higher affinity sites is currently being investigated. In summary, we report here the rank order of affinities of (+)MDA at various brain receptors and uptake sites as being: NE uptake ≥ 5HT uptake = $alpha_2$ adrenergic > $5HT_2$ > $5HT_{1A}$ = Sigma = M_1/M_2 muscarinic > beta adrenergic > $alpha_1$ adrenergic > DA uptake > D_2 dopamine. With respect to (+)MDMA we observed a rank order of: 5HT uptake ≥ NE uptake = Sigma > $5HT_2$ = M_1/M_2 muscarinic ≥ $alpha_2$ adrenergic ≥ $5HT_{1A}$ > beta adrenergic > $alpha_1$ adrenergic > DA uptake = D_2 dopamine. These data suggest that similarities in the effects of MDA and MDMA may be mediated by serotonin receptors, serotonin uptake sites and/or $alpha_2$ adrenergic receptors, while differences in discriminative stimulus properties may relate to their differential affinities at sigma receptors.

336.2 SYSTEMIC MDA AND MDMA, PSYCHOTROPIC SUBSTITUTED AMPHETAMINES, PRODUCE SEROTONIN NEUROTOXICITY. E.O'Hearn*, G.Battaglia, E.B.DeSouza, M.J.Kuhar and M.E.Molliver. Dept. of Neuroscience, Johns Hopkins Univ.Sch.of Med.; and NIDA, Baltimore, MD 21205

Several amphetamine derivatives are reported to have long-term toxic effects upon monoamine neurons. A recent study reports that the psychotropic drug methylenedioxyamphetamine (MDA) selectively reduces serotonin (5-HT) levels in the rat brain [Ricaurte et al, Science 229 (1985)]. We have investigated the cytopathologic effects of systemic MDA and of methylenedioxymethamphetamine (MDMA), another substituted phenethylamine reportedly used for its mood altering effects. Immunocytochemistry of monoaminergic axons was used to identify the specific neuronal elements affected and to characterize the extent, regional distribution, and transmitter specificity of the drug induced neurotoxicity. Rats were administered (±)MDA, (±)MDMA (20 mg/kg) or saline s.c. every 12 hours for 4 days. The rats were sacrificed two weeks later and brain sections were incubated with antibodies to tyrosine hydroxylase (TH) [courtesy of Dr. T. Joh] and to 5-HT. To study acute toxic effects additional rats were killed 1 and 3 days after 4 doses of MDA.

MDMA and, more markedly, MDA produce profound reductions in the density of 5-HT axon terminals, most evident in cerebral cortex, thalamus, olfactory bulb, and striatum but also in hippocampus, hypothalamus, and septum. Ablation of 5-HT axons is as extensive at 1 day as at 2 weeks after injections. In cortex, the fine 5-HT terminals with small varicosities are especially vulnerable, whereas the larger axon terminals with spherical varicosities appear to survive. Remaining 5-HT axons are greatly reduced in number but are increased in diameter and 5-HT staining intensity, probably due to accumulation of 5-HT and other axonal components in fibers after loss of the terminal branches. Similar changes may cause the increased 5-HT staining in the basal forebrain, where dilated, intensely stained 5-HT axons ascend in the medial forebrain bundle. At 1 day survival, the swollen, intensely staining intervaricose segments and giant abnormal 5-HT varicosities in cortex are evidence for acute axonal degeneration. The same drugs do not affect the 5-HT staining or cytology of raphe cell bodies, nor are there changes in the density or morphology of catecholamine (TH-positive) axons. We conclude that MDA and MDMA produce rapid, selective and extensive destruction of 5-HT axon terminals, which may result from the binding of these compounds to the 5-HT uptake sites. The sparing of 5-HT cell bodies and preterminal axons may provide the potential for regenerative sprouting. [Support: NIH NS15199, NS21011, & NS19920]

336.3 DIRECT INTRACEREBRAL ADMINISTRATION OF MDA AND MDMA DOES NOT PRODUCE SEROTONIN NEUROTOXICITY. M.E. Molliver, E. O'Hearn*, G. Battaglia, and E.B. De Souza. (SPON: B. Kosofsky). Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine; and ARC, National Institute on Drug Abuse, Baltimore, MD 21205

Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA, Ecstasy) are psychotropic substituted phenethylamines which are selectively neurotoxic to serotonin (5HT) axon terminals when administered systemically to rats (cf, O'Hearn et al; Yeh et al, this vol). In this study we microinjected MDA and MDMA directly into the cerebral cortex to determine the effects of these amphetamines on neuronal processes and to control local drug concentration. Single stereotaxic microinjections of (±)MDA (n=12) or (±)MDMA (n=13) [3, 10, or 12 ug in 0.3-0.5 ul] were made into right frontal cortex of male rats through 70 um tip glass pipettes. Vehicle control injections of 0.9% NaCl were placed in contralateral cortex (n=16). Microinjections included a pulse of rhodamine labeled latex microspheres to identify the injection site location. To suppress MDA or MDMA oxidation, ascorbic acid (0.1 mg/ml) was added to the drug vehicle in half of the rats (n=12). After survival times of 3 days to 3 weeks, 5-HT and catecholamine (CA) axons were visualized by immunocytochemistry in brain sections incubated with antibodies to 5-HT [courtesy of H. Lidov] and to tyrosine hydroxylase (TH) [courtesy of T.H. Joh].

5-HT and CA axon density surrounding MDA and MDMA injection sites could not be distinguished from that at NaCl control sites. Many fine 5-HT and TH axons could be traced directly up to and through the injection sites. This absence of a cytotoxic effect was found at all survival times and injection dosages. Addition of ascorbic acid to the drug vehicle had no effect. Nissl-stained sections revealed a small zone of gliosis at the injection sites. The cortex around the center of the injection sites (100-200 um zone) in MDA, MDMA, and NaCl injections exhibited a few 5-HT and TH axons with increased diameter and immunoreactivity and, rarely, a very subtle decrease in 5-HT and TH fiber density; more than 300 um away, the cortex was completely normal. The latter findings in MDA, MDMA, and NaCl injected rats are consistent with nonspecific effects induced by the trauma of microinjections. In conclusion, single large doses of MDA or MDMA, administered directly into brain tissue, have no effect on the density, morphology, or staining intensity of 5-HT or CA axon terminals. This indicates that the dramatic neurotoxicity demonstrated after subcutaneously administered MDA and MDMA depends upon systemic administration of these substances. These data suggest strongly that the neurotoxin is a peripheral metabolite of MDA and MDMA, a feature that may prove crucial to elucidate the mechanism of action and to prevent the neurotoxicity of MDA and MDMA.

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336.4 MDA AND MDMA (ECSTASY) INTERACTIONS WITH BRAIN SEROTONIN RECEPTORS AND UPTAKE SITES: IN VITRO STUDIES George Battaglia, Michael J. Kuhar and Errol B. De Souza. Neuroscience Branch, ARC, National Institute on Drug Abuse, Baltimore, MD 21224.

MDA and MDMA are methylenedioxy derivatives of amphetamine and methamphetamine, respectively. These compounds exhibit psychotomimetic and stimulant properties and have been increasingly encountered in illicit drug traffic. Recent data suggest that MDA and MDMA may be toxic to brain serotonin neurons. Although the serotonergic system has been implicated in some of the physiologic and behavioral responses to MDA and MDMA, the precise interactions of these compounds with brain serotonin recognition sites have yet to be elucidated. These studies were designed to delineate an in vitro pharmacological profile of MDA and MDMA on pre- and post-synaptic serotonin recognition sites using radioligand binding techniques.

$5HT_{1A}$, $5HT_2$ and 5HT uptake sites were selectively labelled using ^3H WB4101 (+30 nM prazosin), ^3H-Ketanserin and ^3H-Paroxetine, respectively, in appropriate brain regions. At 5HT uptake sites in frontoparietal cortex, the affinities (K_i's) of (+)MDA (2.1 uM) and (+)MDMA (1.1 uM) were comparable with the S(+) isomer of each being 4- and 8-fold more potent, respectively, than the R(-) isomer. These compounds were 11-13 fold more potent at this site than their respective parent compounds, (+)amphetamine (22.7 uM) and (+)methamphetamine (15 uM). Additionally, S(+)MDMA (0.64 uM) was only 5-fold less potent than serotonin (0.14 uM). With repect to hippocampal $5HT_{1A}$ sites, the affinities of MDA (12 uM) and MDMA (7 uM) were significantly less than those observed at the uptake site. Furthemore, the stereospecificity at $5HT_{1A}$ receptors was the opposite of that at the uptake sites with the R(-) isomer of both MDA and MDMA being more potent than the S(+) isomer. At cortical $5HT_2$ receptors (+)MDA (5.4 uM) and (+)MDMA (4.9 uM) were equipotent and exhibited a sterospecificity similar to that observed at $5HT_{1A}$ receptors.

Of interest, MDA and MDMA appear to have agonistic properties at $5HT_2$ receptors in that they can discriminate high and low affinity components of binding and exhibit GTP-sensitivity.

In conclusion, these data suggest that, in vivo, both MDA and MDMA may exert a potent and preferential effect on the 5HT uptake system associated with serotonin axons and presynaptic terminals. Also, the findings of different potencies and of reverse stereospecificity at uptake sites vs. receptors may help elucidate the primary site(s) responsible for the psychotomimetic effects in man. For example, the S(+) isomer of MDMA and the R(-) isomer of MDA are reported to be the more potent psychotomimetic in man, suggesting that the effects of MDMA may primarily involve 5HT uptake sites while those of MDA may be mediated by its interaction with $5HT_2$ receptors.

336.5 EFFECTS OF MDA AND MDMA (ECSTASY) ON BRAIN MONOAMINERGIC SYSTEMS: IN VIVO STUDIES. S.Y. Yeh*, G. Battaglia, E. O'Hearn*, M.E. Molliver, M.J. Kuhar and E.B. De Souza (SPON: A. Goldberg). Neuroscience Branch, Addiction Research Center, National Institute on Drug Abuse, and Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21224

(+)3,4 methylenedioxyamphetamine (MDA) and (+)3,4 methylenedioxymethamphetamine (MDMA) are compounds which possess psychotomimetic and stimulant properties. These so called "designer drugs" have found increased popularity among recreational drug users. Recent studies suggest that MDA may exert potent neurotoxic effects on brain serotonergic systems (Science 229:986, 1985). Our studies were designed to investigate the effects of in vivo administration of MDA and MDMA on the regional concentration of various brain monoamines and their metabolites as well as on the pre- and post-synaptic binding sites (i.e. uptake sites and receptors).

Male, Sprague Dawley rats were injected s.c. with either saline (1 ml/Kg), MDA or MDMA (20 mg/Kg) twice a day for four consecutive days. Two weeks following the last injection the animals were killed and various brain regions dissected. Brain concentrations of monoamines and their metabolites were measured using RP-HPLC. Radioligand binding techniques were employed to monitor changes in the affinity and/or density of uptake sites and receptors.

MDA caused dramatic decreases in 5HT (65, 26 and 33%) and 5HIAA (58, 64 and 40%) content in frontoparietal cortex (FPC), hippocampus (HIP), and hypothalamus (HYP), respectively. MDMA produced similar but less dramatic decreases in 5HT (42, 15, and 18%) and 5HIAA (46, 55 and 26%) in FPC, HIP and HYP, respectively. These compounds caused smaller changes in striatal 5HT content (10-20%), while the decreases in striatal 5HIAA levels elicited by both MDA (33%) and MDMA (36%) were comparable to that in other regions. In parallel with the observed decreases 5HT and 5HIAA content in FPC, significant and quantitatively similar decreases in the B_{max} of ^3H-Paroxetine-labeled 5HT uptake sites were elicited by both MDA(69%) and MDMA(62%). In contrast, neither MDA nor MDMA elicited any changes in the levels of NE, DA, DOPAC or HVA in any of the brain regions examined. Also, there was no difference in the number of ^3H-Mazindol-labeled NE uptake sites in FPC following either MDA or MDMA administration. Monoamine receptor adaptations to the indirect or direct effects of these compounds are presently being investigated.

In summary, our finding of selective and profound decreases in presynaptic serotonin markers following chronic administration of MDA and MDMA support our immunocytochemical studies which indicate that these changes represent the selective destruction of serotonin terminals.

336.6 NEURONAL RECEPTORS OF ALCOHOL-PREFERRING (P) AND NON-PREFERRING (NP) RATS. D. T. Wong, L. Lumeng*, P. G. Threlkeld*, L. R. Reid* and T. K. Li*. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, and Indiana University School of Medicine, Indianapolis, IN 46223.

Neurochemical studies show that P rats have lower levels of serotonin (5HT) in cerebral cortex and hippocampus and higher levels of norepinephrine in cerebral cortex in comparison with NP rats (Murphy et al, Pharmacol. Biochem. Behav. 16:145, 1982). In the present studies, by means of radioreceptor assays, we found a higher binding of ^3H-5HT to $5HT_1$ receptors in membranes isolated from frontal cortex and hippocampus of 6-month-old P rats with significant increases in receptor numbers (Bmax) of 47% and 90%, respectively, but without change in affinity (Kd). No consistent difference was seen between P and NP rats in Kd and Bmax values for ^3H-ketanserin binding to $5HT_2$ receptors. Similarly, no differences in ^3H-WB4101, ^3H-clonidine and ^3H-dihydroalprenolol binding to α_1, α_2 and β-adrenergic receptors in cortical membranes was observed between these rats. These preliminary findings have been replicated at least once and are consistent with the involvement of the serotonergic system in alochol preference.

336.7 SEROTONIN (5HT) UPTAKE IN BRAIN OF ALCOHOL-PREFERRING (P) AND NON-PREFERRING (NP) RATS. F. P. Bymaster*, D. T. Wong, L. Lumeng* and T. K. Li* (SPON: M. D. Hynes). Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285, and Indiana University School of Medicine, Indianapolis, IN 46223.

Inhibitors of 5HT uptake, including fluoxetine (F), are capable of lowering ethanol intake in P rats (Murphy et al, Alcohol 2:349, 1985). Synaptosomes prepared from cerebral cortex of 8 P and NP rats were compared for their ability to accumulate ^{3}H-5HT (0.04 μM) at 37°C. Both groups exhibited equal rates of ^{3}H-5HT uptake (3.00 ± 0.11 and 3.05 ± 0.18 pmole/mg protein for P and NP rats, respectively), as well as similar Km and Vmax values. F blocked 50% of ^{3}H-5HT uptake in synaptosomes of P and NP at 124 and 114 nM, respectively. Binding of ^{3}H-F at 4°C to 5HT uptake carriers (Wong et al, Drug Dev. Res. 6:397, 1985) in membranes isolated from brain stem, midbrain and cerebellum was compared between P and NP rats. A 15% higher affinity for ^{3}H-F, as indicated by the smaller dissociation constants, was seen in brain stem and midbrain of P rats; a 20% lower number of binding sites for ^{3}H-F was seen only in brain stem of P rats. Preliminary studies consistently show that no significant difference between P and NP rats in ^{3}H-5HT uptake in cortical synaptosomes. A lower number of 5HT uptake carriers was observed in the brain stem of P rats.

336.8 SEROTONIN (5-HYDROXYTRYPTAMINE, 5-HT) HYPERPOLARIZATION OF HIPPOCAMPAL CA_1 NEURONS: PHARMACOLOGICAL CHARACTERIZATION AND POTENTIATION BY ETHANOL. P.H. Wu,* N. Gurevich and P.L. Carlen, Playfair Neuroscience Unit, Toronto Western Hospital, Neurology and Clinical Pharmacology Programs, Addiction Research Foundation, and Departments of Medicine and Physiology, University of Toronto, Toronto, Canada.

5-HT hyperpolarizes hippocampal neurons in vitro. The 5-HT receptor subtypes which mediate the 5-HT hyperpolarization are not known. Although ethanol is known to inhibit 5-HT release and turnover, the mechanism for this interaction at the single neuronal level has not been investigated. We report the pharmacological characterization of 5-HT hyperpolarization and ethanol-5-HT interaction on hippocampal CA_1 neurons.

Hippocampal slices from male Wistar rats (150-200 g) were prepared and perfused with standard artificial CSF or an ethanol (20 mM) containing medium. 3 M KCl microelectrodes (50-180 Meg ohms) were used for intracellular recordings. 5-HT, (±)-8-OH-dipropylaminotetralin (8-OHDPTA), cyproheptadine, mianserin and methysergide were dissolved in the medium and focally applied by pressure ejection.

5-HT (10^{-6} to 10^{-4} M) hyperpolarized (2 to 12 mV) hippocampal CA_1 neurons for 10 sec to a few minutes in a dose-dependent manner. During the 5-HT hyperpolarization, post-spike afterhyperpolarizations were often increased. 8-OHDPTA, a 5-HT_1 partial agonist, produced a weak (≤ 2 mV) hyperpolarizing response at 10^{-5} M. At 10^{-7} M, it did not cause a hyperpolarization, but the spontaneous spiking activity of CA_1 neurons was decreased. Following the 5-HT hyperpolarization, there was often a delayed depolarization (1 to 10 mV) lasting up to 5 minutes, associated with increased spontaneous spiking, which could occur following the 5-HT hyperpolarization without a depolarization. Both the 5-HT hyperpolarization and delayed depolarization were not antagonized by cyproheptadine (10^{-7} to 10^{-5} M) or mianserin (10^{-5} M), putative 5-HT_2 receptor antagonists. The 5-HT hyperpolarization was not potentiated by MK212 a 5-HT receptor agonist. However, methysergide (10^{-4} M), a non-selective 5-HT receptor antagonist blocked the 5-HT hyperpolarization. Our results suggest that the 5-HT induced CA_1 neuronal hyperpolarization is probably mediated through the 5-HT_1 receptor subtype. This is consistent with reports indicating the high localization of 5-HT_1 receptor sites in the hippocampal CA_1 region. Ethanol (20 mM) significantly potentiated the 5-HT hyperpolarization.

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336.9 SEROTONIN MEDIATED SUPPRESSION OF FOCAL EPILEPTIFORM ACTIVITY BY NOXIOUS STIMULATION. P.M. Thompson* and R.S. Neuman. Faculty of Medicine, Memorial University, St. John's, NFLD., Canada, A1B 3V6

Noxious stimulation (NS) suppresses penicillin (PG)-induced focal epileptiform activity (FEA) in the rat (Neuman, EEG Clin. Neurophysiol., in press). The present study investigated the hypothesis that serotonin (5HT) mediates the suppression of FEA by NS. Intracortical recordings (0.5-75 Hz) and PG application by pressure ejection were made using glass microelectrodes positioned 1mm deep in the cortex (layers 4-5). Sprague Dawley rats (both sexes) anaesthetized with urethane were used. NS consisted of mechanical or electrical stimulation. All drugs were administered iv.

Desimipramine (DMI, 3mg/kg), a blocker of norepinephrine (NE) uptake, failed to reduce the suppression of FEA by NS. On the other hand, imipramine (IMI, 3 mg/kg), which blocks uptake of both NE and 5HT, with or without the prior administration of DMI, blocked the suppressant effect of NS. The selective $5HT_{1A}$ agonist, 8-OH-2-(di-n-propylamine)tetralin (DPAT, 0.2-0.5 mg/kg), like IMI, antagonized the suppression of FEA by NS. Baclofen (1-10 mg/kg), a $GABA_B$ receptor agonist, reduced but did not completly abolish NS induced suppression of FEA.

Since IMI but not DMI depresses raphe unit activity (Sheard et al., 1972, Brain Res.) the present results are consistent with the hypothesis that the releaße of 5HT, but not NE, is important for the suppressant effect of NS. DPAT binds to raphe neurones (Verge et al., 1985, Eur. J. Pharm.) which suggests the failure of NS to suppress FEA following DPAT might result from 5HT autoreceptor activation and consequent inhibition of raphe neurones leading to decreased 5HT release. Stimulation evoked 5HT release in cortical slices is reduced by baclofen (Schlicker et al., 1984, N.S. Arch Pharm.), which likely accounts for the action of baclofen in reducing the suppressant effect of NS in the present study. IMI, DPAT and baclofen all appear to reduce 5HT release. Since all three agents antagonize the suppression of FEA by NS the present observations are consistent with 5HT, at least in part, mediating the suppression.

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336.10 EVIDENCE FOR 5-HT-1a RECEPTOR ACTIVATION BY NOVEL ANXIOLYTICS IN THE RAT. James I. Koenig, Herbert Y. Meltzer and Gary A. Gudelsky, Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106

We have shown previously that activation of 5-HT-1a receptors results in 1) a stimulation of corticosterone secretion and 2) a decrease in body temperature in the rat (Abstr. Soc. Neurosci. 11:359, 1985; Pharmacol. Biochem. Behav. 22:489-492, 1985). This contention is based on the ability of the 5-HT-1a agonist 8-OH- DPAT to elicit these responses and the blockade of 8-OH-DPAT-induced hypothermia and corticosterone secretion by spiperone and (-)-pindolol, both of which have high affinity for the 5-HT-1a receptor. Buspirone, gepirone and TVX Q 7821 are non-benzodiazepine anxiolytic agents that have been shown to bind to 5-HT-1a receptors. In the present study, we examined whether these anxiolytic agents also elicit 5-HT-1a receptor-mediated stimulations of corticosterone secretion and decreases in body temperature in the rat. Buspirone, gepirone and TVX Q 7821 each produced a dose-related hypothermic response between 1-10 mg/kg. A decrease in body temperature of approximately 1.5°C was observed 60 min after the administration of 10 mg/kg of each of these agents. The hypothermic responses to buspirone, gepirone and TVX Q 7821 were inhibited 70-80% in animals treated with spiperone (1 mg/kg). Treatment of rats with the 5-HT-2 antagonist ketanserin (1 mg/kg) did not significantly alter the hypothermic responses to any of these anxiolytics. Plasma concentrations of corticosterone were significantly increased in a dose-related manner by buspirone, gepirone and TVX Q 7821. Plasma corticosterone concentrations were 5.6 ± 1.6 ug/dl in vehicle-treated rats and were 29.8 ± 0.8, 20.8 ± 1.9 and 15.8 ± 3.6 ug/dl after 3 mg/kg of buspirone, gepirone and TVX Q 7821, respectively. The corticosterone responses to buspirone, gepirone and TVX Q 7821 were inhibited 80-90% by spiperone (3 mg/kg). Ketanserin (1 mg/kg) had no significant effect on the corticosterone responses to any of the anxiolytic agents. Thus, the effects of buspirone, gepirone and TVX Q 7821 to produce hypothermic responses and elevated plasma concentrations of corticosterone are similar to the actions of the 5-HT-1a agonist 8-OH-DPAT. It is concluded that the treatment of rats with buspirone, gepirone or TVX Q 7821 results in the activation of receptors of the 5-HT-1a subtype. It is of interest to consider the view that activation of 5-HT-1a receptors by these non-benzodiazepine anxiolytics is part of the mechanism through which these agents exert their therapeutic effect.

336.11 IN VIVO NEUROCHEMICAL EFFECTS OF A LONG ACTING SEROTONIN-1A AGONIST. W. C. Boyar*, R. F. Neale*, A. Hutchison* and R. A. Lovell (SPON: B. S. Glaeser). Neuroscience Res., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

A novel benzopyranopyridine, CGS 18660A, with a relative selectivity for serotonin-1A (5HT-1A) binding sites in vitro was studied in vivo by comparison with two known 5HT-1A agonists, 8-hydroxydipropylaminotetralin (8OHDPAT, J. Neural Trans. 55: 169, 1982) and TVXQ 7821 (N.-S. Arch. Pharmacol. 328: 467, 1985). The following amines and metabolites were measured in male Sprague-Dawley rats (200-300 g) by HPLC with electrochemical detection: 5HT, 5-hydroxytryptophan (5HTP), 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) in the frontal-parietal cortex (FPC); 5HT, 5HIAA, dopamine (DA), dihydroxyphenylalanine (DOPA), dihydroxyphenylacetic acid (DOPAC) and HVA in the striatum. The ED25 values for inhibition of 5HTP accumulation in the FPC were 0.08 mg/kg s.c. for 8OHDPAT, 0.1 mg/kg s.c. for TVXQ 7821 and 0.85 mg/kg i.p. for CGS 18660A. Oral ED25 values were 0.5, 3.3 and 2.4 mg/kg, respectively. All 3 compounds increased 5HT and decreased 5HIAA in the FPC. However, at i.p. doses of 10 mg/kg, CGS 18660A and TVXQ 7821, but not 8OHDPAT, increased striatal DOPAC and HVA and FPC HVA. At a high oral dose (10 mg/kg), only TVXQ 7821 increased striatal DOPA accumulation at 1, 2 and 4 hours. Although 8OHDPAT is selective for the 5HT system, it has a short duration of action, lasting only 1 hour after oral dosage. TVXQ 7821 is not selective when given orally, affecting both DA and 5HT systems for at least 4 hours. When orally administered, CGS 18660A is relatively selective for the 5HT system for at least 4 hours. CGS 18660A has the potential for clinical use as a novel, selective and relatively long acting 5HT-1A agonist.

336.12 BEHAVIORAL PHARMACOLOGY OF THE NOVEL 5-HT$_{1A}$ RECEPTOR-SELECTIVE ANXIOLYTIC IPSAPIRONE (TVX Q 7821) J.Traber*, W.U.Dompert*, T.Glaser*, T.Schuurman* and D.G.Spencer (SPON: F.-K. Pierau) Neurobiology Department, Troponwerke, Neurather Ring 1, D-5000 Köln 80, FRG.

Ipsapirone (TVX Q 7821), a pyrimidinylpiperazine derivative, produces anxiolytic-like effects in many animal models of anxiety (Traber et al., Brain Res. Bull., 12:741, 1984). This compound binds with high affinity to the 5-HT$_{1A}$ subtype of 5-hydroxytryptamine (serotonin) receptors, but not to benzodiazepine or GABA receptors. 5-HT$_{1A}$ receptors are predominantly present in the hippocampal formation (excluding dorsal subiculum) of different species. Behavioral experiments point to a complex ipsapirone interaction with the 5-HT system. Although it generalizes in a drug discrimination paradigm to the 5-methoxy-dimethyltryptamine stimulus (see D.G. Spencer, this meeting), it does not evoke any of the elements of the 5-HT syndrome unlike the reference compound 8-OH-DPAT. Prior administration of ipsapirone to rats in the shock-suppressed drinking test resulted in an increased number of punished responses. Moreover, ipsapirone reduced aggressive and fear-related behavior and increased positive social behavior. The latter effect could not only be observed under high light conditions, in which benzodiazepines are also active, but also under low lighting. Since classical anxiolytics like the benzodiazepines often impair memory processes, the effect of ipsapirone on rat working memory in a 8-arm radial maze was studied. Administration of diazepam strongly reduced arm choice accuracy, whereas ipsapirone had no amnesic effect in this test. These data indicate that ipsapirone interacts with 5-HT$_{1A}$ receptors and has a unique and novel pattern of behavioral activity. Thus, ipsapirone could not only be a new tool to elucidate the functional role of central 5-HT receptors, but may also elucidate new neurobiological aspects of anxiety.

336.13 ANTAGONISM OF SEROTONIN-MEDIATED BEHAVIORS IN RATS BY PRETREATMENT WITH THE NONBENZODIAZEPINE ANXIOLYTICS BUSPIRONE OR ISAPIRONE. Irwin Lucki and Hal R. Ward*. Department of Psychiatry, University of Pennsylvania, and the Neuropsychopharmacology Unit, Veterans Administration Medical Center, Philadelphia, PA 19104.

Two similarly-structured azaspiro-decane compounds, buspirone and isapirone (TVX Q7821), have emerged recently as important nonbenzodiazepine anxiolytic compounds. The mechanism of therapeutic action for these compounds is unclear but may involve receptors for serotonin (5-HT). In order to assess their action at 5-HT receptors in vivo, these compounds were studied for their effects on two behavioral responses in rats that are caused by the activation of different types of 5-HT receptors; the 5-HT behavioral syndrome and the head shake response.

The 5-HT behavioral syndrome was produced by administering either 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 3.0 mg/kg) or 8-hydroxy-1-(di-n-propylamino)tetralin (DPAT, 2.0 mg/kg), a selective 5-HT$_{1A}$ receptor agonist. The symptoms of the 5-HT syndrome (forepaw treading, head weaving, resting tremor, hindlimb abduction, postural position, and Straub's tail) are thought to involve the activation of 5-HT$_{1A}$ receptors (Lucki et al., JPET, 1984, 228: 133-139). Neither buspirone nor isapirone administered alone caused the 5-HT behavioral syndrome, although both compounds produced a low outstretched posture similar to that caused by 5-MeODMT or DPAT. The remaining symptoms of the 5-HT syndrome when caused by 5-MeODMT were blocked in a dose-dependent manner by pretreatment with either buspirone or isapirone, with the exception of hind limb abduction. Similarly, pretreatment with either buspirone or isapirone blocked the appearance of the 5-HT syndrome when produced by DPAT.

Head shaking behavior was produced by administering quipazine (2.0 mg/kg). This behavior is caused by the activation of 5-HT$_2$ receptors (Lucki et al., 1984). Both buspirone and isapirone caused a dose-dependent reduction in the head shake response produced by quipazine, although neither drug alone caused shaking.

Administration of either buspirone, isapirone, or their principal common metabolite (1-2(pyridiminyl)-piperazine (1-PP) reduced the locomotor activity of rats, although 1-PP was most potent at causing this effect. However, 1-PP was not effective at blocking either 5-HT-mediated behavioral response, i.e., the 5-HT syndrome or head shake behavior. This indicates that the ability of buspirone and isapirone to antagonize 5-HT-mediated behavioral responses was not due to nonspecific motor-impairing effects of these compounds. The results suggest that diminished function at 5-HT receptors should be considered as a possible mechanism for the therapeutic actions of buspirone and isapirone.

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336.14 SEROTONIN UPTAKE AND IMIPRAMINE BINDING IN PLATELETS ARE CORRELATED IN MAJOR DEPRESSION HERBERT Y. MELTZER, RAMESH ARORA, JOSEPH LOCASCIO*, KEN PRABUCKI* AND JAR-CHI LEE*, CASE WESTERN RESERVE UNIVERSITY, CLEVELAND, OHIO 44106

Platelet serotonin (5-HT) uptake sites and ^{3}H-imipramine binding (IB) sites may be characterized by their number and affinities: K_m and V_{max}, K_d and B_{max}, respectively. The IB sites have been shown to be allosteric regulators of 5-HT uptake. Decreased numbers of 5-HT uptake sites (V_{max}) and IB sites (B_{max}) have been found in depressed patients but no zero order correlations between the two have been observed. No abnormalities in K_m or K_d have been reported. We have found that V_{max} and K_m are positively correlated in normal controls (NC) (rho=0.57, N=158, $p < .0001$) as are K_d and B_{max} (0.42, N=120, $p < 0.0001$). No zero order correlations between K_m and B_{max} or K_d and V_{max} have been observed in NC. Major depressives (MD) shows a significant correlation between K_m and V_{max} (rho=0.43, N=117, $p < 0.0001$) but not between K_d and B_{max} (rho=0.11, N=66). However, K_m and B_{max} are correlated in MD (rho= -0.34, N=58, $p < 0.01$). This correlation persists after treatment with tricyclic antidepressants. It suggests that uptake and IB sites are possibly co-regulated. We hypothesized that the true relationship between V_{max} and B_{max} might be obscured by the influences of K_m and K_d, on the number of uptake and IB sites, respectively. A multiple regression of V_{max} on B_{max}, after covarying out K_m and K_d, respectively, was significant. Whereas the zero order correlation was 0.052 (N=51) between these two variables, we found that V_{max} was highly correlated with B_{max} with the covariate analysis (T=2.71, p=0.009, N=51). This relationship also was found in unipolar and bipolar depressed patients but not in schizophrenics, manics or NC. These results indicate that multivariate methods are needed to assess the relationships of ligand binding site kinetic constants to related biochemical systems such as amine uptake sites. In MD, decreases in B_{max} without compensatory decreases in K_d appear to be the primary abnormality in platelets, with changes in V_{max} and K_m being compensatory to the abnormalities in imipramine binding.

336.15 BIOCHEMICAL AND BEHAVIORAL PHARMACOLOGY OF THE PUTATIVE ANTIDEPRESSANT TIFLUCARBINE (TVX P 4495) T. Glaser*, W.U. Dompert*, T. Schuurman* and J. Traber* (SPON: J. Tautz) Neurobiology Department, Troponwerke, Neurather Ring 1, D-5000 Köln 80, FRG.

In the search for new antidepressive drugs, tiflucarbine (TVX P 4495) has been found to be very effective in animal tests predictive for antidepressive activity in such models as amphetamine potentiation, reserpine antagonism and behavioral despair. Male rats subjected to repeated defeat by dominant rats develop a syndrome characterized by reduced open field activity, social interaction and sexual behavior. Subchronic treatment of such chronically stressed rats with tiflucarbine resulted in a partial normalization of the behavior. In addition, anti-agressive and anxiolytic action has been observed. Biochemical experiments pointed to an interaction of tiflucarbine with the serotonin (5-HT) system. The compound had a moderate (K_i=180 nmol/l) affinity for 5-HT_2 receptors but not for a variety of other neurotransmitter receptors. Furthermore, it potently inhibited 5-HT re-uptake, but not that of noradrenaline or dopamine. Animal behavior studies, such as induction of the 5-HT syndrome or 5-methoxy-dimethyltryptamine drug discrimination experiments corroborated a role of 5-HT in tiflucarbine's mechanism of action. Like some other antidepressants, tiflucarbine downregulated both ß-adrenergic and 5-HT_2 receptors in the rat brain after subchronic administration. Tiflucarbine had a weak affinity for acetylcholine receptors, and a moderate in-vivo pro-cholinergic rather than anticholinergic activity. This differentiates tiflucarbine from many other antidepressants, whose strong anticholinergic actions might be responsible for undesired side effects. The present studies suggest that tiflucarbine is an antidepressive drug with an interesting combination of actions.

336.16 PRECLINICAL EVALUATION OF McN-5652-Z-68 AS A POTENTIAL ANTIDEPRESSANT. R.P. Shank, J.L. Vaught*, C.R. Schneider*, J.R. Mathiasen*, J.F. Gardocki*, C. Gambarana*†, B.E. Maryanoff*, and D.F. McComsey*. Depts. of Biological and Chemical Res., McNeil Pharmaceutical, Spring House, PA 19477, and †Dept. of Psychiatry, Univ. PA Sch. of Medicine, Phila., PA 19104.

McN-5652-Z-68[trans-1,2,3,5,6,10b-Hexahydro-6-[4-(methylthio) phenyl]pyrrolo[2,1-a]isoquinoline perchlorate] is potently active in a variety of tests considered to be predictive of antidepressant activity. In vitro, McN-5652-Z-68 potently inhibits the synaptosomal uptake of 5-HT(K_i ~ 0.6nM) and NE (K_i ~ 3nM), and inhibits the synaptosomal uptake of DA with moderate potency (K_i ~ 40nM). Potent activity in vivo is indicated by results of ex vivo studies (ED_{50} for inhibition of 5-HT and NE uptake were ~2 and ~10 mg/kg p.o., respectively at 2 hr post dosing), and tests in vivo that are indicative of an enhancement of serotonergic function (L-5-HTP induced head twitch in mice and serotonin syndrome in rats). In the mouse head twitch test, ED_{50} values for McN-5652-Z-68 after s.c., i.p., and p.o. administration were 0.01, 0.03 and 0.5 mg/kg, respectively 45 min after dosing. At 6 hr post p.o. dosing the ED_{50} was ~2 mg/kg in the mouse head twitch test. In the rat serotonin syndrome test, the ED_{50} values of McN-5652-Z-68 1 hr after s.c. and p.o. dosing were 0.20 and 1.6 mg/kg, respectively. McN-5652-Z-68 was generally more potent in these tests than other known 5-HT uptake inhibitors. McN-5652-Z-68 effectively prevented tetrabenazine methanesulfonate (TBZ) induced ptosis in mice and rats, but TBZ induced sedation was effectively prevented only for brief periods after dosing (≤1hr) in mice, and at no time period studied in rats. McN-5652-Z-68 displayed some activity in 5-HT_1, 5-HT_2, α_1 and D_2 receptor assays, but in no case was the potency sufficient to indicate an appreciable contribution to the overall pharmacological effects in vivo (i.e., app. K_i > 200nM). In vivo, McN-5652-Z-68 inhibited ^{3}H-cyanoimipramine binding in rat CNS tissues with an ED_{50} of 0.09 mg/kg (i.p.), further demonstrating its high potency as a 5-HT uptake inhibitor. When administered i.p. for 10 days at 10 mg/kg b.i.d., McN-5652-Z-68 caused a highly significant down regulation of the ß adrenergic receptor in rat cerebral cortex. In other studies McN-5652-Z-68 was found to be devoid of anticholinergic and antihistaminergic activity, and was not a behavioral stimulant at pharmacologically relevant doses. Overall, our studies indicate that McN-5652-Z-68 is a highly potent inhibitor of 5-HT uptake with a long duration of activity, and that its potency as an inhibitor of NE uptake is sufficient to contribute appreciably to its total pharmacological activity in vivo.

SEROTONIN: ELECTROPHYSIOLOGICAL STUDIES

337.1 SEROTONERGIC RECEPTORS ON INDIVIDUAL NEURONES IN THE GUINEA-PIG SUBMUCOUS PLEXUS. A.Surprenant* and J.Crist* (SPON:E.Cherubini) Neuropharmacology Lab, MIT, Cambridge, MA. 02139 and Beth Israel Hospital, Harvard Medical School, Boston, MA. 02215

5-HT increases intestinal secretory activity primarily by activating 5-HT receptors on submucous plexus neurones which innervate the mucosa. Ionophoretic application of 5-HT onto submucous neurones has been shown to cause brief depolarizations associated with a conductance increase (Hirst ϵ Silinsky, J.Physiol. 251, 817,'75). In the present study, intracellular recordings were made from submucous plexus neurones and 5-HT was applied by ionophoresis, pressure pulse and superfusion; the effects of 5-HT (and numerous putative 5-HT agonists and antagonists) were examined on membrane potential or membrane current under single-electrode voltage-clamp conditions.

5-HT evoked membrane depolarizations associated with increased membrane conductance in all neurones; depolarizations associated with a decreased membrane conductance were also observed in a majority of cells. The former type of response was brief (minimum durations evoked by 5-HT pressure pulse were 100 ms), increased in amplitude when the membrane was hyperpolarized and showed an extrapolated reversal potential between 0 and -30 mV. The latter type of depolarization was slow (minimum duration 2-10 s) and was found to be due to a decreased potassium conductance. 5-HT also caused presynaptic inhibition of the fast and slow excitatory synaptic potentials and the inhibitory synaptic potential.

Ketanserin, mianserin, spiperone, hexamethonium and cyproheptadine had no effect on their own, or on any of the 5-HT-induced responses; 8-hydroxy-dipropylamino-tetralin (DPAT) and m-trifluoromethylphenylpiperazine (TFMPP) acted as α_2-adrenoceptor antagonists; methysergide and cocaine inhibited 5-HT depolarizations only at concentrations which produced marked local anaesthetic actions. 2-methyl-5-HT produced membrane depolarizations associated with conductance increases; it did not decrease potassium conductance nor cause presynaptic inhibition. ICS-205-930 (20 - 200 nM) specifically blocked this response to 5-HT or 2-methyl-5-HT.

Thus, the serotonergic receptor(s) which mediate potassium conductance decreases and presynaptic inhibition in the guinea-pig submucous plexus are not 5-HT_{1a}, 5-HT_{1b}, 5-HT_2, or 5-HT_M receptors. The 5-HT receptor which depolarizes the membrane via a cation non-specific conductance increase is the 5-HT_M receptor.

337.2 PHARMACOLOGICAL EVIDENCE SUGGESTING AN INVOLVEMENT OF CHLORIDE IN SEROTONIN MEDIATED INHIBITION. J. C. Strahlendorf, M. Lee, J. Netzeband*, R. Cooper, and H. Strahlendorf. Physiology and Medical and Surgical Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

In our initial studies, iontophoretic serotonin (5-HT) elicited one of three effects on cerebellar Purkinje cells (PCs); inhibition, a biphasic response, and excitation (Strahlendorf et al., Brain Res. Bull. 11: 265-269, 1983). These actions of 5-HT appeared to be correlated with the initial firing rates of PCs (Strahlendorf, Exp. Brain Res. 56: 50-58, 1984). Furthermore, inhibitory effects of 5-HT were decreased significantly by continuous application of the chloride (Cl^-) ionophore antagonist picrotoxin, at currents that also induced significant antagonism of GABA-elicited inhibition of PCs (Lee et al., this volume, 1986). Bicuculline failed to induce significant attenuation of 5-HT mediated inhibition. These findings suggested that 5-HT may induce its inhibitory actions through a Cl^- ionophore. In light of this suggestion, we have investigated the effects of sodium pentobarbital (Pb), an agent reported to enhance GABA-activated Cl^-flux (Study and Barker, Proc. Natl. Acad. Sci. 78: 7180-7184, 1981) on 5-HT and GABA-mediated inhibition of PCs in urethane anesthetized rats. Pb at currents that augmented GABA-mediated inhibition enhanced 5-HT mediated inhibition in 24 of 26 PCs tested. Pulsatile iontophoretic applications of 5-HT (10-50 nA for 20-30 sec) induced an average decrease of 11.1 ± 1.2% in spontaneous activity of PCs. Continuous application of Pb (5-25 nA) significantly augmented 5-HT mediated inhibitions of PCs to 30.2 ± 4.2%. In 16 PCs, iontophoretic 5-HT produced excitation and Pb reversed excitation to inhibition in 12 of the 16 neurons. Specifically, in these 12 instances, 5-HT induced an average increase of 24.2 ± 5.7% and in the presence of Pb, 5-HT caused a 21.3 ± 6.3% decrease in spontaneous activity. These findings further substantiate our suggestion that 5-HT mediated inhibition of cerebellar PCs utilizes a Cl^-ionophore, and the observed reversal of 5-HT mediated excitation may further support the existence of more than one 5-HT receptor site. (Supported by NIH Grant RO1 NS 19296.)

337.3 EFFECTS OF SEROTONIN ON PURKINJE CELL FIRING IN THE IN VITRO CEREBELLAR SLICE. E. J. Darrow, H. K. Strahlendorf, J. C. Strahlendorf, and M. Lee. Depts. of Physiology and Neurology, Texas Tech Univ. Hlth. Sci. Ctr., Sch. of Med., Lubbock, TX 79430.

Previous work from our laboratories has demonstrated a complex pattern of responses of cerebellar Purkinje cells (PCs) to microiontophoretically applied serotonin (5-HT) in situ. Initial studies have shown three effects of 5-HT on PC simple spike (SS) activity: inhibition (62% of PCs), biphasic activity (27%), or excitation (11%). These responses appear to be related to basal SS rate. In addition, 5-HT can modulate PC responses to amino-acid neurotransmitters (Lee et al., Brain Res., 1985). Excitatory but not inhibitory actions of 5-HT are blocked by mixed 5-HT_1 and 5-HT_2 antagonists (Strahlendorf et al., Exp. Brain Res., 1984). Excitatory responses are not, however, blocked by putative 5-HT_2 antagonists. In further study of SS activity in vitro, we have compared the responses of PCs to perfusion (10-400 µM) and microiontophoretic application of 5-HT in cerebellar slices. Although PCs respond to both methods of application with either excitation or inhibition, the response characteristics are quite different. Sixty-seven percent of PCs responded to perfused 5-HT with excitation; 33% responded with inhibition. With iontophoresis of 5-HT, however, 65% of PCs responded with inhibition; 32% responded with excitation, and 3% had a biphasic response. In addition, responses of PCs to iontophoretic 5-HT, but not perfused 5-HT, appeared to be rate dependent. Finally, when responses of the same PCs to perfusion and iontophoresis were compared, it was seen that 80% of those cells displaying inhibitory responses to iontophoretic 5-HT showed excitation with 5-HT perfusion, whereas 67% of PCs excited by iontophoresed 5-HT responded also with excitation to perfused 5-HT.

These results suggest the presence of at least two different 5-HT receptor subtypes on PCs. This is supported by the finding that excitatory responses to perfusion of different concentrations of 5-HT demonstrate an atypical dose-response relationship. Excitatory responses are dose related with 5-HT concentration between 10 and 100 µM. A peak excitatory response of 21 ± 2% is observed with 100 µM 5-HT. With 200 and 400 µM 5-HT, however, the response declines to 13%. This suggests that with increasing 5-HT concentrations, the excitatory response may decline because of a subtractive effect of activation of inhibitory receptors. Currently, we are studying the effects of putative 5-HT receptor-specific agonists and antagonists on these responses. Research funded in part by Tarbox Parkinson's Disease Institute, TTU.

337.4 DIFFERENTIAL EFFECTS OF Y-AMINOBUTYRIC ACID (GABA) ANTAGONISTS PICROTOXIN (PTX) AND BICUCULLINE (BIC) ON SEROTONIN (5-HT)-MEDIATED INHIBITION OF PURKINJE CELLS (PCs). H. K. Strahlendorf, M. Lee, and J. C. Strahlendorf. Depts. of Neurology and Physiology, Texas Tech University, Health Sciences Center, Lubbock, TX 79430.

Studies from this laboratory have shown that iontophoretic application of 5-HT induced inhibitory, excitatory, and biphasic effects on PCs (Strahlendorf et al., Exp. Brain Res. 56, 1984). Unlike excitatory responses, inhibitory responses of PCs to 5-HT are resistant to classical 5-HT antagonists. However, the GABA antagonists PTX and/or BIC can antagonize inhibitory effects of 5-HT on neurons of other areas of brain (Mayer and Straughan, Neuropharm. 20, 1981; Segal, Brain Res. 103, 1976). This study was planned to examine and compare the effects of PTX and BIC on inhibitory responses of cerebellar PCs to 5-HT in urethane-anesthetized rats.

Iontophoretic application of 5-HT elicited one of three effects: inhibition, biphasic response, or excitation as shown in previous studies. Only PCs that were inhibited by 5-HT were used in this study. 5-HT (15-50 nA for 20-30 s) induced an average decrease of 18.0 ± 1.1% in the spontaneous activity of PCs. Continuous application of PTX (2-30 nA) attenuated 5-HT-mediated inhibitions of PCs to 8.0 ± 1.1% (n=37). The effect of PTX on 5-HT-mediated inhibitions of PCs was statistically significant ($P < 0.0001$). PTX attenuated 5-HT-mediated inhibition in all cells tested and antagonized inhibitory responses completely in 6 of these cells. The effect of PTX on GABA-induced inhibitions of PCs (n=14) was also significant ($P < 0.0001$). Pulsatile iontophoretic application of GABA (2-20 nA) elicited an average decrease in PC activity of 54.3 ± 8.0%. PTX antagonized GABA-mediated inhibitions to 15.4 ± 2.4%. Continuous application BIC at currents that significantly antagonized GABA-mediated inhibition of PCs (n=37) failed to attenuate 5-HT-mediated inhibitions. Among these 50 PCs tested, BIC attenuated 5-HT-mediated inhibitions insignificantly in 26 cells, had no effect in 15 cells, and potentiated inhibition in 9 neurons. Alone, 5-HT induced an average decrease in spontaneous activity of 17.4 ± 1.1%; in the presence of BIC, it elicited an average decrease of 17.5 ± 2.3%. Thus, BIC caused virtually no change in 5-HT-mediated inhibitions.

In summary, iontophoretic application of PTX antagonized both 5-HT- and GABA-mediated inhibitory responses of PCs; however, BIC antagonized only GABA-mediated inhibitions. (Supported by NIH Grant RO1 NS 19296)

337.5 DIFFERENT SEROTONIN AGONISTS AFFECT THE Ca^{++} DEPENDENT PLATEAU OF ACTION POTENTIALS FROM FROG DORSAL ROOT GANGLION CELLS IN OPPOSITE DIRECTIONS. R. Scroggs* and E.G. Anderson. Dept. of Pharmacology, University of Illinois College of Medicine, Chicago, IL 60612.

Previous work in Aplysia neurons has shown that serotonin (5-HT) blocks a slow K^+ current resulting in an increase in action potential (AP) width and increased Ca^{++} influx (Klein and Kandel, PNAS 75: 3572, 1978). In frog dorsal root ganglion cells, 5-HT decreases the width of the tetraethylammonium (TEA) broadened action potential, probably by decreasing Ca^{++} influx (Holz, Shefner and Anderson, J. Neurosci. 6: 620, 1986). We have now compared the effects of 5-HT with two serotonin agonists which have a high affinity for 5-HT_{1a} binding sites on APs recorded intracellularly from frog dorsal root ganglion cells. Surprizingly, the 5-HT agonists, 8-OH DPAT (+ 8-OH dipropylaminotetralin HBr) and 5 OME DMT (5-methoxy-n, n-dimethyltryptamine oxalate) acted in the opposite manner from 5-HT. Both 8-OH DPAT (20-80 µM) and 5 OME DMT (50 µM) widened the AP plateau induced by TEA, while 5-HT (10 µM) narrowed this plateau. Exposure to 40 µM 8-OH DPAT produced an average increase of 41% ± SE 8.4 in the width of the AP in the presence of 10 mM TEA (11 cells). Application of 10 µM 5-HT decreased the width of the TEA broadened AP by an average of 29% ± SE 6.6 (7 cells). The responses to all compounds were dose dependent, readily reversible and little desensitization was observed. In the absence of TEA, 5-HT was without effect on the AP width, but 8-OH DPAT produced a modest increase in AP width. This increase was more prominent in those cells in which the normal AP was broadened by shallow shoulder on its descending limb.

Two different receptor systems appear to be involved in the actions of 5-HT and 8-OH DPAT. A larger percentage of cells responded to 8-OH DPAT than to 5-HT (85% and 47%, respectively). Of 10 cells exposed to both compounds; 6 were sensitive to both, 2 responded only to 8-OH DPAT and 2 responded to neither substance. Thus, some cells have receptor systems responsive only to 8-OH DPAT.

Concentrations of the Ca^{++} channel blocker Mn^{++}, which only partially block the TEA broadened part of the AP, markedly reduce the ability of 8-OH DPAT to induce AP widening. It appears that 8-OH DPAT affects the opening of a Ca^{++} channel. It is not yet known if this affect is direct or indirect via an action on a K^+ channel. (Supported in part by USPHS NS 17834.

337.6 EFFECTS OF SEROTONIN (5HT) ON MEMBRANE PROPERTIES OF RAT LATERAL SEPTAL NEURONS IN VITRO. Marianne Joels, Joel P. Gallagher, and Patricia Shinnick-Gallagher. University of Texas Medical Branch, Galveston, TX 77550.

The lateral septum of rats is known to receive a serotonergic projection from the dorsal raphe nucleus and displays a high density of binding sites particularly for selective 5HT_1 ligands. We have studied intracellularly the effect of 5HT on membrane properties of neurons in a transverse slice of rat brain containing the septal nuclei. Superfusion of 5HT oxalate (10 µM) on lateral septal neurons induced a hyperpolarization (6.1 ±1.2 mV, mean ± SEM, n=18) of the membrane potential accompanied by a decrease in membrane resistance (23.0± 4.0%, n=21). Neurons that were spontaneously active were inhibited during 5HT application. The 5HT response was rapid in onset, demonstrated little desensitization, and was short lasting. Threshold concentration for the response was 1 µM, maximal hyperpolarization (7.0 ± 0.9 mV, n=9) and decrease in resistance (42.0 ±1.4%, n3) occurred at 30 and 100 µM respectively. At concentrations higher than 30 µM the hyperpolarizations were sometimes masked by a subsequent depolarization. In 5 neurons, only depolarizing responses associated with a decrease in membrane resistance were observed. The onset of the depolarizing response was slow (ca. 1-3 minutes); the response lasted for a considerable time (up to 10 minutes) and in some cases the membrane potential did not return completely to pretreatment level. The intercept of the slopes obtained from current-voltage relationships before and during 5HT superfusion yielded a reversal potential for the hyperpolarizing response at 96.6± 2.6 mV (n=7). Hyperpolarizing responses were also obtained with 5HT pressure-ejected from microelectrodes positioned in the medium near the recording electrode. Data collected with this method revealed reversal potentials at 96.8 ± 3.6 mV. These values suggest that the hyperpolarizing response to 5HT in septal cells is due to an increase in K^+-conductance. The hyperpolarizing responses to superfused or pressure-ejected 5HT persisted in a low Ca/high Mg-containing Krebs solution or in the presence of tetrodotoxin (1 µM), indicating a predominantly postsynaptic site of action. In a limited number of neurons tested, the depolarizing response to 5HT was markedly decreased in low Ca/high Mg solution or during application of tetrodotoxin. We conclude that 5-HT (1-30 µM) hyperpolarizes lateral septal neurons through an increase in K^+-conductance of the postsynaptic membrane. At higher concentrations 5HT evokes a biphasic or exclusively depolarizing response. The ionic mechanism of the depolarizing responses is yet to be determined. (Supported by MH-39163 to JPG)

337.7 IDENTIFICATION OF TWO TYPES OF PERIPHERAL NEURAL RECEPTOR FOR SEROTONIN.
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The enteric nervous system (ENS) has both serotonergic neurons and serotonin (5-HT) receptors. These receptors are found in enteric ganglia and in the mucosa. Stimulation of these "M" receptors in ganglia by 5-HT activates cholinergic neurons, which cause the gut to contract. Activation of mucosal 5-HT receptors stimulates intrinsic enteric afferent neurons that initiate the peristaltic reflex. We have previously reported the existence of specific binding sites for ^{3}H-5-HT (K_D=2.7 nM) in the gut. These sites were localized by radioautography to myenteric ganglia and the intestinal mucosa. Since the structure-activity relationship for the ability of a variety of compounds to antagonize the binding of ^{3}H-5-HT was the same as that for their ability to act as agonists or antagonists of a neuronal response to 5-HT, we concluded that the ^{3}H-5-HT binding sites in the bowel are 5-HT receptors. We now report that there are at least 2 types of 5-HT receptor on enteric neurons, neither of which resembles any 5-HT receptor that has been found in the brain. One causes a slow depolarization of myenteric ganglion cells associated with an increase in input resistance. Agonists at this receptor include, in addition to 5-HT (in order of potency), 5- and 6- hydroxy-[(indolyl-3)-2 ethyl]-4 piperidine (Pharmuka), and 2-methyl-5-HT. These responses are antagonized by N-acetyl-5-hydroxytryptophyl-5-hydroxytrypto-phan amide (5-HTP-DP). High affinity specific binding of ^{3}H-5-HT is antagonized by the agonists and by 5-HTP-DP, but not by ICS 205-930 (Sandoz), a drug reported by Richardson et al. (1985) to be a specific "M" receptor antagonist, or by D-LSD. ICS 205-930 also does not antagonize the slow response to 5-HT. The other enteric neural 5-HT receptor induces a fast depolarization of myenteric neurons associated with a fall in input resistance. These fast responses to 5-HT are antagonized by ICS 205-930. 2-Methyl-5-HT can mimic the fast responses to 5-HT, and this effect of 2-methyl-5-HT is antagonized by ICS 205-930. Thus, one enteric neural receptor for 5-HT appears to have a high affinity for the amine and is labeled by ^{3}H-5-HT. This receptor seems to be responsible for the slow depolarization that is mimicked by indalpines and antagonized by 5-HTP-DP. The other appears to have a lower affinity for ^{3}H-5-HT, and thus is not labeled by that radioligand. This receptor is responsible for the fast depolarization of myenteric neurons that is specifically antagonized by ICS 205-930. Supported by NIH grant # NS 12969 and the P.M.A.F.

337.8 DIFFERENCES IN RESPONSE OF DORSAL AND MEDIAN RAPHE SEROTONINERGIC NEURONS TO 5HT-1 RECEPTOR LIGANDS.
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Recent results indicate that the serotonin 5HT-1 receptor exists in at least two subtypes which have been termed 5HT-1A and 5HT-1B sites, and that there is a good correlation between 5HT-1A agonist action and inhibition of 5HT cell firing in the DRN. A recent report describing the distribution of 5HT-1 receptors (Pazos et al., Br. Res. 346:205, 1985) demonstrates that there is a lower density of 5HT-1 receptors in the median raphe nucleus (MRN) than in the DRN, and that the proportion of 5HT-1B sites may be higher in the former structure. The present study examined the possible functional significance of these differences in binding by determining the action of relatively selective putative 5HT-1A and 5HT-1B ligands on firing rates of 5HT cells in both the DRN and MRN. Rats were anaesthetized with chloral hydrate and a glass microelectrode was lowered stereotaxically to the DRN or MRN. Spontaneously active 5HT units were recorded extracellularly and identified on-line. The location of the recording site was verified histologically at the completion of an experiment. Baseline firing rates were established before test agents were administered intravenously and an ED50, the dose which changed the spontaneous firing rate by 50%, was calculated from a mean dose response plot of 5 separate experiments: one cell was recorded in each animal. TVX Q 7821 and 8-hydoxy-2-[n-dipropylamino]tetralin (8-OH-DPAT) were chosen as selective 5HT-1A ligands, and trifluromethylphenyl piperazine (TFMPP) and a novel pyrroloquinoxaline, CGS 12066B, as selective 5HT-1B ligands. In confirmation of previous reports, DRN 5HT neurons were inhibited by TVX Q 7821 (ED50 of 14 µg/kg) and by 8-OH-DPAT (0.4 µg/kg) and by CGS 12066B (203 µg/kg), but TFMPP did not cause any consistent effects - relatively small changes in firing rate were seen with this compound. In contrast, although MRN 5HT neurons were also inhibited by TVX Q 7821 (30 µg/kg) and by 8-OH-DPAT (6 µg/kg), the ED50 in each case was significantly higher than that recorded for DRN cells. In addition, both TFMPP and CGS 12066B, at low doses, exerted an excitatory action on MRN 5HT units with an ED50 of 12 µg/kg and 5 µg/kg, respectively. Only at much higher doses did these relatively selective 5HT-1B compounds cause an inhibition of MRN 5HT cell firing. Thus the differences in 5HT-1 sites in the raphe nuclei appear to have functional significance for drug responses, and, in particular, the action of 5HT-1B ligands cannot be entirely characterized by their action on DRN neurons. These results may have implications for understanding the mechanisms of action of novel anxiolytics, such as buspirone, which are believed to exert their therapeutic effects by acting at 5HT-1 sites.

337.9 THE PUTATIVE, NOVEL ANXIOLYTIC, TVX Q 7821, DEPRESSES NEURONAL ACTIVITY IN THE DORSAL RAPHE NUCLEUS AND THE HIPPOCAMPAL FORMATION.
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TVX Q 7821 belongs to a new class of putative anxiolytic drugs that do not interact with the GABA-benzodiazepine receptor complex, but rather exhibit specific, high affinity binding to 5-HT$_{1A}$receptors (Peroutka, Brain Res., 344:167, 1985). To investigate the functional significance of this effect, we used single-unit recording techniques to monitor the action of TVX Q 7821 on serotonergic neurons in the dorsal raphe nucleus and on neurons in regions of the hippocampal formation known to receive serotonergic input. All of these recording sites contain high densities of 5-HT$_{1A}$ receptors (Pazos and Palacios, Brain Res., 346:205, 1985).

Adult male rats, anesthetized with urethane, received intravenous injections of TVX Q 7821 in increasing incremental doses beginning with a dose of 31.3 µg/kg. The predominant response in all areas tested was a depression of neuronal activity. All neurons in the dorsal raphe nucleus and dentate gyrus were inhibited by low doses of TVX Q 7821 (median effective dose for a 50% inhibition was 125.0 µg/kg in both areas). The response of CA1 pyramidal cells was more variable but related directly to baseline firing rate. The slowest firing cells were more sensitive than those in the dorsal raphe, whereas fast firing neurons failed to exhibit any consistent change in activity up to a dose of 500.0 µg/kg. The profound effects of TVX Q 7821 on serotonergic neurotransmission may play an important role in the anxiolytic actions of this drug.

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337.10 INHIBITION OF SEROTONERGIC DORSAL RAPHE NEURONS IN THE RAT BRAIN SLICE BY THE 5-HT$_1$ AGONIST 5-CARBOXAMIDOTRYPTAMINE.
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5-Carboxamidotryptamine (5-CONH$_2$-T) is a 5-HT analog which binds to 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (Engel et al., Naunyn Schmiedeberg's Arch. Pharmacol., 1983, 324, 116-124). 5-CONH$_2$-T is a potent agonist in a guinea pig hippocampal adenylate cyclase assay sensitive to 5-HT$_{1A}$ ligands (Shenker et al., Eur. J. Pharmacol., 1985, 109, 427-429). A number of 5-HT$_{1A}$ agonists have been shown to inhibit the firing of serotonergic dorsal raphe (DR) neurons, apparently by acting directly at somatic and dendritic 5-HT autoreceptors on these cells (e.g. Sprouse & Aghajanian, Soc. Neurosci. Abst., 1985, 11, 47; Aghajanian & Lakoski, Brain Res., 1984, 305, 181-185; VanderMaelen & Wilderman, Fed. Proc., 1984, 43, 947). The present study examined the effects of 5-CONH$_2$-T on the firing of rat serotonergic DR neurons in a brain slice preparation.

Extracellular single-unit recordings were made from serotonergic DR neurons in rat brain slices, as previously described (VanderMaelen & Aghajanian, Brain Res., 1983, 289, 109-119). Male albino Sprague-Dawley rats (100-150 g) were anesthetized with chloral hydrate, decapitated, and 400 µM thick frontal midbrain sections cut on a Vibratome. Slices were placed in an atmosphere of 95% O_2, 5% CO_2 and bathed in continuously-flowing artificial CSF of the following composition in mM: NaCl, 130; KCl, 5.0; $CaCl_2$, 1.25; $MgSO_4$, 1.25; $NaHCO_3$, 24; NaH_2PO_4, 1.25; D-glucose, 10. Phenylephrine (10 µM) was always present to provide tonic excitatory input to recorded neurons. Increasing concentrations of 5-CONH$_2$-T were given, with at least 15 min. allowed at each concentration for asymptotic response to be achieved. The concentration-effect curve and IC_{50} value were obtained by computer analysis.

5-CONH$_2$-T quite potently inhibited the firing of serotonergic DR neurons in the rat brain slice. The calculated IC_{50}=28.9 nM. Complete inhibition of firing sometimes occurred with concentrations of 25, 50 and 100 nM. In contrast to some other 5-HT$_{1A}$ agonists (e.g. buspirone, VanderMaelen & Wilderman, Soc. Neurosci Abst., 1984, 10, 259) recovery from the inhibitory effects of 5-CONH$_2$-T was relatively rapid and complete, probably closely paralleling the extracellular concentrations of the drug during wash-out. Since the somatic and dendritic 5-HT autoreceptor appears to be similar to the 5-HT$_{1A}$ but not the 5-HT$_{1B}$ receptor, (Verge et al., Eur. J. Pharmacol., 1985, 113, 463-464; Weissman-Nanopoulos, Neurochem. Int., 1985, 7, 1061-1072), it is concluded that 5-CONH$_2$-T potently inhibits serotonergic DR neurons by a direct agonist action on somatic and dendritic 5-HT autoreceptors located on these neurons.

337.11 INHIBITION OF SEROTONERGIC DORSAL RAPHE CELL FIRING BY 5-HT-1A AGONISTS: STEREOSELECTIVE BLOCKADE BY PROPRANOLOL. J.S. Sprouse* and G.K. Aghajanian (SPON: T. Baker) Dept. of Psychiatry, Yale University Sch. of Med., New Haven, CT 06508.

Previous attempts to block the somatodendritic autoreceptors of serotonergic (5-HT) neurons in the dorsal raphe nucleus with classical 5-HT antagonists have been unsuccessful (J. Neural Trans. 35: 257, 1974). Several lines of evidence now indicate that these autoreceptors are of the 5-HT-1A receptor subtype (Eur. J. Pharmacol. 113: 463, 1985; Soc. Neurosci. Abstr. 11: 47, 1985). Receptor binding studies have demonstrated that the β-adrenoceptor antagonist propranolol (PRO) stereoselectively binds to central 5-HT sites (Nature 267: 289, 1977) and behavioral studies suggest that it may have 5-HT-1A antagonistic properties (Eur. J. Pharmacol. 106: 271, 1985; Br. J. Pharmacol. 84: 473, 1985). The potential usefulness of PRO in electrophysiological studies of 5-HT receptor subtypes has not been previously explored.

Extracellular single-cell recordings in the dorsal raphe nucleus were made from chloral hydrate-anesthetized rats. Compounds were applied by microiontophoresis from 5-barreled micropipettes and included two 5-HT-1A-selective agonists, isapirone (ISA; TVX Q 7821) and 8-OH-DPAT.

At low microiontophoretic currents which alone did not affect spontaneous rate, (-)-PRO reversibly blocked the suppressant effects of ISA. One min of (-)-PRO pretreatment was sufficient to cause a significant blockade of ISA-induced suppression (mean % blockade $\pm$ S.E. = 65 $\pm$ 9, n = 11); 2-10 mins of (-)-PRO exposure typically produced complete blockade. (-)-PRO also blocked the suppressant effects of 8-OH-DPAT; however, longer periods of propranolol exposure were required to achieve blockade. Unlike (-)-PRO, 1-2 mins of pretreatment with (+)-PRO was largely ineffective in antagonizing the action of ISA (12 $\pm$ 6 vs. 53 $\pm$ 9 %, n = 7, $p < 0.002$).

(-)-PRO was a comparatively weak antagonist of 5-HT itself since a discernable blockade (>20%) was achieved in only 5 of 14 cells tested. Mean % blockade in those 5 cells was only 63 $\pm$ 5 despite prolonged exposure to (-)-PRO (mean = 8.8 mins). In the remaining cells, no discernable blockade was observed even after 6-23 mins of (-)-PRO application.

These results indicate that (-)- but not (+)-PRO is an effective antagonist of the suppressant effects of 5-HT-1A agonists on spontaneously firing 5-HT neurons in the dorsal raphe nucleus. The comparatively weak antagonism of 5-HT by (-)-PRO suggests that 5-HT may have actions on dorsal raphe neurons in addition to those at 5-HT-1A sites.

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337.12 INTRACELLULAR RECORDINGS FROM SEROTONERGIC DORSAL RAPHE NEURONS IN UNRESTRAINED CATS: ELECTROPHYSIOLOGICAL CHARACTERIZATION DURING SLEEP. A. Hosseini and M.E. Trulson. Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

The electrophysiological properties of serotonergic dorsal raphe (RD) neurons have been characterized in anesthetized rats (Brain Res., 238, 1982, 463-469). RD neurons exhibit a pronounced post-action potential hyperpolarization followed by a slow inter-spike depolarization. This gradual depolarization continues to the next spontaneous spike. This pattern of activity has been called a "pacemaker potential" and appears to account for the slow, rhythmic discharge pattern of RD neurons. Although the "pacemaker potential" is the most likely explanation for the discharge pattern of serotonergic neurons, it is well-known that anesthetics dramatically alter the membrane properties of neurons (Trends Pharmacol. Sci., 5, 1984, 287-289). Furthermore, it is impossible to examine the membrane properties of neurons during behavior when the animal is anesthetized. Therefore, we developed a technique for recording the intracellular activity of single RD neurons in unrestrained cats. A plate designed to securely hold the slave cylinder of a Trent Wells motorized microdrive was cemented to the cats skull under pentobarbital anesthesia. After complete recovery from the anesthesia the slave cylinder was attached to the base plate. The electrode was 2 mm (o.d.) omega dot glass tube beveled by the thick slurry method of Lederer et al. (Pflugers Arch. 381, 1979, 287-288) with an impedance of 15-30 megohms and filled with 1 M potassium acetate. Six insect pins were positioned 3 mm apart in a hexagonal fashion around th RD to minimize pulsations. RD neurons were impaled by the use of a 2 msec 20V DC pulse which was passed through the breakaway box of a M707 WP amplifier. Only intracellular recordings of neurons with stable spontaneous discharge rates for a given behavioral state (Brain Res. 163, 1979, 135-150) and action potentials of greater than 50 mV were studied. RD cells showed the slow (2.7 spikes/sec), rhythmic discharge pattern previously described. A hyperpolarization of 4-5 mV occurred after each action potential during quiet waking, and there was a gradual ramp of depolarization preceding each spike. As the cat entered slow wave sleep, the overall activity of RD units decreased significantly to 1.6 spikes/sec. During this phase of sleep, the hypolarization following an action potential was greater (8-10 mV) than during quiet waking. When the animal entered REM sleep RD neurons became virtually silent (0.9 spikes/sec). The hyperpolarization following a spike during REM sleep was much larger (12-15 mV) and remained constant at this level for prolonged periods of time. During the few seconds immediately preceding another spike during REM sleep, there was a rapid depolarization to 6-8 mV, and then a slow ramp of depolarization immediately preceeding the spike.

337.13 LATERAL HABENULAR LESIONS ATTENUATE THE SUPPRESSION OF DORSAL RAPHE NEURONAL ACTIVITY DURING REM SLEEP IN UNRESTRAINED CATS. J. C. Joe*, K. Arasteh, A. Hosseini and M.E. Trulson. (SPON. N.H. McArther). Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

Serotonin (5HT)-containing nucleus raphe dorsalis (RD) neurons become virtually silent during rapid-eye-movement (REM) sleep. The significance of this suppression of activity is not known. The RD receives afferent input from many CNS regions, including the cerebellum, caudate nucleus, lateral hypothalamus, spinal cord, prefrontal cortex, and the rostral basal forebrain. However, the most pronounced input to the RD is from the lateral habenula nuclei. Electrical stimulation of the lateral habenula nuclei has been shown to produce a profound inhibition of RD cells. If afferent input to the RD via the habenula nuclei play a role in the suppression of RD cells during sleep, then lesions of the habenula should block this depressant effect. Adult female cats received bilateral lesions of the lateral habenula nuclei. Subsequent histological analysis revealed that the lesions were confined almost exclusively to the lateral habenula nuclei. During the same surgical procedure, the cats received macro- and microelectrode implants as previously described (Brain Res. 163, 1979, 135-150). After recovery from surgery the cats were placed in a recording chamber and allowed to adapt to the novel setting. The microdrive was then slowly advanced, and when a single unit displaying the characteristics of a 5HT-containing RD cell was isolated, the cat was allowed to go through the entire sleep-waking cycle while simultaneously recording unit activity. The data revealed that the spontaneous activity of RD cells was significantly faster in lesioned animals (Mean $\pm$ SEM= 3.11 $\pm$ 0.13, N= 46) than in sham-lesioned control animals (Mean $\pm$ SEM= 2.79 $\pm$ 0.09 spikes/sec, N= 66, $P<0.05$). During REM sleep, the discharge rate of RD cells in sham lesioned cats virtually ceased (0.11 $\pm$ 0.01 spikes/sec), while that of habenula lesioned animals continued at a slightly slower rate than normal (Mean $\pm$ SEM= 1.97 $\pm$ 0.08 spikes/sec). This latter discharge rate is comparable to that seen in normal cats during drowsiness-early slow-wave sleep. These data demonstrate that afferent input to the RD via the lateral habenula exert a tonic suppression on the activity of RD neurons, since the discharge rate was significantly higher following habenula lesions. This is consistent with the finding that RD neurons show a significantly higher discharge rate in vitro than in vivo. Furthermore, the dramatic decrease in RD unit activity observed during REM sleep in normal cats is apparently due, at least in part, to an afferent input that enters the RD via the lateral habenula.

337.14 MICROIONTOPHORESIS IN FREELY MOVING CATS: EFFECTS OF SEROTONIN ON FACIAL MOTONEURONS. D.W. Preussler and M.E. Trulson. Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

Anesthetics have been shown to dramatically alter the outcome of studies investigating the effects of drugs on brain neurons (Trends Pharmacol. Sci., 5, 1984, 287-289). Therefore, it would be of great advantage to be able to apply substances to single neurons in awake, behaving animals. A technique for iontophoretically applying substance to single cells in freely moving rats was recently developed by West and Woodward (Soc. Neurosci. Abstr., 10, 1983, 227). We have developed a modification of their technique to iontophoretically apply substances to brain neurons in awake freely moving cats. A base was implanted on the cat's skull directly over the target area (in this case, the facial motor nucleus), and the cat was allowed to recover from surgery. Four-barreled glass micropipetts were pulled to a fine tip (4-8 μm tip diameter). The electrode barrels were filled with 2 M NaCl saturated with Fast Green dye (recording electrode), 4 M NaCl (for current balancing), serotonin (5HT) creatinine sulfate (0.04 M, pH 4.0), and L-glutamate (0.2 M, pH 8.0). The microdrive was custom made and was shaped such that the 4-barreled micropipet would fit firmly into place. The electrode was inserted into the microdrive, which was then attached to the base. Turning the outer cylinder, which was threaded to the inner cylinder of the microdrive, advanced the microelectrode to the proper depth (up to 8 mm). There was no rotation of the micropipet as it was advanced through the brain. Electrical signals were led from and to the recording, balance and drug barrels via silver wires. The wires were attached to a low noise cable which led to a 24 channel slip ring assembly. Unit action potentials were amplified, filtered and displayed on an oscilloscope. Iontopheretic currents were controlled by Medical Systems Corp. IP-2 iontophoretic pumps. Since facial motoneurons show bursts of activity which are correlated with movement, recordings were performed when the animal became drowsy and showed no movement. During these conditions the neurons displayed little activity. After obtaining a stable baseline, 5HT was iontophoretically applied by 10-100 nA pulses for 10-30 sec. 5HT consistently produced a dosedependent increase in facial motoneuron activity of approximately 5-60 spikes/sec. These neurons were also consistently excited by L-glutamate. Simultaneous application of 5HT and glutamate produced an additive effect. When the cats were anesthetized with chloral hydrate (400 mg/kg, i.p.) iontophoretically applied 5HT no longer excited these cells; however, they could still be excited by glutamate. These data are in contrast to previous studies which reported that 5HT alone had no effect on facial motoneurons in anesthetized rats, and underscore the importance of conducting such studies in unanesthetized animals.

337.15 POSSIBLE INVOLVEMENT OF SEROTONIN AS AN EXCITATORY NEUROTRANSMITTER OF DESCENDING FIBERS PRODUCING SLOW SYNAPTIC POTENTIAL IN THE ISOLATED RAT SPINAL CORD. H. Akagi* and K. Akita* (SPON: M. Otsuka). Department of Neuroscience, Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan.

The dense projection of descending serotonergic fibers to the ventral and lateral horns in the spinal cord has been demonstrated by many histochemical studies. So far, however, the functional role of serotonin (5-HT) in these area of the spinal cord is still unclear; both excitatory and inhibitory functions are postulated. The aim of the present study is to clarify the role of 5-HT contained in these descending fibers in the isolated spinal cord preparation of the neonatal rat.

The spinal cord below thoracic level was isolated from 3-5-day-old rat and perfused with atrificial cerebrospinal fluid containing 3 mM Ca^{2+} and 7 mM Mg^{2+} at 27°C. Responses of lumbar spinal neurons to nerve stimulation or some chemicals were recorded extracellularly from a ventral root (L 4 or L 5) with a suction electrode.

When a single shock stimulus was given to the ventro-lateral area of upper thoracic cord (Th 1-5), spike potentials of fast time courses and a subsequent slow depolarizing response were recorded from the lumbar ventral root. The latter component, the descending slow depolarization, peaked at a latency of 2-5s and lasted 20-30s.

The descending slow depolarization was markedly reduced by methysergide, a 5-HT antagonist, at 20-100 nM whereas the response was not affected by [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]] substance P (20 µM), phentolamine (3 µM) or atropine (1 µM). In the presence of 0.6 µM tetrodotoxin, 5-HT (3 mM) applied by a brief pulses of 0.4-s duration caused a long-lasting depolarization of the ventral root and the response was depressed by 50 nM methysergide.

Treating the spinal cord with 5,7-dihydroxytryptamine (5,7-DHT, a selective 5-HT neurotoxin) in vitro caused a decrease in the amount of 5-HT in the tissue (analyzed by HPLC-electrochemical detection) in concentration- and time-dependent manners. The amount of 5-HT was reduced to less than 10% of the control level by the treatment with 5,7-DHT (30 µM, 2 hr) whereas those of substance P, thyrotropin-releasing hormone (measured by radioimmunoassay), asparatic acid, glutamic acid, glycine and GABA (measured by HPLC-amino acid analyzer) were not affected by the drug. The descending slow depolarization was completely abolished during the application of 5,7-DHT (30 µM, 2 hr) and after the removal of the drug no recovery occurred during the subsequent period of 90 min.

These results suggest that 5-HT released from certain descending fibers produces a slow depolarization in lumbar spinal neurons including motoneurons and/or sympathetic preganglionic neurons.

337.16 EFFECT OF HALLUCINOGENS ON SPONTANEOUS AND EVOKED LOCUS COERULEUS UNIT ACTIVITY IN THE RAT: REVERSAL BY SELECTIVE 5-HT_2 ANTAGONISTS. Kurt Rasmussen and George K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06508.

There are two major types of psychedelic hallucinogenic drugs, the indoleamines (e.g. LSD) and the phenylethylamines (e.g. mescaline, DOM). Despite structural differences between the two groups, a common site of action has been proposed due to their similar effects on autonomic function and behavior. One common action that has been demonstrated for the two types of hallucinogens is in the rat locus coeruleus (LC), where systemic administration of LSD or mescaline has been shown to depress spontaneous activity while simultaneously facilitating activation by sciatic nerve stimulation. Recently, several studies have shown that indoleamine and phenethylamine hallucinogens share the common property of interacting with 5-HT_2 receptors. In light of these findings we examined the ability of the selective 5-HT_2 antagonists LY 53857 and ritanserin to block the effects of LSD, mescaline, and DOM on LC neurons. These two 5-HT_2 antagonists belong to different structural classes and their only known common action is on 5-HT_2 receptors. LC single unit recordings were obtained from chloral hydrate anesthetized male rats and drugs were administered intravenously. As previously reported, systemic administration of the hallucinogens LSD (5-10 µg/kg) and mescaline (2 mg/kg) produced a decrease in spontaneous activity but, paradoxically, facilitated activation of LC neurons by sciatic nerve stimulation. DOM (20-80 µg/kg) was found to have similar effects. Systemic administration of the selective 5-HT_2 antagonists LY 53857 (0.1-0.8 mg/kg) and ritanserin (0.1-0.3 mg/kg) completely reversed both actions of the hallucinogens on the LC and returned the spontaneous and evoked activity to a level that was not significantly different from baseline. In contrast, LY 53857 did not reverse the effects of the stimulant drug (+)-amphetamine (0.5 mg/kg) on the spontaneous or sensory evoked activity in the LC. The iontophoretic application of LSD and mescaline on LC neurons does not mimick the effects of systemically administered LSD and mescaline, and there are few, if any, 5-HT_2 receptors in the LC. Thus, the hallucinogens are most likely acting on afferents to the LC, afferents that are affected directly, or indirectly, by 5-HT_2 receptors. In addition, LY 53857 and ritanserin administered alone produced a slight but significant increase in spontaneous LC activity (although they did not alter evoked LC activity), indicating that these afferents affected by 5-HT_2 receptors may have a low level of tonic activity in the anesthetized rat. We conclude that the common actions of indoleamine and phenethylamine hallucinogens displayed in the LC are mediated via 5-HT_2 receptors; however these receptors are located outside the LC.

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337.17 **ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE EFFECTS OF COCAINE ON SEROTONIN (5-HT) NEURONS.** **K. A. Cunningham and J. M. Lakoski. Dept Pharmacology, Univ Texas Med Branch, Galveston, TX, 77550.**

Biochemical research suggests that, in addition to its ability to stimulate catecholamine neurotransmission, cocaine also alters central 5-HT function (Ross & Renyi, EJP 7: 270, 1969). Therefore, we have begun to explore the effects of systemic and microiontophoretic application of cocaine on the spontaneous activity of 5-HT neurons recorded extracellularly in the dorsal raphe nucleus (DRN) of the rat.

Systemic cocaine elicited a potent (cumulative ED_{50} = 0.65 mg/kg, i.v.), rapid (within minutes), and reversible inhibition of 5-HT cell firing in the chloral hydrate anesthetized rat; the suppression of firing was complete in all animals tested (N=15). In contrast, procaine (N=10) or lidocaine (N=5) did not significantly alter cell firing in cumulative doses up to 16-32 mg/kg (i.v.).

Microiontophoretic application of cocaine (HCl, 0.01 M, pH 4-4.5) produced a current-dependent depression of cell firing in 72% of cells responsive to 5-HT (creatinine sulfate, 0.04 M, pH 4-4.5; N=43). Interestingly, continuous application (2-16 min) of cocaine at low currents (1-8 nA), which initially did not alter firing, gradually produced a significant inhibition in cell firing (N=7). On the other hand, microiontophoretic application of procaine (0.01 or 0.04 M; pH 4-4.5) resulted in depression of firing in only 37% of the 5-HT-sensitive cells tested (N=19).

These results with procaine and lidocaine administration suggest that local anesthetic actions cannot solely account for the observed effects of cocaine. Moreover, the fact that cocaine inhibits 5-HT reuptake suggests that the ability of this drug to depress 5-HT activity is possibly related to a stimulation of autoreceptors in the DRN. Our microiontophoretic findings also suggest that neuropharmacological alterations underlying the suppression of 5-HT cell firing by systemic cocaine occur at the level of the 5-HT cell body. However, in addition to 5-HT, the relative importance of other neurotransmitters found in the DRN must also be considered.

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337.18 SEROTONERGIC DORSAL RAPHE NEURONS: DECREASED RESPONSIVENESS TO 5-MeODMT FOLLOWING LONG-TERM AMPHETAMINE TREATMENT. B.A. Heidenreich* and G.V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Amphetamine suppresses the firing rate of dopaminergic neurons in the substantia nigra and ventral tegmental area. During long-term treatment, this effect diminishes significantly in both sites and often switches to an excitation. In addition, amphetamine pretreatment accelerates the spontaneous activity of these neurons and attenuates their response to challenge injections of a direct-acting autoreceptor agonist. Taken together, these data suggest that long-term amphetamine treatment reduces the sensitivity of dopamine autoreceptors.

We have begun a comparable series of investigations on serotonergic neurons in the dorsal raphe nucleus (DRN). In our initial report, we demonstrated a comparable decline in responsiveness to amphetamine with long-term treatment, although the pretreatment dose required to produce this effect was higher in the DRN than in dopaminergic nuclei (Heidenreich et al., Soc. Neurosci. Abstr., 45:11, 1985). We now report that, like dopaminergic neurons, serotonergic cells lose their sensitivity to a direct-acting autoreceptor agonist. In these experiments, rats were pretreated with saline or 10.0 mg/kg d-amphetamine twice daily for 6 days and then challenged 12 hr later with increasing incremental doses of 5-MeODMT. Whereas saline pretreated rats were extremely sensitive to 5-MeODMT (8 µg/kg or less produced a clear suppression), amphetamine pretreatment significantly attenuated this response and, in some cases, produced an excitation. Spontaneous activity in the DRN also tended to be elevated by amphetamine pretreatment compared to saline controls. It appears, therefore, that a common effect of multiple amphetamine injections in both dopaminergic and serotonergic nuclei is a reduction in autoreceptor sensitivity.

337.19 MODULATION OF SUBSTANTIA NIGRA DOPAMINE NEURONS BY SEROTONIN 5HT-1 RECEPTOR LIGANDS. S.L.Fallon* and C.M.Sinton (SPON: J.A.Norman). Pharmaceuticals Divn., Ciba-Geigy Corp., Summit, NJ 07901.

Recent data show that there is a high density of serotonin 5HT-1B binding sites in the substantia nigra (SN) pars reticulata (Pazos et al., Br. Res. 346:205, 1985). This finding, in conjunction with a known serotoninergic innervation from the dorsal raphe nucleus (DRN) to the SN, suggest that putative ligands at 5HT-1 sites, which affect the firing rate of DRN 5HT cells, are likely to modulate the impulse activity of SN dopamine (DA) cells. The present study examined this possible modulation and evaluated differences between ligands at the two subtypes of the 5HT-1 site, namely the 5HT-1A and 5HT-1B sites. Rats were anaesthetized with chloral hydrate and a glass microelectrode was lowered stereotaxically to the SN. Spontaneously active DA cells were recorded extracellularly and identified on-line; the location of the recording site was later verified histologically. Baseline firing rates were established before test agents were administered intravenously and an ED50, the dose which reduced the spontaneous firing rate by 50%, was calculated from a mean dose response plot from 5 separate experiments: one cell was recorded in each animal. TVX Q 7821 and 8-hydroxy-2-[n-dipropylamino]tetralin (8-OH-DPAT) were chosen as selective 5HT-1A ligands, and trifluromethylphenyl piperazine (TFMPP) and a novel pyrroloquinoxaline, CGS 12066B, as selective 5HT-1B ligands. TVX Q 7821 and 8-OH-DPAT showed variable excitatory and inhibitory effects, though at higher doses inhibition predominated: the ED50 for TVX Q 7821 was not determined as the inhibitory action plateaued at about 30%, and for 8-OH-DPAT the ED50 was about 1 mg/kg. These suppressant effects on firing rate could be reversed by haloperidol. In contrast, TFMPP and CGS 12066B showed consistent inhibitory actions with an ED50 of 190 µg/kg and 110 µg/kg, respectively, and these effects could not be reversed by haloperidol. These data show no correlation with the relative potency of the compounds to reduce DRN 5HT cell firing rate, and suggest that inhibition of a DRN input to the SN may only have relatively minor effects on SN DA impulse flow; for example in causing the excitation seen with low doses of the 5HT-1A ligands. The reversal by haloperidol of the inhibitory actions of the latter compounds on DA cells could be caused by indirect changes in the level of membrane polarization, or by an effect mediated through DA receptors. Significantly, however, these results also suggest that the putative 5HT-1B ligands tested have a direct, relatively potent, action on DA cells in the SN, possibly mediated from the SN pars reticulata. This finding could have impications for dopaminergic side effects of compounds targeted to modulate these 5HT receptors.

TRANSMITTERS AND RECEPTORS IN DISEASE II

338.1 NATURALLY OCCURING ANTI-ACETYLCHOLINE AND ANTI-ANTI-ACETYLCHOLINE IN MYASTHENIA GRAVIS. M.L Souan,M.Geffard,J.Vieillemaringe,P.Lebrun-Grandié and J.M.Orgogozo.Lab.of Neuroimmunology,IBCN/CNRS,Bordeaux 33077-France; Lab.of Neuroendocrinology UA-CNRS Université de Bordeaux I 33405 Talence Cedex France; Centre Hospitalier Universitaire Clinique Neurologique Bordeaux 33077 Cedex-France.

Although the humoral and cellular mechanisms in Myasthenia Gravis (MG) has been studied extensively, the triggering mechanism of the auto-immune process remains unclear. The anti-acetylcholine receptor (ACh-R) antibodies found in the sera of patients suffering from MG can undoubtably damage the neuromuscular junction but what role they play in the onset of the disease? The demonstration of other antibodies in sera of myasthenic patients in relation with idiotypic/anti idiotypic network interactions lead us to test the existence of anti bodies directed against another target, the conjugated ACh itself. Using an adapted ELISA method, we have detected in blood samples higher levels of anti-ACh antibodies in patients suffering from MG than control patients and patients suffering from other neurologic disorder (OND), Parkinson, Epilepsy and Multiple Sclerosis. These levels are usually 3 times higher than the ones found in control and OND patients. This indicates that anti-ACh antibodies occur and increase naturally as the disease progresses. In order to evaluate which antigen is best recognized by"natural anti-ACh antibodies",sera from myasthenic, control and OND patients were preincubated with ACh or analogs of ACh (choline, butyrylcholine, choline-poly-L-Aspartate, choline-poly-L-Glutamate, choline-succinic anhydride-BSA and choline glutaric anhydride-BSA) and tested with our ELISA method. The most immunoreactive compounds were choline-SA-BSA and choline-GA-BSA no matter what sera were used. The human antibody recognition takes place on antigen determinant mimicking ACh but only when it is conjugated. For sera from patients suffering from MG, displacement occurred between 10^{-8} and $5\ 10^{-6}$M, for the other sera between 10^{-8} and $5\ 10^{-4}$M Thus, a better expression of the disease may result through a simultaneous increase of antibody affinity and titer during the course of the disease. The existence of anti-anti-ACh in sera from myasthenic patients was also demonstrated. These immunoglobulins recognized both the antigen-combining site of human anti-ACh antibodies and the ACh-R. This anti-anti-ACh antibody recognition was evaluated with ACh-R from Torpedo Marmorata coated on well plates and with immunocytochemical applications on locust brain. The demonstration of 3 types of antibodies:anti-ACh, anti-anti-ACh and anti-ACh-R in sera of myasthenic patients suggests that the triggering mechanism of the onset of MG could be due to another target than ACh-R. It could be ascribed to either the increasing levels of conjugated ACh or a breakdown in the physiological regulation of the immune system (genetically determined) controlling the idiotypic/anti-idiotypic mechanisms.

338.2 INDUCTION OF EXPERIMENTAL MYASTHENIA IN MICE BY IMMUNIZATION WITH A SYNTHETIC PEPTIDE OF THE ACHR- A.R.Pachner*, F.Brooks*, F.S. Kantor*, P.Wilson*, I.L.Lentz, and F.Hawrot. Departments of Neurology, Medicine, Pharmacology, and Cell Biology, Yale Univ. School of Medicine, New Haven,CT 06510.

We multiply immunized C57Bl/6 mice with a synthetic peptide of the alpha-subunit of AChR from Torpedo californica. This peptide, which has previously been described by Wilson et.al. (Proc.Nat.Acad.Sci.82:8790,1985),corresponds to the sequence 173-204, and has been shown to specifically bind alpha-bungarotoxin. Sera from these mice contained a high titer of antibodies to the peptide as measured by a standard ELISA, as well as antibodies to AChR on the surface of BC3H1 cells; antibody to solubilized, purified Torpedo AChR could not be detected. However, sera from mice multiply immunized with Torpedo AChR had antibodies directed against the peptide.

We then tested the peptide-immunized mice for clinical weakness and defects in neuromuscular transmission as measured by electromyography(EMG). Weakness was detectable in the C57Bl/6 mice after the second and third immunizations and resembled the weakness seen in mice immunized with Torpedo californica. Significant decremental responses characteristic of myasthenia were seen in 83% of immunized mice. The EMG findings were identical to those found in humans with myasthenia and mice with experimental myasthenia: exacerbation with tubocurarine, faradization, and increased body temperature, and improvement with cholinesterase inhibitors.

This is the first demonstration of experimental myasthenia in the mouse induced by the immune response to a synthetic peptide of the acetylcholine receptor, and supports the hypothesis that the "myasthenogenic" immune response in human myasthenia could be induced by a limited number of epitopes of the AChR.

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338.3 MEASUREMENT OF IMMUNOREACTIVE ANGIOTENSIN II LEVELS IN MICRODISSECTED BRAIN NUCLEI OF THE DEVELOPING SPONTANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RAT. J.M. Meyer, D.L. Felten and J.A. Weyhenmeyer, Neural and Behavioral Biology Program and College of Medicine, University of Illinois, Urbana, IL, 61801; Department of Anatomy, University of Rochester School of Medicine, Rochester, NY, 14642.

The underlying cause for the development and maintenance of hypertension in the spontaneously hypertensive rat (SHR) is unknown, however several mechanisms have been proposed including changes in the brain renin-angiotensin system (RAS). The SHR brain shows a broader and more dense distribution of angiotensin II (ANG II)-like immunoreactivity than Wistar Kyoto (WKY) brain, including areas implicated in cardiovascular control (Weyhenmeyer and Phillips, Hypertens., 4:514, 1982).

In the present study, levels of immunoreactive ANG II were measured by radioimmunoassay in specific microdissected nuclei from the brains of newborn (NB-less than 1 week of age), 4-, 8- and 12-week old SH and WKY rats. The nuclei investigated were the paraventricular of the hypothalamus (PVH), nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus nerve (DMN of X), locus coeruleus (LC) and the A1 region of the medulla. A section of cerebellar cortex was used as a control. No difference in ANG II immunoreactivity between SHR and WKY was seen in any of the nuclei in the NB or 4-week old rats. At 8-weeks, the SHR brain had significantly higher ANG II immunoreactive levels in the PVH, NTS and DMN of X when compared to WKY, and at 12-weeks, the PVH, NTS, DMN of X and LC of the SHR brain contained significantly higher levels of immunoreactive ANG II than WKY rat brain. In both strains, ANG II immunoreactive levels increased in each nucleus except the LC, in which levels decreased as the animals aged. No detectable levels of ANG II immunoreactivity were found in the cerebellar cortex in either strain at any age. These data support previous findings from this laboratory that have shown neurochemical differences in ANG II levels between adult SH and WKY rat brain and a concomitant decrease in ANG II receptor levels in adult SHR brain.

The nuclei in this study have been implicated in the neural control of cardiovascular function. The differences in immunoreactive ANG II levels between hypertensive and normotensive strains during development suggests a possible role for ANG II in the pathogenesis of hypertension in the SHR.

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338.4 IS NEUROPEPTIDE Y INVOLVED IN THE PATHOGENESIS OF MAJOR DEPRESSION? - RELATIONSHIP TO MONOAMINE TURNOVER AND CORTICOTROPIN-RELEASING FACTOR. C. Wahlestedt*, R. Ekman, R. Håkanson*, M. Heilig*, E. Rosengren* and E. Widerlöv. Departments of Pharmacology, Psychiatry & Neurochemistry, University of Lund, Lund, Sweden.

The last decades has seen attempts to explain the pathogenesis of major depression in terms of a malfunction of monoaminergic neuronal systems. The recent discovery of a long list of neuropeptides possibly acting as neurotransmitters and/or as monoamine cotransmitters makes it attractive to try to expand the monoamine hypotheseses of depression to include some neuropeptides. The present study evaluates a possible role for neuropeptide Y (NPY) in this context. The CSF concentrations of NPY are elevated in patients with major depression. NPY occurs in a large number of central neurons, in some cases the peptide coexists with noradrenaline (NA) or adrenaline. In the present study we administered NPY intracerebroventricularly in the rat during one week by the use of osmotic mini-pumps. The NA concentrations were elevated in all brain areas. The concentrations of dopamine and its metabolite dihydroxyphenyl acetic acid were unaffected. The serotonin/5-hydroxy indole acetic acid ratio was increased in the hypothalamus. Finally, a connection between NPY and corticotropin-releasing factor (another neuropeptide being elevated in depressed patients) is proposed, because microinjection of NPY into the paraventricular nucleus elevates the plasma concentrations of ACTH and corticosterone in the rat.

338.5 CORTICOTROPIN-RELEASING FACTOR AND DEPRESSION: RELEVANCE OF ANIMAL EXPERIMENTS. R. Ekman, R. Håkanson*, C. Wahlestedt* and E. Widerlöv. Departments of Psychiatry & Neurochemistry and Pharmacology, University of Lund, Lund, Sweden.

Recently, elevated concentrations of corticotropin-releasing factor (CRF)-like immunoreactivity was found in the CSF of depressed patients (Nemeroff et al., Science 226:1342, 1984). This finding may add to our understanding of why a disturbed glucocorticoid homeostasis is a frequent observation in depression. The present study is an attempt to use the rat as an experimental model for research on possible relationships between CRF and depression. In one series of experiments rats were given saline or an antidepressant drug (imipramine or zimeldine twice daily for three weeks). By measuring CRF with a highly sensitive radioimmunoassay (detection limit 4 pg/0.1 ml; IC=50 pg/0.1 ml) in various brain regions it was noted that the CRF concentration in the hypothalamus was lowered in the imipramine- and (less pronounced) in the zimeldine-groups as compared to the salinegroup. CRF was measurable also in other brain areas, but these CRF systems seemed unaffected by the antidepressants. Bulbectomy, which is a fairly well established rat model of depression, did not appear to affect the levels of hypothalamic CRF, although bulbectomized rats have elevated plasmacorticosterone concentrations (Cairncross et al., Psychoneuroendocrinology 4:253, 1979).

338.6 ANTIMYOCLONIC ACTION OF MILACEMIDE IN p,p'-DDT-INDUCED MYOCLONUS IN MICE. C. Hunter*, E. Chung, and M.H. Van Woert, Graduate Program in Neurobiology, Departments of Neurology and Pharmacology. Mount Sinai School of Medicine, New York, NY 10029

The insecticide p,p'-DDT (200 to 600 mg/kg, p.o.)produces generalized, irregular, stimulus-sensitive myoclonus in mice and rats. p,p'-DDT-induced myoclonus in rodents is an excellent animal model for L-5-hydroxytryptophan(L-5HTP)-responsive human intention myoclonus. We previously reported that p,p'-DDT-induced myoclonus in mice is reduced by L-5HTP concomittantly with 5HT uptake inhibitor fluoxetine, other 5HT agonists and clonazepam. Recently, we demonstrated that local stereotaxic unilateral infusions of p,p'-DDT into the medullary reticular formation (MRF) produced generalized, stimulus-sensitive myoclonus similar to that produced by oral administration of p,p'-DDT(Exp. Neurol.85:273, 1984). p,p'-DDT causes repeated neuronal firing in response to a single stimulus which may explain its ability to produce a hyperexcitable state in the MRF. Injections of the glycine receptor antagonists strychnine or brucine into the same MRF sites produced similar myoclonus, while injections of picrotoxin or bicuculline,GABA receptor antagonists were without effect. Therefore we have proposed that MRF neurons may be under glycinergic inhibitory control and that the blockade of this inhibition by strychnine or brucine results in a hyperexcitable foci which is manifested as myoclonus. Based on these observations we were interested in determining whether pharmacological agents which could enhance glycinergic neurotransmission might have antimyoclonic action.

Milacemide(2-pentlyamino-acetamide) is a glycinamide derivative which has been shown to have anticonvulsant actions in a number of animal models of seizures(Arch. int. Pharmacodyn. Ther. 266:239, 1983). In addition to increasing brain glycine levels, milacemide has been shown to potentiate tryptophan-induced behavioral changes. We therefore investigated the effect of milacemide against 500 mg/kg p.o. p,p'-DDT induced myoclonus in mice. Milacemide (300 mg/kg p.o. x 3, every 2 hours) treatment almost completely antagonized myoclonic activity. Pretreatment of mice with p-chlorophenylalanine (400 mg/kg i.p. 24 hours prior) did not alter antimyoclonic action of milacemide.

These results suggest that as opposed to L-5HTP which may act in the Inferior Olive (Neurosci. Lett.24:103-108, 1981), the glycine derived from milacemide could be directly inhibiting reticulospinal neurons which participate in the reticular-reflex myoclonus mediated by DDT.

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338.7 STIMULATION OF CALCIUM UPTAKE IN NEURAL CELLS BY THE ALKYL-ETHER PHOSPHOLIPID PLATELET ACTIVATING FACTOR(PAF). E. Kornecki, D.H. Hardwick*, J. Bergdahl*, R. H. Lenox and Y. H. Ehrlich The Neuroscience Research Unit, Dept. of Psychiatry, University of Vermont, Burlington, VT 05405

Platelet activating factor (PAF) is a naturally occuring phospholipid (1-0-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine). PAF serves a unique function as an extracellular messenger in cell-cell communication in a variety of systems. In addition to being a potent activator of platelets, PAF is known to activate other cells, such as smooth muscle. Our previous studies (Kornecki, et al., Science, 226:1454-56, 1984) have suggested that PAF may play a role in neuronal function. To test this possibility we have examined in the present study the effects of PAF on two clonal cell lines derived from neural tissue; the neuroblastoma X glioma hybrid NG108-15, and the glioma clone C6BU-1. Cells of the clone NG108-15 were grown in culture in a serum-free, chemically defined medium (CDM), as described (Ehrlich et al., Nature, 320:67-70, 1986). Under these growth conditions there is some spontaneous morphological differentiation of the cells (ibid). Addition of PAF significantly accelerated the rate of neurite extension by NG108-15 cells. In CDM, enhancement of neurite extension was seen with as little as 50nM PAF, and maximal effects were measured with 2.5uM PAF. Higher PAF concentrations (>3uM) were cytotoxic to NG108-15 cells grown in CDM. These neurotoxic effects were concentration- and time-dependent, suggesting the initiation of programmed-cell death. To examine the possible mechanisms involved, we have measured the effects of PAF on the level of free calcium ions in intact cells. This was done using the bioluminescent probe aequorin, and measuring aequorin signals with a Platelet Ionized Calcium Aggregometer (PICA, Chrono-Log Corp., PA) at 37°C with cells stirred at 300 RPM. Aequorin was loaded into NG108-15 and C6BU-1 cells transiently permealized by treatment with 5mM ATP in the presence of EDTA. PAF produced a concentration-dependent (0.5-10uM) increase in free ionized calcium inside the cells. This increase was completely blocked by 1mM EGTA, indicating that PAF induces an influx of calcium ions. The results suggest that low PAF concentrations may play a role in neural development, whereas higher concentrations of PAF may contribute to irreversible damage induced in the nervous system by trauma and stroke. The mechanism underlying these effects may involve increased uptake of extracellular calcium ions.

338.8 PERINATAL CEREBRAL HYPOXIA-ISCHEMIA DISRUPTS EXCITATORY NEUROTRANSMITTER RECEPTORS. F. Silverstein*, L. Torke*, J. Barks*, R. Chen*, M.V. Johnston. University of Michigan Medical School, Ann Arbor, MI 48109.

The role of excitatory neurotransmitter release in the pathogenesis of ischemic neuronal injury in immature brain is unknown. In adult stroke models, excessive release of glutamate(GLU) in regions with high densities of GLU receptors may contribute to neuronal vulnerability. Large numbers of GLU receptors are present in the immature brain but their distribution & pharmacology differ from that in mature animals (Greenamyre et al Soc. Neurosci Abstracts 10:225 1984). Although kainate is nontoxic in the immature brain, we found that intra-cerebral quisqualate injection (100 nmol/ul) causes necrosis in striatum & hippocampus in 7 day old rats, suggesting that GLU receptors mediate neuronal injury. To examine the relationship between GLU receptors & selective neuronal damage we used 7 day old rats subjected to unilateral carotid artery ligation(UCL) & exposure to 2.5 hrs of 8% oxygen. This leads to cerebral ischemic injury in forebrain on the side of UCL. We used quantitative in vitro autoradiography with 3H-GLU to measure changes in receptor distribution during 24 hrs after injury. The distribution of cholinergic receptors (3H-QNB) was examined in adjacent sections. Acute ischemic cell damage was assessed by Nissl stain. Side to side differences in 10 regions were compared in lesioned animals & controls. Both GLU & QNB receptors were vulnerable in a regionally selective fashion, but GLU receptors in DG were disrupted especially early & severely. 1 hr after hypoxia few changes were apparent; GLU binding was reduced on the side of UCL only in dentate gyrus(DG) (18±5%, n=7, $p < .01$, t-test) while QNB binding was preserved. Over 24 hrs we found progressive selective reductions in GLU binding ipsilateral to UCL. At 24 hrs, 3H-GLU binding was reduced maximally in DG. (-50±14%, n=15, $p < .005$). Smaller but significant reductions were noted in CA1 (-23%), CA3 (-26%), caudate (-10%), globus pallidum (-15%). At 24 hrs QNB binding was also reduced ipsilateral to UCL: -40% in DG, CA1 & CA3 & -30% in caudate. Histological damage & chronic neuronal changes were observed in regions with extensive receptor loss. Our results support the hypothesis that ischemic release of excitatory neurotransmitters causes selective neuronal injury in the developing brain.

338.9 OXIDATIVE STRESS IN RABBIT RETINA AND LENS FOLLOWING MPTP/MPP^+ TREATMENT. C. G. Wong, T. W. Bruice*, F. Robertson*, and S. Hari*. Dept. Ophthalmology, USC School of Medicine, Los Angeles, CA 90033; Dept. Pathology, UCLA School of Medicine, Los Angeles, CA 90024; Allergan Pharmaceuticals, 2525 Dupont Drive, Irvine, CA 92715.

Oxidative metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by MAO B in brain tissues produces the actual neurotoxic species, 1-methyl-4-phenylpyridinium ion (MPP^+). Oxidative stress induced by MPP^+ to form damaging reactive oxygen species has been suggested as the primary mechanism in producing neuronal cell death. Detoxification of reactive oxygen species such as hydrogen peroxide can occur by reaction with glutathione (GSH) and GSH peroxidase. This process increases production of glutathione disulfide (GSSG). Measuring levels of GSH/GSSG is a method for identifying oxidative stress in vivo. Since catecholamine metabolism in the pigmented rabbit retina is perturbed by MPTP which also appears to damage specific retinal cells (Exp. Eye Res. 40, 509, 1985), further studies were initiated to determine the level of retinal MAO activity, clearance of retinal MPTP, and possible generation of MPP^+. Whether modulation occurs in retinal and lens GSH/GSSG levels after intraocular MPTP/MPP^+ administration was also studied.

MAO activity in the rabbit retina is 11.2 ± 0.86 nmoles tryptamine oxidized/mg protein/hr so that retinal generation of MPP^+ from MPTP is possible. After intraocular (IO) administration of 7.15 nmoles, MPTP could not be detected in the retina 24 hrs later. At that time a peak appeared with an R_f identical to MPP^+. However, MPP^+ could not be detected following incubation of retinal homogenate (1.68 mg protein/ml) with 0.1 mM MPTP. IO injection of 168 nmoles MPP^+ resulted in total retinal necrosis. Cataract formation was also noted in some of the animals.

Retinal GSH levels as measured by HPLC EC detection averaged 45 nmole/mg protein. Current work is underway to determine if a diurnal rhythm in retinal GSH/GSSG levels is present. Data on the modulations of retinal GSH/GSSG after MPTP treatment and of lens GSH/GSSG after both MPTP and MPP^+ administration will be presented.

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339.1 ACETYLCHOLINESTERASE HISTOCHEMISTRY IN THE CEREBRAL CORTEX OF ALZHEIMER'S DISEASE. H. Tago*, P.L. McGeer and E.G. McGeer (SPON: B.R. Sastry). Kinsmen Laboratory of Neurological Research, Dept of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada

Recently, we have demonstrated clear fiber tracts and cells in human post-mortem brains by a new, sensitive histochemical method for acetylcholinesterase (AChE). We report pathological changes of AChE positive structures in the cortex of Alzheimer's brains as compared with controls. Brains of 6 Alzheimer's patients and 3 controls were sampled for biochemical assays and then perfused and postfixed overnight. Frozen sections were stained serially for AChE, modified Bielschowsky and cresyl violet. For AChE histochemistry, free floating sections were incubated in a Karnovsky and Roots' medium diluted 1:100 with maleate buffer, pH 6.0. Reaction products were visualized by intensification with 0.03% diaminobenzidine, 0.3% nickel ammonium sulfate and 0.005% H_2O_2 in Tris buffer (pH 7.4).

By biochemical assay, the Alzheimer's brains had 22% as much ChAT and 43% as much AChE activity in the cortex (average of 7 samples) as the normals. In the cortices of control brains, the AChE positive axons, were mostly perpendicular to the cortical surface and showed clear laminar differentiation with frequent branching and regularly placed small varicosities. In most cases, there were also some positively staining pyramidal neurons mainly in layer III with occasional small positively staining neurons in all layers. Only the cell bodies and proximal dendrites of these neurons were stained. There was a remarkable reduction in AChE-positive fibers in the frontal and temporal lobes in Alzheimer's brains, similar to that seen in rats with electrolytic lesions of the basal forebrain. Such a correlation would suggest that cortical AChE-positive fibers are largely derived from basal forebrain cholinergic neurons. A difference between lesioned rat and Alzheimer's brains, however, was that in Alzheimer's disease the residual AChE-positive fibers also showed pathological changes with disruptions and swellings. Serial sections stained by the Bielschowsky method revealed that these corresponded with classical senile plaques of various sizes. AChE-positive staining tended to be lower in the larger plaques. Developing AChE positive plaques were especially seen in layer I where multiple small plaques were occasionally arranged on a single axon. The results suggest: 1) plaques develop initially from swellings on impaired AChE axons with, usually, multiple sites on a single axon; 2) the altered axon fiber disappears; 3) the plaque continuously expands with material spreading irregularly from the core giving the appearance of an exploding halo.

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339.2 INVOLVEMENT OF THE CHOLINERGIC PONTOMESENCEPHALIC TEGMENTUM IN ALZHEIMER'S DISEASE. R.W. Jacobs, N.J. Woolf, M.C. Hernit*, and L.L. Butcher. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Patients diagnosed with dementia of the Alzheimer type (DAT) consistently demonstrate reduced levels of synthetic and degradative cholinergic enzymes (see Terry and Davies, Ann. Rev. Neurosci., 3: 77, 1980). Correlated with these biochemical reductions, marked cell loss has been noted in the basal forebrain of DAT brains (Price et al., Neurosci. Comm., 1: 84, 1982). Another large group of cholinergic projection cells is found in the pontomesencephalic tegmentum (Woolf and Butcher, Brain Res. Bull., 16: 1986), and we have examined these cholinergic cells, which lie in the pedunculopontine tegmental nucleus (PPT) and the dorsolateral tegmental nucleus (DLT), in acetylcholinesterase (AChE), cresyl violet, and thioflavin-S stained brain sections from demented and normal individuals.

Tissue sections from three DAT brains were analyzed. Cell counts made on AChE-stained sections and compared with those from adjacent Nissl-stained sections revealed that putative cholinergic cells in the PPT and DLT of one of the DAT patients were reduced to approximately 30% of age-matched control cell numbers. The other two DAT brains, as well as brains from two Parkinson's disease dementia patients and one multi-infarct dementia patient, showed little or no cell loss of cholinergic somata in the PPT and DLT. Nonetheless, neuropathologic profiles were more prevalent in the three DAT brains than in brains from patients who were normal or had other types of dementia. Numerous neurofibrillary tangles and/or plaque-like lesions were observed in the PPT of the DAT brains, whereas fewer or none of these neuropathological entities were found in the other brains.

These results suggest that, in addition to the basal forebrain cholinergic system, the cholinergic pontomesencephalic tegmentum may be affected in DAT, although less consistently, as indicated by reduced cell numbers in one affected individual and the increased occurence of neurofibrillary tangles and plaque-like lesions that were evident in all three cases of DAT.

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339.3 CHOLINERGIC AND SOMATOSTATINERGIC NEURITES ARE ASSOCIATED WITH SENILE PLAQUES IN THE SUBSTANTIA INNOMINATA OF ALZHEIMER'S DISEASE PATIENTS. E. Gould*, N.J. Woolf, J.L. Williams*, G. Bruce*+, L.B. Hersh+, and L.L. Butcher. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024 and +Departments of Biochemistry, Physiology and Psychiatry, University of Texas Health Science Center, Dallas, TX 75235.

Senile plaques found in the substantia innominata of patients diagnosed with dementia of the Alzheimer type (DAT) (Jacobs et al., Neurosci. Lett., 56: 347, 1985) and their associations with cholinergic and somatostatinergic neurites were studied immunohistochemically (avidin-biotin peroxidase method) by use of antibodies against choline acetyltransferase (ChAT) and somatostatin (SRIF) combined with thioflavin-S histology for amyloid.

The vast majority of senile plaques in the substantia innominata of DAT brains had ChAT and SRIF neurites embedded in the amyloid mass. Many of the SRIF processes found in the substantia innominata plaques could be traced to SRIF cell bodies. Typically, senile plaques were observed 25-100 µm from SRIF somata. A few ChAT-containing neurites could also be traced to their parent cell bodies.

In contrast to the relationships observed in the substantia innominata, senile plaques found in the amygdala, hippocampus, and frontal and temporal cortices of the same DAT brains were less often associated with ChAT- or SRIF-immunoreactive neurites. Amyloid deposits, as visualized with thioflavin-S, were larger in diameter and more numerous in the amygdala, hippocampus, and cerebral cortex than in the substantia innominata. Additionally, a large number of cells in the substantia innominata were immunoreactive for SRIF, and these neurons exhibited intense immunostaining, whereas fewer SRIF-positive cells were detected in the amygdala, hippocampus and cerebral cortices, and they demonstrated less intense SRIF immunoreactivity.

These results indicate that a greater proportion of senile plaques in the substantia innominata of DAT brains contain ChAT- and SRIF-immunoreactive neurites than do senile plaques in the amygdala, hippocampus, and cerebral cortices of the same brains.

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339.4 NUCLEUS BASALIS MAGNOCELLULARIS (nBM) LESIONS INCREASE CORTICAL SOMATOSTATIN LEVELS WHILE INDUCING CORTICAL CHOLINERGIC HYPOFUNCTION. G.W. Arendash, E.M. Meyer, W.J. Millard, and A.J. Dunn. Division of Physiology and Development, Dept. of Biology, Univ. of South Florida, Tampa, FL 33620 and Depts. of Pharmacology, Pharmacodynamics, and Neuroscience, Univ. of Florida, Gainesville, FL 32610.

Although deficits in cortical cholinergic markers are consistently found in senile dementia of the Alzheimer's Type (SDAT), a dysfunction of cortical somatostatinergic neurons also appears to biochemically characterize the disease. In this context, decreases in cortical somatostatin levels and receptor numbers have both been reported in SDAT. Since nucleus basalis of Meynert (NBM) cholinergic neurons (which normally provide most of the cholinergic innervation to the cortex) degenerate or become dysfunctional in SDAT, excitotoxic lesions of the analogous rat nBM were performed to determine if a loss of cortical cholinergic innervation is involved in the cortical somatostatin dysfunction of SDAT.

Adult male Sprague-Dawley rats were given bilateral infusions of ibotenic acid (5 ug/1 ul) into the nBM and sacrificed ten months later. Cortical somatostatin levels were dramatically increased at 10 months in nBM-lesioned animals compared to sham-lesioned control values (748 ± 77 vs. 362 ± 52 pg/mg tissue). In the same lesioned animals, cortical cholinergic markers were universally decreased (choline acetyltransferase activity down 42%, acetylcholine synthesis down 32%, and high affinity choline uptake down 37%) compared to values in sham-lesioned control animals. In contrast, no significant effects of nBM lesioning on cortical norepinephrine markers (NE, MHPG), dopamine markers (DA, DOPAC, HVA), or serotonin markers (5-HT, 5-HIAA) were found at 10 months after lesioning.

Since acetylcholine is a powerful releaser of cortical somatostatin, these biochemical results suggest that, in SDAT, a dysfunction of cortical somatostatinergic neurons is secondary to a loss of cholinergic innervation to the cortex from the basal forebrain. Additionally, nBM-lesioned animals were memory deficient on a variety of tasks and exhibited significant neuronal losses and SDAT-like neuropathological changes within the cortex (see abstract by Panzarino, Arendash, Mouton, and Gonzalvo). The combined data strongly suggest that a dysfunctional "NBM to cortex" cholinergic pathway is a prime factor in the pathogenesis of SDAT.

339.5 **HIPPOCAMPAL NMDA RECEPTORS IN ALZHEIMER'S DISEASE.** J.W. Geddes and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

A major site of Alzheimer's disease (AD) neuropathology is the hippocampal region, including the subiculum and entorhinal cortex. The neuronal loss in these areas is postulated to functionally disconnect the cortex from the hippocampus, providing a plausible explanation for some of the memory deficits in AD. The N-methyl-D-aspartate (NMDA) subclass of excitatory amino acid receptors are involved in memory formation and in excitotoxin mediated neuronal degeneration. Their status in Alzheimer's disease may therefore indicate (i) the functional capacity of pathways involved in memory (long term potentiation), and (ii) the possible involvement of excitotoxic mechanisms in AD-related neurodegeneration.

We used quantitative in vitro autoradiography to examine the hippocampal distribution and density of NMDA displaceable L-^{3}H-glutamate binding sites which correspond to NMDA receptors (Monaghan and Cotman, J. Neurosci. 5, 2909, 1985). Hippocampal samples were obtained postmortem from 5 neurologically normal and from 5 Alzheimer's disease patients. Postmortem delays ranged from 1.5 to 30h. L-^{3}H-glutamate binding was stable for up to 72 h postmortem. Binding was quantitated in CA3 and CA1 of stratum radiatum and stratum pyramidale, in the inner and outer molecular layers of the dentate gyrus, and in the hilus, stratum oriens, stratum lacunosum-moleculare, and subiculum.

We observed no significant changes in the distribution or density of hippocampal NMDA receptors in Alzheimer's disease. This contrasts to the reported decrease of NMDA receptors in cortical regions (Greenamyre et al., Science 227, 1496, 1985). It appeared that there was a correspondence between the distribution of NMDA receptors and the predilection to AD-related neuropathology in hippocampal subfields. Areas with a high density of NMDA receptors included CA1, subiculum, and outer layers of the entorhinal cortex, all of which show neurofibrillary and granulovacular degeneration in AD. In addition, the dentate gyrus molecular layer, also rich in NMDA receptors, has a predilection for neuritic plaques. The strong correlation of Alzheimer's disease pathology with NMDA receptor density suggests that excitotoxic mechanisms might contribute to the progressive pathogenesis of this disease. Furthermore, in hypoxia, ischemia, hypoglycemia, and epilepsy, NMDA receptor antagonists have been shown to protect against neurodegeneration. It is possible that such agents may eventually have therapeutic value in Alzheimer's disease.

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339.6 SELECTIVE CHANGES IN MU, DELTA AND KAPPA OPIOID RECEPTOR BINDING IN CERTAIN LIMBIC REGIONS OF THE BRAIN IN ALZHEIMER'S DISEASE PATIENTS. J.M. Hiller*, Y. Itzhak* and E.J. Simon. Depts. of Psychiatry and Pharmacology, New York University Med. Ctr., New York, NY 10016.

The total opioid binding levels and levels of the three major types of opioid binding sites were measured in homogenates of various limbic structures from post-mortem brains of Alzheimer's Disease patients and age-matched control individuals. The most consistent finding in Alzheimer's Disease brains was an increase in kappa binding in all six areas of the limbic system examined, with the putamen and caudate regions showing significant increases of 114% and 53%, respectively. In addition, the Alzheimer's Disease putamen showed a significantly higher level of total binding (85% increase). The amygdala of Alzheimer's Disease patients exhibited significantly lower levels of mu and delta binding (41% and 55% decrease, respectively). Total binding and binding to mu and delta receptors in frontal cortex, caudate and hippocampus of Alzheimer's Disease brains was indistinguishable from levels seen in these brain areas from control individuals. It is suggested that the increase in kappa sites could be the result of upregulation due to a decrease in dynorphin, the putative endogenous kappa ligand.

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339.7 EFFECTS OF ESTRADIOL, AF64A, AND POST-MORTEM TREATMENTS ON RAT BRAIN HC-3 BINDING AND ACH SYNTHESIS. R.D. Hautamaki, C. O'Malley*, M. Kelley, G. Arendash, and E.M. Meyer. Department of Pharmacology, Univ. Florida, Gainesville, FL, 32610 and Department of Biology, Univ. South Florida, Tampa, FL.

The loss of functional cholinergic innervation of the cerebral cortex remains one of the earliest neurochemical deficits associated with Alzheimer's disease. [^{3}H]-Hemicholinium-3 is a potential marker for cholinergic terminals in this tissue, since it binds in a saturable, high affinity (Kd = 2-7 nM) manner to choline uptake sites that are specific for cholinergic neurons and rate limiting for ACh synthesis. To determine the potential usefulness of this ligand for diagnosing cholinergic hypofunction clinically, we studied [^{3}H]-hemicholinium-3 binding in rat cerebral cortical membranes after: 1) several treatments that we recently found to decrease (ovariectomy, AF64A) or increase (estradiol) ACh synthesis in this tissue; and 2) several post-mortem treatments to which human tissues are exposed (slow cooling and repeated freeze-thaw procedures). Intracerebral cortical injections of AF64A (2, 1.0 ul bilateral infusions of 1 nmol/ul/hemisphere) reduced high affinity choline uptake and [^{3}H]-hemicholinium-3 binding in this tissue by 42% and 40% when assayed 3 weeks later. Since it has been suggested that post-menopausal women may be more likely to exhibit Alzheimer's disease symptoms, we also studied the effects of ovariectomy and estradiol replacement on cortical cholinergic activity. Ovariectomy reduced cerebral cortical high affinity choline uptake and ACh synthesis by over 50% when measured at least 30 days later; these cholinergic deficits returned to normal after 3 injections of 10 ug/kg of estradiol benzoate, administered subcutaneously every other day. However, no change in newly synthesized [^{3}H]-ACh release was observed with these hormonal alterations. Further, preliminary evidence shows no change in [^{3}H]-hemicholinium-3 binding with these hormonal alterations. Conceivably, AF64A reduces the number of functional cholinergic transporters while estradiol affects the affinity of the transporter for choline in a manner that is not reflected by changes in hemicholinium-3 binding. Slow cooling of intact rat brains, at a rate similar to that of human brain cooling after death at room temperature had no effect on high affinity [3H]-hemicholinium-3 binding for up to 5 hours. One or two freeze-thaw cycles, however, reduced binding by 14% and 45%, respectively. These results suggest that labelled hemicholinium-3 binding may be useful for post-mortem clinical analyses of cholinergic function under some conditions. They also suggest that steroidal hormones may affect the synthesis of ACh in the cerebral cortex to an extent not previously described.

339.8 CHANGES IN GABA-A AND GABA-B RECEPTORS IN HIPPOCAMPUS OF ALZHEIMER'S INDIVIDUALS. DCM Chu, JB Penney and AB Young. Dept. of Neurology and Neuroscience Program, Ann Arbor, MI 48104.

In dementia of the Alzheimer's type (DAT), there is marked cell loss in hippocampal CA1 field, subiculum and entorhinal cortex with relative sparing of CA3 neurons and presubiculum (Hyman, B.T. et al., Science 225:1168, 1984). We have examined the status of GABA-A and GABA-B receptors in hippocampi from 6 individuals diagnosed with DAT upon autopsy and 6 age-matched controls without neurologic disease, using a quantitative autoradiographic method with 3H-GABA as previously described (Chu, D.C.M. et al., Abstr. Soc. Neurosci. 11:725). Saturation binding studies in serial hippocampal sections were achieved by the isotopic dilution of 3H-GABA with nonradioactive GABA to yield a range of 25-1025 nM free GABA. Bound values in pmol/mg protein were obtained densitometrically. Scatchard plots of 3H-GABA binding revealed changes in the Bmax and not in the K_D for both GABA-A and GABA-B receptors as follows:

Receptor	Region of Interest	Bmax(controls)	Bmax(DAT)	%Decline
GABA-A	stratum pyramidale of CA1	3.499±0.421 pmol/mg protein	1.711±0.561*	51%
GABA-B	stratum moleculare of dentate gyrus	1.094±0.154	0.592±0.122*	46%
	stratum lacunosum/ moleculare of CA1	0.846±0.099	0.389±0.074**	54%
	stratum pyramidale of CA1	1.047±0.138	0.343±0.118**	67%

*p<0.05, **p<0.01 by 2-tailed unpaired Student's t-test

The decrease in GABA-A receptor density in stratum pyramidale of CA1 may reflect loss of neurons in CA1, since GABA-A receptors are thought to exist on cell bodies of pyramidal neurons in this region. GABA-B receptors are thought to be presynaptic on terminals of certain proposed glutamatergic pathways. For instance, the CA3 Schaeffer collateral/commissural fibers terminating on CA1 neurons, and the medial perforant pathway terminating in stratum moleculare of the dentate gyrus are both sensitive to the GABA-B agonist, baclofen (Ault, B. and Nadler, J.V., Br. J. Pharmacol. 80:211, 1983). Loss of cells in entorhinal cortex may account for the decline in GABA-B receptors in stratum moleculare of dentate gyrus and in stratum lacunosum of CA1. The losses of GABA-B receptors in CA1 stratum pyramidale are less clear since terminals in CA1 arise from the relatively spared CA3 neurons. Perhaps some GABA-B receptors are located postsynaptically in CA1, or CA3 terminals degenerate with the loss of their target neurons. Supported by USPHS grant NS 15655.

340.1 PHYSIOLOGICAL AND MOLECULAR MEASURES OF ALPHA$_2$ ADRENERGIC RECEPTOR SENSITIVITY IN DEPRESSED PATIENTS DURING ANTIDEPRESSANT TREATMENT. N. Wolfe, A.J. Gelenberg*, B.M. Cohen*. Harvard Univ., Dept. of Psychology, Cambridge, MA 02138.

Mechanisms of antidepressant action were studied using molecular and physiological studies of alpha$_2$ adrenergic receptors in patients with Major Depressive Disorder (MDD) before and after treatment. Patients participated in double-blind trials of imipramine (2.5 mg/kg/day), tyrosine (100 mg/kg/day) or placebo, for four to six weeks. ^{3}H-yohimbine binding to platelet membranes was performed using l-epinephrine as the displacer. Binding was rapid, saturable, highly specific ($\bar{X}$ = 68%), and selective for the alpha$_2$ adrenergic subtype of receptor. No treatment appeared to significantly change alpha$_2$ receptor characteristics as indicated by receptor number (B_{max}) ($F(2,23)=.68$, $p=.52$), ($\log(B_{max})$)($F(2,23)=.56$, $p=.58$) or receptor affinity (K_d) ($F(2,23)=.92$, $p=.42$).

Alpha$_2$ adrenergic receptors were also studied using clonidine-induced hypotension and sedation before and during antidepressant therapy. Clonidine-induced hypotension did not differ significantly following treatment: $F(2,11)=.198$, $p=.82$. Repeated measures analysis of variance revealed no evidence of desensitization in a more detailed examination of diastolic and systolic, lying and seated blood pressure. Clonidine-induced sedation, extracted as a factor-based score from the Visual Analogue Mood Scale, also did not differ following treatment. No measure of alpha$_2$ adrenergic receptor sensitivity was significantly correlated with clinical improvement (change in Hamilton score). However, in the seven patients who participated in both studies, centrally-mediated changes in blood pressure were highly correlated with changes in platelet receptor binding. Mean blood pressure changes were correlated with changes in receptor number (B_{max})($R=.69$, $p=.05$) and receptor affinity (K_d)($R=.80$, $p=.02$). Several factors contributing to these results are discussed.

It is concluded that alpha$_2$ desensitization may not be a major mechanism of action for imipramine or tyrosine. However, studies using two different measures of alpha$_2$ sensitivity (clonidine-induced hypotension and ^{3}H-yohimbine binding to human platelet membranes) might be examining similar underlying phenomena. Because these two separate measures were highly correlated, it is possible that they reflect underlying alpha$_2$ changes in the central nervous system.

340.2 PLATELET α_2-ADRENERGIC RECEPTORS IN DEPRESSED PATIENTS: THE EFFECT OF DESIPRAMINE AND ECT. E.S. Werstiuk, M. Steiner, S.E. Auffarth*, G. Bean. Departments of Neurosciences and Psychiatry, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada.

Supersensitivity of platelet α_2-adrenergic receptors in depression has been suggested, and treatment with tricyclic antidepressants and ECT has been reported by some investigators to decrease α_2-receptor numbers (Bmax) to values of normal controls. These findings however, are controversial. The present study was undertaken to assess the effect of treatment with desipramine (DMI) and ECT on platelet α_2-adrenergic binding parameters in depressed patients.

Subjects for this study were 20 acute psychiatric inpatients who met the Research Diagnostic Criteria for Major Depressive Disorder. 26 normal volunteers served as control subjects. Patients were assigned (on a clinical basis) to treatment with DMI or ECT. Platelet α_2-adrenergic receptor binding was measured pre-treatment and 28 days into DMI treatment; and 48 hours after successive ECTs, as well as 14 days after the last ECT. Platelet membranes were isolated from intact platelets prepared under physiological conditions[1]. ^{3}H-Clonidine (0.1 - 32 nM), an α_2-agonist was selected as ligand, and specific binding was defined as the difference between total bound radioactivity and that not displaced by excess cold clonidine (6.4×10^{-5}M). K_D and Bmax were calculated by the computer program "Ligand"[2]. The values in untreated patients were (Mean $\pm$ S.Dev.) K_D: 1.87 $\pm$ 1.54 nM and Bmax: 156 $\pm$ 88 fmoles/mg protein. Long-term (28 days) treatment with DMI had no significant effect on these parameters K_D: 1.79 $\pm$ 1.30 nM and Bmax: 153 $\pm$ 79 fmoles/mg protein. In control subjects, we found values of K_D: 1.74 $\pm$ 1.24 and Bmax: 141 $\pm$ 72 fmoles/mg protein which were not different from those observed in the patients. Successive ECT treatments resulted in a gradual increase in Bmax values with no significant effect on the K_D.

Our data do not support the proposal that there is a dysfunction of the high affinity state (α_2H) of the platelet α_2-receptor in depression. Similarly, results obtained following long-term DMI and ECT are not consistent with a treatment induced down-regulation of platelet α_2-receptors. Our findings, however, do not exclude the possibility that the functional state of the α_2-receptor may be altered in depression. (Supported by the St. Joseph's Hospital Foundation, Hamilton, Ontario. E.S.W. holds a career award from the Ontario Mental Health Foundation.)

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2. Munson, P.J. and Rodbard, D., Anal. Biochem., 107: 220, 1980.

340.3 BLOOD ELEMENT ADRENERGIC RECEPTORS IN AFFECTIVE PSYCHIATRIC DISORDERS. E.L. Giller, S.M. Southwick* and B.D. Perry. Dept of Psychiatry, Yale University School of Medicine, New Haven, CT 06519 and West Haven VAMC, West Haven, CT 06523

Dysfunction of CNS monoamine systems (NE, 5-HT) has been proposed as a primary etiological factor in major depressive disorders. A variety of clinical and preclinical studies have suggested specific dysregulation of CNS adrenergic receptors (including β- and α_2-adrenergic) as a potential site of pathology. Parallel distribution of α_2- and β- receptors (R) in brain and blood elements (platelet α_2 and lymphocyte β) has allowed evaluation of blood element (BE) Rs using radioligand binding and other biochemical techniques in a variety of psychiatric populations, including major depressives (MDD). These studies, though somewhat inconsistent, indeed suggest differential BE-R regulation in MDD when compared to "controls". Other psychiatric disorders have "depressive" symptoms such as dysphoria, anhedonia, anergy and somatic complaints (e.g., borderline personality disorder, post-traumatic stress disorder, chronic pain syndrome) Within the clinical spectrum many of these disorders are classified as distinct entities yet some would suggest that they are variants of the same underlying pathological process. Indeed traditional antidepressant medications (ostensibly acting on the CNS monoamine systems) are efficacious in these disorders. The current studies were performed to assess potential similarities in BE adrenergic R parameters in these affective disorders relative to MDD and "control".

Subjects were in good physical health, met DSM III and SADS criteria for either MDD, Borderline personality disorder, Post traumatic stress disorder or were 'normal' healthy controls. Subjects were drug free for at least two weeks. Platelets and lymphocytes were isolated from 50 cc of whole blood using standard techniques. Membranes were prepared, washed extensively with EDTA containing buffers and radioligand binding assays were performed in PLTs using ^{3}H-rauwolscine (RAUW) (Eur.J.Pharm. 84:79,1982) and in the lymphocytes using ^{125}I-cyanopindolol (ICYP) (N.Sch.Arch.Pharm. 317:277,1981) For both systems, saturation and competition studies were performed. Epinephrine competition at RAUW sites in the presence and absence of GTP, and isoproterenol comp. at ICYP sites plus/minus GTP revealed two sites of interaction with GTP altering the ratio of high to low affinity agonist sites. Computer resolution of these sites demonstrated no group differences in the estimated K_D values for the sites however, there did appear to be an increased number of agonist high affinity states in the PTSD group. No group differences in RAUW or ICYP saturation parameters were observed, i.e., no K_D or B_{max} differences between groups. The significance of differences in the ratio of high to low affinity states is unclear, however the relationship between changes in ratio of affinity states and the process of R internalization (down-regulation?) has been noted.

More definitive studies are in progress.

340.4 DECREASED ALPHA-2 NORADRENERGIC RECEPTORS IN THE DORSOMEDIAL HYPOTHALAMUS IN RATS FED A HIGH FAT DIET. C.A. Wilmot and B.E. Levin. VA Medical Ctr., Neurology Service, E. Orange, NJ, 07019, Univ. Med. Dent. NJ, Newark, NJ, 07903, and Rutgers Inst. Anim. Behav., Newark, NJ, 07903.

The intake of a high fat (17%)-high calorie (4.6 kcal/g) diet by rats over a 3-month period produces a significant increase in body weight and carcass lipid content in one-half to two-thirds of the animals. In this animal model of diet-induced obesity, selective decreases in the turnover of dopamine (DA) have been reported in the hypothalamic dorsomedial nucleus (DMN) and the median eminence (ME) in the diet-induced obese rats (DIO) compared to rats which eat the same diet but do not become obese, (diet-resistant; DR) and chow-fed (CHOW) rats (Levin et al., Pharmacol. Biochem. Behav. 24: 299, 1986). Norepinephrine (NE) turnover was increased significantly with both DIO and DR rats in the paraventricular nucleus and DMN of the hypothalamus. The following experiments were performed to determine whether there were differences in the levels of noradrenergic and dopaminergic receptors in the DIO, DR and CHOW rats.

Using quantitative autoradiographic procedures, slide-mounted tissue sections from the brains of DIO, DR and CHOW rats were incubated with one of the following ligands: ^{3}H-dihydroalprenolol (DHA), ^{3}H-para-aminoclonidine (PAC), ^{3}H-Prazosin (PRZ), or ^{3}H-spiroperidol (SPIR). Results thus far indicate a significant effect of diet on the number of norepinephrine-displaceable PAC binding sites in the DMN and the area dorsal (DA) to the DMN. Levels for the DIO and DR groups were respectively 60% and 65% of the CHOW group. Also, there was a significant negative linear correlation between the number of PAC binding sites and body weight gain in the DMN and DA ($r=-0.546$, $p<0.035$, and $r=-0.533$, $p<0.041$, respectively, $n=15$). No significant differences between diet groups have been detected in the binding of DHA and SPIR to hypothalamic nuclei. These results suggest that changes in the sensitivity of alpha-2 noradrenergic receptors in the DMN and the DA of the hypothalamus may be related to dietary composition and associated with the degree of body weight gain.

340.5 **EFFECT OF CHRONIC SENSORIMOTOR SEIZURES ON GLUTAMATE BINDING.** E.M. Santori, T. Der, L. Lymer*, D. Zinck*, and R.C. Collins; Depts. Neurology and Neurological Surgery, and the McDonnell Center for the Study of Higher Brain Function, Washington University, St. Louis, MO 63110

The process by which recurrent experimentally-induced focal neocortical seizures progressively increase in severity is an intriguing example of neuronal plasticity. In addition, this type of kindling may have clinical relevance as an animal model of simple partial epilepsy. Lines of evidence have suggested that increases in excitatory glutamatergic neuro-transmission may serve an important role in the genesis of this kindling phenomenon. We have sought to test for postsynaptic changes through an autoradiographic examination of 3H-L-glutamate binding.

Rats were stereotaxically implanted with bipolar electrodes in the cortical forelimb sensorimotor overlap zone. Animals were chronically stimulated with 1-2 second trains of 1 msec pulses delivered at 50 HZ, at currents above the afterdischarge (AD) threshold.

At the time of sacrifice the rats had been subjected to a mean of 39 stimulations. AD duration had increased from an intial mean of 7 seconds to 19 seconds. The seizure response, that initially consisted of mild clonic forelimb movements and upper body bobbing, grew to include rearing, tonic muscle contractions, and balance loss.

The rats were sacrificed 24 hours after their last stimulation. Prior to binding, brain sections were subjected to a defatting xylene dip followed by a buffer prewash. The sections were incubated on ice with 800 nM 3H-L-Glutamate made up in Tris-acetate buffer. The sections were then washed for 15 seconds.

No significant alterations in specific glutamate binding could be demonstrated for the focus or any of its areas of projection:

	Mean fmole/mg tissue ± S.E.M.	
	Control (n=5)	Kindled (n=5)
Focus	536 ± 51	514 ± 49
Caudate	505 ± 45	495 ± 44
Globus pallidus	259 ± 33	242 ± 20
Ventrobasal thalamus	391 ± 26	412 ± 40
Substantia nigra	291 ± 27	333 ± 23

In addition, no clear perturbations of amygdaloid or hippocampal ligand binding could be demonstrated. One kindled subject did have a profound loss of specific binding in the stratum lucidum of the hippocampal CA-3 region.

The strong evidense suggesting that glutamate binds to at least three distinct classes of excitatory receptors complicates the interpretation of these data. Our results do indicate that sensorimotor cortical kindling does not strongly affect the total density of glutamate binding sites in siezure pathways.

340.6 EFFECTS OF TRANSIENT CEREBRAL ISCHEMIA ON EXCITATORY AMINO ACID RECEPTORS IN RAT HIPPOCAMPUS.

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Ten minutes of cerebral ischemia leads to selective degeneration of pyramidal neurons in the CA1 and subicular regions of the septal hippocampal formation. This damage is evident as a delayed neuronal necrosis appearing 3-4 days following the ischemic insult. Several recent lines of evidence have suggested that glutamate (NMDA) receptors may play an important role in the development of the damage.

In order to study possible changes in the properties of glutamate receptors during and following ischemia we have investigated the NMDA sensitive ^{3}H-glutamate-, ^{3}H-AMPA, and ^{3}H-Kainic acid binding to 6 um brain sections using autoradiographic methods.

Male Wistar rats were subjected to 10 minutes of transient cerebral ischemia, induced by bilateral common carotid occlusion combined with hypotension, and allowed to recover for various periods of time (5 minutes-7 days). At the end of the experimental period the rats were either perfusion fixed for later histological evaluation or decapitated, frozen in isopenthane, sectioned at -20°C, and processed for autoradiography. The binding was quantified by computer-aided densitometry.

Our data revealed a biphasic pattern of the AMPA binding. Furthermore the glutamate receptor classes displayed different sensitivities to the ischemic insult. In the early recovery periods the AMPA-binding transiently decreased by 30-40% but returned to control levels at one day following the ischemic insult. In CA1 and subiculum a secondary decrease in binding by 50-60% was noted at 2 days and decreased further up to 7 days following ischemia. This decrease in binding preceded light microscopic evidence of neuronal necrosis. In areas where necrosis was not evident following this period of transient ischemia, such as the dentate gyrus or cerebral neocortex, the secondary decrease in AMPA binding was not observed. The NMDA sensitive glutamate- and kainic acid binding was not affected by ischemia with up to 7 days of recovery.

We conclude that the initial decrease in AMPA-binding reflects a reversible desensitization of the receptors due to the increased extracellular levels of excitatory amino acids during the ischemic insult. The secondary decrease probably is an early sign of irreversible neuronal dysfunction and possibly indicate a site of neuronal injury.

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340.7 DOPAMINE D_1 AND D_2 RECEPTORS IN HUNTINGTON'S CHOREA.

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Huntington's Chorea (HC) is an autosomal dominant neurological disorder characterized by involuntary choreiform movements and dementia. Neuropathologically it is characterized by severe basal gangliar neuronal degeneration. Dopamine D_1 and D_2 receptor alterations in the basal ganglia may be one of the causes of the abnormal motor movements found in HC. Reisine et al. (1977) and Cross and Rossor (1983) have reported decreases in D_2 binding in HC striata. However, in these studies no distinction was made between neuroleptic-treated and untreated HC patients. It is known that chronic pretreatment of rats with dopamine antagonists results in an increase in the number of dopamine binding sites. Cross and Rossor (1983) reported a decrease in D_1 binding sites of HC striata using the nonselective D_1 ligand, ^{3}H-piflutixol. Thus, we conducted an extensive study to investigate the effect of neuroleptics on D_2 receptor densities in HC striata, and to measure D_1 receptor densities in HC striata, using the selective D_1 ligand, ^{3}H-Schering-23390.

The D_1 receptor density (B_{max}) was measured by receptor saturation-type experiments using a range of 100 to 4000 pM ^{3}H-Schering-23390. The D_2 density was measured using 10 to 1000 pM ^{3}H-spiperone. The B_{max} values were determined by Scatchard analysis. Non-specific binding for D_1 and D_2 was defined as that binding inhibited by 1 μM (+)-butaclamol.

The D_2 B_{max} values (pmoles/gm wet tissue) were:

BRAIN REGION	CONTROL	HUNTINGTON'S CHOREA no neuroleptics	with neuroleptics
PUTAMEN	11.3 ± 0.5 (30)	6.7 ± 0.6 (8)	10.3 ± 0.9 (16)
CAUDATE	10.1 ± 0.4 (30)	6.0 ± 0.8 (10)	9.0 ± 1.0 (14)
ACCUMBENS	11.0 ± 0.5 (30)	5.7 ± 0.7 (8)	9.4 ± 1.4 (8)

Values are mean ± S.E.M. Values in parentheses are number of brain samples used in the experiments.

The D_2 densities in all three regions of neuroleptic-free HC brains were reduced by about 40%. This indicates that the disease by itself lowers the D_2 density. The D_2 densities in the neuroleptic-medicated HC brain regions, however, were approximately 36% higher than the neuroleptic-free tissues. This neuroleptic-induced effect of 36% is similar to that seen after long-term neuroleptic medication in general.

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340.8 **INTERACTIONS OF TREMORGENIC MYCOTOXINS WITH GABA RECEPTORS OF RAT BRAIN.**

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Mycotoxins have been implicated in several naturally occurring neurological disorders, primarily of cattle, called staggers. The principal clinical signs of the disease are muscle tremors and hyperexcitability. Mycotoxin-induced tremors in experimental animals were antagonized by injections of γ-aminobutyric acid (GABA), suggesting that GABA receptors may be targets for the toxic action of these mycotoxins.

In this investigation, three tremorgenic mycotoxins (aflatrem, paspalinine, paxilline) and a nontremorgenic mycotoxin (verruculotoxin) were tested for their effects on GABA-induced ^{36}Cl- influx and on ^{35}S-t-butylbicyclophosphorothionate (TBPS), ^{3}H-muscimol, and ^{3}H-flunitrazepam binding to GABA receptors of rat brain. GABA-induced ^{36}Cl- influx into rat brain microsacs was dose dependent, saturable, and had a Hill coefficient of 1.9. The competitive antagonist bicuculline was over 10-fold more potent than (-)bicuculline in inhibiting the influx. It was also inhibited by the noncompetitive antagonists picrotoxinin and TBPS. Inhibition of ^{36}Cl- influx by the tremorgenic mycotoxins had IC_{50} of 38 to 79 μM, while verruculotoxin had no effect. The tremorgenic mycotoxins also displaced ^{35}S-TBPS binding with IC_{50} values ranging from 3.5 to 10.6 μM, and again verruculotoxin had no effect. All mycotoxins had no significant effect on ^{3}H-muscimol binding, while ^{3}H-flunitrazepam binding was enhanced by the tremorgenic mycotoxins, but not by verruculotoxin. The data suggest that tremorgenic mycotoxins inhibit the Cl- channel of the $GABA_A$ receptor by binding at or near the ^{35}S-TBPS binding site, and they allosterically increase the affinity of the benzodiazepine binding site. (Supported in part by NIH grant ES 02594).

340.9 BRAIN [^{3}H]-GABA RECEPTOR BINDING AND PLASMA GABA LEVELS IN RATS WITH THIOACETAMIDE-INDUCED ACUTE HEPATIC ENCEPHALOPATHY. J.E. Maddison*, P.R. Dodd*, M. Morrison*, G.A.R. Johnston, G.C. Farrell* Dept Pharmacology & Dept Medicine, University of Sydney, NSW Australia 2006.

Alteration of GABAergic neurotransmission has been postulated to be involved in the pathogenesis of the neuropsychiatric disturbances that occur in hepatic encephalopathy (HE). It has been hypothesized that gut-derived plasma GABA is increased in HE and crosses an altered blood brain barrier to induce increased density of GABA receptors (Jones et al. Yale J. Biol. Med. 1984 57, 301-316). We have investigated brain GABA receptor binding and plasma GABA levels in a model of acute hepatic encephalopathy in rats. Rats were injected with thioacetamide (TAA) (250mg/kg ip daily for 3 days); controls received an equal volume of vehicle (0.9% NaCl). In addition, a group of rats were neither injected nor handled during the experimental period (non-injected controls). Brain GABA receptor density and affinity was determined in Triton-treated crude whole brain synaptosomal membranes using [^{3}H]-GABA. Plasma GABA was determined by high performance ion-exchange liquid chromatography in the lithium phase with post-column derivitization with o-phthaldehyde. Plasma GABA levels were significantly increased in the TAA treated rats compared with controls (759±227, n=7 vs 226±36 n=9 $\bar{x}$ ± s.e.m. $p<0.005$). However, there was no significant difference in low affinity GABA receptor density or affinity between rats injected with TAA or 0.9% NaCl. The high affinity GABA site was not detectable in most membrane preparations from both the TAA and NaCl treated rats. In contrast, 2 receptor sites were consistently identified in the non-injected control rats. In addition, there was a significant increase in the density of the low affinity receptor in both the TAA and NaCl treated rats when compared with the non-injected controls.

Treatment(n)	High affinity site		Low affinity site	
	K_D (nM)	B_{max}*	K_D (nM)	B_{max}*
NaCl (7)	-	-	120±10	9550±1402
TAA (7)	-	-	107±13	7708±1087^{NS}
Non-Injected(5)	10±3	244±53	297±41	2710±757^{a}

Data expressed as mean ± s.e.m. n=# experiments on membrane preparations from 3-4 pooled brains. ^{a} Significantly different from NaCl $p<0.001$. NS not significantly different. Conclusions: 1. Plasma GABA is increased in rats with TAA-induced hepatic encephalopathy. 2. However, brain GABA low affinity receptor density and affinity is unaltered. 3. Stressful manipulations such as handling and intraperitoneal injection significantly alter brain GABA binding.

340.10 THE EFFECT OF POSTMORTEM FACTORS ON MUSCARINIC RECEPTOR SUBTYPES IN RAT BRAIN. RE Burke, D Greenbaum*. Dept Neurology, Columbia University, NY, NY, 10032.

Studies of neurotransmitter receptor binding in human postmortem brain have made important contributions to the understanding of neurologic diseases. Critical to the interpretation of these studies,however, is information about the effect of postmortem factors on receptor binding. While prior studies support the validity of measuring total muscarinic binding in postmortem brain (Whitehouse et al, 1984), there has been no similar study of the muscarinic receptor subtypes M1 and M2, as defined by high and low pirenzepine (Pir) affinity. We have therefore studied the effect of postmortem delay, storage at 4° and -20° C and multiple freeze-thaw cycles on muscarinic subtypes by assaying [3H]QNB and [3H]Pir binding in rat brain homogenates. In a filtration assay at a concentration of 12 nM, [3H]Pir binds almost exclusively to the M1 site. [3H]Pir binding was performed as described by Watson et al (Life Sci, 1983) in 10 mM NaKPO4 (pH 7.5), incubated at 25°C for 1 hour. [3H]QNB was assayed in parallel for each specimen in the same buffer.

We found that both [3H]QNB and [3H]Pir binding were unchanged compared to fresh tissue following a 4 hour postmortem delay at room temperature, or storage at 4°C for 24 hours, or at -20°C for 4 weeks, or after 3 freeze-thaw cycles. The only change was an increase in [3H]QNB binding, but not [3H]Pir, after 15 hours at room temperature; Scatchard analysis revealed a 20% increase in binding sites with no change in affinity. We interpreted this increase as due to enhanced access of [3H]QNB to sites ordinarily sequestered from the ligand in fresh tissue, following partial tissue autolysis. In this respect [3H]QNB binding in postmortem tissue differs from that of [3H]NMS, which decreases; the difference may be due to different lipid solubilities of the ligands. These results support the validity of measuring [3H]QNB and [3H]Pir binding in postmortem tissue, but they raise concern about the interpretation of the difference between total and M1 binding as an indirect measure of M2 binding.

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340.11 THE RELATIONSHIP BETWEEN STRIATAL DOPAMINE ACTIVITY, GLUCOSE METABOLISM, AND BLOOD FLOW FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA IN THE RAT. M. Y.-T. Globus*, M.D. Ginsberg, R. Busto, S.I. Harik, and W.D. Dietrich. Department of Neurology, University of Miami, School of Medicine, Miami, FL, 33101.

Neurotransmitter release and increased synaptic activity are believed to play a major role in the mediation of ischemic cell injury. The small-to medium-sized striatal neurons are among those known to be selectively vulnerable to ischemia. Dopamine (DA) is a major striatal neurotransmitter. We have recently described that while 45 min of ischemia did not alter striatal levels of DA and its metabolites, 30 min of recirculation increased it significantly (Neurol, in press). We have also shown that, following transient ischemia in the rat, relative glucose hypermetabolism occurs in the striatum, together with hypoperfusion, resulting in marked elevation of the metabolism/flow ratio, and that this correlates with morphological evidence of selective striatal injury (Ann Neurol 18: 470-81, 1985). These results suggest that dopaminergic hyperactivity might be related to the selective vulnerability of striatal neurons to ischemia, either by accentuating postischemic hypoperfusion or by a direct metabolic effect. The present study was undertaken to assess the role of DA in the development of postischemic hemodynamic and metabolic changes in the striatum. To this end we studied, by double-label autoradiography, the effect of striatal DA depletion on the recovery of local brain glucose utilization (lCMRgl) and blood flow (lCBF) after 30 min of global ischemia produced by 4-vessel occlusion in the rat. Unilateral striatal DA depletion (approx. 90%) was produced 2 weeks earlier by 6-hydroxydopamine lesion of the substantia nigra. In order to assess the effectivness of the lesion, and in order to correlate postischemic lCBF and lCMRgl pattern with local DA changes, striatal DA and its metabolite levels were determined using HPLC-EC. Our results show a significant increase in striatal DA metabolism and turnover (as measured by DOPAC/DA and HVA/DA ratios) on the unlesioned side after 10 min (by 300%), 1h (by 400%), 2h and 4h (by 100%) post-ischemia. On the lesioned side DA and its metabolite levels were very low and no significant change was found at any recirculatory time points. lCBF levels were similar in the DA-depleted vs. non-depleted striata at all recirculatory time points. However, the complete normalization of lCMRgl found on the non-depleted striata was significantly supressed on the DA-depleted side after 2 and 4 hrs of recirculation (by 22% and 16% respectively), thus significantly diminishing the degree of lCMRgl/lCBF uncoupling. These results suggest that dopaminergic activity is responsible for postischemic metabolism/flow uncoupling in the striatum and might therefore contribute to selective ischemic injury to this structure.

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340.12 REDUCED STRIATAL DOPAMINE FOLLOWING MICRO-INJECTION OF HERPES SIMPLEX VIRUS IN MICE R.F. Seegal and D.J. McFarland*. Wadsworth Center for Labs and Research, NYS Department of Health, Albany, NY 12201.

Previous work (Seegal et al., 1978; Seegal et al., 1981) indicated that intracerebral (i.c.) inoculation of herpes simplex virus (HSV) into mice alters spontaneous, d-amphetamine-induced locomotor activity and mortality induced by exposure to high levels of d-amphetamine. The underlying basis for these behavioral and toxicological events is thought to involve alterations in the catecholaminergic systems of the brain. However, inconclusive results have been reported when central nervous system tissue was analyzed for either activity of tyrosine hydroxylase or concentrations of biogenic amine metabolites (Lycke and Roos, 1974; Bak et al., 1979; Elizan et al., 1982).

BALB/C mice were stereotactically injected in striatum with either 100 plaque forming units of the MacIntyre or MP strain of HSV or the control vehicle. The injection volume in all instances was 0.1 µl. Animals were killed three days later by cervical dislocation and the striatum rapidly dissected. The tissue was weighed and immediately homogenized in 20 volumes of ice-cold perchloric acid containing 100 mg/l of EGTA.

Concentrations of dopamine (DA), homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by high-performance liquid chromatography with electrochemical detection.

Concentrations of DA in the MP and MacIntyre injected mice were significantly lower than in the vehicle injected animals ($p<.02$). HVA concentrations in striatum were significantly elevated in only the MP inoculated animals ($p<.01$). Ratios of HVA to DA, a recognized measure of neuronal turnover, (Westerink, 1979) were also shown to be elevated in MP inoculated mice when compared with MacIntyre or control animals. No differences were noted in striatal serotonin concentrations. Immunoperoxidase staining in separate animals indicated that the infectious process was restricted to the site of injection.

These results provide a neurochemical basis for the observed behavioral changes previously reported by Seegal et al., (1978; 1981) and others. Significant differences in HVA concentrations and HVA/DA ratios between the two virus strains suggest either that the neurochemical actions of these two strains of HSV differ in the mouse or that the rate at which viral pathogenesis developed differed between the two virus strains.

340.13 STRIATAL ACETYLCHOLINESTERASE NEURONS AND TYROSINE HYDROXYLASE IMMUNOREACTIVITY ARE PRESERVED IN HUNTINGTON'S DISEASE.
R.J. Ferrante, N.W. Kowall, E. Ross, J.B. Martin, E.P. Richardson, Jr., Massachusetts General Hospital,Boston, MA. 02114

In Huntington's disease (HD) and in normal brain, the striatum displays patches of low-acetylcholinesterase (AChE) activity with an intervening matrix of high-AChE activity. The high-AChE matrix zone is decreased in area in HD, while the size and total area of low-AChE patches remain within normal limits (J. Neuropathol. Exp. Neurol., abstract in press). In contrast to the persistent AChE pattern in HD, patchy enkephalin immunoreactivity (ir) is absent in the dorsal striatum (J. Neuropathol. Exp. Neurol., abstract in press).

In order to characterize further the preserved patch-matrix pattern in HD, we examined the striatum in 8 cases of HD and in 12 normal age-matched controls using immunohistochemistry for tyrosine hydroxylase (TH) to demonstrate catecholiminergic projections, and AChE enzyme-histochemistry to identify AChE positive-neurons.

In the normal human striatum, TH ir was distributed in an irregular pattern. Comparison of TH to AChE activity in adjacent sections showed that dense TH ir was confined to the high-AChE matrix. In HD, the correspondence of TH ir to the AChE matrix persisted. The area of TH ir was decreased in size without any alteration in intensity of activity. Cellular AChE activity was predominantly found in large striatal neurons (25±4 μm in maximum diameter). They were distributed throughout the striatum in both high- and low-AChE compartments. Their density was significantly greater in both the caudate nucleus (HD: 600±82 cells/cm^2; control: 168±36, $P<0.001$) and putamen (HD: 645±122 cells/cm^2; control: 230±43, $P<0.005$) in HD. This reflects the combined effects of tissue shrinkage and neuronal sparing.

The persistence of TH staining confirms the preservation of the patch-matrix pattern in HD. The increased AChE neuronal density suggests that this subset of chemically distinct neurons is resistant to the destructive process in HD.

340.14 SEROTONIN AND TYROSINE-HYDROXYLASE IMMUNOREACTIVE FIBRES AND TERMINALS IN SPINAL CORD OF GUINEA PIGS DURING CHRONIC RELAPSING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS.
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Chronic relapsing experimental allergic encephalomyelitis (CrEAE) is an inflammatory autoimmune disease of the central nervous system. CrEAE is accompanied by demyelinating lesions and exhibits distinct relapses and remissions. Because of its histological and clinical similarity to multiple sclerosis (MS), CrEAE is regarded as a better model of MS than acute EAE.

We previously have observed distinct signs of damage to both bulbospinal catecholamine-containing and serotonin-immunoreactive (5HTIR) axons and terminals in the spinal cord of rodents with acute EAE. The purpose of this study was to examine the extent of damage in supraspinal 5HTIR and tyrosine-hydroxylase-immunoreactive (THIR) fibres and terminals in guinea pigs affected with CrEAE.

CrEAE was induced in juvenile Hartley guinea pigs by inoculation in the nuchal area with homologous spinal cord in complete Freund's adjuvant. Control animals received similar inoculations devoid of spinal cord tissue. Animals sensitized with CNS tissue showed signs of the first attack of paralysis between 15 and 25 days after inoculation. This was followed by a period of complete recovery lasting from 10-20 days before the next clinically distinct relapse occurred. Sections from cervical, thoracic and lumbar cords were processed for 5HT and TH immunoreactivity using the PAP method.

Our results demonstrate that CrEAE, during both relapse and remission, is associated with marked loss of axons and terminals immunoreactive for 5HT and TH, the severity of which increased in the cervico-lumbar direction. Thus, the lumbar cord appeared virtually denuded of 5HTIR neuropil. The few 5HTIR terminals which remained in thoracic and lumbar cord were distorted and swollen. A parallel but less marked depletion of THIR axons and terminals was evident at both levels. By contrast, the cervical cord did not display any detectable change in immunoreactivity for either 5HT or TH. The immunohistochemical changes evident in caudal spinal cord remained essentially unaltered irrespective of whether animals were in remission or relapse. No signs of either axonal regrowth or proliferation of surviving terminals were found. It is interesting to note that rats which recovered from acute monophasic EAE show re-innervation of thoracic and lumbar cord by 5HTIR terminals (White et al., Neuroscience, 1985). We conclude that (i) no correlation exists between the degree of bulbospinal monoaminergic fiber loss and the motor deficit of CrEAE; (ii) recovery of caudal spinal motor function is not associated with the regeneration or sprouting of the bulbospinal fibers.
(Supported by The Multiple Sclerosis Society of Canada)

340.15 SYNAPTOSOMAL UPTAKE AND RELEASE OF SEROTONIN IN CEREBRAL ISCHEMIA AND POSTISCHEMIA OF GERBILS.
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Serotonin (5-HT) has been implicated in the pathogenesis of at least three major cerebrovascular disease processes (ischemia, vasospasm and migraine). Therefore, we investigated the uptake and release of 5-HT by synaptosomes obtained from gerbils cerebral cortex, striatum and hippocampus in ischemia and post-ischemia. Respective sham operated gerbils served as controls.

As a model for this study, we used common carotid artery occlusion for 15 min with or without 1 hr of reflow. Crude synaptosomal fractions were isolated by the method of Booth and Clark (Biochem. J. 176: 365, 1978). Uptake and release of 5-HT were measured according to the modified procedure of Kuhar (Life Sci. 13: 1623, 1973). Aliquots of synaptosomal suspension (0.2 ml containing 1-5 mg of proteins) were incubated in freshly prepared Krebs-Henseleit solution (1 ml final volume) with 1.9×10^{-9} M of ^{3}H 5-HT at 37°C and 0°C for 5 min. The reaction was terminated by passing half of the sample through GF/B filters. The other half of the sample was reincubated for 10 min and the reaction was terminated for the release as for the uptake. The uptake (pmol of ^{3}H 5-HT/mg proteins/min) was calculated as the difference between values obtained at 37°C and 0°C while the difference between ^{3}H 5-HT uptake and the remained radioactivity (after 10 min of reincubation) served as a measure of the release.

After 15 min ischemia, uptake was significantly decreased 38 and 42 per cent in cortical and hippocampal but not in striatal synaptosomes. In contrast, in the reflow period, a significant decrease of uptake was observed in the striatum and to a lesser degree in the cortex as compared to that in ischemia. However, hippocampal uptake remained the same as in ischemia alone. In ischemia, release of 5-HT was significantly increased by 50, 130 and 90 per cent above the control in cortical, striatal and hippocampal synaptosomes, respectively. After 1 hr of reflow, the increase of 5-HT release persisted but to a lesser extent in the cortex and the striatum while there was no change in the hippocampus as compared to that in control.

These data are in agreement with the previously reported impairment of serotonin turnover and marked accumulation of water under the same conditions (Cvejic et al, Acta Neuropathol. Berl. 51: 71, 1980). Thus, these findings provide further evidence for the involvement of this neurotransmitter in the formation of ischemic brain edema. Moreover, the observed regional differences in cerebral uptake and release might be one of the factors responsible for the selective vulnerability of ischemic injury.

340.16 ALTERATIONS IN PLATELET 5-HYDROXYTRYPTAMINE UPTAKE AND MONOAMINE OXIDASE ACTIVITY IN PREMENSTRUAL SYNDROME.
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Premenstrual syndrome (PMS) is characterized by the occurrence of various psychological and somatic symptoms beginning several days prior to the onset of menses and terminating with menses. Pharmacological, biochemical and clinical evidence suggest that many of the individual symptoms associated with PMS may be related to an alteration in central serotonergic neuronal function. Because blood platelets and brain serotonergic neurons have been demonstrated to have many pharmacological and biochemical similarities, the platelet was utilized in the present study as a model to determine possible alterations in serotonergic mechanisms in individuals with a diagnosis of PMS. Healthy volunteers between the ages of 22 and 42 years were requested to complete daily anxiety assessment scales and a menstrual symptom diary for three complete menstrual cycles. The Minnesota Multiphasic Personality Inventory was performed in the follicular phase and again in the luteal phase in each subject. PMS patients and control subjects were selected for the study based on the results of these tests. Blood samples were collected on Day 5 and Day 23 of the third and/or fourth cycle. Platelet monoamine oxidase (MAO) activity, uptake of 5-hydroxytryptamine (5-HT) into platelets, 5-HT content in platelets and plasma total and free tryptophan levels were determined for each subject.

The V_{max} of 5-HT uptake in platelets from PMS patients was significantly decreased on Day 23 compared to control subjects. There was a smaller decrease in V_{max} of PMS patients compared to controls on Day 5. The K_m of 5-HT uptake was higher on Day 23 than Day 5 in PMS patients. Platelet MAO activity was significantly increased on Day 23 in PMS patients compared to Day 5. There was no difference in plasma total and free tryptophan levels between PMS patients and control subjects on either day. The decrease in 5-HT uptake correlated with the appearance of symptoms, suggesting that altered central serotonergic neuron activity may underlie or be associated with these symptoms. The increase in MAO activity during the period of high anxiety in the luteal phase in PMS patients may reflect enhanced serotonergic neuron activity. Any alteration in neuronal or platelet 5-HT function does not appear to have resulted from changes in substrate availability since plasma free tryptophan levels did not differ between the two groups.

340.17 GABA Stimulated ^{36}Cl Influx in the Genetically Epilepsy-Prone Rat. S. Wieland[1], A. Istock*, P.D. Weeks*, and H.E. Laird II. [1]Dept. of Psychology; Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ. 85721.

Genetically epilepsy-prone rats (GEPR) have been widely used as an animal model of epilepsy. The underlying mechanism(s) for the seizure-prone state in these rats is unknown. Recent published reports have suggested that the GABA/BZ complex may be involved in this genetically based phenomenon. This study is an attempt to evaluate the function of the GABA/BZ complex in non-previously convulsed GEPR-9 rats.

GABA stimulated ^{36}Cl influx was measured in GEPR-9 and Sprague-Dawley (SD) controls by a modification of the method of Harris et al. (Science,228,1985). In this procedure, particulate preparations of cortex from seizure-naive GEPR-9s (the rat line having the most severe seizure episode) and seizure-naive controls were incubated (3 sec.) with ^{36}Cl (100 nCi) in the presence or absence of graded concentrations of GABA (10-1000 uM). The incubation was terminated by dilution with ice-cold buffer (Krebs-Ringer, pH 7.5) and rapidly filtered through GF/C filters.

The results showed that GABA stimulated ^{36}Cl influx was dose dependent in both types of rats. However, the GEPR-9s were less responsive to GABA-stimulated Cl^- influx as shown by a lower absolute maximal ^{36}Cl influx (40% less) at saturation. In addition, the GEPR-9s reached maximal Cl^- influx at a lower GABA concentration (100 uM) as compared to SD controls (500 uM). No differences in basal ^{36}Cl influx were observed between GEPR-9 and controls. These data are similar to those reported by Yu et al. (Fed. Proc. ABST No. 2395, 1986) in DBA 2/J mice.

Our observations are consistent with previous work in seizure-experienced GEPR-9s showing that they were less sensitive to iontophoretically applied GABA in the inferior colliculus (Gehlbach and Faingold; Fed. Proc. ABST No. 569, 1984). Our observation coupled with the work of Gehlbach and Faingold suggest that the differences seen in the GEPR-9's GABA stimulated Cl^- influx is genetically determined and not due to previous convulsive activity.

Supported by a grant from the Epilepsy Foundation of America and a BRS Grant from the College of Pharmacy, University of Arizona.

340.18 TIME EFFECT ON NORMAL HUMAN INTACT PLATELET ^3H-IMIPRAMINE BINDING. A. Goziotis and S.W. Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

If the platelet ^3H-imipramine binding site is to be used as a possible biochemical marker in psychiatric studies, the discrepancies and wide range of reported values in the literature must be accounted for. Traditional assay procedures using platelet membrane preparations have been shown to contain varying proportions of intracellular protein contamination, possibly invalidating the protein reference which assumes membrane origin. Friedl and Propping (Psychopharmacology, 80:96, 1983) proposed the use of intact blood platelets to minimize the binding variation. We have now completed a nine-month study on intact platelet ^3H-imipramine binding in 12 normal subjects sampled monthly. Using this improved assay, both B_{max} and K_d for ^3H-imipramine binding still exhibited large month-to-month variation (a low of 1010 fmoles/10^9 platelets and a high of 1430 fmoles/10^9 platelets). In comparison with values reported in other longitudinal studies using platelet membrane preparations (Egrise et al., Neurophysiology 10:101, 1983; Whitaker et al., Psychiat. Res. 11:127, 1984; Tang and Morris, Psychiat. Res. 16:141, 1985), no consistent seasonal trend is observed.

The result of this and other longitudinal studies raise an important question regarding the validity of reported clinical data without control for the temporal influence. Furthermore, the substantial interindividual variation observed in the present study, and the varying degrees of change in B_{max} values over time within each subject, may limit the clinical usefulness of this biochemical parameter.

340.19 TRITIATED IMIPRAMINE BINDING TO PLATELETS IS MARKEDLY REDUCED IN ELDERLY DEPRESSED PATIENTS. D.L. Knight,* K.R.R. Krishnan,* D.G. Blazer* and C.B. Nemeroff. (SPON: D. Kimmel, Jr.). Dept. Psychiatry and Pharmacol., Duke Univ. Med Ctr., Durham, NC 27710.

The number of ^3H-imipramine binding sites (Bmax) in platelets has previously been reported to be reduced in depressed patients when compared to normal controls (Raisman et al., Psychopharmacol. 75:368, 1981; Briley et al., Science 209:303, 1980). In most of the studies thus far conducted, the patient populations have consisted primarily of young and middle-aged depressed patients (<50 years old). In the present study, the number (Bmax) and affinity (Kd) of platelet ^3H-imipramine binding sites was measured in four groups: 1) normal subjects under 50 years of age (n=25); 2) normal subjects greater than 60 years (n=12); 3) depressed patients (DSM-III criteria for major depression) less than 50 years old (n=24); and 4) depressed patients 60 years of age or older (n=17). A fifth group, comprised of patients with other neuropsychiatric disorders (n=9) including Alzheimer's disease and schizophrenia, were also studied. All subjects were drug-free for at least five days prior to venipuncture. The ^3H-imipramine binding assay, as well as the method of platelet preparation, was that of Briley et al. (1980) with minor modifications. The Bmax, but not the Kd, of ^3H-imipramine binding was reduced in both the young and elderly depressed patients when compared to their appropriate age-matched controls. The reduction in Bmax in the elderly depressed patients was of a much greater magnitude than that observed in the younger depressed patients; there was little overlap in the Bmax values in the elderly normal controls and the elderly depressed patients. There were no age- or sex-related differences in Kd or Bmax in the normal controls or in any of the patient groups. No alterations in ^3H-imipramine binding were observed in patients with Alzheimer's disease or schizophrenia. These findings confirm and extend previous observations that the number of platelet ^3H-imipramine binding sites are reduced in patients with major depression. The robust nature of the reduction of binding sites in the elderly depressed patients and the normal number of binding sites in other neuropsychiatric disorders such as Alzheimer's disease are concordant with the hypothesis that this laboratory test may have diagnostic utility in depression in the elderly. (Supported by NIMH MH-40159).

340.20 THE EFFECTS OF INTRATHECAL ADMINISTRATION OF SEVERAL INHIBITORY AMINO ACIDS AND AN EXCITATORY AMINO ACID ANTAGONIST IN THE SPASTIC MUTANT MOUSE, D.K. Boyd, P.A. Boxer, P.J. Barr*, G.W. Campbell, S.M. Yuhas*, and R.J. Anderson. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105

The genetically mutant mouse SPA/SPA exhibits neurological signs that resemble those seen in spasticity. These include extension and tremor of the limbs, toe walking, loss of righting reflex and rigidity of the back and tail. These animals have a severe deficiency in the number of glycine receptors in the brain and spinal cord, and it has been hypothesized that this is responsible for many of the abnormalities that are observed. Consequently, increasing spinal cord levels of glycine, other inhibitory neurotransmitters or blocking excitatory neurotransmission might ameliorate the degree of neurological impairment. Since the amino acids (glycine, GABA, serine, taurine) and the amino acid antagonist, 2-amino-7-phosphono-heptanoic acid (APH), do not cross the blood brain barrier (or do so marginally), it is difficult to determine whether they would have any beneficial effects using conventional routes of administration.

To circumvent this problem a 5 microliter injection was made between the fourth and fifth lumbar vertebrae. The effect of various drugs was assessed by rating limb tremor and toe walking by three independent observers at two minute intervals for 14 minutes following injection. The drugs tested were glycine (300 mM), GABA (100 mM), serine (300 mM), taurine (300 mM) and APH (100 mM).

Intrathecal injections of saline produced improvements in both limb tremor and toe walking compared to the pre-injection baseline. Therefore, the actions of all other compounds were compared to the saline control group. Glycine, serine and taurine accentuated both toe walking and limb tremor as compared to the saline control. GABA had only a slightly beneficial effect on toe walking but no effect on limb tremor. APH had no effect on either of these parameters.

These data indicate that direct administration of glycine onto the spinal cord has a deleterious effect in the spastic mouse. Serine, a possible precursor of glycine and taurine a putative inhibitory neurotransmitter, likewise worsen the deficits seen in the spastic mouse. GABA, an inhibitory neurotransmitter, and APH, a glutamate antagonist, have virtually no effects on the spastic mouse when injected intrathecally.

341.1 THE VENTRAL STRIATUM IS THE "VISCERAL" STRIATUM. E.J. Neafsey and R.R. Terreberry. Department of Anatomy, Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL 60153.

Recent anatomical and physiological studies by our laboratory have implicated the infralimbic region of the rat medial frontal cortex (MFC) in control of visceral function (Terreberry and Neafsey, BR 278, 1983; Hurley-Gius and Neafsey, BR 365, 1986), suggesting this cortex functions as a "visceral" motor cortex. The present study examined the efferent projections from this cortex, focusing especially on the pattern of terminations in the striatum. Stereotaxic injections of 0.03 ul of 1% WGA-HRP were made in 30 rats anesthetized with ketamine HCl (100 mg/kg). After a 48 h survival, the animals were deeply anesthetized, perfused with 1% glutaraldehyde and 1.25% paraformaldehyde, and the brains removed. Fifty um frozen sections were cut and processed for HRP histochemistry using the TMB method of Mesulam. The sections were then examined under bright field, dark field and polarized light for the presence and location of label within the striatum.

Injections which involved the ventral infralimbic region of the MFC resulted in bilateral, anterograde, "terminal-like" labeling in the nucleus accumbens, the olfactory tubercle, and the medial portion of the caudate-putamen. Label in the olfactory tubercle avoided the islets of Calleja and the ventral pallidum, but was found in the "cell bridges" connecting the nucleus accumbens with the olfactory tubercle. These results agree with the findings of Jayaraman in the cat (Neurosci. Abstr. 11, 1985) and those of Newman and Winans in the hamster (JCN 191, 1980).

The nucleus accumbens and olfactory tubercle have been termed the "ventral" or limbic striatum by Heimer and his coworkers (TINS 5, 1982). Our findings suggest that this "ventral" striatum functions, at least in part, as a "visceral" striatum, wired into the forebrain in parallel with more dorsal "somatic" striatum (caudate-putamen) rather than in series with it.

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341.2 THE EFFERENT CONNECTIONS OF THE MEDIAL PREOPTIC NUCLEUS. R.B. Simerly and L.W. Swanson. Howard Hughes Medical Inst. and Salk Inst., La Jolla, CA 92138.

The medial preoptic nucleus(MPN) has been implicated in a number of neuroendocrine, autonomic, and somatomotor functions related primarily to reproduction, thermoregulation, and fluid balance. The afferents of the MPN suggest that the nucleus is in a unique position to integrate olfactory, visual, auditory, and tactile sensory information together with the influence of circulating gonadal hormones(Simerly and Swanson,1986). Further, these inputs are distributed differentially within the nucleus. As a first step toward determining the functional systems impacted by this sensory information we have examined the projections of the MPN by using the anterogradely transported lectin phaseolus vulgaris leucoagglutinin(PHA-L). Over 60 male Sprague-Dawley rats received injections of PHA-L into the MPN and the brains were processed as described elsewhere(Gerfen and Sawchenko,1985).

The MPN was found to project extensively to widely distributed regions in both the forebrain and brainstem, most of which supply inputs to the nucleus.The important neuroendocrine role of the MPN is underscored by its extensive projections to all parts of the periventricular zone of the hypothalamus including the anteroventral periventricular nucleus, and the paraventricular(PVH) and arcuate(ARH) nuclei of the hypothalamus, and a role in autonomic function is indicated by projections to such regions as the dorsal and lateral parvicellular parts of the PVH, the nucleus of the solitary tract, and the lateral part of the parabrachial nucleus. Other projections of the MPN suggest that the nucleus may participate in the initiation of specific motivated behaviors. Inputs to nuclei of the medial zone of the hypothalamus such as the ventromedial and dorsomedial nuclei may relate to the control of reproductive and ingestive behavior respectively, but the possible function of the strong projection to the ventral premammillary nucleus is unclear. Execution of these behaviors may involve activation of somatomotor regions via projections to the substantia inominata, perifornical region, peripeduncular, and pedunculopontine nuclei. Similarly, MPN efferents to regions that project to the spinal cord such as the periaqueductal gray, the laterodorsal tegmental nucleus, certain medullary raphe nuclei, and the magnocellular reticular nucleus may also be involved in this function. Finally, the MPN may influence a wide variety of physiological mechanisms and behaviors through its massive projections to areas like the ventral lateral septum, the bed nucleus of the stria terminalis, the supramammillary nucleus, and the ventral tegmental area, all of which have either direct or indirect connections with multimodal association areas of the cerebral cortex. Although the PHA-L method does not demonstrate unequivocally differential projections of each subdivision of the MPN, our results suggest that each subdivision projects in a unique pattern. In general, the central part of the MPN has the most extensive connections with the telencephalon(including limbic regions), as well as to those regions known to contain large numbers of cells that accumulate gonadal steroids; the medial part of the MPN appears to be primarily involved in neuroendocrine functions by way of strong connections with the PVH and ARH; and the lateral part of the MPN has the most extensive projections to regions thought to be involved in general behavioral arousal such as the lateral and posterior hypothalamic areas, the periaqueductal gray, and regions of the caudal brainstem.

341.3 CONNECTIONS OF THE BASAL FOREBRAIN WITH THE AMYGDALA IN THE RHESUS MONKEY. K. K. HREIB AND D. L. ROSENE . Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Injections of retrograde tracers (HRP and fluorescent dyes,FD) or anterograde tracer, radioactively labelled amino acids (RLAA), where made in the amygdala and in nuclei of the basal forebrain (BF): the medial septum (MS), the vertical and horizontal nuclei of the diagonal band (VDB, HDB) and the nucleus basalis (NB). An RLAA injection in the ventral part of the MS and the VDB resulted in dense label over the anterior amygdaloid area (AAA), the cortical nuclei (CN) and the cortical-amygdaloid transition area (CTA) and lighter labelling over the central (CE) and accessory basal (AB) nuclei. However an RLAA injection in NB resulted in dense label over the dorsal part of laterobasal nucleus (LB) as well as the AAA, CN, CE, and lighter labelling over the ventral part of LB. A large HRP injection in the amygdala confirmed all the anterograde results. More limited injections of FD in the CN labelled neurons in all four BF nuclei but an injection in the AAA and dorsal AB labelled neurons mainly in the NB, and an injection in ventral LB labelled neurons mainly in the lateral part of NB. Thus the AAA, CN and CE receive projections from all four nuclei while the AB and LB receive projections mainly from the NB.

To investigate the possible reciprocity of amygdaloid efferents to the BF, RLAA injections were made at different loci in the amygdala and three HRP injections were made at different loci in the BF. An RLAA injection restricted to the AAA and the dorsal AB produced labelling over the VDB, HDB and NB. An HRP injection into the NB labelled cells in the AAA and the AB, but an injection into the VDB-HDB labelled cells only in AB. Furthermore, no labelled cells were observed in the dorsal parts of the lateral (LT) or LB nuclei after any of the HRP injections, but a large number were found in ventral LB and a few in ventral LT after HRP injections in NB or VDB-HDB. A large RLAA injection that involved the mediobasal nucleus (MB), CTA, AB, and ventral LB resulted in label over the NB and HDB. All HRP cases showed labelled neurons in the AB while labelling in CTA was observed only from injections involving MS or VDB-HDB. Finally an RLAA injection into the CN and CTA showed labelling in the NB and some in ventrolateral VDB and both nuclei contained HRP labelled neurons after HRP injections into VDB-HDB or NB. Thus the amygdaloid projections to the BF originate from the same nuclei that receive BF afferents but there is no tight reciprocity amoung individual nuclei.

Since the BF nuclei which project to the amygdala have been implicated in both psychiatric (aggression) and cognitive (memory) disorders that are known to depend upon the amygdala as well, the interconnections between these two structures may play a critical role in these functions.

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341.4 CONNECTIONS BETWEEN THE INTERPEDUNCULAR NUCLEUS AND THE BASAL FOREBRAIN. B. Fass and R.P. Vertes. Anatomy Dept., UMDNJ/Rutgers Med. Sch., Piscataway, NJ 08854; Mercer Univ. Med. Sch., Macon, GA 31207

The interpeduncular nucleus (IPN) is organized into seven subnuclei which can be recognized on the basis of morphological features (1). In addition, there is evidence for a heterogeneous organization of the IPN's connections. For example, afferents to the IPN originating from the diagonal band nucleus (NDB) are: 1) differentially distributed across IPN subnuclei; and 2) most heavily concentrated in the apical subnucleus and least concentrated in the lateral subnucleus (2). Recently, Vertes (3) found evidence that the IPN projects back to the NDB. The present study further analyzed the subnuclear organization of these reciprocal connections.

Adult male albino rats were given injections of WGA-HRP into specific regions of the basal forebrain. The brains were processed with TMB as the chromogen, to reveal anterograde terminal labeling and retrograde cell labeling. Injections were aimed at: 1) medial septum (MS) and vertical limb of NDB (vNDB); 2) lateral septum (LS); 3) horizontal limb of NDB (hNDB); or 4) substantia innominata (SI) and magnocellular preoptic nucleus (MPN).

The major findings of this study are: 1) apical, central, and lateral subnuclei contain the highest concentration of neurons projecting to MS/vNDB; 2) apical, central, and rostral subnuclei contain the highest density of efferents from MS/vNDB; 3) apical, lateral, and intermediate subnuclei contain the highest concentration of neurons projecting to LS; 4) LS does not project back to IPN; 5) apical, central, and lateral subnuclei contain the highest concentration of neurons projecting to hNDB; 6) central and rostral subnuclei contain the highest density of efferents from hNDB; 7) IPN does not project to SI/MPN; 8) central, intermediate, and rostral subnuclei contain the highest density of efferents from SI/MPN; and 9) rostral subnucleus does not contain neurons which project to the basal forebrain. These findings indicate a heterogeneous distribution of projection neurons and efferent terminal fields across IPN subnuclei. The IPN and regions of the basal forebrain share reciprocal connections which may serve as a substrate through which these structures directly influence each other's functional activity.

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(1) Hamill and Lenn, J. Comp. Neurol., 1984, 222, 396-408.
(2) Hamill and Fass, Neurosci. Lett., 1984, 48, 43-48.
(3) Vertes, Neurosci. Abstr., 1985, 11, 1224.

341.5 ESTRADIOL CONCENTRATION BY SUBSTANCE R IMMUNOREACTIVE NEURONS OF THE HYPOTHALAMIC VENTROMEDIAL AND ARCUATE NUCLEI. T.R. Akesson and P.E. Micevych. Dept. of Anatomy and Medicine and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

An abundant population of substance P immunoreactive (SPir) neurons can be found in the rat mediobasal hypothalamus. SP has been shown to be an excitatory peptide in the spinal cord and amygdala, however a role in the hypothalamus has not yet been established. A growing body of evidence does suggest that SP may participate in reproductive functions. Intraventricular injections of SP have been shown to facilitate release of luteinizing hormone in the female rat and neuroactive fragments of this peptide disrupt (prolong) normal estrous cyclicity. Numbers of SPir neurons in the arcuate nucleus vary with the estrous cycle and subcellular alterations in these cells suggest increased ribosomal activity during proestrus and estrus. SP content of the mediobasal hypothalamus has also been shown to vary with the estrous cycle. We have used the combined techniques of steroid autoradiography and immunohistochemistry to test for estrogen concentrating capacity among hypothalamic neurons which express SP.

Autoradiograms were prepared according to the method of Morrell and Pfaff (Meth. Enzymol., 103:639, '83) with some modifications. They were postfixed in Bouin's solution, developed in D-170 (Kodak), photofixed in 10% sodium thiosulfate, and tissues were incubated with primary antiserum at room temperature for 48 h. Location of primary antibodies was visualized with the ABC method (Vector). This method has also allowed us to successfully visualize immunoreactive cholecystokinin and estrogen accumulating cells. Estrogen (E) concentrating cells were identified using a computer-assisted grain counter. Distribution of SP- and E-labeled neurons appeared to be comparable to results we have obtained from tissue optimally processed for immunohistochemistry or autoradiography separately.

SPir neurons were found at all levels of the hypothalamus. Substantial numbers of labeled cells were found in the preoptic area, lateral hypothalamus, dorsomedial, ventromedial, arcuate, and ventral premammillary nuclei. Occasional cases of coexistence were found in the lateral hypothalamus and a small but consistently double-labeled population was discovered in the arcuate nucleus. The majority of neurons which coexpress SP and receptors for E, however, were located in the ventrolateral part of the ventromedial nucleus. These results provide anatomical support for the hypothesis that discrete subpopulations of SPir neurons may be functionally linked to estrogenic regulation of gonadotropin secretion and/or reproductive behavior.

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341.6 LOSS OF SEPTO-HIPPOCAMPAL CONNECTIONS FOLLOWING TEMPORARY BLOCKADE OF AXON TRANSPORT IN THE FORNIX/FIMBRIA. B. D. Fantie, R. H. Dyck and R. J. Sutherland. Dept. of Psychology, The University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

The hippocampal formation receives input from cholinergic and noncholinergic neurons of the medial septal nucleus and nucleus of the diagonal band of Broca. Septal cholinergic neurons have been shown to retrogradely transport nerve growth factor from terminal fields in the hippocampal formation and, following coronal transections that include fornix/fimbria, a substantial proportion of both cholinergic and noncholinergic septal neurons degenerate. We examined two questions: 1. Does the maintenance of septo-hippocampal connections require continuity of axonal transport through the fornix/fimbria? and 2. Do cholinergic and noncholinergic neurons differ in their response to axonal transport blockade.

Colchicine, a tubulin-binding agent, was injected (1 µg/µl physiological saline) unilaterally into the fornix/fimbria. At least two weeks after colchicine treatment, the rats received injections of a retrogradely-transported fluorescent dye, True Blue (0.2 µl), into the ipsilateral dorsal hippocampus. One week later the rats were treated with diisopropyl fluorophosphate then sacrificed 5 hours later. The brains were sectioned at 40 µm through the medial septum, diagonal band, and hippocampus. Fluorescent cells were photographed and counted. The sections were then stained for AChe using a modification of the Karnovsky and Roots method and rephotographed. The numbers of fluorescent cells in the medial septal nucleus and nucleus of the diagonal band which were AChe-positive or AChe-negative were then calculated. Control rats were treated identically except that colchicine was not injected.

Consistent with other results, we found that only a minority of cells in the septum projecting to the hippocampus were AChe-positive (40-50%) in control rats. In contrast, a much greater proportion of cells projecting to the hippocampus were AChe-positive (65-75%) in rats treated with colchicine. In addition, there were fewer retrogradely-labelled septum neurons. Both effects of colchicine--the increase in proportion of cholinergic to non-cholinergic cells and the reduction in number of cells projecting to the hippocampus--were more apparent in the nucleus of the diagonal band than in the medial septal nucleus.

The results suggest that there is a preferential loss of noncholinergic septo-hippocampal connections following temporary blockade of axonal transport in the fornix/fimbria. One interpretation is that noncholinergic septal neurons, particularly those in the nucleus of the diagonal band, are more dependent upon a continuity of retrograde transport of trophic substance(s) through their axons in the fornix/fimbria than are cholinergic neurons.

341.7 PROJECTIONS OF AMYGDALOID NEURONS CONTAINING SOMATOSTATIN, VASOACTIVE INTESTINAL PEPTIDE, OR GABA. A.J. McDonald Dept. of Anatomy. Univ. of So. Carolina Sch. of Med., Columbia, SC 29208.

The present study examined the axonal projections of amygdaloid neurons containing GABA, somatostatin (SOM), or vasoactive intestinal peptide (VIP). Sprague-Dawley rats (n=43) received injections of WGA-HRP into telencephalic and diencephalic areas known to receive amygdaloid projections. After 24 hr animals were perfused with 2.5% paraformaldehyde-0.5% glutaraldehyde. Brains were processed for HRP histochemistry (stabilized TMB protocol of Rye et al., '84) followed by PAP immunohistochemistry (primary antibodies obtained from Immuno Nuclear Corp.).

With injections into the bed nucleus of the stria terminalis (BST) or medial hypothalamus numerous SOM-positive retrogradely labeled neurons (SOM-HRP cells) were seen in the amygdala. Most of these double labeled cells were located in the medial nucleus and intra-amygdaloid portion of the BST (BSTia). Smaller numbers of SOM-HRP cells were observed in the anterior amygdaloid area, basomedial nucleus, and medial subdivision of the central nucleus. With lateral hypothalamic injections SOM-HRP neurons were also seen in the lateral subdivision of the central nucleus. Occasional double-labeled VIP-HRP neurons were observed in the anterior amygdaloid area or BSTia with hypothalamic and BST injections.

Injections into the cerebral cortex or striatum retrogradely labeled numerous cells in the lateral and basolateral amygdaloid nuclei (L-BL) but, with the exception of an occasional SOM-HRP neuron, no double-labeled cells were observed. Likewise, no double-labeled neurons were observed in L-BL with injections into the mediodorsal thalamus, hypothalamus, or BST. The results of experiments in which small iontophoretic injections were made into individual amygdaloid nuclei suggest that axons of most SOM, VIP, and GABA containing cells in L-BL do not extend more than 500 µm from the cell body of origin. There is evidence, however, for a somatostatin containing projection from the lateral nucleus to the basomedial nucleus. In addition, a few SOM-positive neurons appear to project axons to the central nucleus.

Thus, the results of this study suggest that most SOM, VIP, and GABA containing neurons in L-BL are involved in intra-amygdaloid connections while in the corticomedial amygdala a significant number of SOM containing neurons project to the BST and hypothalamus. Supported by NIH Grant NS 19733.

341.8 COEXPRESSION OF OPSIN-LIKE AND VIP-LIKE IMMUNOREACTIVITY IN CSF-CONTACTING NEURONS OF THE AVIAN BRAIN. P. Horvath*[1], P. Witkovsky[2], M. Lehman[3], and R. Silver[1]. [1]Barnard College, NY, NY 10027; [2]New York University Medical School, NY, NY; [3]University of Cincinnati, College of Medicine, Cininatti, Ohio 45221.

CSF-contacting cells in both septal and tuberal areas in the brain of the ring dove (Streptopelia roseogrisea) labeled by RET-P1 (gift of C.J. Barnstable), a monoclonal antibody which reacts with inner and outer segment membranes of retinal photoreceptors in a variety of vertebrates (Fekete and Barnstable 1983, J. Neurocytol 12: 785-803). Most septal RET-P1-positive cells are roughly spherical in shape with diameters of ~7-10um. Many have dendritic processes penetrating the ependyma, and bear a knob-like terminal (~2.5um) suspended in the lateral ventricle. The cells also emit other processes. Some form a plexus in the region of cell bodies, while others radiate to the overlying neuropil. The cell bodies are suenpendymal and lie on the medial wall of the lateral ventricle extending dorsally ~600um from the ventral tip of the ventricle. RET-P1-positive cells in the tuberal area have similar morphological characteristics to their counterparts in septal area. The population of stained cells in the tuberal area is 1/6 that of the septal population, and extends ~500um along the ventricle. Immunoreactive fibers are seen in the area bounded by the medial aspect of the lateral ventricle in the septal region. A few scattered fibers project dorsally to the hippocampus. Many diffuse immunoreactive fibers are widely distributed in the diencephalon from the level of the septum to the level of the infundibulum. Additionally, a discrete band of labeled fibers occurs in the external layer of the median eminence. Preliminary studies involving Japanese quail (Coturnix coturnix) and the domestic Cayuga duck (Anas platyrhynchos) suggest that the septal area of these species also contains RET-P1-positive cells. The infundibular region remains to be studied. Use of an immunofluorescent double-label technique involving rhodamine and FITC labels demonstrates that RET-P1-positive cells coexpress VIP-like immunoreactivity. VIP-positive cells in other brain areas are not RET-P1-positive.

We are presently examining the ultrastructure of these cells. Such studies, together with physiological experiments are being pursued to probe the possible role of these cells as "deep encephalic photoreceptors."

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341.9 ORGANIZATION OF PYRAMIDAL CELL AXONAL COLLATERALS IN FIELD CA3 OF THE RAT HIPPOCAMPUS. N. Ishizuka, K. Krzemieniewska and D.G. Amaral (SPON: J. Lindstrom). The Salk Institute, La Jolla, CA 92037.

The distribution of local collaterals arising from the pyramidal cells of the CA3 region of the rat hippocampus have been studied by two methods. In the first, single pyramidal cells in the hippocampal slice preparation were intracellularly injected with HRP. The whole, 400µm thick slice was reacted for the demonstration of the enzyme and the plexus of axonal collaterals was plotted using a three dimensional computer reconstruction system. The second method involved the stereotaxic placement of small, iontophoretic injections of the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L) into different portions of field CA3. Animals were allowed to survive for 7-10 days and 30µm sections through the hippocampus were immunohistochemically processed for the localization of the tracer.

In adddition to the well known Schaffer collaterals which project to field CA1, each pyramidal cell had approximately 4 additional local collaterals originating from the parent axon. Regardless of the position of the cell body of origin, some of these collaterals extended towards field CA1 while others projected back towards the dentate gyrus and ramified either in stratum oriens or in stratum radiatum of field CA3. No collaterals entered any portion of the dentate gyrus. The collaterals from pyramidal cells located near the dentate gyrus traveled mainly in the superficial aspect of stratum radiatum and few entered stratum oriens. The pyramidal cells located in the mid and distal portions of field CA3, in contrast, gave rise to extensive axonal plexuses both in stratum oriens and in stratum radiatum. The position and number of presumed presynaptic varicosities along the collaterals were also determined; as many as 2300 varicosities were observed on the collaterals of one cell. The collaterals were clearly not confined to the thickness of the slice since most were cut at one of the slice surfaces. The PHA-L preparations confirmed the observations from the intracellular preparations and further demonstrated that the CA3 collateral plexus can extend for several millimeters along the septotemporal axis of the hippocampus. These observations provide evidence that CA3 pyramidal cells are potentially influenced by other pyramidal cells from all transverse positions of the same hippocampal level via local collaterals and by more distant pyramidal cells through the longitudinal association bundle.

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341.10 SYNAPSIN 1 IMMUNOHISTOCHEMICAL ASSESSMENT OF DEVELOPMENT IN THE HYPOTHALAMUS IN THE RAT. M.F. Bernstein and R.Y. Moore, Depts. of Neurology and Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Synapsin 1 is a neuron specific phosphoprotein which is an endogenous substrate for cyclic AMP-specific and Ca/calmodulin dependent protein kinases. Synaptic 1 is present in terminals of the peripheral and central nervous systems where it is located in the membrane of small synaptic vesicles (DeCamilli et al., 1979; 1983; Navone et al., 1984). In a previous study we used synapsin 1 immunoreactivity as a marker for synapse development in the rat hypothalamus (Bernstein and Moore, 1985). The development of synapsin 1 immunoreactivity in the suprachiasmatic nucleus exhibited a distinctive pattern that differed from adjacent anterior hypothalamus and correlated exactly with the development of synapses demonstrated by electron microscopy. Thus, synapsin 1 immunoreactivity appears to be an excellent marker for synapse formation. In addition, in our prior study, there was some indication that perikaryl labeling preceded that of synaptic fields suggesting that one stage of development might be marked by high levels of synapsin production in a neuron before its terminal fields are elaborated.

In the present study we examined the development of synapsin 1 immunoreactivity in the paraventricular (PVN) and supraoptic (SON) hypothalamic nuclei. These nuclei are derived from the germinal epithelium between embryonic days 13 (E13) and E15 (Altman and Bayer, 1978), and both contain subfields with oxytocin- and vasopressin-containing neurons (Sawchenko and Swanson, 1982,1983,1984). Synapsin 1 immunoreactivity was studied in PVN and SON from E18 to P6. In PVN there is a steadily increasing number of synapses from E18 to P6. At E19 the first distinct synaptic fields appear in dorsal and medial PVN and they become more heavily labeled and expand in size with age. By P6 the density of synapsin 1 immunoreactivity approximates that seen in the adult. Large numbers of PVN perikarya exhibit immunolabeling at E18-E19 suggesting that these neurons are producing terminal axonal arborization shortly thereafter. In SON there is some labeling of synapses at E18 and the nucleus becomes progressively and uniformly more densely labeled until P6. Perikaryal staining is noted only rarely in SON.

In summary, SON and PVN are innervated progressively through late fetal and early postnatal life. Perikaryal staining is widespread in PVN but very uncommon in SON, indicating that this is not a reliable indicator of synapse formation. We are grateful to Drs. Paul Greengard and Charles Ouimet for providing the synapsin 1 antibody. Supported by NIH grant NS-16304.

341.11 **IMMUNOHISTOCHEMICAL MAPPING OF ADENOSINE DEAMINASE-CONTAINING NEURONS AND FIBERS IN THE RAT BRAIN.** **Wm. A. Staines*, P.E. Daddona* and J.I. Nagy; Department of Physiology, University of Manitoba, 770 Bannatyne Ave., Winnipeg, Manitoba Canada R3E 0W3.**

We have reported previously on the immunohistochemical detection of adenosine deaminase (ADA) in neurons in the posterior hypothalamus and several other structures of the rat brain (Nagy et al., Science 224 (1984) 166-168). We now have completed a detailed mapping of the distribution of ADA-immunoreactive (ADA-IR) neurons and fibers in the entire brain of this species. ADA was found to have a highly restricted localization, being selectively expressed within neurons of only a few, well-defined brain structures and often by only subpopulations of neurons within these structures.

The most prominent group of cells stained were those of the tuberomammillary nucleus of the hypothalamus. In addition, positive neurons were seen in the anterior olfactory nucleus, the anterior, posterior, retrosplenial and entorhinal cortices, the medial border of the striatum, the olfactory tubercle, the septum, the lateral extreme of the horizontal limb of the diagonal band, the central nucleus of the amygdala, the lateral hypothalamus, the parafasicular nucleus, superior colliculus, central grey, medullary reticular formation and preganglionic parasympathetic nuclei.

ADA-IR fibers and varicosities appeared to fall into two categories. (1) A very striking network of long, large-caliber varicose fibers, apparently arising from the cells of the TM, showed dense ramification throughout the hypothalamus and more sparse distribution throughout most other regions including cortex, striatum, septum, midline thalamic nuclei and diverse regions within the midbrain and brainstem. The hypothalamic origin of this class of fiber was confirmed in preliminary experiments involving electrolytic lesions of the TM. Some reductions in the density of ADA-IR fibers ipsilateral to the lesions are seen in temporal and frontal coritical areas, bed nucleus of the stria terminalis and anterior medial hypothalamic areas. (2) ADA-IR presumptive terminal fields of a fine, puncture nature were seen in a number of nuclei but were particulary dense in the dorsal midbrain, medial habenula and substantia nigra. These latter two are correlated with the presence of positive cells in the septum and striatum.

The documentation of neurons and fibers containing ADA is expected to aid studies of the functional significance of this enzyme in relation to adenosine metabolism in the CNS.

341.12 THALAMOCINGULATE PROJECTIONS AS REVEALED BY RHODAMINE MICROSPHERES USED AS A RETROGRADE TRACER. Kyoi Horkawa* and Ervin W.Powell. Dept. of Neurosurgery, University of the Rukyus, Okinawa, Japan and Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

This study identifies neurons of the anterior thalamic group of nuclei projecting to specific areas of the cingulate gyrus. Although the connection is well known, there are differences in reports of specific projections. The anterior and posterior parts of areas 24 and 29 were injected with 1µl of a 100% solution of rhodamine microspheres. Seventy-two hours later the rat (n=11) was perfused through the left ventricle of the heart with a 10% solution of formaldehyde. The brain was frozen and 30 µm sections were cut, mounted and dried on slides. The sections were studied using a Leitz microscope equipped with epifluorescence and a PLOEMOPAK M_2 filter. Results are compared in Table 1. The anteroventral (AV) and anteromedial (AM) nuclei project to anterior and posterior areas of region 29. We found no projections from anterodorsal (AD) and AV nuclei to anterior area 24. The density and topography of labeled neurons differed in the thalamic nuclei studied. Furthermore, the percent of neurons retrogradely labeled in the anterior nuclei totaled 111% for AD; 113% for AV; 205% for AM. Therefore, we suspect that collateralization exists from the anterior nuclei to areas of the cingulate gyrus. Labeled neurons were not present in the lateral dorsal and dorsal medial nulcei of the thalamus using this tracer.

TABLE 1

THALAMIC PROJECTIONS TO CINGULATE GYRUS

	Area 24 anterior	Area 24 posterior	Area 29 anterior	Area 29 posterior
AD	- - N - -	- - N - O	R N D F O	R D N F O
AV	- - N - -	- - N - O	- D N F O	- D N F O
AM	R D N F O	R D N F O	- - N F O	- - N F O

R = Rose and Woolsey; D = Domesick; N = Niimi et al.; F = Finch and Delian; O = Ours

341.13 THE TOPOGRAPHY AND FINE STRUCTURE OF SUBICULAR CORTEX PROJECTIONS TO THE MAMILLARY NUCLEI IN THE RAT. G.V. Allen* and D.A. Hopkins. Dept. of Anatomy, Dalhousie University, Halifax, Canada, B3H 4H7.

The mamillary body (MB) constitutes a major hypothalamic link between cortex (subicular) and brain stem (dorsal and ventral tegmental nuclei) for afferent information relayed to the thalamus and cingulate cortex. However, little is known regarding the ultrastructure of afferent fibers in the MB. In order to elucidate the synaptic organization of subicular afferents to the MB, 5% wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was injected in different regions of the subicular cortex in 23 male Wistar rats. After 1-2 days the animals were perfused and the brains processed for HRP histochemistry using tetramethyl benzidine (TMB) and cobalt chloride-glucose oxidase enhanced diaminobenzidine (DAB) for light and electron microscopy.

Following localized injections of WGA-HRP in the subicular cortex, anterograde labeling was found to be topographically distributed in the MB. Injections in the rostral parts of the subicular cortex resulted in anterograde labeling in the dorsal MB, bilaterally. Caudal and ventral subicular injections produced anterograde labeling in more ventral regions of the MB, bilaterally. In addition, injections in lateral subicular cortex produced label in the medial subdivision of the posterior MB which contrasted with medial subicular injections which resulted in anterograde labeling in the lateral as well as medial posterior MB, bilaterally. Anterograde label was seen in the lateral mamillary (LM) nucleus, primarily ipsilaterally, after injections in parasubiculum and caudoventral presubiculum but no anterograde label was observed in the LM following injections of WGA-HRP in the dorsal presubiculum.

At the electron microscopic level anterograde label was best visualized following stabilization of TMB reaction product by subsequent application of the DAB histochemical procedure. Electron dense reaction product was found in synaptic boutons which were mainly characterized by round vesicles and asymmetrical synaptic junctions. Labeled terminals were observed to synapse primarily on dendritic processes while only occasional labeled terminals were observed in contact with neuronal somata. The characteristics of these terminals contrast with those of axon terminals of ascending projections from the brain stem tegmentum which consisted of axosomatic and axodendritic synapses with pleomorphic vesicles and symmetrical junctions (Allen and Hopkins,'84).

In summary, specific groups of subicular neurons project topographically to the MB. Subicular afferent fibers in the MB comprise a distinct class of axon terminals which contrasts in type and location with synapses formed by brain stem tegmental afferent projections to the MB. Supported by MRC of Canada.

341.14 COMBINED ELECTROPHYSIOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES OF HUMAN HIPPOCAMPUS FROM PATIENTS WITH TEMPORAL LOBE EPILEPSY. K.R. Erickson*, M.J. Kelly, K.D. Laxer*, T.J. Rosenbaum*, and O.K. Ronnekleiv (SPON: O.S.M. Marin). Dept. of Physiology, Oregon Health Sciences University, and Neurophysiology Laboratory, Good Samaritan Hospital, Portland, OR 97201.

The in vitro slice technique has been applied to hippocampal tissue in many species, but no reported studies have focused specifically on human hippocampus using combined electrophysiological and immunocytochemical techniques. In this study, human hippocampus was obtained from patients undergoing temporal lobectomy for control of refractory partial complex seizures. The anterior 1.5-2.0 cm of hippocampus was removed for seizure control. Part of this tissue was immediately placed into cold, oxygenated artificial cerebrospinal fluid (ACSF) and 450 µm-thick transverse slices were made through the hippocampus. Some slices were immediately fixed in 4% paraformaldehyde (phosphate buffered to pH 7.4). 10 µm cryostat sections were made, and the sections were processed immunocytochemically using the avidin-biotin method. Primary antisera to the following substances were used at a 1:1500 to 2000 dilution: substance P, neuropeptide Y, somatostatin, serotonin, dynorphin, enkephalin, and VIP. Other hippocampal slices were transported in ACSF to the recording chamber and maintained for 10 hrs while electrophysiological studies were carried out. Electrophysiological recordings were made in the CA3 and CA1 cell layer with stimulation of the dentate for orthodromic activation and the alveus/fimbria for antidromic activation of pyramidal neurons. Upon locating viable cells, norepinephrine (NE), acetylcholine (Ach) and serotonin were applied in nanodrop quantities via micropipettes. Robust intracellular activity was recorded in the pyramidal cell layer up to 10 hrs following surgical removal. Cells showed spontaneous activity ranging from 3-15 Hz, and were antidromically driven by alveus stimulation. Ach depolarized and increased the firing frequency in several cells, and NE mostly inhibited cell firing. Immunocytochemical staining revealed intense labeling of cell bodies and fibers for substance P, somatostatin, and neuropeptide Y. A high concentration of dynorphin-positive fibers were observed in the CA3 cell layer, and large caliber enkephalin fibers were observed in the dentate cell layer. Both CA1 and CA3 neurons appeared to be heavily innervated by serotonin fibers. These studies indicate that combined electrophysiological and immunocytochemical studies are possible on human pathological tissue, and such studies should yield some information on the basic cellular pathophysiology underlying epilepsy.(Supported by NIH grants: HD 19905 to MJK; HD 16793 to OKR; AG 05354 to KRE)

341.15 LOCAL CIRCUIT INTERACTIONS BETWEEN ORIENS/ALVEUS INTERNEURONS AND CA1 CELLS: PAIRED INTRACELLULAR RECORDINGS AND ELECTRON MICROSCOPY. J.-C. Lacaille, D.D. Kunkel,* P.A. Schwartzkroin. Dept. Neurological Surgery, Univ. of Wash., Seattle, WA 98195.

To further determine the role of interneurons in the CA1 region of hippocampus, we examined the synaptic interactions between oriens/alveus (O/A) interneurons and neurons in stratum pyramidale of hippocampal slices of guinea pigs, first, with simultaneous intracellular recordings from O/A interneurons and CA1 cells, and second, with electron microscopy of HRP-injected O/A interneurons.

(1) Electrophysiology: Tests for synaptic interactions were performed in 53 pairs of cells (O/A interneurons: RMP -57 ± 14 mV; spike amplitude 58 ± 11 mV). In 29 of 45 pairs (64%) of O/A interneuron-CA1 pyramidal cells tested, EPSPs were seen in O/A interneurons immediately following depolarization-induced action potentials in CA1 pyramidal cells (unitary EPSP mean amplitude 2.16 ± .95mV; mean peak latency 3.83 ± .92 ms; p (failure) 0.34). In 10 of these 29 pairs, EPSPs were sometimes large enough to fire action potentials in the interneurons (mean latency from pyramidal cell spike to A/O spike 3.79 ± 1.24 ms).

Stimulation of A/O interneurons produced fewer synaptic interactions in pyramidale neurons. In 3 of 42 O/A interneuron-CA1 pyramidal cell pairs, small amplitude inhibitory postsynaptic potentials (IPSPs) were seen in pyramidal cells during depolarization-induced firing of O/A interneurons. These IPSPs were sufficient to inhibit CA1 action potentials normally evoked by rheobasic current injection. In 1 of 6 pairs of O/A interneuron-str. pyramidale interneurons tested, IPSPs were also observed in the pyramidale interneuron during a burst of action potentials in O/A interneurons. In one O/A interneuron-CA1 pyramidal cell pair, reciprocal interactions were found. Stimulation of the pyramidal cell produced EPSPs in the interneuron, and stimulation of the interneuron produced an IPSP in the pyramidal cell.

(2) Electron microscopy: Following HRP injection (positive current pulses: 3-5 nA, 20 msec, 33 Hz, for 2-10 min.), 4 O/A interneurons with well-filled processes were recovered and processed for EM. HRP-filled fusiform somata and dendritic processes were oriented parallel to the alveus at the O/A border. The axon left the soma, coursed parallel to the alveus, then turned and descended toward str. pyramidale. HRP-filled symmetric synaptic contacts were seen on pyramidal dendrites in stratum oriens and radiatum, as well as on pyramidal cell somata. Similar HRP-filled symmetric contacts were made on non-pyramidal cell dendrites in str. oriens.

These electrophysiological and anatomical results suggest that O/A interneurons are directly excited by CA1 cells, and, in turn, inhibit these cells. Thus, they may mediate feedforward and feedback inhibition of CA1 cells.

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341.16 **NEW CONNECTIONS OF THE SUBFORNICAL ORGAN OF THE RAT: PROJECTIONS TO THE AMYGDALA, CORTEX AND THALAMUS.** M.L. Weiss and R.R. Miselis. Depts. of Biology, Animal Biology and the Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104-6045.

The subfornical organ (SFO) mediates the drinking, vasopressin and pressor responses to peripherally administered angiotensin II. The neuroanatomical substrate which subserves these responses has been described previously (Miselis et al., '79; Miselis, '82). We sought to confirm and extend previous descriptions of additional projections of the SFO (Weiss and Miselis, '84; Lind and Swanson, '85) using new and sensitive tracers cholera toxin conjugated to HRP (CT-HRP) and Fluoro-gold. Injections of CT-HRP into the SFO produced all of the known efferent connections of the SFO: the magnocellular nuclei of the hypothalamus (including the accessory areas), parvocellular subnuclei of the paraventricular nucleus, nucleus medianus (NM), organum vasculosum of the lamina terminalis, bed nucleus of the stria terminalis (medial or parastrial and lateral subdivisions), specific regions of the lateral hypothalamus (LH) and the medial and lateral preoptic areas (MPO/LPO), anteroventral periventricular nucleus (AVPV), paraventricular nucleus of the thalamus, substantia innominata, zona incerta and suprachiasmatic nucleus. In addition, we saw projections into the infralimbic cortex (ILC), the central medial thalamus, and the central and medial nuclei of the amygdala. A few fibers were detected in the arcuate nucleus and the median emminence. Several of these projections were confirmed by injections of either CT-HRP or Fluoro-gold into their respective terminal areas. Afferents to SFO were found in the dorsal medial hypothalamic nucleus, lateral parabrachial nucleus, NM, AVPV, LH, MPO, periventricular stratum, dorsal and medial raphe nuclei and medial septum. Newly described afferents were found in the ILC, locus coeruleus and the tuberal supraoptic nucleus. These findings suggest that the SFO is intimately connected with a set of structures which modulate endocrine, autonomic and behavioral aspects of homeostasis. Supported by GM 27739 and MH 15092.

341.17 **SEPTAL PROJECTIONS TO ACCESSORY MAGNOCELLULAR NEURONS OF THE PREOPTIC-HYPOTHALAMIC REGION.** D.A.Scott*, M.L.Weiss and R.R.Miselis. (SPON: R.O.Davies). Depts of Biology, Animal Biology and Institute of Neurological Sciences, Univ. of Penn., Phila., PA 19104.

The role of the septum in fluid balance has been unclear. Past lesion studies implicated it as a source of inhibition to thirst or a facilitator of antidiuresis. These studies were done without knowledge of the neural circuitry emanating from the subfornical organ, nucleus medianus or the organum vasculosum which lie in the lamina terminalis just behind the septal nuclei. Therefore, it is not certain whether the results were due to inadvertent damage to structures of the lamina terminalis or the septal nuclei (Eng and Miselis, '81). With the use of tritiated amino acids and horseradish peroxidase (HRP) it had been found that the septum did not project directly into the classical magnocellular [supraoptic (SON) and paraventricular (PVN)] nuclei of the hypothalamus indicating that the septum's influence on vasopressin secretion would have to be multisynaptic. However, the SFO had been found to have the most prominent projections to the SON and PVN as well as to all the accessory magnocellular (AcM) nuclei of the preoptic-hypothalamic region (Miselis et al, '79; Miselis '82) suggesting a more direct influence on vasopressin secretion compared to the septum. In the current study we reexamined the septal projections to the preoptic-hypothalamic region using wheat germ agglutinin or cholera toxin (CT) conjugated to HRP with particular attention to the apparent terminal fields over the AcM nuclei. We find that the septum projects heavily into the vicinity of the SON and PVN such that it outlines the boundaries of these nuclei without entering them (Oldfield and Silverman, '85). However, there was significant apparent terminal field labelling overlying the AcM nuclei that was independently identified by CT-HRP injections into the posterior pituitary. These included the following AcM areas: nucleus of the anterior commissure, nucleus circularis, posterior fornical nucleus and the nucleus of the medial forebrain bundle (Peterson, '66). There were additional magnocellular neuronal collections projecting to the posterior pituitary gland which were in restricted parts of the periventricular stratum (Kelly and Swanson, '80) that also had apparent terminal field labelling originating from septal injections. Our findings suggest that septal projections may have direct contact with the cells of the AcM areas; and, therefore, may directly affect vasopressin secretion. Double labelling studies are underway to provide definitive confirmation. The distribution of AcM neurons labelled by posterior pituitary injections of CT-HRP suggests that many of the SFO's projections to the preoptic-hypothalamic region may be to AcM neurons for the purposes of modulating posterior pituitary secretions rather than affecting other functions often associated with these specific areas. Supported by NIH grant GM 27739.

341.18 ANATOMICAL EVIDENCE FOR OLFACTORY INPUT TO THE SUPRAOPTIC NUCLEUS OF THE RAT: AN ANTEROGRADE STUDY EMPLOYING THE LECTIN PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). D.E. Hollowell*, K.G. Smithson* and G.I. Hatton (SPON: G.Richmond). Neuroscience Program & Department of Physiology, College of Osteopathic Medicine, Michigan State University, E. Lansing, MI 48824.

Terminal degeneration has been seen lateral to the more ventral and caudal portions of the supraoptic nucleus (SON) following lesioning of the rat main olfactory bulb (R.C. Switzer & L. Heimer, unpublished). It has been suggested that both osmoregulation and maternal behavior may be influenced in part by olfactory bulb input to the hypothalamus. Therefore, one might expect to find inputs from the olfactory bulb to cells that synthesize and release vasopressin and oxytocin, both of which are produced by cells within the SON. We have investigated this proposed connection by injection of the anterogradely transported lectin, Phaseolus vulgaris leucoagglutinin (PHA-L; Brain Res., 290: 219, 1984) into the main olfactory bulb.

Discrete iontophoretic injections of PHA-L were stereotaxically placed into the main olfactory bulb. The animals were allowed to survive for 7-14 days after which their brains were fixed by transcardial perfusion, embedded in polyethylene glycol, and sectioned at 15-25 um. The presence of the lectin was detected immunocytochemically with an avidin-biotinylated peroxidase procedure.

Stained fibers in the area of SON were most often seen passing laterally to the nucleus as they continued to more posterior structures, possibly coursing in the median forebrain bundle. Many of these fibers gave rise to collateral branches whose putative terminals ended along the lateral and more posterior margin of the SON. Additionally, a smaller percentage of terminal profiles were also observed within the nucleus itself. Putative terminals were also observed in the tuberal portion of SON.

These results confirm observations seen with terminal degeneration studies. Additionally, they provide new information by demonstrating a direct connection between the main olfactory bulb and the supraoptic nucleus. Further evidence for this connection is provided by data obtained from an electrophysiological investigation of this pathway (see Dougherty et al. abstract). Supported by NIH grants NS16942 & NS01940 and by a fellowship from the Medical Scientist Training Program to KGS.

341.19 EVIDENCE FOR ZONA INCERTA/PERIFORNICAL PROJECTIONS TO THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI OF RAT HYPOTHALAMUS. K.G. Smithson* and G.I.Hatton (SPON: L.I. O'Kelly) Neuroscience Program & Dept of Physiology, College of Osteopathic Medicine, Michigan State University, E. Lansing, MI 48824.

Recent research has led to the suggestion that local neuronal circuits connect the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei to adjacent brain areas. Support for this hypothesis is provided by the presence of neurons which send axon collaterals to terminate within nearby areas. Furthermore reciprocal projections between PVN neurons and neurons of the perifornical region (Brain Res. Bull., 14: 123, 1985) as well as between the SON and the lateral hypothalamus (Neurosci., 11: 169, 1984) have been demonstrated.

Efferent projections of cells from areas that receive axon collaterals from PVN neurons were studied using the anterogradely transported lectin, Phaseolus vulgaris leucoagglutinin (PHA-L) essentially according to the technique of Gerfen and Sawchenko (Brain Res., 290: 219, 1984). Discrete iontophoretic injections of PHA-L were stereotaxically placed in an area immediately posterior to the PVN, dorsal to the fornix, and which included anteromedial portions of the zona incerta.

Two prominent sets of labeled fibers originated from these injection sites. The most promineht pathway traveled anteriorly from the injection site, taking a course medial and ventral to the fornix. As this group of fibers approached the base of the brain it turned laterally appearing to join the medial forebrain bundle. Throughout the course of this pathway many terminal profiles were observed. Of particular interest, with respect to the magnocellular neuroendocrine system, was a dense plexus of terminal profiles seen to decorate the lateral borders of PVN and lateral and medial borders of SON. A small percentage of these terminals were found within each magnocellular nucleus. The second group of fibers ran anteriorly from the injection site, but passed lateral and ventral to the fornix to join the medial forebrain bundle. This group of fibers also appeared to contribute to the terminal profiles surrounding SON laterally.

These observations provide additional evidence for the existence of local circuits involving the perifornical region and the PVN. It appears that SON may also participate in this circuit, however, a reciprocal projection from SON to the perifornical region remains to be described. Supported by NIH grants 16942 & 09140 and by a fellowship from the Medical Scientist Training Program to KGS.

341.20 SYNAPTICALLY MEDIATED RESPONSES RECORDED INTRACELLULARLY IN RAT SUPRAOPTIC NUCLEUS INDUCED BY LATERAL OLFACTORY TRACT STIMULATION. P.J. Dougherty*, Q.Z. Yang and G.I. Hatton (SPON: J. Zacks). Neuroscience Program, Michigan State University, East Lansing, MI 48824

Lesion of the main olfactory bulb in rat results in terminal degeneration lateral to the more ventral and posterior portions of the supraoptic nucleus SON (R.C. Switzer & L. Heimer, unpublished). Anterograde transport of lectin injected into the main bulb have confirmed and extended this finding (see Hollowell et al. abstr.). There have been suggestions in the lore and literature over the years that the olfactory system may play roles in both water balance and maternal behavior. Thus, inputs from this system to the cells that synthesize and release vasopressin and oxytocin might have been expected. We electrophysiologically investigated this direct ipsilateral projection from the bulb to the SON region using brain slices. Horizontally cut 500 μm thick slices included the basal hypothalamus and ventral cortex extending several millimeters anterior to the optic chiasm. Transillumination of such slices clearly reveals the compact lateral olfactory tract (LOT) coursing posteriorly as well as the full extent of the SON. One bipolar stimulating electrode was placed in the LOT about 2 mm anterior to the SON; a second one was placed away from the LOT, equidistant from the SON as a current spread control. SON neurons were impaled with 0.25 M LiAcetate/5% Lucifer Yellow-filled (LY) electrodes and postsynaptic responses to extracellular electrical stimulation (25-150 μA, 0.1 ms) were recorded. Synaptic responses from a total of 27 cells were studied in slices from 24 rats (8 each: lactating, virgin females and untreated males). LOT stimulation evoked short latency (1-2 ms) excitatory and inhibitory synaptic responses in SON neurons. In 1 neuron from a lactating and in 2 from virgin rats, IPSPs were evoked. One of these cells fired action potentials (APs) with a 35-40 ms latency after evoked IPSPs. The majority of responses were excitatory, consisting of: APs following each stimulation (@ 50 but not 100 Hz); trains of APs after single stimulations; only one EPSP per stimulation; or EPSPs leading to APs with increased current. LY injections revealed that single as well as dye coupled neurons in all groups were among the cells synaptically influenced by stimulation of this pathway. In no case did stimulation via the control electrode result in responses. Identification of the peptide contents of the LY-injected cells has not yet been done. These results support the existence of a mono- or disynaptic (or both) olfactory input to the SON. Supported by NIH grant NS16942

341.21 DIRECT EVIDENCE FOR ELECTRICAL COUPLING AMONG RAT SUPRAOPTIC NUCLEUS NEURONS. G.I. Hatton and Q.Z. Yang. Neuroscience Program, Michigan State University, East Lansing, MI 48824

Transfer of the fluorescent dye, Lucifer Yellow (LY), from one intracellularly injected neuron to another has been used as indirect evidence for the existence of electrotonic interactions between such dye coupled cells in a number of brain areas. Direct evidence for electrical coupling obtained by recording from two coupled cells at once has been gained for hippocampal neurons, but not for hypothalamic magnocellular neuroendocrine cells (MNCs). Reasons for this are that quality impalements are difficult to maintain and that MNCs are often coupled via distal dendrites with cell bodies 100-200 μm apart. Since interpretations of the functional significance of dye coupling depend upon its faithfully representing electrical coupling, we attempted to obtain direct evidence for electrotonic interactions among MNCs. Coronally cut slices of male rat hypothalamus were prepared as in our previous studies. Pairs of SON MNCs were impaled using microelectrodes filled either with 3M KAcetate or 5% LY-.25M LiAcetate. Taken as evidence for electrical coupling were: membrane depolarizations in one cell that were synchronized with spontaneously occurring action potentials in the other cell; synchronous changes in the two membrane potentials with current injection; and ability to evoke action potentials in both neurons with current injection in one. Five dual impalements produced pairs of electrically coupled cells. In each case that there was evidence for electrical coupling, dye coupling resulted from LY injection via one of the electrodes. All cells were dendrodendritically coupled. That the two electrodes were not in the same neuron was evident since membrane responses between coupled cells were never symmetrical, current injections in cell A of a pair could evoke action potentials in cell B, but not vice versa, and interelectrode distances were always too great to allow both electrodes to impale one cell. Capacitative coupling between the electrodes was ruled out by the following observations: cells in the same tissue penetration as the coupled cell showed no responses to membrane voltage changes in the primary cell; no responses were seen when the second electrode was placed extracellularly or in the medium; and similar coupling potentials were also seen when one cell was recorded without a second electrode present. These results indicate that electrical coupling exists among MNCs in brain slices and that it is reflected in dye coupling.

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BLOOD BRAIN BARRIER II

342.1 ASTROGLIA INDUCES CHANGES IN TIGHT JUNCTIONS OF CEREBRAL ENDOTHELIUM IN VITRO. J.-H. Tao-Cheng, Z. Nagy* and M.W.Brightman*. Lab. of Neurobiology,NINCDS,NIH,Bethesda,MD 20892.

Does the astrocyte, the nearest neighbor of the cerebral capillary, influence the formation of tight juctions (TJ) between the endothelial cells? Cerebral capillaries have zonular TJ, whose strands anastomose extensively without free ends, and are devoid of gap junctions. Cerebral endothelium in culture also has TJ but their frequency and extent are greatly reduced, especially in subcultures. We have examined more than 20 different subcultures of cerebral endothelium at different stages from 6 adult beef brains and TJ were infrequent in all of them. Even though our subcultures of endothelial cells, identified by their uptake of fluorochrome labeled, low density lipoprotein, were highly enriched and homogenous in cell type, only a small proportion (5-10%) of cells had TJ. The TJ usually consisted of short segments of anastomosing strands with many free ends. There was usually more than one TJ patch in a membrane; these patches could be very close to each other yet clearly separated. Many gap junctions lay between the TJ strands. The frequency, length and complexity of the TJ usually increased with time; beyond 15 days in vitro, the TJ occasionally consisted of a few, very long anastomosing strands enclosing many gap junctions. However, these TJ were still separated from other patches of TJ in the same membrane. To see if astrocytes influence the formation of endothelial TJ, we co-cultutered astroglia with cerebral endothelium. In 9 different endothelium subcultures grown with primary cultures of astroglia from 2 day old rats, the frequency, length, width and complexity of the TJ were markedly increased. The area of gap junction enclosed by the TJ strands was greatly reduced. Significantly, the TJ formed continuous, long networks rather than separate patches. The changes appeared as early as 6 days when, in one set, the average TJ length and width were 3.7μ and 0.7μ in the co-culture, and 1.4μ and 0.2μ in the control. These changes also increased with time. In one 17 day old co-culture, the average TJ length and width were 7.7μ and 2.3μ, while those in the control of the same age were 4.8μ and 0.5μ. Secondary, enriched, astroglial cultures induced similar changes. Fibroblasts, smooth muscle cells and conditioned medium from astroglial cultures had no effect on the TJ. Astroglia did not induce TJ formation in beef pulmonary or aortic endothelium, or in human umbilical endothelium. In summary, when cerebral endothelial cells are grown with astroglia, TJ morphology approaches that of cerebral capillaries in situ. The trend toward continuity of the TJ in co-cultures is what would be expected of a blood brain barrier seal. Further, gap junctions, absent from endothelial cells of cerebral capillaries in situ, are diminished markedly in number upon the addition of astroglia. Thus, the cerebral endothelial TJ in vitro is "normalized" in the company of astroglia.

342.2 IMMUNOLOGICAL DEFENSE OF THE BRAIN: ROLE OF THE CHOROID PLEXUS. James A. Nathanson and Linda L.Y. Chun*. Program in Neuroscience, Harvard Medical School, Department of Neurology, Massachusetts General Hospital, Boston, MA 02114

Because the brain lacks a true lymphatic system, it is unclear how, during disease states, peripheral lymphocytes initially recognize foreign antigens present in the CNS. Furthermore, because the brain parenchyma is largely isolated from the systemic circulation by the blood-brain and blood-CSF barriers, it is unclear how antigen-presenting cells (possibly microglia and astrocytes) present in the CNS can initially interact with lymphocytes located in the periphery.

Foreign substances present in brain extracellular fluid are known to move by bulk flow into the CSF, which itself passes through the ventricular system into the cisterna magna. The present study demonstrates that the choroid plexuses may be capable of detecting foreign antigens present in CSF and communicating this information to the periphery. Choroid plexus tissue not only contacts CSF in the ventricles but is strategically present at the narrow ventricular foramina through which CSF must pass. Also, choroid epithelial cells form an interface between CSF and periphery, since their apical side faces CSF and their basal side faces fenestrated peripheral capillaries.

In this study, highly enriched suspensions of dissociated Balb/c mouse choroid plexus epithelium were irradiated to prevent cell division and co-cultured with a T helper cell clone (O3, L3T4$^+$, Ly2-) in the absence or presence of the antigen, chicken ovalbumin. In the presence of antigen, choroid epithelial cells and T lymphocytes developed numerous activated centers, associated with a marked increase of tritiated thymidine incorporation, indicative of lymphocyte proliferation. Proliferation was blocked by a monoclonal antibody to Ia^d, and cell markers demonstrated that the activated centers contained lymphocytes and choroid epithelial cells but no macrophages or astroglial cells. Because choroid epithelium has access to peripheral lymphocytes and is capable of taking up proteins and virus-sized particles from the CSF, our studies suggest that the choroid plexus may be involved in immunological surveillance in the CNS.

342.3 DIFFERENCES IN STRUCTURE AND TRANSPORT PROCESSES OF CAPILLARY SYSTEMS BETWEEN TWO HYPOTHALAMIC NUCLEI IN THE RAT BRAIN

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The prinicipal function of capillary systems in most CNS areas is to deliver substrates such as glucose and oxygen to neural tissue. In the hypothalamus, however, the capillary systems may not only subserve this delivery function but also participate in the "sensing" of blood-borne information such as plasma osmolality and circulating levels of hormones. Accordingly, we hypothesized that the structural and transport properties of capillary beds would not be the same among hypothalamic nuclei and tested this hypothesis by comparing the morphological and physiological features of the capillary systems of two hypothalamic nuclei, the supraoptic nucleus (SON) and ventromedial nucleus (VMH), and of one non-hypothalamic structure, the inferior colliculus (IC). The experiments were performed on Sprague-Dawley rats using several radioactive tracers, quantitative autoradiography, computer-assisted image processing, and light and electron microscopic morphometry. The following parameters were assessed: 1) capillary blood flow; 2) transcapillary influx constant for AIB (Ki); 3) capillary permeability-surface area product (PS) for AIB; 4) capillary transit time of albumin; 5) capillary profile density, surface area, and volume fraction from light micrographs; 6) frequency of vesicles, intercellular junction, and mitochondria within capillary endothelial cells from electron micrographs. The ranking of capillary surface area, volume fraction, and profile density was: SON > IC > VMH. Despite the greater "capillarity" of the SON, the rate of blood flow was similar to that of the VMH and about one-half that of the IC. Analysis of the mean microvascular transit times of albumin and of the Ki and PS products for AIB plus comparisons of the radiotracer and morphometrically determined capillary blood volumes indicate that about 80% of the VMH capillaries, 50% of the IC capillaries, and 30% of SON capillaries were perfused in control rats during the period of these experiments. The Ki and PS products for AIB were very low and quite similar among IC, SON and VMH and indicated the tightness of their capillaries. The endothelial cells of the SON, VMH, and IC were unfenestrated and contained similar numbers of mitochondria, but the frequency of endothelial cell vesicles was several-fold higher in SON than in VMH and IC. The capillary beds of the SON, VMH, and IC apparently differ with respect to volume fraction, surface area, blood flow, proportion of perfused capillaries, and number of microvesicles. These variations in capillary systems may well reflect differences not only in local metabolism and substrate supply but also in the sensing of blood-borne information.

342.4 COMPUTER SIMULATION OF BLOOD-BRAIN BARRIER TRANSPORT.

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Kinetic analysis of facilitated transport across the blood-brain barrier (BBB) in vivo is complicated by the fact that two membranes are involved. When standard techniques of measuring the uptake of a substrate are used, the apparent kinetic constants of the the overall system may not accurately reflect the kinetics of the carriers in each individual membrane. For instance transport by the monocarboxylic acid (MCA) system can be described by two components, one with Michaelis-Menten kinetics and the other non-saturable. During brain maturation the activity of the MCA system decreases markedly. This is accompanied by an expected decrease in Vmax but an unexpected increase in affinity. The question arises whether the change in Km implies the presence of more than one transport system or whether it can be predicted by a single carrier present on both membranes.

We developed a mathematical model of the BBB to help predict its kinetic behavior and to assist in the analysis of data. The basis of the model is a capillary-tissue unit consisting of three compartments: the capillary lumen, the endothelial cell, and the cerebral tissue space. The compartments are separated by concentric endothelial cell membranes. Michaelis-Menten transport kinetics can be assigned to each membrane. The effects of blood flow and concentration gradients along the length of the capillary are taken into consideration. Due to the complexities of the model, numerical solutions are used. For the MCA system the model predicts that one carrier species is sufficient to explain the experimental observations. By increasing the density of carriers in only one of the two membranes, while keeping the affinity of the carrier constant and equal in each, the apparent Vmax and Km will both increase. A Kd or non-saturating component of BBB transport is not predicted mathematically to result from having two facilitated transport systems in series when each transport system has similar Vmax and Km.

The model can be applied to brain uptake index experiments to predict and quantify the effect of having unlabeled substrate endogenous to the cerebral tissue compartment transported into the capillary lumen, where it mixes and competes with labeled substrates for uptake. Also, the model predicts that if the same Vmax and Km are assigned to each membrane, the apparent Vmax will be smaller than the assigned Vmax and the apparent Km will be larger than the assigned Km.

In conclusion this model of BBB transport is generally applicable and can describe diverse experimental settings, such as the brain uptake index technique and continuous infusion methods.

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342.5 COMPARISON OF [14C]UREA AND [14C]SUCROSE AS INDICATORS OF REGIONAL BLOOD-BRAIN BARRIER PERMEABILITY USING QUANTITATIVE AUTORADIOGRAPHY.

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Blood-brain barrier integrity can be evaluated in brain regions as small as 100 um^2 using the intravenous administration method and quantitative autoradiography (QAR). In most studies, a cerebrovascular permeability-surface area product (PA) is determined from the unidirectional uptake of a slowly-penetrating tracer into each brain region. This analysis requires that there is minimal net diffusion of tracer between adjacent brain regions and that influx across cerebral capillaries is the predominant route for tracer uptake into the brain. These requirements, however, are not met by several commonly-used tracers, such as 22Na, 36Cl and [14C]AIB, which have significant CSF components of uptake into the brain. Therefore, we examined the regional brain uptakes of [14C]urea and [14C]sucrose to determine if either of these compounds would provide a suitable autoradiographic tracer of cerebrovascular PA.

Regional brain uptake was determined using awake, Long-Evans rats (250-350 g). Each rat received ~1 mCi of [14C]urea or [14C]sucrose as a bolus injection into the femoral vein. Blood samples were collected at fixed times from the femoral artery for 15-30 min, when the rat was killed. After a CSF sample was obtained from the cisterna magna, the brain was removed from the skull and frozen at -50° C. Regional brain concentrations of radiotracer were determined using QAR, whereas plasma and CSF concentrations were measured by scintillation counting. Cerebrovascular PA products were calculated from the parenchymal brain content of tracer divided by the integral of plasma concentration.

Cerebrovascular PA in frontal cortex equaled $1.04 + 0.05 \times 10^{-5}$ s^{-1} (mean + SEM, n=3) for [14C]sucrose and $1.01 + 0.07 \times 10^{-4}$ s^{-1} for [14C]urea, and did not differ significantly from values obtained by tissue dissection and scintillation counting. PA values for both tracers were ~3-fold greater in gray matter than in white matter, consistent with the ~3-fold greater capillary surface area in gray matter. However, the pattern of uptake of [14C]urea suggested significant uptake into CSF with subsequent diffusion of tracer from CSF to brain. [14C]Urea concentrations were ~2-fold greater in brain tissue immediately adjacent to ventricular or subarachnoid CSF. In contrast, brain [14C]sucrose concentrations were not elevated in brain tissue adjacent to CSF.

These results suggest that [14C]sucrose is a suitable tracer for autoradiographic determination of local cerebrovascular PA. [14C]Sucrose, unlike [14C]urea or 36Cl, does not have a significant CSF component of uptake into the rat brain.

342.6 COMPARISON BETWEEN A QUARTERNARY AMMONIUM CHOLINESTERASE INHIBITOR AND [14C]SUCROSE AS MARKERS FOR STUDYING BLOOD-BRAIN BARRIER PERMEABILITY CHANGES.

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The most frequently employed markers for studying blood-brain barrier (B-BB) permeability changes in experimental animals are non-metabolized radiotracers.The radiotracer [14C]sucrose has been used successfuly to determine vascular permeability-area product (PA) in different cerebral regions of rats (Ohno et al.,Am. J. Physiol. 235:H299,1978). In order to extend the choice of available markers in B-BB studies, a new approach based on the anti-cholinesterase (anti-ChE) activity of quarternary molecules was suggested and demonstrated with respect to phospholine iodide and MEPQ (Ashani et al.,Life Sci., 36:493,1985). To further substantiate the applicability of these quarternary anti-ChE compounds in B-BB research, we compared the permeability of osmotically-induced barrier opening to both MEPQ and [14C]sucrose administered simultaneously to the same rat. Adult rats (CD , Charls River, U.K.) weighing 300-400g were anesthetized with sodium pentobarbital and catheters were placed in the left common carotid artery and the left femoral artery. Five and 60 minutes following injection of 50% glycerol-saline solution into the exposed carotid artery, the rats were administered a mixture of MEPQ(15 μg/Kg) and [14C]sucrose (7.5 μCi) as a bolus via the carotic catheter. Blood samples were collected from the femoral artery. Percent of brain ChE inhibition and PA values for [14C]sucrose were measured as described before (Ashani et al. and Ohno et al.,respectively). In control animals where glycerol was replaced by saline, both the left and right hemispheres of the cortex and the brain stem displayed the same PA values [5(±2)x10^{-6} sec^{-1}]. Brain ChE inhibition in these regions was found to be practically zero. Five and 60 minutes following the application of the osmotic load the PA values increased up to 200x10^{-6} sec^{-1} and 60x10^{-6} sec^{-1} in the left and right cortex,respectively. An average of 70% inhibition of ChE activity in the left cortex and 30% enzyme inhibition in the contralateral side were obtained in good correlation with the PA values reported above. The barrier permeability was less damaged in other brain regions such as the brain stem, cerebellum or the hypothalamus as expressed in the corresponding PA values and percent inhibition of brain ChE activity. B-BB permeability of control animals in which the markers mixture was administered six hours following the saline increased significantly. The results clearly indicate a correlation between penetration of labeled sucrose and MEPQ through the osmotically-damaged B-BB.

342.7 ION SELECTIVITY OF THE CRAYFISH BLOOD BRAIN BARRIER: AN ELECTROPHYSIOLOGICAL STUDY. A.M.Butt*, P. Hargittai* and E.M. Lieberman. (SPON: W. R. Wooles) Dept. Physiology, Sch. Med., East Carolina U., Greenville, NC 27834.

Previous studies from this laboratory (Lieberman et al., Biophys J., 46,146a, 1985) presented evidence that the perineurium surrounding the ventral nerve cord (blood brain barrier) is highly selective to the major ions, being 10 times more permeable to K^+ than Cl^- and practically impermeant to Na^+.

The ventral nerve cord is removed intact from the animal with special regard to avoiding physical stretch and exposure to air, thus maintaining the integrity of the permeability properties of the sheath. Using standard electrophysiological techniques the perineurium and the medial giant axon are impaled. In this way the potential across the sheath and the axon membrane can be monitored by differential recording.

In control solution (190mM Na, 5.4mM K crayfish physiological solution) there is no potential across the sheath. Exchange of the control solution with one containing 100mM [K] (Na substituted) causes a rapid change in potential to +42±2mV, inside positive, peaking within 10 seconds. This is followed by a fall in potential with a plateau value of approximately +25mV. The peak potential is used to evaluate ion selectivity of the barrier.

The permeability selectivity sequence for several monovalent cations is $K^+ = Rb^+ >> NH_4^+ > Cs^+ = Li^+ > Na^+ > Tris^+$. Based on the effect of Na depletion, and Cs^+, Li^+, and $Tris^+$ substitution on the action potential these ions are effectively impermeant within a time frame of 30 minutes exposure.

The potential response of the perineurial sheath to 100mM Rb^+ is of particular interest. Although the peak response is the same (40±1mV) as in 100mM K, the plateau is much lower (11±1mV). In several experiments spontaneous spikes occurred at this potential while the preparation was continuously bathed in Rb solution. Current-Voltage relations of the sheath showed a negative resistance region in the range of 10-15mV. The additional spikes had the same approximate amplitude and time course of the initial spike. The removal of Ca^{2+} from 100mM K solution greatly depressed the spike behavior of the barrier and slowed the potential response.

The data suggest that the permeability across the perineurial sheath of the crayfish nerve cord is modulated by K-selective channels. These channels appeared to be voltage and ion sensitive, yet were not affected by 30 minutes exposure to Ba^{2+} (2-5mM), TEA (1mM) and 3,4-DAP (0.5mM).

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342.8 THE EFFECTS OF DISULFONIC STILBENE AND PROSTAGLANDIN E2 ON CSF PRODUCTION RATE IN ADULT RATS. Q.S. Deng and C.E. Johanson, Dept. of Pharmacology, Univer. of Utah, Salt Lake City, UT 84132 and Dept. of Neurosurgery, Brown Univer. & Rhode Island Hospital, Providence, RI 02903.

Previously we have shown that 1) DIDS (disulfonic stilbene) inhibited and PGE_2 stimulated chloride transport in the rat choroid plexus-CSF system (Fed. Proceed. 44(5): 1746, 1985 and 45(4): 892, 1986); and 2) DIDS also inhibited sodium movement from blood into the choroid plexus(CP)-CSF system (Fed. Proceed. 45(4): 892, 1986. Since Cl is the primary anion and Na the main cation in the CSF secretory process, drugs that affect either Cl or Na transport likely alter CSF production. The purpose of this study was to investigate the effects of DIDS and PGE_2 on CSF formation rate.

Ventriculo-cisternal perfusions were carried out on male rats weighing 150-300 g. All animals were anesthetized with ketamine HCl. The ventricular system was perfused with artificial CSF at 18 ul/min through a 27-gauge needle in the lateral ventricle of the left cerebral hemisphere. The outflows over 15-min intervals were collected from a 22-gauge needle in the cisterna magna. ^3H-inulin (3 uC/ml CSF) and drugs were added to the CSF stock solution on the perfusion day. Steady-state conditions were generally achieved after an hour of perfusion. The radioactivity of the perfusate was determined by liquid scintillation.

DIDS inhibited CSF production rate by 25-30%, by reducing Cl as well as Na transport. The primary in vivo effect of DIDS appears to be inhibition of $Cl\text{-}HCO_3$ exchange in the choroid plexus. This is consistent with our earlier finding that DIDS and SITS (another stilbene) reduced Cl-36 uptake and steady-state distribution in the in vitro choroid plexus.

PGE_2 boosted CSF production in the adult rats by nearly 30% by accelerating Cl movement into both CP and CSF, compared to control. Although PGE_2 may directly stimulate the anion exchange system, it is not possible to rule out prostaglandin-induced augmentation of blood flow to the choroid plexus.

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342.9 CHOROID PLEXUS ANTIPORT AND SYMPORT MECHANISMS THAT DRIVE THE SECRETION OF CEREBROSPINAL FLUID. C.E. Johanson, Q.S. Deng and V.A. Murphy.* Dept. of Neurosurgery, Brown Univer. & Rhode Island Hosp. Providence, RI 02902 and Dept. of Pharmacology, Univer. of Utah, Salt Lake City, UT 84132

To test for carrier transport of Na-22 and Cl-36 from blood into the choroid plexus(CP)-CSF system, we have analyzed the kinetics of distribution (0 to 1 hr) of these ionic tracers in adult male Sprague-Dawley rats. Steady-state C-14 dimethadione (DMO) distribution was used to estimate pH and bicarbonate (calculated) gradients across the basolateral (plasma-facing) and apical (CSF side) membranes of the choroidal epithelium. Drugs were administered in doses to give final concentrations of 0.01 to 1 mM in the plasma or cerebrospinal fluid. Such doses cause 30-50% alteration (maximal) in CSF secretion rate.

Amiloride and acetazolamide maximally inhibited Na uptake by CP and CSF (cisterna magna) from the plasma containing a stable level of radioisotopic Na-22; conversely, insulin tended to enhance uptake of Na. The disulfonic stilbene, DIDS, drastically reduced Cl-36 permeation into the CP epithelium and secreted fluid; in contrast, neither furosemide nor bumetanide inhibited Cl movement from blood into the CP-CSF system. PGE_2, a well known stimulator of anion (Cl and HCO_3) exchange in other tissues, augmented the penetration of Cl-36. It is concluded that Na and Cl movement, blood into choroid plexus, occurs primarily by the parallel operation of basolateral Na-H and $Cl\text{-}HCO_3$ exchangers rather than by tightly coupled Na-Cl cotransport (symport).

Apical ion extrusion mechanisms underlying CSF secretion are also being considered. DIDS, an inhibitor of $Na\text{-}HCO_3$ extrusion by symport in other tissues, depressed the Na-22 and Cl-36 transport, choroid cell to CSF; but it apparently did not inhibit the Na-K exchange pump (because neither CP [K] nor [K]/[Na] was altered). Transmembrane HCO_3 gradients were also consistent with the working hypothesis that apical membrane $Na\text{-}HCO_3$ symport is the main Na-pumping mechanism directly involved in fluid generation. On the other hand, the Na-K exchanger has at least a permissive role, i.e. in maintaining transmembrane gradients for Na sufficient for normal operation of the other Na-translocating mechanisms. This model does not preclude the possibility that some apical efflux of Cl and/or HCO_3 occurs by diffusion via channels, from the choroid cell into CSF.

Supported by NS 13988-09.

342.10 ATRIOPEPTIN RECEPTORS IN BLOOD-BRAIN AND BLOOD-CSF BARRIERS. L. Steardo,* C.J. Owen,* E.J. Hunnicutt, and J.A. Nathanson, Dept. of Neurol., Harvard Med. School, Mass. Gen. Hosp., Boston, MA 02114.

Atriopeptins (AP's), synthesized by atrial cardiocytes, are thought to regulate intravascular volume. They appear to be released into the circulation in response to stretching of the cardiac atria by fluid overload, and cause a rapid and intense diuresis and natriuresis through direct and possibly indirect effects on the kidney. Despite the well-known anatomical and physiological similarities between choroid plexus epithelium and renal tubule cells, there have been as yet no reported studies examining the possible effects of AP's on CSF production or CNS water balance. Both the epithelial cells of the choroid plexus (constituting the blood-CSF barrier) and the endothelial cells constituting the blood-brain barrier have potential access to AP's from the systemic circulation. In addition, these tissues could conceivably receive innervation from recently discovered AP-containing neurons. Because of this, we have undertaken experiments to determine 1) if receptors for AP's might exist in brain barrier tissues, 2) whether these receptors are coupled to a second messenger system, and 3) if AP's might exert physiological effects on brain-barrier tissues.

Recent evidence suggests that AP receptor occupancy is associated with the intracellular production of cyclic GMP. Therefore, to determine whether AP receptors are present in brain-barrier tissues, we examined the ability of AP's to activate guanylate cyclase in membrane fractions of rabbit or porcine choroid plexus and cerebral microvessels (prepared by fragmentation and density gradient centrifugation). Rat atrial peptide (Ser-Leu-Arg-Arg-APIII) caused significant activation of guanylate cyclase both in rabbit microvessels (Vmax=190% of control; K_a=0.3 nM) and in choroid plexus (Vmax= 155% of control; K_a=50 nM). Stimulated activity was, however, very low in pia-arachnoid and in dural sinus. Characterization of stimulation in choroid plexus revealed that AP fragments 1-11 and 13-28 were much less active than the intact peptide, similar to the situation which we observed in particulate preparations of kidney. Guanylate cyclase present in broken cell preparations of purified choroid epithelium showed greater responsiveness to AP than did fractions enriched in choroid vessels, suggesting that the secretory epithelium, itself, contains AP receptors. In physiological studies, under conditions of constant ICP, ventricular-cisternal perfusion with rat atrial peptide caused a significant decrease (as much as 75%) in CSF formation rate without a significant change in peripheral blood pressure. This result, together with the above data demonstrating the existence of receptors and second messenger systems for AP's on brain-barrier tissues, suggests that AP's may affect the water permeability (and/or content) of the brain/CSF compartments.

342.11 BLOOD-BRAIN BARRIER MONOAMINE OXIDASE: [^{3}H]PARGYLINE BINDING TO CEREBRAL MICROVESSELS. R.N. Kalaria, M.J. Mitchell* and S.I. Harik. Department of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

In addition to the structural characteristics of the mammalian blood-brain barrier (BBB), manifested by capillary endothelial cells with tight intercellular junctions and small pinocytotic vesicles, there is accumulating evidence suggesting the existence of a biochemical component of the BBB. An example of the latter is the finding of monoamine oxidase (MAO) in cerebral microvessels from a variety of mammalian species, which can degrade monoamine neurotransmitters which may escape the structural barrier under physiological or pathophysiological conditions. We measured MAO of the BBB by assessing the specific binding of [^{3}H]pargyline to cerebral microvessels isolated from the cerebral cortex of man, rat, mouse, guinea pig, pig, and rabbit. [^{3}H]pargyline binding to cerebral microvessels from all these species was compared to the binding results of the mitochondrial-enriched P_2 fraction of cerebral cortical and liver samples. [3H]pargyline binding to tissue preparations was irreversible and linear with the amount of tissue protein in the incubation mixture. Specific binding was defined as the difference between total binding and nonspecific binding, assayed in the presence of excess (20 μM) unlabeled pargyline. Also, the relative concentration of the two forms of MAO, A and B, were assessed by the relative inhibition of [^{3}H]pargyline binding by unlabeled deprenyl and unlabeled clorgyline, respectively. We find that rat and rabbit cerebral microvessels exhibit higher capacity for pargyline binding than human, mouse, guinea pig, and pig cerebral microvessels. In all species, brain microvessel MAO B exceeded MAO A, the ratio of B to A being highest in rat microvessel preparations (4:1). [^{3}H]pargyline binding to the P_2 fraction of the cerebral cortex and liver preparations did not show the marked interspecies differences that were noted for cerebral microvessels. Solubilization of brain microvessels after pargyline binding, followed by polyacrylamide gel electrophoresis revealed two radioactive peaks corresponding to molecular weights of 60,000 and 55,000 consistent with previous studies. The radioactivity in the MAO B peak was several-fold greater than that of MAO A in rat cerebral microvessels. These results indicate that cerebral microvessels from various mammalian species vary in their content of MAO and that the BBB of humans appears relatively poor in MAO activity. This finding may have important basic and clinical implications regarding the entry of MAO substrates from blood-to-brain.

342.12 THE EFFECT OF FATTY ACIDS ON [^{3}H]-OUABAIN BINDING TO CEREBROVASCULAR ($Na^+ + K^+$)-ATPase. Mary Lou Caspers and Paula Grammas*. Dept. of Chemistry, Univ. of Detroit, Detroit, MI 48221 and Dept. of Pathology, Wayne State Univ., Detroit, MI 48201.

The effect of fatty acids on [^{3}H]-ouabain binding to cerebrovascular ($Na^+ + K^+$)-ATPase was investigated because non-esterified fatty acids are known to possess detergent-like properties which can disrupt cellular membranes and the functioning of membrane-bound enzymes and receptors. In addition, fatty acids can serve as an energy source for brain endothelial cells. A microvessel preparation was obtained from the cerebral cortices of 24 rats. The binding of [^{3}H]-ouabain to microvessel ($Na^+ + K^+$)-ATPase was measured at 37°C for 15 min in an assay mixture containing 75 - 100 μg microvessel protein, 100 mM Tris HCl (pH 7.4), 200 mM NaCl, 10 mM $MgCl_2$, 5 mM ATP, 10 μM bovine serum albumin and 30 - 40 nM [^{3}H]-ouabain. Non-specific binding, measured in the presence of 50 μM unlabeled ouabain, was less than 2% of total binding. Under these assay conditions, Scatchard analysis yielded a B_{max} of 11.1 pmol/mg and a K_D of 46 nM; the latter is consistent with the presence of the high affinity ($\alpha+$) form of the ($Na^+ + K^+$)-ATPase in cerebral endothelium. The results indicate that arachidonic acid (0.1 - 100 μM), when added at the time of assay, stimulated [^{3}H]-ouabain binding. An increase in binding of 12% was noted at 1.0 μM arachidonic acid. The saturated fatty acid palmitic acid did not significantly stimulate [^{3}H]-ouabain binding. 4-Pentenoic acid, a compound known to inhibit the β-oxidation of fatty acids, caused no significant stimulation of [^{3}H]-ouabain binding to cerebral microvessel ($Na^+ + K^+$)-ATPase when added in the range 1 μM to 1 mM. However, at 10 mM 4-pentenoic acid, [^{3}H]-ouabain binding was increased by 51%. In summary, these data suggest that long chain, polyunsaturated fatty acids, but not saturated fatty acids, stimulate [^{3}H]-ouabain binding to cerebrovascular ($Na^+ + K^+$)-ATPase in a dose dependent manner. Therefore, these fatty acids may be involved in the regulation of blood-brain barrier ($Na^+ + K^+$)-ATPase and may play a role in the cerebral disfunction associated with diseases such as hepatic encephalopathy and Reye's Syndrome where elevated plasma levels of non-esterified fatty acids are prominent. (Supported in part by USPHS HL 23603.)

342.13 THE COMPARATIVE EFFECT OF 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T) AND QUINOLINIC ACID ON 5-HYDROXY-3-INDOLEACETIC ACID (5-HIAA) TRANSPORT BY THE RABBIT CHOROID PLEXUS. C.S. Kim*, R.F. Keizer* and G.R. Breese. (SPON: M.I. Gage). Depts. of Neurology, Psychiatry and Biol. Sci. Res. Ctr., Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514.

Quinolinic acid is present in the brain of various animal species including man and is known to produce motor excitement and clonic convulsions (Lapin, J. Neural Transm. 42:37-43, 1978). Patients with hepatic coma have elevated 5-HIAA and quinolinic acid in cortex (F. Moroni et. al., Neurosci. Abs. 11: 821, 1985), suggesting an interaction of quinolinic acid with the active pump mechanism for 5-HIAA located at the choroid plexus (CP). For this reason, the effect of quinolinic acid and 2,4,5-T on the transport of 5-HIAA from choroid plexus was compared to actions produced by octanoic acid, an organic acid previously shown to inhibit the uptake of 5-HIAA (Kim et. al., Brain Res. 310:149-153, 1984). The action of 2,4,5-T on this system has not been investigated. Young adult albino rabbits of either sex were sacrificed by decapitation. Lateral and fourth ventricular CP (LVCP, FVCP) were removed and individual CP were placed in 2.5 ml of artificial CSF of control or experimental media containing ^{14}C-5-HIAA 0.02 μCi/ml (spec. act. 60 mCi/mmol). After 5 min incubation, T/M ratio of 2.2±0.4 (S.D.) and 2.1±0.5 were achieved in LVCP and FVCP, respectively, in control media. When the incubation medium contained either 0.1 mM or 1.0 mM 2,4,5-T, the uptake of ^{14}C-5-HIAA was reduced in a dose-related manner (66% and 76% in LVCP; 57% and 67% in FVCP, respectively). Similar results were obtained with octanoic acid. However, treatment with quinolinic acid at the same concentration did not inhibit ^{14}C-5-HIAA uptake by either FVCP or LVCP. The present study demonstrates that quinolinic acid in contrast to 2,4,5-T or octanoic acid does not interfere with the uptake of 5-HIAA by choroid plexus through a mechanism known to be a major route for excretion of 5-HIAA from the brain in vivo. Supported in part by NIH grants: HD-03110 and ES-03458.

342.14 CALCIUM REGULATION IS ABSENT IN THE FROG PERIPHERAL NERVE DURING CHRONIC CHANGES IN PLASMA [Ca]. K. C. Wadhwani, H. Levitan, S. I. Rapoport. Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892.

The stability of calcium in the vertebrate peripheral nerve after acute alterations of [Ca] in the external medium in vitro has been known for sometime. However, whether peripheral nerve regulates calcium following chronic changes in serum [Ca] has not been studied. To investigate if calcium is regulated in peripheral nerve during chronic hypocalcemia and hypercalcemia, calcium contents in the whole sciatic nerve, desheathed nerve and the perineurial sheath of male Rana pipiens were measured as a function of the concentrations of ionized calcium in the plasma. Chronic hypocalcemia was induced by parathyroidectomy. Chronic hypercalcemia was induced by intramuscular injection of 300,000 IU of vitamin D_3, and by bathing the frog in tap water containing 50 mM $CaCl_2$ for up to 14 days. Blood and nerve tissue were collected for analysis of total calcium by atomic absorption spectrometry. Plasma ionized calcium was measured by ion selective electrode. The calcium contents of whole nerve, desheathed nerve, and the perineurial sheath varied linearly with the amount of ionized plasma calcium, which ranged from 0.3 mM to 8 mM but was not linearly related to total plasma calcium. Plots of tissue calcium vs. plasma ionized calcium yielded correlations (r) of 0.97, 0.96 and 0.86 and slopes of 0.72, 0.71 and 1.72 [(mmol/kg wet weight)/(mmol/l)] for whole nerve, desheathed nerve and perineurial sheath, respectively ($p<0.001$). In contrast, the calcium content in the cerebrum of the same animals did not change when plasma ionized calcium varied from 0.5 mM to 1.5 mM confirming results in rats by Murphy et al. (Fed. Proc. Abstr. 45:184, 1986). Our results provide no evidence for calcium regulation in the frog peripheral nerve during chronic hypocalcemia and hypercalcemia.

342.15 REGIONAL BLOOD-BRAIN BARRIER TRANSPORT OF GLUCOSE DURING ACUTE AND CHRONIC HYPERGLYCEMIA IN RATS. S.I. Harik, K.A. McCracken*, and J.C. LaManna. Dept. Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106.

Glucose diffusion across the blood-brain barrier is facilitated by means of a specific carrier. It is of interest to determine if the transport characteristics of this system can be affected by chronic changes in blood levels of its substrate. To study the role of glucose levels in regulating the blood-brain glucose transporter, male Wistar rats (300 g) were made hyperglycemic by treatment with streptozotocin (SZ) (60 mg/kg in citrate buffer, i.p.) and kept for 2 or 4 weeks before study. Littermates, given buffer only, were used as controls. SZ-treated rats became hyperglycemic within a few days and continued to have blood glucose levels of 25-50 mM for weeks following a single dose of SZ. These rats lost about 10% of their initial body weight in the first week, and failed to gain weight after that, whereas their littermates gained body weight by about 25% over the same period.

At the end of 2 or 4 weeks, SZ-treated rats and their littermate controls were anesthetized with chloral hydrate in order to place an arterial catheter via the external jugular vein, and a femoral arterial cannula. Unidirectional glucose transport and regional brain blood flow was determined in awake, restrained rats by the dual-label indicator fractionation method using [^{14}C]butanol and [^{3}H]D-glucose. Non-specific glucose uptake was corrected by subtraction of the calculated [^{3}H]L-glucose space. Control rats were made acutely hyperglycemic by i.p. administration of 50% dextrose in water to raise their blood glucose levels to about that of the SZ-treated rats at the time of the study.

Both acute and chronic hyperglycemia were associated with a 25% reduction in regional blood flow when compared to normoglycemic controls. A somewhat larger reduction was seen in the 2 week chronic hyperglycemic group, especially in the hippocampus, cerebellum, and striatum. Unidirectional flux of D-glucose was not significantly different in any brain region between acute hyperglycemic rats and SZ-treated rats at either time period. Therefore, we were unable to detect any decrease in the glucose transport capacity in response to chronic hyperglycemia. However, the [^{3}H]L-glucose space was significantly lower (by 25%) in both chronic hyperglycemic groups compared to acute hyperglycemic rats. Thus, while there does not seem to be a regulation of the glucose transporter with chronic exposure to high blood glucose levels, there does seem to be a physiological attempt to limit the brain exposure to glucose through control of capillary blood volume and flow.

AUDITORY SYSTEM: PERIPHERY

343.1 EAR-CANAL RECORDED DISTORTION PRODUCTS IN RABBIT: SITES OF ORIGIN. G.K. Martin, B. Stagner, R. Probst, A.C. Coats* and B.L. Lonsbury-Martin*. Dept. of Otorhinolaryngology & Communicative Sciences, Baylor Col. of Med., Houston, TX 77030.

Ear-canal recorded distortion products (DP) have predictable mathematical relationships to their primary tones, f_1 and f_2, and, thus, promise to provide frequency-specific information concerning the state of the cochlea following experimental manipulation. However, it is not clear whether the emitted difference tones (DT) $2f_1$-f_2 and $2f_2$-f_1 solely reflect basilar-membrane non-linearities at the sites of primary tones or if the DP site makes a significant contribution to the retrograde signal. Results from studies involving suppression and adaptation of otoacoustic emissions and N_1 responses indicate that the emitted $2f_1$-f_2 (cubic-difference tone, CDT) can be attributed almost exclusively to the primary-tone site. However, other emission data implicate reemission of the response from the DP site as a potential contributor to response magnitude. In the present work, we attempted, in rabbit, to establish the precise contributions of the basilar-membrane regions involved in generating emitted DPs using suppression and temporary threshold shift (TTS) techniques. Suppression contours (6-dB reduction criteria) were obtained for 2-and 4-kHz CDTs and the associated $2f_2$-f_1 DT with primary-tone levels of 65 dB SPL (f_2/f_1 ratio=1.25). The same DPs were examined in TTS experiments using pure tones (90 dB SPL, 3 mins) either at the DP, f_1, or f_2 frequency, or 1/2-octave below these frequencies. Results for both CDTs, indicating that suppression was maximum when the suppressor tone was near the f_1 or f_2 frequency, with no suppression occurring at the CDT frequency, implicated the primary-frequency regions as major contributors to the emitted CDT. Contrasting results were obtained for the $2f_2$-f_1 DT in that maximal reduction occurred when basilar-membrane regions slightly basal to the DP site were affected by our procedures. Following brief TTS episodes, CDTs were significantly reduced to as low as 10% of their original amplitudes and recovered slowly, depending on exposure frequency. For both CDTs, exposure 1/2-octave below f_2 produced the greatest reduction in emitted-response amplitude, with exposures at f_1 and 1/2-octave below f_1 producing the next greatest response reduction. In summary, the results presented here infer that the ear-canal recorded CDT is primarily a reflection of cochlear activity generated at primary-tone sites within the organ of Corti, while the $2f_2$-f_1 DT reflects activity above the primary tones and nearer to the DP place. Consequently, depression of the CDT following cochlear insult seems to reflect hair-cell damage in the region of the primary tones while changes in $2f_2$-f_1 implicate the DP site as contributing to a reduced ear-canal recorded response.

343.2 STEREOSPECIFICITY OF THE OTOTOXIC EFFECTS OF THE LOOP DIURETIC INDACRINONE. L. P. Rybak and C. A. Whitworth*. Depts. of Surgery and Pharmacology, SIU Sch. of Med., Springfield, IL 62708.

Indacrinone (MK-196) is a loop diuretic which consists of a racemic mixture. The (-)-enantiomer, known as MK-199, is a very potent loop diuretic (Blaine et al., Clin Exp Hypertension A4: 161-176, 1982). By contrast, the (+)-enantiomer (MK-197) is a much weaker diuretic and may act on a different site in the nephron. The purpose of this study was to evaluate the individual enantiomers in the chinchilla model to determine whether these compounds affect auditory function and whether a difference in ototoxic potency exists. Chinchillas weighing 400-600 grams were anesthetized with ketamine 45 mg/kg IM, followed by pentobarbital 30 mg/kg IM. A jugular vein was cannulated for intravenous injection of diuretic. The auditory bulla was opened to expose the round window membrane. A microelectrode was inserted into scala media, through the basilar membrane to monitor the endocochlear potential (EP). The compound action potential (CAP) of the eighth nerve was monitored at the round window by using a tungsten wire connected to a preamplifier (WPI DAM 60). The CAP was further amplified and displayed on an oscilloscope, and a permanent record was made using a computer averager, with an x-y plotter. Auditory stimuli consisted of 0.1 millisecond clicks, delivered at 5 per second, through a David Kopf, hollow, chinchilla ear bar. CAP threshold and input-output functions were determined prior to drug administration. After a stable EP and CAP threshold were obtained, a solution of either enantiomer was injected over a dosage range of 10-150 mg/kg. The EP was monitored continuously and the threshold and 30dB suprathreshold CAP responses were repeatedly tested at various intervals. Very little change of EP or CAP was noted in animals receiving the (+)-enantiomer. On the other hand, chinchillas injected with the (-)-enantiomer were found to have a dose related reduction in both CAP and EP, with a disproportionately greater effect on the CAP. These findings suggest the possibility that the diuretic receptor in the kidney and the receptor mediating ototoxicity in the cochlea, may have similar steric requirements for interacting with loop diuretics.

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343.3 FOURIER ANALYSIS OF INNER HAIR CELL BASOLATERAL MEMBRANE RECEPTOR POTENTIALS. D.C. Mountain*+ and A.R. Cody* (SPON: R. Remmel). MRC Neurophysiology Group, School of Biological Sciences, Univ. of Sussex, Falmer, Brighton, Sussex BN1 9QG U.K.
+Dept. of Biomedical Eng., Boston Univ., Boston, MA 02215

The basolateral membrane potential of inner hair cells (IHC) was calculated by subtracting the extracellular potential (EC) measured near the cell from the intracellular receptor potential. Stimulus frequencies ranged from 60 Hz. to 4 kHz. The magnitude and phase of the fundamental of the basolateral membrane potential was calculated using Fourier analysis.

The magnitude of the receptor potential for frequencies above 250 Hz grew and then saturated with increasing sound pressure level (SPL). For very low frequency stimuli the magnitude growth was more complex, with significant departures from linearity occuring at receptor potential levels which would be in the linear range for high frequency stimuli. These same stimulus conditions produced considerable harmonic distortion. In some cases the energy in the harmonics exceeded that in the fundamental component.

The phase of the fundamental referenced to the SPL showed considerable variability across animals varying from 100 to 270 degrees. The phase was also compared to the phase of the extracellular potential which is believed to be largely an outer hair cell (DHC) response. In approximately 1/2 the animals studied the IHC response led the EC response by at least 130 degrees for low SPL stimuli. In many cells the phase varied by as much as 150 degrees with SPL.

In a number of cells the calculated receptor potentials were greater than the extracellular potentials suggesting that the complex behavior at low frequencies reflects the mechanical input to the cell. Further support for this hypothesis was obtained from measurements of the resistance changes associated with low frequency acoustic stimulation.

343.4 DIFFERENTIAL DISTRIBUTION OF F-ACTIN IN HAIR CELLS IN THE GUINEA PIG COCHLEA. L. Carlisle*, P.R. Thorne*, G. Zajic*, J. Schacht, R.A. Altschuler, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109

The distribution of filamentous (F)- actin in hair cells of the guinea pig organ of Corti was examined using rhodamine - labelled phalloidin (a specific marker for F-actin). The entire cochlear spiral was examined in surface preparations and cryostat sections after aldehyde fixation. Both living and fixed isolated hair cells were also examined. All preparations were incubated in phalloidin, rinsed and examined under epifluorescent illumination.

Previous studies (Drenckhahn et al. 1985, Zenner 1986) have described labelling in stereocilia, the cuticular plate and synaptic region of hair cells and Slepecky & Chamberlain (1985) found similar results with antibodies to actin. We confirm and extend these results in our studies. We observed a "scalloped" appearance of cuticular plate label, as the F-actin only extended to the edge of the hair cell at intermittent points. Zenner (1986) also described a centrally located bundle of F-actin filaments extending from the cuticular plate into the perinuclear region. Our systematic examination of the entire organ of Corti, using surface preparations, revealed a differential distribution of this structure in hair cells along the cochlear spiral. This spiralling filamentous network of F-actin labelling was greatest in hair cells in the upper turns of the cochlea and decreased in the lower turns. It was observed in all hair cells in the apical turn. Towards the base, along the cochlear spiral, this labelling of F-actin decreased and then disappeared initially in the first row of outer hair cells and then in the second row. By the first (most basal) turn of the cochlea only minimal labelling was observed in the third row and none in the first two rows. In the most basal portion of the first turn there was no label in any hair cells. This filamentous network of labelling was only occasionally observed in inner hair cells. It is interesting that this distribution is similar to the pattern of afferent innervation of outer hair cells and the converse of their efferent innervation. This study adds another observation to the increasing list of differences between basal and apical cochlea.

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343.5 PATHOLOGICAL CHANGES IN THE CYTOPLASM OF AUDITORY HAIR CELLS AND NERVES WITH NOISE-INDUCED TEMPORARY AND PERMANENT DEAFNESS. M. J. Mulroy. Dept. of Anatomy, Sch. of Med., Medical College of Georgia, Augusta, GA 30912.

Exposure to loud noise can cause structural changes to both the input (sensory hair bearing pole) and output (neural, synaptic pole) regions of auditory hair cells. This study compares the cytological changes occurring in the neural pole of auditory hair cells during temporary threshold shift (TTS) with the irreversible changes occurring during permanent threshold shift (PTS). Two ears with temporary threshold shifts were prepared by measuring their cochlear potentials immediately before exposing them to broadband noise at a level of 87 dB SPL for 2 and 16 minutes. This exposure resulted in a decrease in the cochlear potential which did not recover to it original amplitude until after a recovery period of at least 30 minutes. The ears were then exposed to the same noise again and perfused with fixative within 5 minutes after the noise exposure, presumably during a period of temporary deafness. One ear was exposed to broadband noise at a level of 106 dB SPL for 2 hours and fixed after a recovery period of 29 days, presumably during a period of permanent deafness. Semiserial transmission electron micrographs through the neural pole of the free-standing hair cells of these ears were prepared and analyzed. Hair cells fixed during temporary deafness had an increase in the number and volume of clear vacuoles in their neural pole compared with unstimulated controls. Their nerve terminals were swollen and had more disrupted cell membranes than controls. Hair cells fixed during permanent deafness were very pathological in appearance. They were shrunken, their cytoplasm and nucleoplasm were extremely electron dense, and their mitrochondria swollen compared with TTS and control hair cells. Like the TTS hair cells the PTS hair cells had an increase in their cytoplasmic vacuoles. I interpret the increased vacuoles in the neural pole of TTS hair cells to be the cytological sign of reversible metabolic failure of the synaptic end of the hair cell during TTS. I can not be certain whether the PTS hair cells examined in this study had stabilized or whether they would have been resorbed if the recovery period had been longer. Examining PTS ears with much longer recovery periods will resolve this issue. Supported by NIH Grants NS 22105 and 18871.

343.6 EFFECTS OF EXCITATORY AND NON-EXCITATORY SUPPRESSOR TONES ON TWO-TONE RATE SUPPRESSION IN AUDITORY NERVE FIBERS. J.A. Costalupes, N.C. Rich and M.A. Ruggero. Dept. of Otolaryngology, Univ. of Minnesota, 2630 University Ave. SE, Minneapolis, MN 55414.

Previous studies have shown that the dynamic range portions of tone-evoked rate-level functions can be shifted to higher tone levels in the presence of wideband noise. Such dynamic range shifts can extend the overall operating range of rate responses of auditory nerve fibers. It has been shown that dynamic range shift is attributable primarily to two-tone suppression produced by the background noise and is limited to those cases where the noise spectrum level is sufficiently high to excite the fiber. To date, however, most studies of two-tone suppression have considered the properties of suppressor tones that do not excite the fiber being studied. The purpose of this study is to compare the properties of two-tone suppression produced by excitatory suppressor tones with those of two-tone suppression produced by non-excitatory suppressor tones.

Recordings were made from individual auditory nerve fibers in anesthetized chinchillas. Test stimuli were 200-ms tones at fiber best frequency (BF) presented once per second. Analyses are based on rate-level functions for BF tones in the presence of suppressor tones at selected frequencies and intensities. Suppressor tones were gated simultaneously with test tones. For each rate function, intensity of the suppressor tone was held constant while the BF tone level was varied.

Results show that addition of a non-excitatory suppressor tone (i.e., one that did not evoke a rate change by itself) to the BF tone resulted in an approximately parallel shift of dynamic range to higher BF tone levels with no change in low-level or saturated response rates. Addition of an excitatory suppressor tone (i.e., one that did evoke a rate change by itself) resulted in greater shifts of dynamic range and in reduced slope of the dynamic range portion of rate functions. The rate of shift (log-log coordinates) is an approximately linear function of the intensity of the suppressor tone for both excitatory and non-excitatory suppressor tones. Preliminary results suggest that the rate of shift for above-BF suppressor tones is approximately .45 dB of shift per dB increase in suppressor tone level. For below-BF suppressor tones, the rate of shift is approximately 1.17 dB per dB. These results indicate that properties of excitatory suppressor tones are consistent with those of non-excitatory suppressor tones.

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343.7 DETERMINATION OF THE MAXIMUM INTEGRATION TIME IN THE AUDITORY NERVE OF THE TREEFROG, E. COQUI. R. Dunia and P.M. Narins. Interdepartmental Neuroscience Program and Department of Biology, University of California, Los Angeles; Los Angeles, Calif. 90024.

Neural processing occurs in two distinct domains: the time domain, and the frequency domain. The most common methods of describing the auditory nerve are by its frequency characteristics. However, much information about the auditory nerve can be obtained by describing its temporal features. One such feature is the maximum integration time, or temporal integration. This study involves calculating the maximum integration time through a systematic determination of thresholds for tones of various durations.

Adult E. coqui (3-7g) were anesthetized with nembutal (1.5ul/g; 50 mg/ml) and immobilized with curare (1.5 ul/g; 20 mg/ml). A small diameter hole drilled through the roof of the mouth exposed the auditory nerve as it entered the brain. Intracellular recordings were made with 3 M KCl filled glass micropipettes (30-50 MΩ).

The paradigm used to determine the maximum integration time was the rate-intensity function (RF). In this procedure, a constant duration tone (closed field) is played at various sound levels (0-110 dB SPL) in 3 dB increments produced in a random order at the rate of 1/500 ms. The spike rate is measured as a function of of the stimulus intensity. The procedure is repeated four more times so that each RF is the average of five individual determinations. The duration of the tone was systematically varied from 25-450 ms., changing after the completion of each set of five individual determinations. Various levels of continuous white noise (20-50 dB/Hz) were then added and the entire procedure was repeated in the presence of the background noise.

The criterion for determination of threshold in each averaged RF was the point at which the local slope of the curve differed significantly ($p<.01$) from the slope of the baseline. We then plotted the threshold as a function of tone duration for each auditory neuron.

In the pooled data set of 400 threshold determinations from 80 auditory neurons, the calculated maximum integration time was 275 ms. With the addition of continuous noise, the maximum integration time decreased slightly to 225 ms. These results are consistent with others' results for the maximum integration times of this frequency range in mammals and humans, and indicate no significant differences despite the differences in the auditory systems of the various species.

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343.8 KYNURENIC ACID SUPPRESSES THE COMPOUND ACTION POTENTIAL (CAP) OF THE GUINEA PIG COCHLEA. R. P. Bobbin and G. Ceasar*. Kresge Hearing Research Lab., Dept. of Otorhinolaryngology, L.S.U. Medical Center, New Orleans, LA 70119.

Evidence exists to suggest that the hair cell transmitter in octavolateralis systems is an excitatory amino acid such as glutamate (Bobbin et al., In Auditory Biochemistry; Edited by Drescher, D. G., pp. 102, Thomas, 1985). However there are few chemicals that are effective in blocking transmission between the hair cells and the auditory nerve. Notably without effect are the NMDA-type of excitatory amino acid antagonists, i.e., alpha-aminoadipate and 2-amino-5-phosphonovalerate. This study addresses the question of whether kynurenic acid affects the compound action potential of the auditory nerve (CAP).

Perilymphatic spaces of guinea pig cochleae were perfused with Ringer solutions containing 0.6 to 10 mM concentrations of kynurenic acid (Sigma) at a rate of 2 µl/min. for 10 min. (Bledsoe, et al., Hear. Res. 4:109, 1981). Immediately after each period of perfusion the CAP, cochlear microphonics (CM) and the summating potential (SP) evoked by 10 kHz tone bursts of varying intensity were recorded from a wire inserted in the basal turn scala vestibuli. These responses were compared to those elicited immediately after perfusion with normal Ringer solution. The crossed olivocochlear bundle was stimulated, and the endocochlear potential (EP) together with the evoked slow potential change produced in the scala media (COCP) were recorded in some animals. The effect of 5 mM kynurenic acid was tested on the EP and COCP and compared to the effects of Ringer solution.

Kynurenic acid (0.6-10 mM) reversibly suppressed the CAP, but did not affect the CM and SP (n=5). Maximum CAP suppression occurred at about 5 mM kynurenic acid and was equivalent to more than a 40 dB reduction in sound intensity. Kynurenic acid (5mM) reduced the EP slightly (10mV) and reversibly, but did not affect the COCP.

These results suggest that kynurenic acid, which has blocking effects at non-NMDA type of excitatory amino acid receptors, blocks receptors which are involved in the generation of the CAP, but not receptors used to generate the efferent response. Consistent with this possibility L-glutamic acid-diethyl ester, an additional non-NMDA type blocker, has been reported to suppress the CAP (Bobbin et al., ARO Abstracts, pp. 27-28, 1981). Future experiments will examine the pharmacology of kynurenic acid in greater depth.

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343.9 LOCALIZATION OF PUTATIVE SPIKE INITIATING SITES WITHIN THE LAGENA OF THE TELEOST FISH, HELOSTOMA TEMINCKI William M. Saidel, Dept. of Anatomy, Georgetown Univ., Schools of Med. and Dent., Washington, D.C. 20007

Single myelinated afferent axons to the lagenar sensory epithelia of fish innervate many hair cells. These axons are spatially distributed with a large diameter class forming synaptic relations with hair cells in the central epithelium and a small diameter class primarily synapsing with hair cells in the peripheral epithelium.

A question of functional interest is raised by the innervation pattern. Does a single hair cell generate a spike in an axon as a consequence of a suprathreshold post-synaptic potential (PSP) or do many hair cells generate sub-threshold PSP's which summate at an axonal spiking zone? The cytochemical procedure of Quick and Waxman (J. Neurocytol., 1977, 6:555-570 and J. Neurol. Science, 1977, 31:1-11) was used to study this question as it selectively stains spiking sites of peripheral and central myelinated axons.

In lagenar sensory epithelium, blue precipitate, characteristic of positive staining, was found only in the central epithelial region in two strata, a basal level corresponding to intraepithelial nodes of Ranvier and an apical level, corresponding to the strata in which hair cells synapse with 8th nerve afferents. Nodal labeling was found in all diameter axons external to the sensory epithelium.

By TEM, some of these positive staining sites identified hair cell synapses. These synapses had multiple dense bodies, each surrounded with synaptic vesicles, and the post-synaptic axons were invaginated into their respective pre-synaptic hair cells. At other nearby afferent synapses, the post-synaptic axons did not stain.

The results of this examination suggests a difference in spike initiation of lagenar axons. (1) The spike train of small diameter fibers in the peripheral region apparently results from summation of subthreshold PSPs from many hair cells at a spike initiating zone located some distance from the epithelium. (2) Some synapses onto large diameter fibers in the central epithelium appear to have the capacity for generating spikes in an afferent fiber. Only a few of the many hair cells synapsing on these fibers stained. Consequently, for the large diameter fibers, some synapses may generate spikes while others do not. Perhaps in this class of lagenar fibers, irregularity of observed interspike intervals might be a consequence of combining sub- and supra-threshold PSPs.

(Supported by NIH and ONR)

343.10 NEURONAL INNERVATION OF INDIVIDUAL SENSORY HAIR CELLS IN THE SACCULAR EPITHELIUM OF THE EAR OF A TELEOST FISH. A. N. Popper, W. M. Saidel, M. Uhlman*. Dept. of Anatomy and Cell Biology, Schools of Med. and Dent., Georgetown Univ., Washington, D. C. 20007.

The saccular sensory epithelium of the ear of the oscar, Astronotus ocellatus contains tens of thousands of sensory hair cells and is innervated by both afferent and efferent neurons. The number of sensory hair cells greatly outnumbers the neurons by a ratio exceeding 25:1 (Popper, A. N. and B. Hoxter, Hearing Res., 15:133,1984). While individual neurons must innervate many hair cells, a characterization of afferent and efferent synapses on single hair cells is lacking.

We have analyzed individual hair cells in Astronotus for afferent and efferent synaptic characteristics. Saccular epithelia from several fishes of the same size (to approximate the same number of hair cells in each epithelium) were cut into small pieces and serially cross-sectioned in a transverse plane at approximately 70 - 80 nm. Sections were mounted on grids, and viewed by TEM. Photographs of the sections were used to reconstruct the types and extent of synapses on each hair cell.

F1 and F3 sensory hair cell have afferent and efferent synapses, although the number of synapses of each type varies among cells. The number of afferent synapses on a single cell ranges from 1 to at least 6, while the number of efferent synapses ranges from 1 to at least 10. Approximately 75 per cent of the cells examined (N=17) have two to three times more efferent than afferent synapses. In the other 25 per cent of cells the number of afferent and efferent synapses are approximately equal.

The positions of afferent and efferent synapses on individual cells differ. Efferent synapses are distributed more widely than afferent synapses along the length of the cell. Afferent synapses are found along the base of the cell, and along the basal sides of the cell membrane to a level just below the nucleus. Efferent synapses are found from the base of the cell and along the cell membrane to a level corresponding to the middle of the nucleus.

The presence of efferent synapses on every sensory hair cell, despite the few efferent fibers, suggests CNS modulation of hair cell response. Multiple afferent synapses suggest a complexity in coding of the afferent information and its transmittal to the CNS. Although data are from a limited number of cells, our results indicate considerable inter-cell variability of neuronal innervation within small saccular regions as well as along the saccular epithelium. This variability differs from the more regular organization of innervation patterns in some tonotopically organized auditory epithelia and may suggest that there is other than tonotopic organization in the saccule of Astronotus. At the same time, we suggest that the extensive afferent and efferent innervation in the Astronotus saccule is likely to indicate peripheral computation of information regarding the acoustic stimulus. (Supported by NIH and ONR)

343.11 PROJECTIONS OF TYPE II NEURONS FROM THE GUINEA PIG COCHLEA. M.C. Brown, Research Laboratory of Electronics, M.I.T., and Eaton-Peabody Laboratory, Massachusetts Eye & Ear Infirmary, Boston, MA 02114.

Although the projections of the more numerous type I ganglion cells from the mammalian cochlea have been extensively studied, the peripheral and central projections of type II cells are less well known. The present study describes the peripheral and the initial portion of the central projections of type II cells at the light microscopic level, after labeling with extracellular injections of horseradish peroxidase into the spiral ganglion. The focal injections were confined to a narrow region of the basal turn in guinea pigs (7 to 275 ganglion cells labeled).

Labeled type II ganglion cells were distinguished by their small size (avg. soma silhouette area=100, st. dev.=19 sq. microns), compared to the larger type I cells (avg. area=216, st. dev.=40 sq. microns) and because their thin central and peripheral processes were about equal in diameter near the soma, whereas type I cells had central processes greater in diameter than peripheral processes. In eight cases, type II ganglion cells could be traced peripherally to outer spiral fibers (OSFs) contacting outer hair cells, whereas 11 type I cells contacted single inner hair cells immediately radial to the injection site. Thus the guinea pig innervation plan is similar to that of the cat (Kiang et al., *Science* 217:175, 1982).

Each of the 34 reconstructed OSFs spiralled basally within the organ of Corti 90 to 600 um before contacting outer hair cells, thus the topographic map from organ of Corti to the ganglion is offset for type II cells, relative to type I cells. Most of the OSFs contacted outer hair cells within one row and the OSF characteristics were row-dependent. Third-row fibers showed the most extensive innervation and the longest spiral and termination lengths. For instance, OSFs from the first row contacted between 5 and 12 outer hair cells whereas those from the third row contacted between 14 and 28 outer hair cells. Of 69 OSFs traced to a predominant row of innervation, 46% contacted first-row outer hair cells, with the remainder distributed to the second or third rows. Even though the number of OSFs contacting the third row seems to be fewer than the number contacting the first row, the difference in the number of terminals formed leads to the conclusion that there are more afferent boutons on the third row than on the first row of outer hair cells.

Labeled axons from type II cells were traced centrally through serial sections of a block containing the cochlea, auditory nerve and cochlear nucleus. Type II ganglion cells sent thin axons (avg. diam. = 0.45 um) centrally into the auditory nerve to travel with the thick central axons of the type I ganglion cells (avg. diam. = 2 um). The thick axons exhibited periodic constrictions interpreted as nodes of Ranvier whereas the thin axons did not. Most of the type II axons (23/24) were unbranched within the nerve and could be followed into the cochlear nucleus before fading. Five type II axons traced deeply into the interstitial portion of the cochlear nucleus bifurcated within the region of the thick-fiber bifurcation. Three descending and one ascending branches could be traced into the postero-ventral and antero-ventral divisions of the cochlear nucleus, respectively, and these coursed with the labeled thick fibers, although in no cases was the complete central projection obtained (due to fading of the reaction product within the axon). Although preliminary, these results suggest that the initial projection of type I and II cells from spiral ganglion to cochlear nucleus is topographically similar. This implies that the topographic map from the organ of Corti to the cochlear nucleus is slightly offset for the two types of neurons. Conduction times calculated from the respective fiber diameters would lead us to expect type II information to take more than 4 msec longer than type I information to travel from the periphery to the bifurcation point. The differences in peripheral innervation and axonal caliber suggest greatly different functions for the type I and II afferent neurons. (Supported by NIH grants T32 NS-07047 and PO1 NS-13126.)

343.12 A MONOCLONAL ANTIBODY (RT-97) LABELS TYPE II NEURONS OF THE SPIRAL GANGLION. A.M. Berglund and D.K. Ryugo. Dept. Anatomy and Cellular Biology, Harvard Med. School, Boston, MA 02115 and Eaton-Peabody Laboratory, Massachusetts Eye & Ear Infirmary, Boston, MA 02114.

Spiral ganglion neurons in the mammalian cochlea have been classified on the basis of morphological characteristics of their cell bodies and/or the pattern of hair cell innervation (e.g., Kiang et al., in L. Bolis et al., eds., Comp. Physiol. of Sensory Systems, p. 143, 1984). Type I neurons have relatively large cell bodies, innervate inner hair cells, and comprise 95% of the ganglion population. Type II neurons have relatively small cell bodies, innervate outer hair cells, but represent only 5% of the population. While cytological criteria enable one to identify each ganglion cell as type I or type II, the infrequent type II neurons are intermixed with the more prevalent type I neurons and are therefore difficult to detect. As a result, cell counts and population studies would be facilitated if type II neurons could be selectively and distinctly labelled.

Light and electron microscopic observations indicate that the type II cell bodies contain an abundance of neurofilaments and a paucity of ribosomes. The histological affinity of type II cell bodies for neurofilament stains (Kiang et al., ibid) is consistent with the specific labelling by protargol of proteins that form neurofilaments (Gambetti et al., Science, 213:1521, 1982). In this context, we hypothesized that the cell bodies of type II neurons might be preferentially labelled by the neurofilament antibody, RT-97 (Wood and Anderton, Biosci. Reports, 1:263, 1981).

Cochlear tissue of rat and cat was first stained with toluidine blue in order to identify type I and type II cell bodies. Photomicrographs and camera lucida drawings provided records of cell types and their position in the ganglia. The toluidine blue was then washed away and the tissue processed by immunocytochemical methods. Type II cell bodies were distinctly stained whereas all other cells were unstained or only lightly stained. In this way, we directly demonstrated that type II neurons defined by basic dye criteria are the same as those distinguished by neurofilament content. Studies involving type II cell counts, plots of their distribution within the ganglion, or morphometry will be greatly expedited by this simple and reliable method.

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343.13 COCHLEAR INNERVATION IN THE MONGOLIAN GERBIL. S.M. Echteler and K. Ohlemiller*. Auditory Physiology Laboratory. Department of Neurobiology and Physiology. Northwestern University, Evanston, IL 60201.

The pattern of innervation of mammalian inner (IHCs) and outer (OHCs) hair cells is known only in general terms and for a relatively few species. Moreover, many previous studies of cochlear innervation have employed the Golgi method, which works best in young animals and may not completely reflect the pattern of cochlear innervation in mature mammals. Recently, extracellular horseradish peroxidase labeling methods have been applied to the adult auditory periphery in chinchillas (Schmeidt and Feng, Hearing Res., 2:79, 1980), cats (Kiang et. al. Science, 217:175, 1982), and mice (Berglund and Ryugo, Soc. Neurosi. Abstr., 11:246, 1985). While these studies reveal a complete segregation of afferent and efferent innervation to adult IHCs and OHCs, they also suggest that significant differences may exist in the precise pattern of innervation of cochlear receptors in cats and rodents.

As part of a combined structure/function study of the auditory periphery in the Mongolian gerbil, HRP (10% w/v) was iontophoresed (+ 2-3 uA DC current for 20-30 mins.) into the cochlear nerve. Four to eight hours after injection, animals were perfused through the heart and cochlea with 2.5% glutaraldehyde in 0.1 M phosphate buffer. Following decalcification (0.1 M EDTA) for 2-4 days, cochleae were reacted with diaminobenzidine, embedded in araldite and viewed as surface preparations.

Bipolar neurons traced to OHCs have small (less than 10 ums) spherical cell bodies lying, almost exclusively, at the periphery of the spiral ganglion, near the intraganglionic spiral bundle. The peripheral processes of these cells are thin (less than 0.5 um) and, after crossing the tunnel of Corti, they spiral basally for 200 ums or more before terminating on 5-10 OHCs.

Radial fibers (RFs) projecting to IHCs arise from larger bipolar neurons. After passing through the habenula perforata, a single RF may: 1) form a single bouton on the nearest IHC, 2) course for short distances apically or basally forming pronounced varicosities, suggestive of en passant synapses, on up to three IHCs or 3) branch to innervate two adjacent IHCs.

Cochlear efferent fibers innervating OHCs have also been labeled. Preliminary reconstructions reveal that these neurons innervate receptors along restricted (250 ums or less) portions of the basilar membrane.

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343.14 ELECTRON-MICROSCOPIC OBSERVATIONS ON AN HRP-FILLED, PHYSIOLOGICALLY-CHARACTERIZED MEDIAL OLIVOCOCHLEAR AXON IN THE GUINEA PIG COCHLEA.
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There is substantial evidence that the efferent innervation of the cochlea is provided by two systems of olivocochlear (OC) neurons, lateral and medial, which have separate distributions in the brain stem and which synapse in the organ of Corti, respectively, with radial afferent fibers and outer hair cells. However, it is still not clear whether the post-synaptic targets of the lateral and medial systems may be broadly viewed as being mutually exclusive or partially overlapping.

In the present study, we examined the fine structure of a medial OC axon which had been impaled by a microelectrode inserted into the intraganglionic spiral bundle of an anesthetized guinea pig, its physiological characteristics determined (CF 11 kHz, responsive to ipsilateral sound), and its peripheral segment filled with HRP by iontophoresis (see Robertson, D., Hear. Res. 15:113, 1984, for details). Subsequent histological processing and light-microscopic examination showed that this fiber, before terminating in relation to outer hair cells, coursed for some 80 μm within the inner spiral bundle (ISB), where it displayed varicosities quite reminiscent of the pre-synaptic specializations of lateral OC axons which travel in the same region.

Ultrastructural study of this fiber showed that it was myelinated up to the habenula perforata and that the varicosities in the ISB contained a dense accumulation of synaptic vesicles, partially masked by the HRP reaction product, but identical in appearance to those in terminals contacting outer hair cells. The labeled varicosities partially enveloped and were in apparent synapse with small, but as yet unidentified, axons in the ISB.

Although these observations indicate that the same medial OC axon that innervates outer hair cells may also form synapses in the ISB, such synapses may not be typical of all medial OC fibers, because many pass directly to the outer hair cells without coursing in the ISB (Liberman, M.C., Hear. Res. 3:189, 1980).

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344.1 ENDBULBS OF HELD IN A COCHLEAR NUCLEUS, NUCLEUS MAGNOCELLULARIS IN THE RED-EARED TURTLE, CHRYSEMYS SCRIPTA ELEGANS. R.H. Browner and D.M. Pierz. Department of Anatomy, New York Medical College, Valhalla, N.Y. 10595.

Forty adult turtles were impregnated with the Golgi-Kopsch method. The Golgi impregnated brains were embedded in Araldite and sectioned in coronal and horizontal planes at 120 μm. The inner ear in 30 turtles was injected with horseradish peroxidase (HRP) to determine the connections of auditory ganglion cells and its types of ending in the cochlear nuclei (nuclei magnocellularis and angularis). The turtles were anesthetized with a combination of nitrous oxide, halothane, and oxygen. The temporal muscle was removed over the temporal bone and the inner ear exposed by drilling. A glass micropipette, with a 20 μm tip, was placed in the inner ear. A 30% solution of HRP was iontophoretically injected for 15 minutes into the inner ear. The bony opening was capped with dental cement and the animal was moving within 15 minutes following surgical closure. Five days following the injection the turtles were killed with an overdose of Nembutal. The turtles were transcardially perfused with Reptilian Ringers followed by a fixative of 1.25 glutaraldehyde and 1.0 paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were prepared for HRP histochemistry. The sections were cut at 40 μm in the coronal plane and then reacted with either BHDC or TMB. The sections were counterstained with Neutral Red.

Eighth nerve fibers entered the brain stem dorsolaterally and coursed to both cochlear nuclei. They entered the nucleus magnocellularis and spread throughout the nucleus. A predominant 8th nerve ending was the endbulb of Held. There were 3 modifications of endbulbs of Held. One was of an enlarged cup which surrounded the neuron for at least one half of its soma. It had no additional appendages. A second had an enlarged terminal with 2 to 3 thickened appendages arising from its base. The third was hand-like. It had a wide portion with 3 to 4 finger-like expansions arising from the edge of the expanded area. These expansions arched around the soma. All the endings were presynaptic to moderately sized bushy neurons.

The presence of endbulb of Held in the turtle, bird (Jhaveri and Morest, JCN, 7:809, '82; SN, 11, '85), and mammal (Ryugo and Fekete, JCN, 210:239-257) is further evidence for similarities in the amniote auditory system. (Supported by the Culpeper Foundation, 48-320).

344.2 ^{14}C-GLYCINE RELEASE IN THE GUINEA PIG COCHLEAR NUCLEUS AFTER AXOTOMY OF COCHLEAR AND CENTRIFUGAL FIBERS. C. Staatz-Benson and S.J. Potashner, Dept. Anatomy, Univ. Connecticut Health Center, Farmington, CT 06032.

We previously presented evidence that glycine may be a transmitter in the cochlear nucleus (CN), as the CN contains mechanisms for the synaptic release and the high-affinity uptake of glycine (Neurosci. Abs., 11: 1048, 1985). The present study was performed to determine if the axonal endings that release glycine in the CN are contributed by centrifugal projections to the CN from other brain stem auditory nuclei, by cochlear nerve fibers, or by cells intrinsic to the CN.

The electrically-evoked, calcium-dependent release of exogenous [14C]glycine (GLY) was measured, in vitro, in the three major subdivisions of the guinea pig CN, the anteroventral (AVCN), posteroventral (PVCN), and dorsal (DCN) cochlear nucleus. For comparative purposes, we measured the release of exogenous [3H]-D-aspartate (D-ASP), a non-metabolizable marker taken up and released by the axonal endings of glutamatergic and aspartatergic neurons. Release was compared using three test groups: unlesioned controls; after destruction of the left cochlear nerve by cochlear ablation; and after section of the centrifugal projections to the right PVCN and DCN that course in the trapezoid body and the acoustic striae. In this last group, most of the centrifugal projections to the right AVCN were spared.

Two days after section of the trapezoid body and the acoustic striae on the right, GLY release was depressed in the right PVCN (by 41%) and DCN (by 37%), when compared to unlesioned controls. GLY release in the right AVCN, and in all three CN subdivisions on the left, was similar to that of the unlesioned controls. D-ASP release was unaffected by this lesion. Two to four days after ablation of the left cochlea, GLY release was indentical to that of the ulesioned controls. By contrast, D-ASP release was depressed in the left AVCN (by 78%), PVCN (by 80%), and DCN (by 60%), but not in the CN subdivisions on the right side.

These findings suggest that a proportion of the glycinergic axonal endings in the PVCN and the DCN are probably contributed both by centrifugal projections from other brain stem auditory nuclei and by cells intrinsic to the CN. They also suggest that although the cochlear nerve may use L-GLU/L-ASP as transmitters, it may not contain glycinergic fibers.

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344.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF GLYCINE IN THE COCHLEAR NUCLEUS AND SUPERIOR OLIVARY COMPLEX OF THE GUINEA PIG. R. J. Wenthold, R. A. Altschuler, D. Huie*, M. H. Parakkal*, and K. A. Reeks*. Lab. of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892, and Kresge Hearing Research Inst., Ann Arbor, MI 48109.

Our recent studies on the immunocytochemical distribution of the glycine postsynaptic receptor (Altschuler et al., Brain Res. 369:316, 1986), showed a dense, widespread distribution of receptors in the cochlear nucleus (CN) and superior olivary complex (SOC), supporting previous findings that suggested glycine is a major neurotransmitter in these nuclei. We have now studied glycine immunoreactive labeling to further characterize glycinergic synapses in the CN and SOC. Antibodies, made in rabbits against glycine conjugated to BSA with glutaraldehyde, were affinity purified by passing through a column of glycine conjugated to ovalbumin. Bound antibodies were eluted with acid, neutralized and passed through a column containing GABA conjugated to BSA. The unbound fraction was used in these studies. Immunoblot analysis using ovalbumin conjugates of several amino acids showed intense reaction with glycine, minor reactions with alanine and beta-alanine, and no significant reactions with other amino acids. For immunocytochemical studies, guinea pigs were perfused with PBS, followed by 4% paraformaldehyde containing 0.25% glutaraldehyde. Labeling was determined using both immunofluorescence and immunoperoxidase techniques on vibratome sections.

In the CN immunoreactive cell bodies were most abundant in the dorsal cochlear nucleus (DCN), with labeled cells found in both superficial and deep layers. In the ventral cochlear nucleus (VCN), only scattered immunoreactive cells were seen. At least two populations were present; larger cells were most abundant in the caudal-AVCN while smaller cells were often found in the granule cell cap. In the SOC intense cell labeling was seen in the medial nucleus of the trapezoid body (MNTB), while only scattered cell labeling was present in the lateral superior olive (LSO). Puncta resembling presynaptic terminals were present throughout the cochlear nucleus. In the VCN this labeling was often present around neuronal cell bodies resembling patterns seen using anti-GABA antibodies. Heavy punctate labeling was present in the LSO. The glycine immunoreactive cell bodies in the superficial DCN appeared similar to a class of cells labeled with anti-GABA and anti-GAD antibodies (Wenthold et al., Brain Res. In Press). The possible coexistence of glycine and GABA in these cells was studied using rabbit anti-glycine antibodies and guinea pig anti-GABA antibodies. These studies reveal that some neurons in the superficial DCN are immunoreactive to both antibodies. The present studies add support that glycine is a neurotransmitter in the cochlear nucleus and SOC. Some glycinergic inputs to the cochlear nucleus may originate with the immunoreactive cells in the DCN. The intense labeling of cells in the MNTB and terminals in the LSO is consistent with this pathway being glycinergic.

344.4 DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE COCHLEAR NUCLEUS COMPARED AMONG THREE STRAINS OF RAT. D.A. Godfrey, L. Carlson*, and C.D. Ross. Dept. of Physiology, Oral Roberts Univ., Tulsa, OK 74171.

Recent results have suggested that sensory systems of albino animals may have peculiar features (e.g. Creel, Pharmacol. Biochem. Behav. 12(1980)969). Therefore, distributions of choline acetyltransferase (ChAT) activities in the cochlear nucleus of 2 pigmented rat strains - Long Evans Hooded and Norway Brown - have been measured for comparison to that previously measured for an albino strain - Spraque-Dawley. Transverse sections at 3 rostrocaudal levels through the cochlear nucleus in each rat were microdissected, and all sample locations mapped. Overall comparisons among the 3 rat strains are contained within the table below, with ChAT activities, in μmol/kg dry wt/min at 38°C, shown as mean ± S.E (no. of rats) for each region.

Region	Spraque-Dawley Albino	Long Evans Hooded	Brown Norway
Granular lat. to PVCN	221±39(4)	181±25(3)	246±14(3)
Granular dorsolat. to AVCN	333±57(7)	275±20(3)	264±7 (3)
DCN molecular layer	242±26(6)	126±31(3)	191±33(3)
DCN fusiform soma layer	432±31(6)	322±31(3)	403±13(3)
DCN deep layer	318±20(3)	246±31(3)	293±20(3)
PVCN	317±15(5)	195±8 (3)	224±20(3)
AVCN caudoventral	343±41(4)	234±23(3)	225±48(3)
AVCN caudodorsal	498±42(4)	326±60(3)	280±32(3)
AVCN rostral	424±37(7)	387±36(3)	322±32(3)
IN	43±14(5)	22±5 (3)	43±4 (3)
Acoustic striae	226±34(4)	159±29(3)	199 (2)
Trapezoid body	123±34(4)	89±21(3)	77±9 (3)

Activities of ChAT are consistently somewhat higher in the albino than in the pigmented strains, but the relative distribution among regions is similar in all 3 strains. Highest activities tend to be in rostral and caudodorsal regions of the anteroventral cochlear nucleus and in the fusiform soma layer of the dorsal cochlear nucleus. There may be some differences between the two pigmented strains, in that activities tend to be higher caudally in Brown Norway than in Long Evans Hooded, but higher rostrally in Long Evans. At a more detailed level, several trends were similarly observed in all 3 strains. In the dorsal cochlear nucleus, ChAT activities were higher in deeper vs. more superficial parts of the molecular layer and, usually, in more superficial vs. deeper parts of the fusiform soma layer. In the anteroventral cochlear nucleus, ChAT activities were higher dorsolaterally than ventromedially. Overall, the data show minor rather than major differences among the 3 rat strains. (Supported by NIH Grant NS17176).

344.5 ENDBULBS OF HELD AND SPHERICAL CELLS: ACTIVITY-DEPENDENT VARIATIONS IN MORPHOLOGY. T.E. Benson, S. Sento*, and D.K. Ryugo. Dept. of Anatomy and Cellular Biology, Harvard Med. School, Boston, MA 02115, and Eaton-Peabody Laboratory, Massachusetts Eye & Ear Infirmary, Boston, MA 02114.

In the ventral cochlear nucleus (VCN) of cats, type I auditory nerve (AN) fibers give rise to large, axosomatic endings called endbulbs of Held. These endings contact spherical cells and are thought to convey with great fidelity the activity of the presynaptic fiber. Spike discharges occur in single units of the VCN in the absence of controlled acoustic stimuli. Most of this spontaneous activity (SR) is dependent on the SR of the AN (Koerber et al., Exp. Neurol., 6:119, 1966). In this context, it may be inferred that the SR of an individual AN fiber is a rough indicator of both its general activity level (Liberman, J. Acoust. Soc. Am., 63:442, 1978) and that of its postsynaptic spherical cell. Given that type I AN fibers can be segregated into separate functional groups on the basis of SR, we investigated the extent to which SR differences might be reflected in the structure of endbulbs and their postsynaptic targets.

In the present study, selected pairs (n=9) of physiologically characterized AN fibers from 5 cats were intracellularly injected with horseradish peroxidase. By using fibers that were from the same animal and within an octave of each other in characteristic frequency, we examined morphological parameters with respect to differences in SR. We determined silhouette areas and perimeters for "coded" terminal endbulbs and the area of their postsynaptic targets, the somata of spherical cells. The proportion, endbulb area divided by perimeter, produced a "form factor" reflecting geometric complexity. Values of this factor separated endbulbs of low (≤18 spikes/sec) and high (>18 spikes/sec) SR fibers into two nonoverlapping groups regardless of variations in endbulb size. Furthermore, spherical cells postsynaptic to low SR endbulbs were significantly smaller than those postsynaptic to high SR endbulbs (Smirnov test, $p < 0.001$).

These observations reveal that low SR fibers terminate in complicated endbulbs that contact relatively small spherical cells, whereas high SR fibers give rise to relatively simple endbulbs that contact larger spherical cells. Some of the effects of low SR fibers on their postsynaptic targets, when compared to those of high SR fibers, appear to be mimicked by effects of experimentally-induced deprivation (e.g., Benson et al., J. Neurosci., 4:638, 1984). Finally, the data argue against fibers of different SR groups converging onto the same spherical cell.

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344.6 A MORPHOMETRIC ANALYSIS OF THE ANTEROVENTRAL COCHLEAR NUCLEUS IN TWO MOUSE MODELS OF PRESBYCUSIS. J.F. Willott, L.M.Jackson* and K.P.Hunter*. Dept. Psychol., Northern Ill. Univ., DeKalb, IL 60115.

The dimensions and volume of the anterior ventral cochlear nucleus (AVCN), the number of AVCN neurons, and the size of both soma and nuclei (in Nissl-stained tissue) were determined in two mouse models of age-related hearing loss: The C57BL/6J strain, which undergoes progressive chronic sensorineural hearing loss with onset during young adulthood and the CBA/J which demonstrates only moderate hearing loss with onset late in life. Frontal and horizontal AVCN sections, as well as cochleas, were analyzed in 4 C57 age groups (1-,7-,12-,19+ months) and in 3 CBA age groups (1-, 10-,22- months). No significant changes were observed in either AVCN dimensions, volume, or total number of neurons as a function of age in either strain. In aging C57 mice, however, AVCN multipolar cells show a slight decrease in size, but spherical and globular cells increase in size. The increase in cell size--at least in spherical cells--is due primarily to an increase in soma size, as indicated by nucleus to cytoplasm ratios. The size increases are most consistent in the dorsal (high frequency) region of the AVCN, and are correlated with loss of spiral ganglion cells which is most severe in the basal (high frequency) region of the cochlea. In aging CBA mice, all 3 AVCN cell types tend to decrease in size. The data indicate that aging is associated with different central anatomical consequences depending on AVCN cell type, genotype, and/or the type of peripheral hearing loss involved.

The C57 and CBA AVCNs also differ in several aspects irrespective of age. The volume of AVCN and number of AVCN neurons are significantly greater in C57 mice. This is due, in part, to the fact that the ventral portion of the C57 AVCN is wider, medio-laterally, than the CBA AVCN.

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344.7 DUAL ORIGIN OF NEURONS IN THE DORSAL COCHLEAR NUCLEUS. F.W. WILLARD AND G.F. MARTIN.[1] Department of Anatomy, University of New England, Biddeford, Maine 04005 and The Ohio State University, Columbus, Ohio 43210.[1]

The dorsal cochlear nucleus (DCN) in the opossum is composed of four layers. Fundamental to this arrangement are two types of macroneurons: principal cells and giant cells. Various forms of microneurons are found surrounding these larger cells. Previous studies suggested the rhombic lip as the source of DCN neurons (Taber Pierce, J. Comp. Neurol. 1967). However recently we have shown that the macroneurons migrate from a medially located cytogenetic zone into the DCN over the presumptive dorsal acoustic stria (DAS). We now report on a Golgi study of the DCN which investigates the differentiation of its microneurons.

An age-graded series of pouch-young opossums has been prepared using the Rapid-Golgi and Stensaas variant of the Golgi-Kopsch technique. Sections were taken in several planes at 80-100 um thickness.

At 25-30 mm snout-rump length (SRL) macroneurons with thin, varicose dendrites were present within the DCN or the presumptive DAS. These cells were embedded in a matrix of primitive neuroepithelial (PNE) cell processes. The PNE cell somata were aligned along the ventricular surface and their processes, covered with thin filapodia, extended ventrally into the brain stem. By 40 mm (SRL) the macroneurons were found within the DCN and had thick, tapering dendrites. Many PNE cells had lost the distal portions of their ventrally oriented process and had now developed gnarled and recurving processes. In the 68 mm (SRL) animal, the macroneurons had filapodia and rudimentary dendritic appendages. Few PNE somata were located near the ventricular lining, however many presumptive microneurons with gnarled processes were now found scattered throughout the nucleus. By 80 mm (SRL) both macro- and microneurons had recognizable dendritic spines. Finally by 160 mm (SRL) the adult-like dendritic morphology of the DCN neurons was evident and no PNE cells were definable.

Our observation of an age-graded transition of rhombic lip PNE cells into microneurons, coupled with the previously documented migration of macroneurons from a medially-position cytogenetic zone (Willard and Martin, J. Comp. Neuol. 1986), supports the notion that the neurons of DCN have a dual origin. [Support: BNS-8309245 and NS-10165 (GFM) and NSO 6829-02 (FHW)]

344.8 EFFECTS OF LOCAL INJECTIONS OF LIDOCAINE IN THE COCHLEAR NUCLEUS ON RESPONSE PROPERTIES OF TYPE IV UNITS IN CATS. W.P. Shofner and E.D. Young. Dept. of Biomedical Engineering, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

In unanesthetized, decerebrate cats, Type IV units are recorded from the principal cells of the dorsal cochlear nucleus (DCN) (Young, E.D., Brain Res., 200:23, 1980). Type IV units are characterized by their nonmonotonic best frequency (BF) rate vs level functions; they give excitatory responses to BF tones near threshold, but give inhibitory responses to BF tones at 30-40 dB above threshold. One source of inhibitory input to Type IV units comes from DCN interneurons which possess Type II response properties (Voigt, H.F. and Young, E.D., J. Neurophysiol., 44:76, 1980). However, electrical stimulation of the auditory nerve evokes inhibitory responses in both Type II and IV units implying that part of the inhibitory input to Type IV units comes from additional sources besides Type II interneurons (Shofner, W.P. and Young, E.D., J. Neurophysiol., 54:917, 1985). Therefore, we investigated whether projections from the anteroventral cochlear nucleus (AVCN) to the DCN are a source of inhibitory input to DCN as concluded by Evans and Nelson (Exp. Brain Res., 17:428, 1973).

Adult cats were decerebrated at the level of the midbrain. The cerebellum was aspirated in order to view the lateral free surface of the DCN and its attachment to the cerebellum. Broken tipped glass micropipettes coupled to a Hamilton syringe were inserted into the AVCN through the junction between the DCN and cerebellum. Responses of Type IV units were studied before and after 100-300 nl injections of 2% lidocaine hydrochloride.

When the pipette was located in the caudal AVCN, there is a weakening of the inhibitory responses of Type IV units after lidocaine is injected. These weakened inhibitory responses are seen as an increase in discharge rate, which in some cases increase to rates above spontaneous activity. Moreover, the weakened inhibitory responses are observed at frequencies near BF, but not at frequencies off-BF. These results suggest that the AVCN provides BF inhibitory input to the DCN, but does not provide inhibitory sideband input. It is unknown whether AVCN projections form inhibitory synapses on DCN principal cells (Type IV units) or form excitatory synapses on DCN interneurons (Type II units). When the pipette and spread of lidocaine were near auditory nerve fibers and granule cell domains, we observe a loss of acoustically driven responses and spontaneous activity. In one cat, the pipette was placed in the deep DCN, and for one AVCN Type I/III unit there was an increase in the excitatory discharge rate near BF, but not off-BF following injection of lidocaine.

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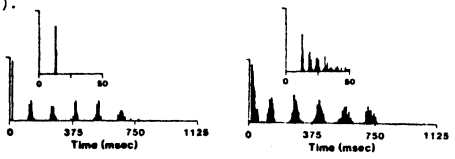
344.9 DEVELOPMENT OF TEMPORAL RESPONSE PROPERTIES IN THE AUDITORY PERIPHERY OF CATS. E.J. Walsh and J. McGee*. Depts. of Surgery and Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62702.

Neurons located in the auditory nerve and the cochlear nucleus of kittens between birth and 14 postnatal days respond "rhythmically" to tone-burst and broad-band stimuli. "Rhythmic" responses are characterized by an onset discharge burst consisting of one to several action potentials, followed by alternating bursts of discharge activity and quiet intervals. Specific temporal discharge patterns depend on the type of neuron considered and its location. Temporal patterns of auditory nerve fibers exhibit a pronounced onset peak which decays rapidly to near spontaneous rates, with subsequent discharge bursts occurring approximately every 100 msec throughout the duration of stimulation. The temporal pattern of evoked discharge activity in the auditory nerve of immature kittens is relatively homogeneous. Certain neurons recorded from the cochlear nucleus, on the other hand, often respond with a single action potential at stimulus onset, remain quiescent for as long as 100 msec, and display discharge burst patterns with a frequency similar to that noted for auditory nerve fibers, but with more precise rhythmic discharge characteristics. Other cochlear nucleus neurons recorded from young animals exhibit temporal discharge patterns during the first 50 msec of stimulation that are similar to the complex responses found in adult animals, followed in time by rhythmic bursting activity that resembles the temporal response profile of auditory nerve fibers. As a rule, discharge burst frequency remains stable regardless of stimulus frequency and intensity, although slight deviations from this behavior have been noted. The majority of neurons in the auditory periphery exhibit the same burst frequency throughout development, however, exceptions are occasionally observed. The percentage of neurons responding rhythmically decreases as kittens age, so that bursting discharge patterns give way to more sustained patterns of activity by the end of the second postnatal week. Examples of poststimulus time histograms from cochlear nucleus neurons are illustrated. (Supported by a grant from the Deafness Research Foundation).



344.10 THE REPRESENTATION OF THE STEADY-STATE VOWEL SOUND /ɛ/ IN THE TEMPORAL DISCHARGE PATTERNS OF CAT ANTEROVENTRAL COCHLEAR NUCLEUS NEURONS. C.C. Blackburn* and M.B. Sachs. Dept. of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The encoding of spectrally complex, speech-like sounds in populations of auditory-nerve fibers has been studied in some detail. We have undertaken a series of experiments designed to investigate the transformations of the auditory-nerve code which take place in the cochlear nucleus. Since morphological analyses make it clear that this nucleus consists of a complex of subsystems, each with its own structure, response characteristics, and connections, it is important to be able to identify and segregate subpopulations in an analysis of processing in this structure. Studies coupling HRP-labeling with electrophysiological recording indicate that in the anteroventral division of the cochlear nucleus (AVCN), the three major electrophysiological unit types observed can largely be correlated with the three major morphological cell types, with primary-like units recorded from spherical bushy cells, primary-notch units recorded from globular bushy cells, and chopper units recorded from stellate cells.

Platinum-iridium microelectrodes were used to characterize single units in the AVCN of nembutal-anaesthetized cats and to study the responses of these units to the synthetic vowel /ɛ/, which has formants at 0.512, 1.792, and 2.432 kHz. Units were characterized on the basis of their PST histograms in response to short tone bursts at characteristic frequency (CF) and their inter-spike interval regularity. Primary-like and primary-notch units with CFs near the formant frequencies showed strongly phase-locked responses to those formants, as evidenced by their Fourier transforms. Chopper units with CFs near the second and third formants showed little or no phase-locking to the vowel formants. Estimates of the population responses to the vowel were made by computing average localized synchronized rate (ALSR) profiles for each unit type, using data pooled across cats with similar thresholds. ALSR is the average phase-locked response at a particular harmonic of the stimulus among the units with CFs near that hormonic, and thus reflects phase-locking information, frequency encoding, and average rate within a population of related units. ALSR profiles of primary-like and primary-notch populations had well-defined peaks corresponding to the second and third formant frequencies of the vowel, while ALSR profiles of chopper units did not. Thus, temporally encoded information from auditory-nerve firing patterns in response to complex stimuli is preserved in primary-like and primary-notch populations but is seriously degraded in chopper populations. (Supported by NIH grants NS12112, NS07283, and NIMH grant MH18030.)

344.11 RESPONSES TO PARALLEL FIBER STIMULATION IN SLICES OF THE GUINEA PIG DORSAL COCHLEAR NUCLEUS. P.B. Manis* (SPON: W.E. Brownell). Depts. of Otolaryngology-Head & Neck Surgery and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Recent anatomical studies have provided important insights into the cytoarchitecture and ultrastructural organization of the molecular layer (ML) of the dorsal cochlear nucleus (DCN). Several anatomical features, including a longitudinally oriented parallel fiber system, are particularly reminiscent of the cerebellar ML. However, little is known about the electrophysiology of the parallel fiber system of the DCN. We have examined extracellular field potentials and single unit responses produced by direct parallel fiber stimulation in vitro. Slices of guinea pig DCN 300 µm thick were cut parallel to the strial axis of the nucleus and maintained in mammalian slice Ringers at 34° C. The superficial parallel fibers were stimulated with a bipolar electrode placed against the pial surface of the slice. Extracellular recordings were made with saline-filled glass pipettes 0.5-1 mm from the stimulation site.

Parallel fiber stimulation produced a short-latency, primarily negative triphasic wave, 1-3mV in amplitude in the ML. This volley was followed by a small negative wave 5-10 msec in duration, which was selectively abolished by blocking synaptic transmission with a low-Ca^{++} solution. When paired stimuli were given 15 msec apart, potentiation of the second (test) response was revealed. The field potential in the pyramidal cell layer (PCL) was smaller and more complex. An initial fast Ca^{++}-independent negative wave was followed by a fast negative and slow positive Ca^{++}-dependent wave. The falling phase of the positive wave usually had a brief negative deflection. The PCL positive wave was also potentiated by paired stimuli.

The responses of single units in the PCL to parallel fiber stimulation were also studied. A single stimulus to the parallel fibers usually resulted in one action potential in each of the 5 units recorded to date. However, equal amplitude paired stimuli with intervals between 5 and 50 msec revealed significant facilitation occuring during the test stimulus. When the conditioning stimulus was subthreshold, the cells responded to nearly every test stimulus. With suprathreshold conditioning stimuli, two to four action potentials were evoked by the test stimulus.

In summary, DCN field potentials evoked by parallel fiber stimulation closely resemble those reported following local stimulation of cerebellar parallel fibers. Substantial potentiation of both synaptic field potentials and single-cell responses was revealed using paired stimuli. The results suggest that DCN parallel fibers are excitatory to dendritic elements of the molecular layer and to single cells of the PCL.

344.12 ANATOMICAL AND PHYSIOLOGICAL STUDIES OF A CLASS OF WIDE DYNAMIC RANGE NEURONS IN THE CAT COCHLEAR NUCLEUS. W.S. Rhode* and P.H.Smith. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, Wisconsin 53706.

Recordings from the cat ventral cochlear nucleus (VCN) reveal a variety of response patterns to a series of 25 msec. tone pips. The most frequently observed poststimulus time histogram (PSTH) response has been designated onset-chopper (O_c) because of the abrupt onset peak followed by 1 to 3 less well timed peaks in the response. This pattern has been observed in both the pentobarbital anesthetized and the unanesthetized preparations.

This neuronal category exhibits several very unique response properties: 1) the most broadly tuned (widest frequency range) units, 2) the widest rate-intensity functions often up to 80 dB SPL, 3) the lowest standard deviation of the first spike latency (.09 vs. .73 msec. for eighth nerve fibers), 4) intracellular depolarizations whose amplitude increases with increasing stimulus intensity.

Neurons displaying these properties were labeled intracellularly with horseradish peroxidase and subsequently analyzed. These large stellate cells, located in the the PVCN near the entrance of the eighth nerve root, have a few large dendrites which branch sparingly yet traverse much of the dorsoventral extent of the PVCN. The myelinated axon gives rise to a number of collaterals some of which branch extensively in the vicinity of the parent soma and dendrites and terminate on the cell bodies and large dendrites of neighboring neurons. Other collaterals project to other regions of the ventral cochlear nucleus and toward the dorsal cochlear nucleus. At the electron microscopic level the collateral terminals observed thus far contain pleomorphic vesicles. The cell body is covered with synaptic terminals many of which contain large round vesicles and are presumed to originate from eighth nerve fibers, while flat and pleomorphic vesicle terminals are also seen.

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344.13 MULTICHANNEL UNIT RECORDINGS FROM AVCN IN GUINEA PIG D.J. Anderson and B.M. Clopton. Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, Michigan 48109

A dorsal-to-ventral penetration of the anteroventral cochlear nucleus (AVCN) was made using a passive silicon substrate multichannel electrode with four sites (BeMent, S.L. et al., IEEE Trans. Biomedical Engineering, BME 33:230, 1986). Site spacings utilized at various stages of this study were 30, 50 and 100 microns arranged either parallel or perpendicular to the axis of penetration. Site areas were 60-100 sq. microns with impedances of 5-10 Mohms at 1 kHz. Sites not recording spike activity were used as references for the active sites to reduce the large field potential which often interferes with unit isolation in AVCN. Using this technique, up to three units could be well isolated simultaneously. Penetrations across isofrequency planes using longitudinally spaced sites sampled adjacent units in a tonotopic sequence thus affording an opportunity to observe statistical relationships among closely spaced cells having overlapping primary afferent inputs.

Spontaneous rate and responses to gated sinusoids and synthesized complex periodic sounds were observed. Analysis methods include post stimulus and interval histograms for individual cells and cross correlograms for pairs of cells. It was determined in this preliminary study that having variety of site spacings and orientations was valuable for discovering the encoding strategies of small communities of neighboring cells. There is a trade-off between having wide site spacings, such as 100-200 microns, which yields independent spikes on each channel but a lower probability of association, versus closely spaced sites which increases the probability of interrelationships but also increases the amount of cross talk among channels. The alignment of the sites can compensate for restricted angles of approach to the nucleus. Our continuing efforts to optimize both the electrode design and signal processing and analysis procedures needed for a multichannel electrode array have been a major component of this study and will be discussed.

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344.14 INTRACELLULAR RESPONSES TO PURE TONES FROM IDENTIFIED NEURONS IN THE VENTRAL COCHLEAR NUCLEUS OF THE GERBIL. J.Feng*, E.-M. Ostapoff, S. Kuwada and D.K. Morest, Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

This is a preliminary report on the electrophysiology and morphology of cochlear nucleus neurons in young adult Mongolian gerbils (Meriones unguiculatus). Acoustic repoonses were determined intracellularly and impaled cells were injected iontophoretically with HRP. We have reconstructed 9 labeled cells from 25 intracellularly recorded units.

Under pentobarbital and ketamine anesthesia, a hole was made in the temporal bone internal to the arc described by the superior semicircular canal. Beveled micropipettes, filled with 4% HRP, traversed the paraflocculus en route to the cochlear nucleus. Acoustic stimuli were generated digitally and delivered through a sealed, calibrated system. The intracellular responses were recorded on FM tape and subsequently digitized. Brain stems were cut on a Vibratome (50 µm), reacted with DAB, and processed for both light and electron microscopy. Cells were reconstructed from complete series of sections at 600-1200X.

In the rostral part of the cochlear nerve root region we recovered a globular bushy cell and a stellate cell. Both gave sustained responses to tone pips. The best frequency of the bushy cell was 9-10 KHz. Although the stellate cell was tuned to low frequencies (700 Hz), it did not exhibit phase-locked discharges. The other 7 cells were located in the caudal part of the cochlear nerve root region. Two were octopus cells. Of these, one showed a sustained depolarization for the duration of the tone burst, but each stimulus only evoked one action potential. The responses of the other are still being analyzed. Three were stellate cells. Two were characterized as having "chopper" response patterns. One was a very large stellate, with a best frequency around 5 KHz, and at the lower end of its frequency range (2 KHz) was an indication of phase-locked discharges. The third stellate cell had an unusual, unidirectionally oriented dendritic field with a length of about 400 µm and large claw-like endings. It showed a robust and sustained depolarization and discharge pattern to acoustic stimulation. The last 2 recovered cells were large elongate neurons. One of these has a best frequency around 6 KHz and generally resembled a pauser type discharge. The response type of the other elongate cell was unclear.

In general, this preparation offers relatively long, stable recordings and a high yield of recovered cells. One can use this to advantage in analyzing the relationship between physiological responses to acoustic stimuli and synaptic organization.

Supported by NIH grants NS14347 and NS18027.

344.15 INTRACELLULAR MEASUREMENTS OF TIME CONSTANTS IN ACOUSTICALLY RESPONSIVE NEURONS IN GERBIL COCHLEAR NUCLEUS. H.F. Voigt and T.A. McMullen*, Departments of Biomedical Engineering and Otolaryngology, Boston University, Boston, MA 02215.

We have used single electrode intracellular recording techniques to measure both acoustic responses and voltage responses to injected current pulses from neurons in the cochlear nucleus of barbiturate-anesthetized young adult gerbils (Meriones unguiculatus; Tumblebrook Farms). Micropipette electrodes, filled with 4 molar potassium acetate, were selected for rise times of less than 300 us in response to current pulses of +1 to -1 nA. Electrodes were passed through the paraflocculus and into the cochlear nucleus via the transbulla approach described by Frisina et al. [Hearing Research 6 (1982) 259-275]. Under computer control, a series of nine, 50-ms duration current pulses were delivered to the electrode for the purpose of either injecting current to an impaled cell or testing the electrode characteristics. Voltage responses were digitized and stored for analysis.

Estimates of the time constants of the intracellular voltage decay curve were computed as a first step in determining the electrotonic characteristics of acoustically responsive cells. The decay curve was approximated as a sum of exponentials. The time constants were estimated by fitting a linear-quadratic spline to the logarithm of the decay curve [Azarro et al., IEEE Transactions on BME, BME-28 (1981) 755-760]. In order to insure that the time constants of the intracellular decay curve were sufficiently different from the time constants of the electrode response, records of the microelectrode pulse response were obtained before intracellular penetration and after loss of intracellular contact. These records were analyzed with the same algorithm. Time constant estimates were calulated for cells that responded to acoustic stimulation. In about half of the cells, the estimated membrane time constants were between 1 and 2 ms. For the other cells, time constant estimates were less than 1 ms and were not significantly different from those of the electrode response alone. (This work was supported by NSF.)

344.16 IS THERE TUNING OF NEURONAL INTERACTIONS IN RAT DORSAL COCHLEAR NUCLEUS? J.A. Kaltenbach, P.M. Gochin, and G. L. Gerstein. Dept of Physiology, Univ. of Penna. Phila., PA 19104

The expression of a synaptic connection between neurons is not fixed but depends on a variety of factors. This can be demonstrated physiologically by cross-correlation analysis which plots the probability of firing of one neuron as a function of time following the firing of another neuron. Indeed, two interconnected neurons may show signs of near synchronous firing which varies across the stimulus spectrum. For example, recent studies in our laboratory suggest that the strength of an excitatory connection between neurons in auditory cortex may be "tuned" to location of a sound source (1) even though single neurons appear to show no signs of location selectivity. The presence of "tuned interactions" implies that certain stimulus parameters may be represented by the synchronous firing of groups of neurons rather than by the firing patterns of single neurons. In addition, direct interactions have also been observed to exhibit preferences for specific sequences of tone bursts (2).

An issue which needs to be explored is whether the sort of tuning observed in the synchronous firing between neurons can be demonstrated in the frequency and intensity domains. It is this domain in which single neurons display frequency threshold tuning. It is thus of interest to know how the areas in which interactions occur between neurons compare with frequency response areas of individual neurons. To address this issue we are recording the simultaneous activity of multiple neurons in the rat dorsal cochlear nucleus in response to each of 64 different frequency/intensity combinations which define the response area of each unit. A cross correlation histogram is then collected for each pair of neurons at each of the 64 stimuli and these are assembled into a cross correlation map (see 3). This in turn can be split into a map for direct neuronal interaction and a map for shared input. In both cases we can define the frequency and intensity areas over which that class of interaction between two neurons is distributed. We describe the structure of these "interaction areas" and report the relationships these have to frequency response areas seen in single units.

(1) Bloom, M. and Gerstein, G., Neurosci. Abs. p.245, 1984.
(2) Espinosa, I. and Gerstein, G., Neurosci. Abs. p.245, 1984.
(3) Voigt, H. and Young, E., Exp. Brain Res. 60:594-598, 1985.

Supported by NIH NS07719, and Systems Development Foundation 0013.

344.17 STIMULUS LINKED TEMPORAL CHANGES IN INTERACTIONS OF NEURONS IN RAT DORSAL COCHLEAR NUCLEUS. P.M. Gochin, J.A. Kaltenbach and G.L. Gerstein, Dept. of Physiology, Univ. of Penna., Phila. PA 19104

It has long been established (Gerstein, 1970) that direct synaptic interactions can be detected in the firing of simultaneously recorded neurons in cat dorsal cochlear nucleus. More recent studies have described interactions of such neuron pairs (Voigt & Young, 1980) which suggest participation of type II/III neurons in the formation of the receptive field properties of type IV neurons. Furthermore, it has been observed that patterns of connectivity may be stimulus dependent (Voigt & Young, 1985). No investigations have been conducted, however, which attempt to relate the PST histogram patterns established by Pfeiffer (1966) to neural interactions.

The present series of experiments investigates the details of interactions of neuron pairs in the rat dorsal cochlear nucleus with respect to PST structure. In addition, the effects of binaural stimulation and stimulus frequency on interaction profiles are examined. The cross correlation histogram, commonly used in evaluation of neuron interactions, is inadequate to study dynamically changing modes of interaction within a short time span, such as the 50 msec pure tones used in this study. Joint peri-stimulus scatter diagrams (Gerstein & Perkel, 1969; Abeles 1982), however, provide a graphic means for detailed examination of the time course of relationships between two and three neurons.

Preliminary results show that the mode of interactions within neuron pairs recorded on the same electrode can differ in accord with differences in PST patterns. Shift controls confirm that the observed correlations are direct interactions, and not simply due to changes in firing rate. In some instances, stimulus properties influenced the modes of interactions, whereas in other cases the interaction patterns were stimulus invariant. Our results suggest that neuronal interactions within the dorsal cochlear nucleus could play a role in the commonly observed PST patterns, and may explain some of the variations in these patterns that are observed when stimulus conditions are altered.

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344.18 EXCITATORY INTERCONNECTIONS IN THE MOUSE COCHLEAR NUCLEI. Robert E. Wickesberg*, Judith A. Hirsch and Donata Oertel. Dept. of Neurophysiology, University of Wisconsin - Madison, Madison, Wisconsin 53706.

The cochlear nuclear complex, where the VIIIth nerve synapses, is a complicated, interconnected structure. Lorente de Nó described a two-way connection between the dorsal cochlear nucleus (DCN) and the anteroventral cochlear nucleus (AVCN) (Laryngoscope, 48: 327-350, 1933). An inhibitory influence that the AVCN has on the DCN was demonstrated by Evans and Nelson (Exp. Brain Res., 17: 428-442, 1973). We report here that the DCN provides an excitatory input to the AVCN.

The neuronal circuitry of the cochlear nuclear complex was studied by recording intracellularly from neurons in brain slices of mouse cochlear nuclei. The recordings of the synaptic responses to electrical stimulation of the auditory nerve were made from cells in the AVCN in slices containing portions of the DCN. The tissue slices were made with a single, parasagittal cut 200 μm to 300 μm below the pial surface.

The excitatory input from the DCN to the AVCN appeared as long-latency EPSP's in response to stimulation of the auditory nerve stump. These late EPSP's were not seen in preparations lacking the DCN. If the stimulating electrodes were placed directly on the DCN, the EPSP's could be elicited without activating the VIIIth nerve inputs to the AVCN neuron.

The addition of 1 mM glutamate to the bathing saline produced trains of EPSP's in cells in the AVCN. Neurons in the AVCN were not themselves excited by bath-applied glutamate, they showed no change in input impedance, and the glutamate-induced EPSP's were not altered by current pulses delivered through the recording electrode. Since the glutamate-induced EPSP's were not present when the DCN was missing, these findings suggest that glutamate activates DCN neurons, which in turn excite AVCN neurons.

The application of 1 mM kynurenic acid blocked the long-latency EPSP's, but not short-latency EPSP's, after electrical stimulation of the auditory nerve. Kynurenic acid also antagonized the EPSP's evoked by stimulating the DCN directly or by applying 1 mM glutamate.

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AUDITORY SYSTEM: HIGHER CENTERS

345.1 CO-LOCALIZATION OF ^{3}H GABA AND GABA-LIKE IMMUNOREACTIVITY IN SUPERIOR OLIVARY NEURONS RETROGRADELY LABELED FROM GUINEA PIG COCHLEAR NUCLEUS. R.L.Saint Marie, E-M. Ostapoff, and D.K. Morest. Dept. of Anatomy, Univ. Conn. Health Center, Farmington, CT 06032

It has been proposed that GABAergic neurons in the superior olivary complex (SOC), projecting to the cochlear nucleus (CN), can take up ^{3}H-GABA and transport label retrogradely from injections in the CN (Ostapoff et al., SN Abstr. 11: 1051, '85). The present study is to test this proposal and to compare two methods for localizing GABAergic neurons: immunocytochemistry and high affinity uptake with transport. Following injections of ^{3}H-GABA in CN, individual semi-thin plastic (Epon) sections, or adjacent pairs, of the SOC were prepared for autoradiography and immunostained with antisera to a GABA-glutaraldehyde-BSA conjugate (Altschuler, et al., SN Abstr, 11: 1048,'85). Preliminary findings indicate that two-thirds of the retrogradely radiolabeled axons and periolivary neurons were also GABA immunoreactive.

The autoradiographic results duplicated the previous ones. Radiolabeled cells were present in all of the periolivary nuclei, with some nuclei more heavily labeled than others. No neurons were labeled in the medial (MSO) and lateral (LSO) superior olivary nuclei or medial nucleus of the trapezoid body (MNTB).

The postembedding technique of GABA-like immunostaining yielded light-to-very-dark staining of cell bodies and dendrites. Axons and terminals always stained darkly. Many fusiform cells in LSO exhibited moderate-to-dark immunostaining, as did small cells in the hilus and a few large cells in MSO. Many cells in the ventral trapezoid (VNTB) and dorsal periolivary nuclei exhibited a range of immunoreactivities, as did fewer cells in other periolivary nuclei. A very few large neurons in MNTB and dorsomedial periolivary nucleus were lightly labeled. A few small cells in the periphery of these two nuclei were moderately stained. Immunostained terminals were most abundant in VNTB, rare in LSO, and intermediate in number in the other olivary nuclei.

Two-thirds of the radiolabeled cells (those projecting to the cochlear nucleus) were also immunostained. Double labeled cells appeared in each periolivary nucleus that had radiolabeled cells. Many double labeled axons projected through the ventral acoustic stria. One-third of the radiolabeled cells bodies and axons were considered to be immunonegative. Some of these were heavily labeled, indicating that the radiolabel was not immunodetectable in these cells. Either some non-GABAergic neurons are accumulating and rapidly transporting radiolabel from the CN, or some GABAergic periolivary neurons do not have immunodetectable levels of GABA in their soma and axons. Nevertheless, the finding of double labeled neurons strengthens the hypothesis that these neurons form GABAergic projections to the CN.

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345.2 EXCITANT AMINO ACID RECEPTOR TYPES IN THE LATERAL SUPERIOR OLIVARY NUCLEUS. D.M. Caspary and C.L. Faingold. Dept. of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708.

Principal cells of the lateral superior olivary (LSO) nucleus receive a direct excitatory input from the ipsilateral ventral cochlear nucleus (VCN) and an inhibitory input from the contralateral VCN via a secure synapse in the medial nucleus of the trapezoid body. These LSO neurons are thought to code for the localization of sound in space, are excited by ipsilateral acoustic stimuli, and inhibited by binaural stimuli (IE). Ipsilateral acoustic stimulation at characteristic frequency (CF) results in a phasic "chopper" response which is suppressed by like binaural stimulation at CF. These neurons are also sensitive to interaural time and intensity differences. The inhibitory neurotransmitter mediating binaural inhibition is thought to be glycine (Moore and Caspary, J. Neurosci., 3:237-242, 1983) while the neurotransmitter mediating the ipsilateral excitatory response remains to be identified. LSO principal cells in 24 anesthetized chinchillas were identified by characterizing response properties, horseradish peroxidase (HRP) injection and track reconstruction. Iontophoretic application of excitant amino acids and their receptor antagonists were used in attempts to block and mimic ipsilateral tone-evoked activity in 34 neurons displaying "on-chopper" response patterns and binaural inhibition. Effects of N-methyl-D-aspartate (NMDA) and its receptor antagonist DL-2-amino-5-phosphonovalerate (APV) were compared to the effects of quisqualate and cis-2,3,piperidine-dicarboxylate (PDA). PDA reportedly acts as an antagonist at quisqualate receptors as well as NMDA and kainate receptors. Iontophoretic application of PDA reduced ipsilateral tone-evoked activity (a reversible 20% or greater effect) in 90% (9/10) of LSO neurons examined compared to 50% (9/18) with APV. Eighty-five percent (16/19) of LSO neurons examined were sensitive to iontophoretic application of quisqualate which shifted rate-intensity functions to the left, while only 44% (8/18) of the LSO neurons examined were sensitive to NMDA. The effectiveness of PDA relative to APV suggests that if an excitatory amino acid receptor mediates the ipsilateral excitatory response of LSO IE neurons it is unlikely to be of the NMDA receptor type but rather of the quisqualate or kainate receptor type. (Supported by NIH grant NS 15640, and a gift from the Pearson Family Foundation.)

345.3 ASSYMETRY IN THE BILATERAL PROJECTIONS OF THE LATERAL SUPERIOR OLIVARY NUCLEI TO THE INFERIOR COLLICULUS IN THE CAT. Craig K. Henkel and Ami Shneiderman. Department of Anatomy, Bowman Gray School of Medicine, NC 27103.

Cells of the lateral superior olivary nuclei (LSO) are excited by ipsilateral sounds and are sensitive to interaural intensity differences which represent the ipsilateral hemifield; however, the axonal projections of LSO end bilaterally in the inferior colliculus. There is very little information about how these two representations of activity in LSO are processed that would account for response properties of cells in the inferior colliculus. Thus, we examined the distribution of LSO projections in the cat using anterograde tracing methods to compare the organization of the crossed and uncrossed representation of LSO in the inferior colliculus. Either wheat germ agglutinin conjugated with horseradish peroxidase or tritiated leucine was injected in LSO and the distribution of the projections was compared by carefully matching the inferior colliculus on both sides. The results showed that although both the ipsilateral and contralateral projection shared a common topographic arrangement there were distinct differences in the pattern of distribution within the central nucleus. First, the area of terminal fields on each side was measured and expressed as a ratio of contralateral area relative to the ipsilateral area. Interestingly, a trend was found in which the ratio in cases with injections in the low frequency lateral limb of LSO was 0.4 to 1.0 and increased to more than 2.0 with more medial injections. Second, regardless of the tracer being used, the uncrossed projection exhibited a distinct banding pattern in the central nucleus comprised of dense sheets of labeling flanked by unlabeled or very lightly labeled bands. In the frontal plane these dense bands extended from ventrolateral to dorsomedial approximately 1.3-1.6 mm and were 150-200 μm thick. The crossed projection was more uniformly distributed although embedded in the projection area were denser bands of labeling resembling the more distinct ipsilateral pattern. When the ipsilateral and contralateral bands were compared by superimposing carefully matched sides it was found that they were not homotopic. Reconstruction of the patterns suggested that there were alternating ipsilateral and contralateral bands. This heterotopic pattern was confirmed in sections processed for both labels in a second group of experiments with LSO injections on both sides. These differences in the uncrossed and crossed projections of LSO in two important anatomical domains are most likely related to differences in the intrinsic connectivity of these projections related to their functional role in the inferior colliculus. Supported in part by NIH Grant NS 18627.

345.4 QUANTITATIVE EM STUDIES OF AXOSOMATIC SYNAPTIC TERMINALS IN THE RAT LATERAL SUPERIOR OLIVARY NUCLEUS. M.A. Casey. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

This study is part of an ongoing investigation into the effects of aging on the morphology of the rat auditory pathway. Young adult (3 months of age) male Fischer 344 albino rats obtained from the NIA contract-supported colony were perfused with mixed aldehydes, and brainstem blocks containing the lateral superior olivary nucleus (LSO) were prepared routinely for transmission electron microscopy. Photographic montages of principal cells were constructed at a final magnification of 15,000X for morphometric analysis of axosomatic terminals. Thus far, 25 neurons from 3 animals have been analyzed. The pooled data show that about 75% (mean = 76.4, SEM = 1.4, N = 25) of the perikaryal surface of principal cells is apposed to synaptic terminals, almost all (97.5%) of which contain pleomorphic synaptic vesicles and form slightly asymmetric membrane specializations. Of the 474 axosomatic terminals examined so far, 8 (1.7 %) contained large spherical vesicles, and 4 (0.8%) contained small, densely-packed spherical vesicles. While the terminals with large spherical synaptic vesicles rarely form axosomatic synapses, they frequently form axodendritic synapses in the LSO neuropil. Dendritic spines in the neuropil of the LSO are postsynaptic only to terminals with large spherical vesicles and asymmetric synapses. Terminals containing pleomorphic vesicles also synapse in the neuropil, but mainly terminate on large dendrites. These preliminary findings, along with recently published studies, suggest that synaptic relationships in the LSO are remarkably similar among Fischer 344 rats, Sprague-Dawley rats (White, Neurosci. Abstr. 9:765, 1983), and cats (Cant, JCN 227:63, 1984; Helfert and Schwartz, JCN 244:533, 1986). Questions now being addressed include: 1) Does the synaptology in nuclei of the superior olivary complex differ significantly between albino and pigmented rats (i.e., Creel et al., Brain Res. 260:1, 1983; Conlee et al., JCN 225:141, 1984), and 2) Do auditory structures in pigmented rats exhibit aging changes similar to those reported in albino rats (Casey and Feldman, Neurobiol. Aging 3:187, 1982; JCN 232:401, 1985).

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345.5 ULTRASTRUCTURAL CHARACTERIZATION OF OLIVOCOCHLEAR EFFERENT NEURONS IN THE GERBIL LATERAL SUPERIOR OLIVE LABELED RETROGRADELY BY UPTAKE OF ^{3}H-D-ASPARTIC ACID AND/OR HORSERADISH PEROXIDASE FROM THE COCHLEA. R.H. Helfert[1,2], I.R. Schwartz[2], and A.F. Ryan[3]*. [1]Dept. of Anatomy and [2]Div. of Head & Neck Surgery, UCLA Sch. Med., Los Angeles, CA 90024; and [3]Div. of Otolaryngology, VA Med. Ctr. and UCSD Sch. Med., La Jolla, CA 92093.

To study the fine structure of olivocochlear neurons residing in the lateral superior olive (LSO), Mongolian gerbil cochleas were perfused with artificial perilymph containing ^{3}H-D-aspartic acid (DASP) and/or horseradish peroxidase conjugated to wheat germ agglutinin (HRP). The transverse brainstem sections were developed for HRP using diaminobenzidine as the chromogen, enhanced with cobalt chloride; and were then prepared for light and electron microscopic autoradiography.

Two populations of olivocochlear neurons were identified within the neuropil of the LSO, based on their differential labeling patterns. One population of neurons contained both HRP and DASP, while the second population labeled with HRP only. From a sample of 31 perikaryal profiles representing 18 individual neurons that labeled with both tracers, all were small (≤18 μm) and fusiform, possessed nuclei with multiple deep nucleoplasmic infoldings, and contained a scant cytoplasm with few, if any, Nissl bodies. Less than 12% (N=31, $\bar{x}$=5.5%, s=3.9) of the surface of any given profile was apposed to axosomatic presynaptic terminals, all of which contained small, round vesicles with symmetrical synaptic specializations. Roughly one half of these terminals also contained one or more dense core vesicles. The dendrites of this neuronal class receive a small number of axodendritic synaptic terminals (usually 1 terminal per dendritic cross section). The vast majority of these terminals also contained an abundance of small round vesicles, with a few dense core vesicles. Very rarly were other types of terminals seen on the dendrites (i.e., those with large round, flat, or pleomorphic vesicles). These "double labeled" neurons can be classified as small neurons, using the criteria previously established for typing LSO neurons (Helfert & Schwartz, Neurosci. Absts., 1985).

Light microscopic data suggests that a few neurons in the LSO may be retrogradely labeled with HRP only. These cells have a distribution similar to that of class five neurons (op. cit.), which can only be identified with certainty at the electron microscopic level. Preliminary electron microscopic study of HRP and double labeled material has not yet been able to confirm the identity of the larger HRP labeled neurons, although further analysis is in progress.

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345.6 ASCENDING AND DESCENDING INPUT TO SUBDIVISIONS OF RAT INFERIOR COLLICULUS. J.R. Coleman, P. Cash*, T. Kobus* and W.J. Clerici. Departments of Psychology and Physiology, University of South Carolina, Columbia, South Carolina 29208.

The inferior colliculus (IC) is a major structure of the central auditory pathway which may be divided into a central nucleus, an external cortex and a dorsal cortex. The input to these regions of IC was the focus of this study. We used the horseradish peroxidase (HRP) method for retrograde and anterograde analysis in 16 Sprague-Dawley rats. Animals survived 18-30 hrs and coronal brain sections cut at 40 um were processed for HRP histochemistry. Labelled cells were examined microscopically and the position of these cells relative to cytoarchitectonic boundaries confirmed with a pantographic system.

Following microiontophoretic injections into IC numerous sites within the brainstem and neocortex were retrogradely labelled depending on the location of the injection. When the central nucleus was injected labelled cells were observed in several brainstem auditory groups. Particularly evident was bilateral labelling of the lateral superior olivary nucleus, and ipsilateral labelling of the medial superior olivary nucleus and superior periolivary nucleus. Labelled cells were observed bilaterally in the lateral nucleus of the trapezoid body, and ipsilateral ventral nucleus of the trapezoid body. No label was seen in the medial nucleus of the trapezoid body. Label in the dorsal and ventral cochlear nuclei was mostly on the side contralateral to the injection. Labelled cells were observed in all subdivisions of the contralateral IC. Little or no label was observed in neocortex following injections of central nucleus. When the injection was confined to the external cortex of IC only very sparse labelling was observed in the contralateral cochlear nuclei and none in the superior olivary complex. In this case labelled neurons of the ipsilateral, and to a lesser extent, contralateral nucleus of the IC were observed along with those in other collicular subdivisions. HRP reaction was found in the parabrachial nucleus and deep superior colliculus, as well as in areas 41 and 39 of auditory cortex. HRP injections of dorsal cortex of IC resulted in light labelling of neurons of the lateral superior olive (mainly ipsilateral) and in subdivisions of the olivary complex, and some label in the contralateral dorsal and ventral cochlear nuclei. Bilateral label was observed in subdivisions of IC and dorsal nucleus of lateral lemniscus. Numerous clusters of labelled cells in layer V of cortical areas 41, 20 and 36 appeared after IC dorsal cortex injection. Anterograde label from auditory cortex injections was heaviest in the caudal part of dorsal cortex of IC. Labelled cells of the contralateral dorsal column nuclei and trigeminal nucleus were observed only after external cortex injection. These results show differential inputs to subnuclei of the IC. (Supported by NS-20785)

345.7 DIFFERENTIAL PROJECTIONS TO MONAURAL AND BINAURAL REGIONS OF THE MUSTACHE BAT'S INFERIOR COLLICULUS. L.S. Ross* and G.D. Pollak, Dept. of Zoology, University of Texas, Austin, TX 78712.

The central nucleus of the inferior colliculus (ICc) in the mustache bat has a division devoted solely to processing the 60 kHz component of its echolocation call. This enlarged isofrequency region, the dorsoposterior division (DPD) is further characterized by a spatial segregation of monaural and binaural response types. This segregation consists of a dorsal monaural region and four binaural regions; two EI regions located laterally and ventromedially, and two EE regions located ventrolaterally and dorsomedially. The isofrequency DPD receives bilateral projections from the lateral superior olives (LSO) and the dorsal nuclei of the lateral lemniscus (DNLL), contralateral projections from the cochlear nucleus, and ipsilateral projections from the medial superior olive (MSO) and the ventral and the intermediate nuclei of the lateral lemniscus (VNLL & INLL).

In other mammals, each of these lower brainstem auditory nuclei possesses a characteristic tonotopic organization and aural properties which are either monaural or binaural. Cells which project to the DPD form segregated sheets of neurons in the lower monaural and binaural nuclei. These sheets of cells presumably represent the corresponding 60 kHz representation in the lower nuclei.

Here we report on the projections to the different aural regions within the DPD. The boundary of an aural region was determined physiologically, and a small (200 microns) deposit of HRP was made iontophoretically within a restricted portion of that aural region. Deposits in monaural regions of the DPD resulted in labeling of cells in the monaural nuclei of the brainstem, including the cochlear nucleus, the VNLL, and the INLL. However, there appears to be a differential projection pattern within this large monaural region. The rostral portion receives its input primarily from the VNLL and the INLL, while the caudal portion receives projections primarily from the dorsal cochlear nucleus and the VNLL. We have also begun to trace the projections to the ventromedial EI region. The projections to this region come primarily from the DNLL bilaterally, with minor projections ascending from the ipsilateral INLL and the contralateral LSO. A noteable feature of this projection pattern was the absence of afferents from the MSO. These results demonstrate that monaural regions within an isofrequency region can be further characterized by the subset of nuclei that provide the dominant input. Our preliminary data suggest that this is also true for the binaural regions.

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345.8 BINAURAL PHYSIOLOGY OF ISOFREQUENCY EI NEURONS IN MUSTACHE BAT'S INFERIOR COLLICULUS. J. Wenstrup, Z. Fuzessery, and G. Pollak. Department of Zoology, University of Texas, Austin, Texas 78712.

Single unit responses to dichotic stimuli were obtained from the 60 kHz region of the mustache bat's inferior colliculus (IC). The immediate goal of the study was to examine the range of binaural responses among EI neurons in one isofrequency representation of IC. A broader goal was to understand variations in EI properties that may underly the encoding of spatial location.

Responses to interaural intensity disparities (IIDs), obtained from 125 EI units, were characterized by three features: 1) The strength of inhibition was expressed as the maximum percentage of inhibition elicited by ipsilateral sound. About 60% of EI units were suppressed by 90% or more. 2) The sharpness of inhibition was expressed as the IID range over which the response decreased from 80% to 20% of the maximum response. Nearly 70% of the units had "80-20% IID ranges" of 15 dB or less. 3) The cutoff IID was assessed as the IID at which the response was suppressed by 50%. There was a broad range of cutoff IIDs, from -17 dB (ipsilateral intensity less than contralateral intensity) to +30 dB (ipsi greater than contra). Since these features do not co-vary within our sample of EI units, the neural mechanisms determining these dimensions of IID sensitivity may differ.

The effect of changes in stimulus intensity upon unit responses were studied. Many 60 kHz EI units display sharply non-monotonic rate/intensity functions; they responded best at 10-20 dB above threshold, and over half were reduced to 50% below their maximum response at 30 dB above threshold. In 72 units, IID functions were obtained at more than one contralateral (excitatory) intensity. Most units (74%) displayed limited changes in the strength of inhibition as a function of intensity, so that the maximum strength of inhibition changed by less than 25% across the intensity range tested. Where a change was noted, most units (67%) displayed a reduction in the strength of inhibition at higher intensities. Regarding the IID cutoff, most units (62%) showed a limited shift in the IID cutoff value as a function of intensity. In units for which the IID cutoff did change as a function of intensity, it generally shifted to a more negative cutoff (i.e., cutoff at lower relative ipsilateral intensities).

These IID response features shape, in part, the responses of EI units to free-field sound. Variations among units in the strength of inhibition, the sharpness of cutoff, and the effects of stimulus intensity may add "noise" in the encoding of spatial location, whereas variations in IID cutoff may encode spatial location. The effect of these IID response features upon the response to free-field sounds can be modeled using a predictive method outlined previously (Fuzessery and Wenstrup, *Soc. Neurosci. Abstr.* 11:734, 1985). Supported by grants from NIH.

345.9 RESPONSES TO AMPLITUDE-MODULATED SOUNDS IN THE INFERIOR COLLICULUS OF THE MUSTACHED BAT. H.D. Lesser, R.D. Frisina, and W.E. O'Neill. Center for Brain Research, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Rapid changes in sound amplitude, amplitude modulation (AM), are important features of communication sounds, and may be especially significant to echolocating mammals in pursuit of flying prey. The mustached bat (Pteronotus parnellii) biosonar signal contains a long constant-frequency component, the echo of which can be amplitude-modulated by the beating wings of insects; behavioral studies have shown that these bats are especially attracted to fluttering insects. However, the encoding of amplitude-modulated sounds in the auditory system has not yet been investigated. The intent of the present study was to record the responses of inferior colliculus neurons to AM frequencies that might normally be encountered by a bat hunting flying insects.

We recorded from 92 single units with best frequencies (BF) in the 59-64 kHz (CF_2) range in 8 awake animals. Stimuli had BF carriers and consisted of both pure tones and sinusoidal AM (33-800 Hz, 79% modulation) at intensities from threshold to 90 dB above threshold. Synchronous responses to AM were analyzed by computing the first Fourier coefficient (r) from period histograms. For 73% of the units, plots of r vs AM frequency showed a lowpass characteristic. The other units had non-monotonic r vs AM functions. The predominance of maximal synchronous responses at low AM frequencies agrees with previous findings in rat (Rees,A., Møller,A.R., Hearing Res., 10:301, 1983). Analyses of average firing rate showed a wider variety of response patterns. The majority of units (92%) showed changes in average rate in response to AM; most units (71%) showed enhanced firing rates, whereas 29% were suppressed. Plots of average rate vs AM frequency for facilitated units showed several unit types. Some had a best amplitude for AM-responsiveness (BAM) near threshold, others had BAMs at moderate intensities, and several had high intensity BAMs. Individual units were tuned to AM frequencies throughout the AM frequency range tested, a finding consistent with previous results in the cat (Schreiner,C., Langner,G., Soc. Neurosci. Abstr., 10:395, 1984). For example, some units showed vigorous responses to low-frequency AM and were suppressed at high frequencies; others responded conversely. Most units had on, off, on-off or chopper post-stimulus-time histogram response patterns. All choppers showed AM responsiveness whereas some of the on, off and on-off units were not AM responsive.

We are presently exploring which features of the AM stimuli are critical to the production of the responses we observed.
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345.10 STRUCTURAL COMPONENTS OF BINAURAL INFORMATION PROCESSING IN THE AUDITORY MIDBRAIN. PHYSIOLOGY AND ANATOMY OF HRP-INJECTED CELLS IN THE CAT AND GERBIL. D.L. Oliver, S. Kuwada, R. Batra, T.R. Stanford, & C. Henkel. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

The inferior colliculus (IC) integrates binaural inputs from superior olivary and lemniscal sources as well as monaural inputs from the cochlear nucleus. These inputs converge in the central nucleus of the IC and continue to primary auditory cortex via a synapse in the medial geniculate body. This central pathway may carry a variety of binaural information. In contrast, pathways from the cortex of the IC and the tegmentum of the IC are directed at non-primary auditory cortical areas. The functional differences between these pathways are unclear. Are similar responses found in these distinctive midbrain regions? Are their responses related to either the afferents of the IC or to interconnections within the midbrain?

Intracellular recording and injection of HRP addresses directly the relationship of cell types in the IC's subdivisions to the information they process. The approach was to record the intracellular responses of neurons in the IC of barbiturate anesthetized animals to acoustic stimuli followed by iontophoresis of HRP into the impaled cell. In most cases, the responses to monaural and binaural tones were determined. Sensitivity to FM and AM signals and to interaural time (phase) and intensity differences was assessed whenever possible. Histological preparation included fixation in mixed aldehydes, Vibratome sectioning, and an intensified DAB reaction.

Twenty-six units from 13 experiments were recovered for anatomical study. Numerous additional units were studied but not injected. In the central nucleus, both disc-shaped and stellate neurons were driven by a wide variety of binaural or monaural pure-tone stimuli. Injected cells included a small stellate, possible Golgi II cell, with an elaborate axonal arbor near its dendritic field. Axons from larger stellates sometimes extended over much wider areas quite distant from the dendrites. In the cortex, where most neurons are stellate cells, units also responded well to pure tone stimuli; however, some units responded to FM sweeps in preference to pure tones. Most well-driven injected stellate cells were deep in the dorsal cortex where they may receive direct inputs from cochlear nucleus afferents. In the tegmentum of the IC, units responded to FM stimuli; not all responded to pure tones. Axons of injected cells with dendrites in the IC had direct projections to the intercollicular tegmentum and cuneiform nucleus. HRP-filling of IC cells and axons may show how auditory information is distributed from the central nucleus to the cortical and tegmental pathways.

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345.11 AGING AND BASAL METABOLISM OF GLUCOSE IN THE CENTRAL AUDITORY SYSTEM OF RATS AND MICE. K.P. Hunter*, J.F. Willott, W.J. Clerici, and J.R. Coleman (SPON.: C.L. Lints). Dept. of Psychol., Northern Ill. Univ., DeKalb, IL 60115 and Depts. of Psychol. and Physiol., Univ. South Carolina, Columbia, SC 29208.

Basal rates of glucose metabolism were evaluated in aging mice and rats using 2-deoxy-D-glucose (2DG) autoradiography, and analyzed with optical densitometry. Male Sprague-Dawley rats in 2 age groups--"young" adults (8-16 mo.) and "aged" adults (27-36 mo.) were injected (i.v.) with tritiated 2DG, placed in quiet for 45 min., and sacrificed. X-ray film autoradiographs were prepared from serial frontal sections (20um), and optical density was converted to tissue concentrations of tritium. In comparing dorsolateral, middle, and ventromedial regions of the inferior colliculus (IC), 2DG incorporation was greatest in the ventromedial regions of young rats. No significant differences in 2DG levels were found between young and old groups. However, there was a trend toward increased utilization of glucose in aged rats, especially in the dorsolateral and middle IC.

"Young" (1-2 mo.), "middle-aged" (1-year) and "aged" (2-years) mice of the C57BL/6J and CBA/J strains were used. Adult C57 mice undergo progressive sensorineural hearing loss with profound impairment by the second year of life; CBA mice maintain good hearing until very late in life. Mice were injected (i.p.) with 14C-2DG, placed in quiet for 45 min., and sacrificed. Autoradiographs were prepared from 30um frontal sections. 2DG densities of the anterior ventral cochlear nucleus (AVCN) and IC were determined relative to densities of the trigeminal nerve. In all subject groups there was relatively more 2DG incorporation in the IC than in the AVCN. No significant differences in 2DG labeling were observed as a function of age or strain. However, in the CBA strain there was a trend toward increased labeling in both the AVCN and IC in the oldest age group.

In summary, aging, with or without presbycusis, is not associated with a decrease in basal levels of glucose uptake by central auditory neurons; in fact, there is a suggestion of slightly enhanced activity.

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345.12 NEURONS OF THE RAT MEDIAL GENICULATE BODY: GOLGI OBSERVATIONS. W. J. Clerici, J. R. Coleman, A. J. McDonald, M. Miller* and R. Thompson*. Departments of Psychology, Physiology and Anatomy, University of South Carolina, Columbia, South Carolina 29208.

Three major divisions of the medial geniculate body (MGB) can be identified in the cat and tree shrew based on observations of morphology and density of perikarya and neuropil in silver impregnated material. The present work confirms the existence of characteristic neuronal features in ventral (MGv), dorsal (MGd) and medial (MGm) divisions of the rat MGB prepared with the rapid Golgi method. Adult Sprague-Dawley rat brains were perfused with 4% paraformaldehyde and blocked into 3 mm slabs which were postfixed in osmium tetroxide, placed in .75% silver nitrate solution and embedded in paraffin. Frontal and horizontal sections of 150 um thickness were inspected for silver stained neurons; every third section (50 um) was stained with thionin for cytoarchitectural examination. Separate brains were prepared with hematoxylin for study of myeloarchitecture.

MGv occupies the ventrolateral region throughout most of the MGB and is composed of densely packed medium and small sized, relatively darkly staining somata in Nissl preparations. In Golgi stained material principal neurons of MGv have characteristic small rounded somata from which tufts of dendrites forming acute angles to one another emerge before branching. Small stellate neurons are also found in MGv. MGd is situated superior to MGv and occupies the caudal pole of MGB where fibers of the brachium of the inferior colliculus are also observed. Hematoxylin and Golgi stains reveal a dense band of fibers which separates MGd from MGv. Both round and fusiform cell bodies of loosely packed neurons are found in MGd; the most prominent neurons have medium to large sized somata with an extensive radiate dendritic pattern that occupies a spheroidal field. Primary dendrites of these neurons leave the perikarya in various directions and branch evenly and at wide angles. MGm is located medial to MGv and composed of medium and large sized multipolar neurons of low packing density. Principle neurons with both tufted and radiate dendritic patterns are observed in MGm. Our observations suggest that the auditory thalamus of rat shows features typical of other mammalian species. (Supported by NIH grant NS-20785.)

345.13 ORGANIZATION OF BINAURAL INTERACTIONS IN THE HIGH FREQUENCY REPRESENTATION OF THE VENTRAL NUCLEUS OF THE CAT'S MEDIAL GENICULATE BODY. T.J. Imig and A. Morel*. Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

We have studied the organization of binaural interactions exhibited by single units and clusters of units in the high frequency representation (best frequencies > 5kHz) of the ventral nucleus of barbiturate anesthetized cats. Tone pips were delivered to each ear using a closed system, and sound pressure levels at each were independently controlled. Unit responses were recorded using glass insulated tungsten microelectrodes which passed vertically through the ventral nucleus. At each recording site in the ventral nucleus, units' best frequencies were determined first. Next, best frequency tone pips were delivered separately to each ear, and finally to both ears simultaneously. Interaural intensity disparities as great as 30 dB were introduced in order to study binaural interactions. A great majority of neurons exhibited one of two types of responses: 1) EE responses in which stimulation of both ears together produced a greater response than stimulation of either ear alone, or 2) EI responses in which stimulation of the contralateral ear was excitatory and simultaneous stimulation of the ipsilateral ear reduced or inhibited the response. An electrode passing through the ventral nucleus typically encountered sequences of neurons exhibiting similar binaural interactions, and sequences extended for distances ranging from several hundred micrometers to over one millimeter. Our most complete maps exhibit consistent patterns of binaural interactions in the ventral nucleus. The vast majority of neurons in the dorsorostral quadrant of the high frequency representation exhibit EE responses, although EI neurons are found along the rostral pole of the nucleus. In the ventral half of the high frequency representation, the vast majority of neurons exhibit EI responses. In the dorsocaudal quadrant, EE and EI neurons occupy segregated regions. These physiological maps of EE and EI neurons are largely consistent with patterns of retrograde thalamic labeling resulting from HRP injections into EE and EI bands in AI (Middlebrooks, J.C. and J.M. Zook, Neurosci. 3:203-224, 1983). (Supported by NINCDS NS 17220)

345.14 THE AUDITORY THALAMUS OF THE MUSTACHED BAT: CONVERGENT INPUT AND COINCIDENCE OF EXCITATION FROM THE ORIENTATION SOUND AND ECHO. J.F. Olsen* and N. Suga. Dept.Biol., Washington U., St. Louis, MO 63130

The orientation sound (pulse) of the mustached bat consists of four harmonics; each harmonic consists of a constant-frequency component followed by a frequency-modulated component (FM). Many neurons in the medical geniculate body (MGB) are facilitated by combinations of FM components in pairs of synthetic orientation sounds (pulses) and echoes (Olsen, J. F. and N. Suga, Soc. Neurosci. Abstr. 9:768, 1983.). These "FM-FM" neurons are tuned to the combination of the FM component of the fundamental of the pulse with the FM component of the second, third, or fourth harmonic of the echo. Thus, each FM-FM neuron is tuned to two different frequency bands, approximately harmonically related. Echo delay, the cue used by bats to determine target range, is an important parameter to facilitate FM-FM neurons. The best delay (BD), which varies among FM-FM neurons, is the echo delay that produces the largest, facilitated response. In this report, evidence is presented for a scheme in which combination-sensitivity in the frequency and time domains is produced by a convergent projection to the MGB. The responses of thalamic FM-FM neurons to synthetic pulses and echoes were recorded extracellularly. We found 56 FM-FM neurons that responded, although poorly, to single FM components; these same FM components, when presented together at the best delay (BD), maximally facilitated the neuron's response. The BD of these neurons is correlated strongly with the latency of the response to thepulse FM component ($r = 0.88$), but only weakly with that of the response to the echo FM component ($r = 0.14$). The BD is correlated best with the difference between pulse and echo response latencies (slope = 0.91, $r = 0.96$; $p < 0.001$). Thalamic FM-FM neurons were found only in the dorsal and medial divisions of the MGB. Iontophoretic injections of wheat germ agglutinin-conjugated HRP (WGA-HRP) placed within these regions (5 hemispheres) produced anterograde label within layer IV of auditory cortex. Based on cytoarchitecture and physiological maps of cortex in the injected hemisphere, anterograde label appeared to be confined to cortical areas containing FM-FM neurons. Retrogradely-labeled cells were found within the central nucleus of the IC (ICC), where they formed at least two distinct bands. These data support the hypothesis that the harmonic sensitivity of thalamic FM-FM neurons is formed by a convergence of afferents from the ICC, a structure known to be tonotopically organized (Zook, J. M. et al., J. Comp. Neurol. 231:530, 1985), but lacking combination-sensitive neurons (O'Neill, W. E., J. Comp. Physiol. 157:797, 1985). The near-perfect correlation between delay-dependent facilitation and the coincidence of pulse- and echo-evoked excitation appears to result from a delay intrinsic to the part of the ascending auditory pathway in which the fundamental of the orientation sound is represented. (Supported by PHS R01-NS17333).

345.15 ANATOMICAL ORGANIZATION OF THE MEDIAL GENICULATE BODY OF THE MUSTACHED BAT.

M.L. Zettel*, R.D. Frisina and W.E. O'Neill. Center for Brain Res., Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Neurophysiological studies of the mustached bat (Pteronotus parnellii) auditory cortex have revealed that neurons are specialized for encoding specific features of echolocation signals. Virtually all the information passing between the brainstem and auditory cortex is processed in the medial geniculate body (MGB). Despite the importance of this thalamic nucleus for auditory signal processing, its anatomical organization in the mustached bat has not been the subject of any published reports. To remedy this, we have investigated the structure of the MGB in 7 animals (14 MGBs) using Golgi-Cox, Nissl, Weil and Kluver-Barrera stains.

The location, major divisions and neuronal types of the mustached bat MGB are similar to those of cat and man as described by Winer (Advances in Anatomy, Embryology & Cell Biology, 86, 1986; Hearing Res., 15:225, 1984). Despite basic similarities, some organizational differences exist. 1) In mustached bats the inferior colliculus (IC) lies more dorsal than in other mammals and therefore the brachium of the IC (BIC) enters the MGB from a dorsocaudal direction. Thus, the interstitial nucleus of the BIC lies dorsocaudal to the MGB. 2) In coronal sections the prominently laminated ventral division extends more dorsally and occupies a relatively larger area than in other mammals. In horizontal and sagittal sections, anteriorly the ventral division laminae appear continuous with laminae in the auditory radiation (AR) although the cell density is decreased in AR. 3) Along the entire anterior-posterior extent of the medial aspect of the medial division, a distinctive region exists containing primarily oval-shaped neurons with soma sizes of approximately 15 X 35 μm. These neurons have tufts of dendrites extending from the long axis of the soma that course up to 250 μm dorsomedially or ventrolaterally in coronal sections, and anteromedially or posterolaterally in horizontal sections. The stratified orientation of the somata and dendrites gives a highly laminated appearance to this region, especially in Golgi material. These neurons resemble the wide-field neurons found in the cat medial division, although there they are not segregated from other neurons and have a different orientation. In this "wide-field" nucleus of the mustached bat medial division, a few large stellate cells with truncated dendritic fields and Golgi type II cells are also found. Although it is not yet clear what the functional significance is of these differences in the mustached bat MGB, they are probably related to the decoding of certain aspects of the echolocation signals upon which this species relies so heavily for its survival.

[Supported by NSF and NIH]

345.16 NEURAL STRUCTURES MEDIATING ACOUSTIC AND TACTILE STARTLE REFLEXES AND THE ACOUSTICALLY-ELICITED PINNA RESPONSE IN RATS : ELECTROLYTIC AND IBOTENIC ACID STUDIES. J.V.Cassella and M.Davis. Dept. of Psychiat., Yale Univ. Sch. of Med., New Haven, CT, 06508.

The mammalian startle reflex, characterized by a distinctive pattern of whole-body muscular responses, is elicited by a sudden, intense auditory or tactile stimulus. A notable component of the startle reflex in rats is a flexion of the ears, the pinna response. The amplitude of startle and the pinna response can be modified by various behavioral and pharmacological manipulations. In order to study the cellular mechanisms underlying these behavioral changes it is necessary to know the neural pathways mediating these responses. Electrolytic lesion and stimulation techniques suggested that a primary acoustic startle circuit in the rat consisted of the auditory nerve, ventral cochlear n.(VCN), n. ventral lateral lemniscus (VLL), n. reticularis pontis caudalis (RPC), and spinal neurons (Davis et al., J.Neurosci., 1982,3,791-805). However, with these techniques it was not possible to differentiate between fibers of passage and cell bodies at each of these nuclei. The present study employed the selective cell body neurotoxin, ibotenic acid, to reexamine the pathway proposed by Davis et al. In addition, the involvement of the VLL and RPC in tactile startle and the acoustically-elicited pinna response was examined using electrolytic, and subsequently, ibotenic acid lesions.

Acoustic and tactile startle responses were measured in rats 1 day before and 3 and 10 days following surgery. Thirty white noise bursts or 30 air puff stimuli were presented at a 20-sec interstimulus interval on each test session. Startle was measured by an accelerometer attached to a specially designed cage. Pinna responses were elicited acoustically and recorded on videotape while the animal was held in a restraining cage. A frame by frame analysis of the videotape during the presentation of each stimulus was conducted and the response for each pinna was scored as 0 or 1. Electrolytic lesions were made by passing 0.1mA anodal DC current for 30 or 60 sec. Ibotenic acid was made in phosphate buffer (pH=7.4) at a concentration of 10 μg/1 μl. Lesions were produced by bilaterally infusing 0.5 μl of this solution over a 12-min period.

Electrolytic as well as ibotenic acid lesions of the VCN, VLL, or RPC eliminated acoustic startle responses. Electrolytic or ibotenic acid lesions of the VLL or RPC also eliminated tactile startle. Electrolytic lesions of the VCN or VLL but not the RPC eliminated the acoustic pinna response. These results support earlier suggestions of a primary acoustic startle pathway. Further studies will employ ibotenic acid to determine the complete stimulus-response pathways for each of these behaviors.

345.17 THE CONTRIBUTION OF THE LATERAL LEMNISCUS TO THE DESCENDING AUDITORY PATHWAY John M. Zook Dept. of Zoological & Biomedical Science and the College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701.

Three cell groups are found within the ascending auditory fibers of the lateral lemniscus in the mammalian brainstem. While these cells mainly project to the inferior colliculus, there is a small but significant descending projection which has not been adequately described in any mammal. In the mustache bat, Pteronotus parnellii, the nuclei of the lateral lemniscus are unusually well defined and conveniently located for electrophysiological and anatomical study.

Single and double barrel glass micropipettes, filled with either a tracing substance (PHA-L tritiated leucine or horseradish peroxidase) or a metal stimulating electrode, were introduced into the nuclei of the lateral lemniscus. With these multipurpose electrodes unit responses to sound stimuli could be characterized. In some cases electrical current was passed at a specific lemniscal locus while recording from units in the cochlear nuclei. It was thus possible to test whether lemniscal stimulation could alter the response of cochlear nuclei units to sound stimuli. In all cases the recording and/or stimulation sites were marked with one or more anatomical tracing substances.

Of the three lemniscal nuclei, the intermediate nucleus makes the greatest contribution to the descending auditory pathway, both physiologically and anatomically. A consistent effect of stimulation was an inhibition of the normal response to sound for a small number of units within the anteroventral cochlear nucleus (AVCN) ipsilateral to the stimulation site. The AVCN does not project to the intermediate nucleus on the same side of the brain. However, anterograde transport from deposits at stimulation sites reveal a small, but consistent and topographically organized descending projection to the ipsilateral AVCN. A descending projection to the core of the dorsal cochlear nucleus is also seen in these cases. Following small deposits of HRP in the intermediate nucleus of the lateral lemniscus, labeled cells are always found at sites in the contralateral AVCN that are the mirror image of the target sites of descending projections within the ipsilateral AVCN. Descending projections were also seen from both the intermediate and ventral nuclei of the lateral lemniscus to periolivary cell groups of the superior olivary complex. Discrete descending projections were also observed to ipsilateral medical and lateral superior olive and contralateral lateral superior olive. Further experiments will attempt to link these anatomical findings with unit response properties.

Supported by NIH Grant NS20986, OURC Grant 756 and the Ohio University College of Osteopathic Medicine.

346.1 GAD IMMUNOCYTOCHEMISTRY IN THE CEREBRAL CORTEX OF THE MUSTACHE BAT, PTERONOTUS P. PARNELLII. D.C. Fitzpatrick* and O.W. Henson, Jr.* (SPON: C. PRICE). Dept. of Anatomy, Univ. of North Carolina, Chapel Hill, NC 27514.

The physiology of the auditory cortex of the mustache bat has been described by Suga and his colleagues (J. Neurophysiol. 47:225-255, 1982; 49:1573-1626, 1983). However, little is known concerning the cellular arrangement of the cortex of this species. The purpose of this study is to describe the pattern of glutamic acid decarboxylase (GAD) labelling in the cortex. GAD is a good marker for GABA-ergic neurons and terminals which provide the bulk of intracortical inhibition in mammals. Thus, the pattern of GAD labelling can provide information regarding basic processes occurring in the cortex.

Animals were perfused with either a paraformaldehyde (4%)-Zn salicylate (0.5%) or a sodium periodate (.1M)-lysine (.1M)-paraformaldehyde (2%) mixture. The cortex was sectioned at 25µm using a vibratome and the sections were incubated with an antibody against GAD (Oertel, W.H., et al., Neurosci. 6:2715-2735). Cortical sections were then lightly counterstained.

For the purposes of this evaluation the cytoarchitectural layers of the mustache bat cortex are defined in terms of four zones: a) zone 1 is equivalent to layer 1; b) zone 2 corresponds to a distinct, superficial band of layer 2 cells; c) zone 3 combines the remainder of layer 2, layer 3, and layer 4 (the latter is small in this animal, even in primary sensory areas); and d) zone 4 corresponds to layers 5 and 6; it can be distinguished from zone 3 because cell density is reduced.

GAD labelling of cell bodies and axonal terminals was found in all zones but the pattern of labelling varied among zones. Zone 1 showed considerable labelling of terminals in its superficial half with fewer labelled terminals in its deeper portion. A large proportion of cell bodies in zone 1 were labelled; these appeared to be relatively small cells. Zone 2 contained very few labelled cells but many cells were outlined by labelled terminals; this feature was also characteristic of the remaining zones. Zone 3 had sparse labelling of mostly small cells in its upper half; the lower half was characterized by extensive labelling of both small and large cells. Zone 3 also showed a subtle band of increased terminal density which was visible under darkfield microscopy; this field corresponded to the area of greatest cellular labelling. By contrast, terminal labelling in zones 2 and 4 was homogeneous. Zone 4 showed labelling primarily in small cells.

These results indicate that the broad outlines of GAD labelling in the mustache bat cortex are similar to those seen in other mammals but that there are differences in details. Quantitative data, and a comparison of these results with other species will be presented. (Supported by USPHS Grants NS12445 and NS19031)

346.2 TONOTOPIC ORGANIZATION OF GERBIL AUDITORY CORTICES MAPPED WITH 2-DEOXYGLUCOSE. H. Scheich, P. Heil* and G. Langner. Zoological Institute, Technical University, 6100 Darmstadt, FRG

The organization of auditory cortex of awake gerbils (Meriones unguiculatus) was mapped with 14-C-deoxy-2-fluoro-D-glucose (2DG) 18µCi/animal using stimuli emitted from a loudspeaker such as narrow-band frequency and amplitude modulated tone bursts with varying intervals and tone bursts with alternating frequencies. Methods corresponded to those in Gonzales-Lima and Scheich, Brain Res. 344, 70-88 (1985). With these types of stimuli and not with continuous tones bands of strong 2DG labeling were obtained in the area of auditory cortex which shifted position as a function of tone frequency. This suggests that auditory cortex may take little interest in lasting auditory stimuli. Tonotopic bands in one dimension took a roughly dorsoventral course and in the other covered all layers of cortex. At least two regions of auditory cortex were identified showing parallel but mirror imaged tonotopic organization. In a posterior region the progression from low to high frequencies was from caudal to rostral and in an anterior region the converse was found. Thus the two regions appeared to fuse in the high frequency range. The similarity of this organization in cat (Knight, P.L., Brain Res. 130, 447-467 (1977)), and guinea pig (Hellweg et al., Exp. Brain Res. 29, 467-474 (1977)) led us to name the posterior region AI and the anterior region anterior auditory field (AAF). The spatial resolution for frequencies was higher in AI than in AAF and best between 500 Hz and 8 kHz. Isofrequency contours diverged in the ventral half of the two auditory fields, which may provide better resolution for complex parameters. The dorsoventral extent of AI as seen with 2DG was about 3 mm and that of AAF about 1.5 mm centered about the middle of AI. The largest rostrocaudal width of the two fields together was about 4 mm. In most cases stimulation with one frequency yielded, beside a band of strong labeling, several weaker band along other frequency contours, suggesting preferred interactions.

It is noteworthy that multiple bands of more ore less weak labeling were obtained in animals left without any stimulation. Such bands rarely covered the whole dorsoventral extent of an isofrequency contour and had a "discontinuous" appearence. Similar labeling with little relationship to stimulus frequency was also seen in animals hearing continuous tones. Roughly homogeneous spontaneous labeling across AI and AAF (no bands) was obtained only in some animals with ear plugs. This suggests that self-stimulation and possibly non-auditory excitation of cortex are contributing factors but clearly weaker in their effects than the described time varying signals.

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346.3 DYNAMIC CHANGES IN CORTICAL CONNECTIVITY DURING SOUND LOCALIZATION. M. J. Bloom and G. L. Gerstein. Depts of Anesthesia and Physiology Univ. of Penna. Phila., PA 19104

We previously reported (1) that fine localization of sound might be an emergent property of the correlated firing of at least two cells. Much effort has been applied to extending the conclusions beyond simple response planes and to find presentation formats for the results.

Ablation studies (2) show that Al is necessary for cats to localize sound, but no single cells with such selectivity have been found in Al.

Extracellular potentials were recorded from Auditory cortex of cats using a bundle of tungsten microelectrodes. Dichotic tone pips were delivered through matched earphones at 2 per second. Spike trains from up to 16 cells were analyzed using cross-correlations and the results were used to construct response planes and functional wiring diagrams. The diagrams show all interactions among all pairs separated for each stimulus.

Previous results showed cross-correlation had sharper tuning for position than individual cell PSTs. Now, further analysis of all pair interactions in an assembly show that the patterns of whole-assembly "wiring" differ with stimulus location, but we do not have a parametric measure of the "wiring" to assess whether spatial tuning occurs. Further, the functional wiring of the assembly does not change in the same way for positions simulated by Interaural Amplitude Differences, Interaural Time Differences, or both. Individual cells and cell pairs appear to serve multiple functions; the functional connectivities are different for different stimuli. Stimuli were presented either in long blocks of the same stimulus or in interleaved sequences of different stimuli. The changes of effective connectivity were observed in both situations, i.e. a time scale from minutes to less than one second.

These results suggest that a single neuron can participate in different sub-assemblies when different localization cues are presented. Presumably each such sub-assembly selectively activates different target cells.

(1) BLOOM, M. and GERSTEIN, G. Multineuron analysis shows spatial tuning not found in Single Unit Responses. Neurosci. Abstracts 10:245, 1984.
(2) JENKINS, W. and MERZENICH, M. Role of Cat Primary Auditory Cortex for Sound-Localization Behavior. J. Neurophys. 52:819, 1984.

Supported by NIH NS00859, NS05606, and Systems Development Foundation 00013.

346.4 SINGLE CELLS' TONE SEQUENCE SELECTIVITY IS POOR WHEN COMPARED WITH THAT OF CELL PAIRS FUNCTIONALLY CONNECTED. I.E. Espinosa and G.L. Gerstein, Univ. of Pennsylvania, Dept. of Physiology/G4, Phila., PA 19104

We have previously shown significant differences in the crosscorrelation of auditory cell pairs responding to three-tone sequences, suggesting that melody discrimination is possibly reflected in their functional connectivity (1). To complement these results we studied the single cells' role in melody selection. We compared areas under PST histograms for each one of the 19 different stimuli included in 6 different tone sequences. Data consisted of 26 single neuron and 6 multiple (unseparable spikes) neuron recordings [These had been recorded in 3 groups for our earlier cell pair study (1)]. For PST's calculated for 194 ms, 4 single cells and 4 multiple cells (25%) showed significant increase in response (selectivity) to a specific tone sequence.

Thus, single cells are capable of identifying and selecting a tone sequence. However, our previous results (1) suggest that the interaction between neurons enhances such selectivity. Namely, pair selectivity is higher (46%) and can be shown even before the 3rd tone in a sequence has ended. Single cell selectivity needs a longer time to show up. Also, a selective single cell does not determine the selectivity of a pair in which it participates: the selected melody may be different for the single cell and for the cell pair. There are also selective single cells which do not participate in the selective pairs in a group. Thus, single cell selectivity and cell pair selectivity seem to be distinct coding mechanisms. This is possibly due to different cell processes taking place both during and after the stimulus. As we have suggested (1), this mechanism might take part in a short-term memory process.

It is likely that higher order crosscorrelations would produce significant differences that could be interpreted as a faster selective mechanism. Probably the brain is using a fair amount of connectivity and redundancy to produce fast and reliable decision making algorithms, as Abeles has suggested in his synfire chain models (2).

(1) Soc. Neurosci. Abstr. 11: 249, 1985.
(2) Abeles,M., LOCAL CORTICAL CIRCUITS, Springer 1982.
(Supported by NIH NS05606, The Systems Development Foundation and CONACyT)

346.5 TERMINAL ARBORIZATION OF AFFERENTS TO AUDITORY CORTEX : A COMPARISON OF THALAMOCORTICAL AND CALLOSAL AXONS USING THE PHA-L METHOD. N.T. McMullen and E.M. Glaser. Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

We have studied the terminal arborizations of thalamocortical(TC) and callosal(CC) axons to the auditory cortex of the NZW rabbit using the anterograde tracer PHA-L (Gerfen and Sawchenko, 1984). Iontophoretic injections of PHA-L into the ipsilateral medial geniculate body (MGB) or supragranular layers of contralateral auditory cortex(AC) were made using glass micropipettes (7-15 um tip diameter) with intermittent positive current of 1-3 uA for 20-30 minutes. The anterogradely transported lectin was immunocytochemically visualized using the ABC method to yield a Golgi-like image of afferent axons. Large injections into the MGB or AC yielded dense terminal arborizations which permitted population analyses of cortical laminar termination. Smaller injections labeled a sparse population of afferent axons which were then followed in serial sections from the white matter to their cortical terminal zones. In some cases, single afferents were reconstructed from serial sections using our image combining computer microscope. Auditory cortical injections of PHA, in addition to labeling the callosal pathway, also labeled corticogeniculate and corticocollicular afferents. TC afferents remained unbranched until they penetrated the grey matter and terminated in layers VI, III/IV and I with the most dense terminal field in III/IV. Associated with the dense arborization in III/IV were sparse vertical sprays of axonal fascicles to II/III. Large injections into the MGB labeled cortical "patches" of vertically arborizing, bouton-studded terminal axons in III/IV that formed strips elongated in the anterior-posterior plane. Single MGB axons may course diagonally for long distances in the cortical grey before branching and terminating. Small injections of PHA-L into the supragranular AC labeled mirror-image foci in the contralateral cortex. Fascicles of callosal axons traversed the ventralmost portion of the white matter and terminated in the form of vertical axonal columns. Callosal axons occasionally branched repeatedly in the white matter which in some cases gave rise to pially-directed offshoots. A single callosal afferent may therfore innervate more than one cortical patch. The major termination of callosal axons occurred as a vertical spray of bouton studded axons which spanned from layers I-IV. Although most callosal axons remained in the white matter before turning pially to terminate, others traveled diagonally in the grey matter for long distances (3-5 mm) unbranched before terminating in a cluster of vertically-oriented axons in I-IV. (Supported by NIH Grants NS17861, IR24RR02169 and the Deafness Research Foundation)

345.6 THALAMOCORTICAL AFFERENTS ONTO IDENTIFIED NEURONS OF THE PRIMARY AUDITORY CORTEX OF THE RHESUS MONKEY: AN ULTRASTRUCTURAL ANALYSIS. P.B. Cipolloni, D.N. Pandya and V.M. Knowlton.* Boston University School of Medicine, Boston, MA 02118 and ENR Memorial Veterans Hospital, Bedford, MA 01730.

The thalamocortical afferent projections to the primary auditory cortex of the rhesus monkey have previously been demonstrated at the light microscopic level (Pandya and Rosene, Neurosci. Abst. 9: 954, 1983). The present ultrastructural study demonstrates thalamocortical afferents forming synapses with identified neurons in this primary auditory cortex using the Golgi-EM technique (Fairen, et al., J. Neurocytol. 6: 311-337, 1977) in combination with lesion-induced degeneration.

Following lesions of the medial geniculate body, degenerating axon terminals were found predominately in cortical layer IV with some also found in lower layer III and upper layer V. Portions of eight identified neurons, which included five spinous and three smooth cells, found in these layers were examined in serial thin sections. The five spinous include a layer III pyramidal neuron, a layer IV "star" pyramidal neuron, and three layer VI pyramidal neurons. The thalamocortical synapses, all of which formed asymmetric synapses with their postsynaptic elements (Colonnier, Brain Res. 9: 268-287, 1968), were onto spines of the apical shafts or basal skirts of these cells. No contacts on their somata were found and none would be expected since the somata of pyramidal neurons do not form asymmetric synapses. These three smooth neurons included a layer IV bipolar, a layer V bipolar and a layer IV smooth bitufted neuron. The thalamocortical synapses of these neurons were onto their dendrites and somata.

These results indicate all the neurons which have post-synaptic elements in the field of termination of thalamocortical axonal afferents that form asymmetric synapses may do so with these afferents. Thus, our results in the monkey cortex are in agreement with similar studies in other lower animals (e.g. Peters, et al., J. Neurocytol. 8: 331-357, 1979 and Ichikawa, et al., Brain Res. 345: 87-101, 1985.

This work was supported by NIH grants NS20967 and NS16841 and by the Veterans Administration.

345.7 TWO EFFERENT POPULATIONS IN LAYER V OF RAT AUDITORY CORTEX: CELLS PROJECTING TO THE INFERIOR COLLICULUS AND CONTRALATERAL CORTEX. Kate D. Games and Jeffery A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We injected HRP into the ipsilateral inferior colliculus and contralateral auditory cortex in adult rats to compare the distribution and somatodendritic profiles of the cells of origin in layer V of the auditory cortex. The pattern of retrograde labeling in the cochlear nuclei or in the medial geniculate complex served as a check on the locus of injection.

HRP injections in the contralateral auditory cortex labeled neural somata in homotopic regions of the opposite cortex. Many small, polymorphic neurons were labeled in layer V, and in layers II, III, IV, and VI. Many of the labeled layer V cells had a triangular somatic shape and a primary dendrite oriented toward the pia; their somatodendritic profiles resembled those of small pyramidal cells in layer V; however, the retrograde filling of their apical dendrites rarely extended beyond layer IV. Other labeled cells had a more fusiform profile and could not be definitively classified as pyramidal cells.

In contrast to this pattern, injections in the inferior colliculus consistently labeled layer V cells with larger, triangular somata and whose robustly filled apical dendrites often extended to layer I. The form of these cells matches that of large pyramidal cells in Golgi material. Labeled cells were also observed in layer VI. The smaller cells observed were not labeled by HRP injections in the inferior colliculus.

HRP injections in the ipsilateral inferior colliculus and contralateral cortex resulted in overlap of laminar origins and cell populations in layer V. Inferior colliculus cells were significantly larger ($P > .005$, $df = 130$, t: 6.59; $X = 196$ μm^2, s.d. = 50 μm^2) than commissural cells ($X = 147$ μm^2, s.d. = 32 μm^2). The morphology and somatic areas of commissural neurons in layer V did not differ significantly from that of retrogradely labeled commissural neurons in the other cortical layers.

The spatial distribution of commissural and tectal cells was plotted across layer V. Thirty-six percent of the commissural neurons were found in the upper one-fourth, which contained only 7% of the cells projecting to the inferior colliculus. The number of corticotectal cells increased to 33% in the next quarter; the lower half of layer V contained more tectal (60%) than commissural (39%) cells.

We conclude that layer V represents at least two efferent systems besides its participation in ipsilateral corticocortical pathways. These systems have a degree of sublaminar and cytological segregation which implies that they are anatomically distinct. This research was supported by USPHS Grant R01 NS16832, and SIB Training Grant USPHS 5-T32-GM07379.

346.8 CELLS OF LAYER V IN CAT PRIMARY AUDITORY CORTEX (AI). Jeffery A. Winer, Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The neurons and intrinsic organization in layer V of cat primary auditory cortex (AI) were studied in Golgi, Nissl, and plastic-embedded semi-thin preparations. The goal of these studies is to classify these cells with sufficient precision to ultimately understand their functional role in corticocortical, commissural, or corticofugal projection systems, and to identify which type(s) take up [^{3}H]gamma-aminobutyric acid (GABA) or have an intrinsic role.

Layer V has an outer half (layer Va) in which medium-sized cells and neuropil-rich zones predominate between clusters of neurons, while layer Vb contains the full range of cell sizes and a more dispersed pattern of neuropil organization. Seven types of neurons were recognized in Golgi preparations on the basis of their somatodendritic form and their axonal organization. These include (1) *medium-sized pyramidal* and (2) *large pyramidal* cells whose primary apical dendrite often extended to the superficial layers, and whose axon had many local branches, some entering the white matter; (3) *star pyramidal* cells had a stellate-shaped dendritic field and an axon like that of other pyramidal cells; (4) *inverted pyramidal* cells were quite common in layer Vb [R.A. Code, J.A. Winer, *Anat. Rec.*, 1985, 212:226A-227A, and in preparation; J.A. Winer, *Neuroscience*, 1986, in press] and had an axon which could often be traced to the white matter; both (5) *small multipolar* and (6) *medium-sized multipolar* cells have smooth or sparsely spinous dendrites and local axonal branches; (7) *bitufted* cell dendrites have moderately spinous dendrites arising from each somatic pole and forming vertical tufts. Some of these neurons (e.g., the medium-sized and star pyramidal cells) resemble comparable types of cells in other layers of AI (layer III [J.A. Winer, *J. Comp. Neurol.*, 1984, 229:476-496] and II [J.A. Winer, *J. Comp. Neurol.*, 1985, 238:10-37), while others (e.g., the inverted pyramidal cells) appear to be unique to layer V. This raises the possibility that both common and unique neuronal populations coexist within each layer of AI. It remains to be demonstrated if the presumptively corresponding classes of neurons in the different layers have analogous patterns of connectivity or equal affinity for neurotransmitter candidates.

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346.9 GAD IMMUNOREACTIVITY IS CORRELATED WITH FUNCTIONAL INHIBITION IN THE AUDITORY SYSTEM OF THE BARN OWL. C. Carr, N. Brecha and M. Konishi. Division of Biology, Caltech, Pasadena CA 91125, and Department of Medicine, UCLA, Los Angeles, CA 90024.

Single unit studies have demonstrated that inhibitory processes mediate many binaural interactions. At least part of this inhibition may be GABAergic in nature, as inhibition in some auditory nuclei is correlated with the presence of immunoreactivity to the GABA biosynthetic enzyme, glutamate decarboxylase (GAD). In this study a specific antiserum to GAD was used to determine the distribution of GABAergic cells and axon terminals in auditory brainstem and midbrain nuclei of the owl.

The barn owl uses binaural time and intensity differences for sound localization, and processes these time and intensity cues in parallel channels. Eighth nerve afferents innervate the intensity coding nucleus angularis (NA) and the time coding nucleus magnocellularis (NM). The intensity pathway includes the superior olive (SO), the ventral part of the nucleus of the lateral lemniscus(LLv) the posterior part of the ventral nucleus of the lateral lemniscus (VLVp) and part of the central nucleus of the inferior colliculus (ICC). The projections of the time coding pathway are to nucleus laminaris (NL), the anterior part of VLV (VLVa), and part of ICC. Once in the inferior colliculus, time and intensity pathways recombine to compute the location of the auditory stimulus in the space-mapped region of the inferior colliculus (ICX).

Moiseff and Konishi (J.Neurosci. 3:2553, 1983) classified neurons in the auditory system on the basis of response types, and described inhibitory responses (EI,II,OI,OO) in VLV and IC. This physiological inhibition correlates with the distribution of GABAergic cell bodies observed in the present study.

	intensity				time				both	
	NA	SO	LLv	VLVp	NM	NL	VLVa	ICC	ICX	OT
response	E	EE,EO	EO	EI	E	EE	EE	EE,EI,OO	OO,II	OO
GAD+ve somata	-	+	+	+++	-	-	++	+++	++	-
GAD+ve terminals	++	++	++	++	+	+	++	+++	++	++

Each auditory nucleus receives a characteristic innervation by GABAergic terminals. All neurons of the time pathway receive large perisomatic GABAergic terminals of as yet unknown function, while the intensity pathway is characterized by more GABAergic neurons and finer terminals distributed throughout each nucleus. In addition colchicine treatment has unmasked a number of GABAergic cell types in VLV, ICC and ICX.

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346.10 AN IMMUNOHISTOCHEMICAL MARKER FOR A FUNCTIONAL PATHWAY IN THE AUDITORY SYSTEM OF THE BARN OWL. T. Takahashi, C. E. Carr, N. Brecha and M. Konishi. Division of Biology, Caltech, Pasadena, CA 91125, and Department of Medicine, UCLA, Los Angeles, CA 90024.

Interaural time and intensity differences, the primary cues for sound localization in the barn owl, are processed in separate pathways. Nucleus laminaris (NL) is the site at which the timing of auditory signals from the two sides are compared. It is thus the origin of the pathway devoted to the processing of interaural time differences. We report here that vitamin D-dependent calcium binding protein (CaBP)-like immunoreactivity is congruent with the terminal fields of NL and is therefore a useful marker for the time pathway.

Our antiserum was monospecific for CaBP. CaBP-positivity was assessed by staining with the peroxidase-antiperoxidase and avidin-biotin methods. Serial dilutions confirmed that the degree of staining is primary antibody dependent.

NL innervates the entirety of nucleus ventralis lemnisci lateralis (VLV) pars anterior and the central "core" of the central nucleus of the inferior colliculus (ICc). CaBP-immunoreactivity, which consisted of a plexus of fibers ending in bouton-like swellings usually around ghosts of somata, conformed precisely to the terminal field of NL. By contrast, immunoreactivity was absent in the posterior division of VLV and from the "shell" which surrounds the core in ICc. The latter regions are innervated in their entirety by the projections of nucleus angularis, a cochlear nucleus and the origin of the pathway devoted to the processing of interaural intensity differences.

Somata of NL were also CaBP-positive. A restricted lesion in NL resulted in a localized loss of CaBP-positive terminals in those regions of ICc and VLV that are innervated by the lesioned area of NL. In adjacent sections processed by the Fink-Heimer method, degenerating axons were observed in the region of the deficit in immunoreactivity. The simplest explanation is that the immunoreactive fiber plexus is the terminals of axons originating in NL.

Earlier studies showed that injections of tritiated proline into the cochlea labels the targets of nucleus angularis and is thus a method for the delineation of the intensity pathway. The CaBP-immunoreactivity described above provides a complementary technique for the delineation of the time pathway.

346.11 THE EFFECTS OF KAINIC ACID LESIONS OF THE SUPERIOR OLIVARY NUCLEUS ON SOUND LOCALIZATION BY THE FERRET. G.L. Kavanagh* and J.B. Kelly (SPON: D.C.S. Roberts). Dept. of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

The superior olivary complex provides the first opportunity in the auditory pathway for binaural interaction. Physiological and anatomical studies have shown that cells within the superior olivary complex are binaurally innervated and are sensitive to small differences in stimulation of the right and left ear. Section of the trapezoid body results in impaired sound localization under conditions in which binaural cues are important. Thus, the superior olive is implicated by several lines of evidence as an important structure for sound localization. Direct study of the functional contribution of the superior olive, however, has been limited by the fact that damage to the superior olivary complex using the ablation technique brings with it massive disruption of fibers of passage within the trapezoid body itself. Therefore, interpretation of the behavioral deficits which result from such lesions is difficult. In the present study, we have examined the effects of unilateral lesions of the superior olivary complex using the neurotoxin, kainic acid, to destroy cells in the superior olivary complex without massive destruction of fibers in the trapezoid body. Injections of 5 nmoles of kainic acid in 1 µl of Locke's solution were made with a micropipette inserted just above the olivary complex. Animals were allowed 10-12 days to recover from transient effects, and were tested in a semicircular apparatus to determine sound localization ability at various positions in the azimuth plane. Severe impairments in sound localization were found with the most prevalent deficits appearing ipsilateral to the lesion site. The results, generally, confirm expectations that the superior olive is important for sound localization.

This work was supported by grant No. 7654 from the Natural Sciences and Engineering Research Council of Canada.

346.12 REPRESENTATION OF SOUND FREQUENCY IN THE PRIMARY AUDITORY CORTEX OF THE FERRET. P.W. Judge*, J.B. Kelly and D.P. Phillips. Departments of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6, and Dalhousie University, Halifax, Nova Scotia, B3H 4J1.

The ferret (Mustela putorius) is a highly altrical carnivore with a relatively late postnatal maturation of auditory function. The species is, therefore, particularly well suited for developmental studies of the auditory system (Moore, 1982), and has been used in physiological investigations of sensory development (King et al., 1985). Also, the ferret has been employed recently in a number of behavioral studies of hearing. For example, lesions of auditory cortex have been shown to result in impairments in sound localization with deficits in the contralateral sound field (Kavanagh and Kelly, 1986). In the present study, we have used neurophysiological mapping techniques to determine the response properties of cells in the auditory cortex of the ferret. We have found neurons within the middle ectosylvian gyrus of the barbiturate-anesthetized animal which exhibit short latencies (12-19 msec) and narrow tuning to sound frequency. Neurons or clusters of neurons with similar best frequencies are organized into isofrequency contours. These contours are oriented in a roughly rostral to caudal alignment with high frequencies represented dorsally and low frequencies represented ventrally. Thus, the middle ectosylvian gyrus has a cochleotopic representation similar to that found in the primary auditory cortex of other mammalian species. There was no evidence of any frequency reversals within the cortical area sampled, which suggests that we have mapped a single auditory field corresponding to AI in the cat. The question of whether other frequency organized fields are present in areas outside the middle ectosylvian gyrus has yet to be resolved. The present study has demonstrated the presence of a single frequency organized primary auditory area in the middle ectosylvian gyrus. The data provide a general framework for further work on the function, physiology, and development of auditory cortex in the ferret.

This work was supported by grants No. 7654 to J.B. Kelly and No. U0442 to D.P. Phillips from the Natural Sciences and Engineering Research Council of Canada.

346.13 THE EFFECTS OF PRIMARY AUDITORY CORTICAL LESIONS ON SOUND LOCALIZATION BY THE FERRET: STIMULUS DURATION. J.B. Kelly, P.L. Stanton*, and S.J. White*. Dept. of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

Previous studies have shown that lesions of the primary auditory cortex in the ferret result in impaired sound localization (Kavanagh and Kelly, 1985). In behavioral tests with a single click presented at the beginning of each trial, ferrets with unilateral lesions of auditory cortex are incapable of responding correctly to sounds located within the contralateral sound field. No deficits are found in the ipsilateral field. Following bilateral lesions impairments in localization are found on both left and right sides of the test apparatus. In contrast, no deficits in sound localization are found when clicks are repeated at a rate of 2/sec throughout a trial. These results suggest that the effects of auditory cortical lesions on sound localization are systematically dependent on stimulus duration. We have tested this idea in the present study using ferrets with either unilateral or bilateral lesions of the primary auditory cortex. The animals were tested in an apparatus designed to assess sound localization ability at different positions in the horizontal plane. Thus, each animal was tested separately in both left and right lateral fields with single bursts of broad band noise. Four different durations were used, viz., 60 msec, 30 msec, 15 msec and 7 msec. The results generally confirmed the outcome of our previous studies by showing that unilateral lesions of the primary auditory cortex result in impaired localization in the contralateral hemifield, and that bilateral lesions result in impairments in both hemifields. The impairments were most evident, however, with stimuli of very brief duration, and were not reliably found with durations of 30 or 60 msec. The improved performance with longer durations was not associated with head movement within the sound field. Videotapes of the animals' behavior during the test sessions indicated that the latency to execute a movement was in every case greater than 60 msec.

This work was supported by grant No. 7654 from the Natural Sciences and Engineering Research Council of Canada.

346.14 THRESHOLDS FOR PURE TONE DETECTION BY THE FERRET. J.C.H. Dalton, G.L. Kavanagh*, and J.B. Kelly. Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

The ferret (Mustela putorius) has been the subject in a number of recent studies of the central auditory system. The importance of auditory structures for sound localization has been investigated by traditional behavior/ablation techniques. The physiological responses of cells in the inferior colliculus and auditory cortex have also been examined. In addition, ferrets have been employed in developmental studies of hearing, a research area for which they are particularly well suited by virtue of their relatively late postnatal maturation. The increasing use of the ferret for research in auditory neuroscience has encouraged us to determine the animal's sensitivity to pure tones using behavioral testing procedures. Absolute thresholds for a wide range of frequencies were obtained by the method of limits for two adult male ferrets. The resulting audiograms showed that ferrets are sensitive to sounds between 16 Hz and 48 kHz. The upper and lower limits of hearing as determined by the often used 60dB SPL cut-off criterion were 44 kHz and 36 Hz respectively. The greatest sensitivity was found for sounds in the 8-12 kHz region. Individual animals showed narrow tuning to frequencies within this range with a maximum sensitivity of -6 to -10 dB SPL. The ferret is similar to other carnivore species in having a very broad range of hearing. The average audiogram is almost identical to that reported for the dog, but is generally elevated in comparison with the cat. The upper limit of hearing is somewhat less than reported for the cat. Further specific comparisons can be made with the racoon, and least weasel. In general, the ferret has excellent hearing over a broad range of frequencies, and is a good animal for further research on the mammalian auditory system.

This work was supported by grant No. 7654 from the Natural Sciences and Engineering Research Council of Canada.

346.15 PRECISION OF SOUND LOCALIZATION AND COORDINATION OF EYE AND PINNA MOVEMENTS BY CATS PRESENTED WITH VISUAL AND AUDITORY TARGETS. P.Hartline, D.Northmore*, Eye Research Institute, Boston, MA 02114

Cats readily learn to look toward a light or sound source in the frontal quadrant; the latter reveals that auditory localization can be precise to ±2-3 degrees. Pinna movements that are highly correlated with target position occur more or less simultaneously.

A cat, its head fixed in front of an array of LEDs, was trained by turning on LEDs singly and following LED offset with liquid food. The cat learned to look toward the visual target (via saccade[s]) after a few hours' experience. Subsequently, hidden loudspeakers in line with selected LEDs played brief noise bursts 100-300 msec before the corresponding LED was illuminated; reward followed LED offset. The cat rapidly learned to look toward the source of sound. The left eye and pinna were monitored using an electromagnetic search coil for the eye and another coil fixed to the pinna. The pinna's predominant motion within the forward quadrant, (rotation about 2 axes), was digitized along with eye orientation.

Visual targets (up to ±20° eccentricity): Angular scatter of fixations after several training sessions seldom exceeded ±2°, a figure that could be reduced by rewarding consistent performance. Even when the cat had not been exposed to the auditory stimuli, visual saccades were usually accompanied by nearly simultaneous high velocity movements of the pinna, with changes of azimuth and elevation as if to place the pinna in a preferred position for each target location. However, the mapping of target location to pinna orientation seems to have less than unity magification (less than 0.5 for elevation). Scatter of pinna orientations for a given LED was equivalent to ±5° target uncertainty.

Auditory targets (up to ±20° eccentricity): After several training sessions on the auditory task, a 50 msec noise burst evoked rapid ballistic pinna and (saccadic) eye movements. The scatter of sound-evoked visual fixations was ±2-3°, double that for LED-evoked fixations; the mean position fixated was the same for each speaker as for the corresponding LED. The distribution of pinna fixations was similar to that for visual targets.

Was the cat looking at the remembered position of the LED associated with each speaker (possibly identified by a non-spatial cue)? Apparently not, since it correctly looked toward interleaved novel speaker locations, none of which was followed by an LED cue.

Latencies: For visual or auditory tasks, most eye movement latencies fell between 80-140 msec. For the visual tasks, most pinna latencies fell between 80-100 msec, while for auditory tasks, the latencies were 60-80 msec. There was not a stereotyped relationship between latency of eye and pinna motion; in some cases, eyes or pinna but not both were moved. These results indicate a degree of independence of the decisions to command eye and pinna movements toward visual or auditory targets.

346.16 GABA-ERGIC NEURONS IN AUDITORY NEOCORTEX : QUANTITATIVE IMMUNOCYTOCHEMICAL ANALYSIS USING COMPUTER ASSISTED MICROSCOPY. J.P. Ross*, N.T. McMullen, M.E. Blue, M. Molliver and E.M. Glaser (SPON: D. Ruchkin). Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, MD 21201.

We have used computer microscopy to quantitatively analyze the laminar distribution and cell areas of GAD and GABA positive cells--putative inhibitory local circuit neurons--in rabbit auditory cortex. Neurons were immunocytochemically labeled for the synthetic enzyme GAD (Oertel et al., Neurosci., 1981) or GABA (Altschuler et al., Soc. Neuro., 1985). A region of auditory cortex 4 mm in pial length was mapped in 30-40 um thick coronal sections using our Image Combining Computer Microscope which included digitization of the contours of the area of interest, identification and marking of GAD+ neurons at high magnification and digitization of cross-sectional soma area. Sections stained for GAD and GABA are qualitatively similar in their distribution of labeled neurons. Two sections each from 2 animals stained for GAD have been quantitatively analyzed. Major findings were: a) GAD+ cells are present in all cortical layers (I-VI); b) GAD+ cells are most numerous in the supragranular layers 200-400 um beneath the pia and are especially concentrated at the I/II border; c) in terms of cross-sectional area, the largest GAD+ cells (250-500 um2) are preferentially distributed in lamina III/IV and upper layer V, the major terminal zone of afferents arising from the medial geniculate body; d) in terms of soma morphology and laminar distribution, the largest GAD+ cells located in III/IV are identical to the major nonpyramidal cell type in these layers--the large spine-free cell described in previous quantitative Golgi studies (McMullen, et al., JCN, 1984); e) unlike the largest GAD+ cells that are distributed in III/IV, the smallest GAD+ cells (50-125 um2) have a bimodal distribution in the supra- and infragranular layers. The smallest GAD+ cells are most numerous in layers II and upper layer III and correspond to those layers containing the greatest variety of nonpyramidal cells described in earlier Golgi studies (McMullen and Glaser, JCN, 1982). The laminar distribution of these putative inhibitory neurons differs markedly from those described in other sensory cortices, e.g., visual and somatosensory. These results imply that local circuit neuron distribution may have significant species as well as sensory modality differences. (Supported by The Deafness Research Foundation and NIH Grants NS 17861 and IR24RR02169)

346.17 SOURCE LOCALIZATION OF LONG LATENCY AUDITORY EVOKED MAGNETIC FIELDS IN HUMAN TEMPORAL CORTEX. D.L. Arthur*, G. Sullivan*, E.R. Flynn & S.J. Williamson+. Los Alamos National Lab, Physics Division, Los Alamos, NM 87545 & +New York University, Dept. of Physics, New York, NY 10003.

Studies of the neuromagnetic field evoked by auditory stimuli have the capability of localizing sources of the various evoked components in different regions of the primary sensory cortex. The present study was designed to serve two functions: first to independently replicate and extend previous reports on source localization of long latency magnetic field responses (P50m, N100m P200m, SFm) and secondly to provide additional data about the frequency dependence of these responses.

Measurements were carried out in a magnetically shielded room with four subjects. Auditory stimuli were presented binaurally to the subjects by means of standard plastic airline headsets. Two different frequencies of acoustic stimuli were used: 250 and 2500 Hz, presented randomly and equiprobably. The magnetic field was measured with a second order gradiometer coupled to a DC SQUID. The output voltage from the SQUID electronics was applied to a bandpass filter set to 0.01 to 50 Hz and the averaged waveforms were digitally filtered from 0 to 25 Hz. Extensive field maps were obtained over the left hemisphere in three subjects and over the right hemisphere in the fourth. The latency and amplitude of the evoked response was measured at about 40 positions targeting the auditory cortex.

In all subjects good quality AEFs were obtained. The amplitude of the P50m, N100m, P200m and SFm had two clear extrema of opposite polarity located at the estimated ends of the Sylvian fissure. To determine the source location of the cortical activity evoked by the tones, a current dipole in a conducting sphere was used as a theoretical model. The theoretical field was calculated and the observed fields were computed. The parameters determining the dipole were then adjusted by computer until the sum of the squared deviations was minimal. By this technique, it was found that the N100m could be distinguished in location from the P200m in 3 out of 4 subjects. The only frequency dependence that was observed was an increase in depth of the P50m to the higher frequency tone in 3 out of 4 subjects.

346.18 AUDITORY CHARACTERISTICS AND SEXUAL DIMORPHISM IN THE GYPSY MOTH, Lymantria dispar, (Lepidoptera: Lymantriidae). B. Cardone* and J.H. Fullard. (SPON: P. Brawley). Department of Zoology, Erindale College, University of Toronto, Mississauga, Ontario. L5L 1C6. Canada.

The auditory characteristics of two populations of gypsy moth were examined, (colony and wild). L. dispar exhibits morphological and behavioural sexual dimorphism. Females are much larger than males and are flightless while males actively search for female pheromone trails. Since various moths possess auditory organs to detect the echolocation signals of sympatric, insectivorous bats, and show resultant avoidance behaviours, the auditory characteristics of the gypsy moth and sexual dimorphisms were investigated.

Thresholds to pure tones of 5-150 kHz were recorded using extracellular techniques. Two auditory cells A_1 and A_2, and a putative proprioceptor B cell were identified. Both sexes have functional tympanic organs with females exhibiting highest sensitivity to 10-30 kHz and males exhibiting greatest sensitivity at 20-70 kHz. Audiogram comparisons of wild males and females (Figure 1) reveal statistically significant differences between thresholds (indicated by *) at 5-15 kHz, 30-120 kHz, and 130-140 kHz with females less sensitive at all but the lowest frequencies. Mean best frequency data (Figure 2) reveal females to be sensitive to lower frequencies. We suggest that male gypsy moths need their ears to detect the echolocation calls of sympatric bats and are tuned to their frequencies (30-60 kHz). Flightless females are not exposed to predation pressure and as a result have degenerate ears.

Comparisons of colony raised versus wild gypsy moths showed that audiograms of colony males exhibit greater variability. This suggests an artificial source of auditory degeneration due to the absence of exposure to bat predation over multiple generations of laboratory breeding. We caution against using colony animals when testing for responses depending upon naturally occuring situations.

Fig.1

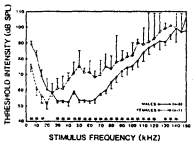
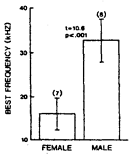


Fig.2



346.19 PERCEPTUAL ORGANIZATION OF SIMPLE AND COMPLEX ACOUSTIC SIGNALS IN THE BUDGERIGAR(MELOPSITTACUS UNDULATUS). R. Dooling, T. Park*, S. Brown*, and K. Okanoya*. Psychology Department, University of Maryland, College Park, MD 20742.

The budgerigar is a highly vocal and social parrot with a complex vocal repertoire. In this species, learning is involved in the development of vocalizations and there is evidence for unusual frequency resolving power in the spectral region important for vocal communication.

In the following experiments, budgerigars were trained with operant conditioning techniques to discriminate among pairs of acoustic stimuli. In this "Same\Different" discrimination task, response latency was used as a measure of perceptual similarity. Response latencies were then analyzed using multidimensional scaling(MDS) procedures to produce a spatial representation of the perceptual similarities among stimuli.

Results for simple sounds such as pure tones which vary along the dimensions of frequency, intensity, or duration indicate that the budgerigar is far more sensitive to frequency changes than intensity or duration changes. MDS analysis also permits construction of pitch scale and loudness scales for the budgerigar. These results are compared to pitch and loudness scales for the human.

Results for complex sounds such as calls drawn from the vocal repertoire of the budgerigar indicate that there are natural perceptual categories for the functional classes of these calls (ie. food-begging calls, alarm calls, contact calls). Humans show the same perceptual categories for these calls but the relation among categories is different for the two species. The acoustic dimensions accounting for the spatial arrangement of complex contact calls are quite clear for the human but not for the budgerigar. These results indicate that budgerigars and humans attend to different features of the complex budgerigar contact call. Tests with computer-modified calls show that budgerigars probably use a combination of features in discriminating among these complex species-specific acoustic signals.

Work supported by NIH grants to RD.

346.20 EFFECTS OF SEX AND HORMONAL STATE ON ACOUSTIC RESPONSIVENESS IN THE HAMSTER. O.R. Floody and R.D. Lisk*. Dept. of Psychology, Bucknell Univ., Lewisburg, PA 17837 and Dept. of Biology, Princeton Univ., Princeton, NJ 08544.

Semi-quantitative [^{14}C]2-deoxyglucose (2DG) autoradiography was used to map the neural responses of golden hamsters to ultrasounds resembling the calls used for communication during courtship and mating. One study examined the processing of ultrasounds and ambient noise by estrous females, some of which were deafened or hemideafened with plastic ear plugs. Comparisons of relative levels of 2DG uptake failed to reveal responses specific to the ultrasounds. However, hemispheric differences were pronounced, especially in the hemideafened subjects. For the ventral cochlear nucleus (VCN), 2DG uptake was elevated contralateral to the plug and ipsilateral to the effective stimulus. In contrast, uptake by more rostral structures (dorsal n. of the lateral lemniscus = DNLL; ventral n. of the lateral lemniscus = VNLL; central n. of the inferior colliculus = CIC; medial geniculate n.) was elevated ipsilateral to the plug and contralateral to the stimulus.

A second experiment examined responses to unilaterally presented sounds in groups of intact or castrated males and females. Effects of sex, hormonal state and hemideafening were sought in the auditory system and in forebrain areas rich in hormone-concentrating neurons. As before, relative levels of 2DG uptake differed across the hemispheres for auditory structures including the VCN, trapezoid body, VNLL, DNLL, and CIC. In addition, intact females showed higher levels of uptake than males in the DNLL, auditory nerve (AN), and lateral lemniscus (LL). Females also tended to show higher anterior hypothalamic uptake than males, but just in the hemisphere contralateral to the stimulus. Together with previous behavioral data, these results suggest that male and female hamsters differ in acoustic responsiveness, and partly because of hormonal effects at relatively peripheral sites in the central auditory system. (Supported by MH-08070 and MH-33191 from the N.I.H. and PCM-7812582 from the N.S.F.).

347.1 **THE EFFECTS OF CLICK POLARITY ON PERIPHERAL AND BRAINSTEM AUDITORY RESPONSES IN BIRDS.** K. Borg*, H. Brown-Borg*, M. Beck (SPON: M. Dosiak) Dept. Animal Science, UNL. and T.A. Jones, Dept. Oral Biology, U.N.M.C., Lincoln, Ne 68503.

There is compelling evidence to suggest that in mammals afferent auditory neurons are activated preferentially during one-half cycle of a sinusoidal stimulus (Davis 1976). Inverting the polarity of sinusoidal click stimuli under these circumstances should produce latency shifts in the compound action potential which approximate one-half cycle of an equivalent sinewave. In the present study, peripheral and brainstem auditory responses (PBAR's) were recorded in White Leghorn chicks in order to explore the possible existence of a similar 'rectification' or phase-preference in the avian cochlea. Far-field PBAR's were recorded in response to either rarefaction or condensation clicks, (512 samples were averaged, the EEG was amplified X10K and filtered LF=100, HF=10KHz). Latencies and amplitude of both peripheral (P1a and P2a) and central (P3a) PBAR components were measured in eight 2-week-old chicks. The acoustic output of the stimulus driver was monitored using a calibrated microphone to verify that click inversion produced equivalent (although inverted) stimulus amplitudes and symmetries. Condensation clicks produced the shortest latencies in all animals (condensation: P1a= 1.18 ± 0.138, P2a= 1.44 ± 0.110, P3a= 2.67 0.166; rarefaction: P1a= 1.56 0.107, P2a= 1.89 0.064, P3a= 3.02 ± 0.116 msec). Significant differences between responses to rarefaction and condensation clicks were produced in all peaks (P<.001). Mean latency shifts obtained were: P1a= 0.374, P2a= 0.372 and P34a= 0.352 msec. Using the mean across all peaks, the shift would correspond to that expected from a pure sinewave stimulus of approximately 1.4kHz. These results are consistent with the hypothesis that the cochlea of the bird, like that of the mammal, produces neural activation preferentially during one half-cycle of a stimulus. Unlike the mammal however, it appears that this preference is for the period corresponding to a condensation in the ipsilateral external auditory meatus.

347.2 **ORIGINS OF NEURAL ACTIVITY IN BRAINWAVES OF NORMAL AND AUDIOGENIC MUTANT CHICKS** H.M. Brown-Borg* and M.M. Beck, Department of Animal Science, University of Nebraska-Lincoln, and T.A. Jones, Dept. of Oral Biology, University of Nebraska Medical Center, Lincoln, NE 68583.

In previous work, it has been shown that paroxysmal (px) chicks show extensive and progressive degeneration of central auditory and vestibular nuclei and fiber tracts (Beck et al., 1983). Additionally, a more recent study has shown that ABRs recorded from the scalp of the px chick range from essentially normal to severely abnormal in comparison to those of non-px siblings (Beck and Jones, 1984). In an attempt to determine which auditory neurons contribute to the abnormal waveforms seen, studies were conducted to identify the origins of the major components of the peripheral and brainstem auditory responses (PBARs) in px chicks. Two and three-wk-old normal and px chicks were anesthetized and presented with a rarefaction click stimulus to the right ear, the left being occluded with petroleum jelly. Major auditory projections were identified surgically using one of three knife cuts (rostral coronal, sagittal, and uni- and bi-lateral VIIIth nerve), all confirmed histologically. No changes in response waveform were detected following rostral coronal sections, indicating that higher-order pathways (above midbrain) do not contribute significantly to the PBARs. Sagittal sections interrupted either the crossed dorsal cochlear tract (Cod) or the trapezoid (CTz) fibers. Cod-section decreased (P<.05) amplitudes of peaks after P2B but interruption of the CTz alone did not change waveform morphology. In normal chicks, uni- and bilateral VIIIth nerve sections, separating the nerves near the points of insertion into the brainstem, left peaks P1 and P2A intact, suggesting that these early potentials do not rely on central projections. Peaks subsequent to P2A (P2B - P5A) appear to represent activity of central auditory pathways, primarily the crossed dorsal and uncrossed fibers projecting from the cochlear nuclei to higher order ipsilateral and contralateral brainstem relays. Comparisons of our results with those of mammalian lesion studies show differences between effects of CTz lesions, suggesting that the avian Cod, connecting ipsi- and contra-lateral nuclei laminaris may carry fibers homologous to those found in the mammalian ventral trapezoid striae. Effects of VIIIth nerve sections differed in px chicks as compared to normals in that in normals, N2A but not P2A was significantly reduced in amplitude whereas in px the opposite was found. In either case, dependence on central neurons did not extend beyond components of wave 2. (Supported by University of Nebraska Agricultural Research Division Funds (13-061) and by UNMC College of Dentistry Research Grants 83-25, 84-30-60, 85-37.)

347.3 RAT AUDITORY CORTEX: PRIMARY EVOKED RESPONSES AND THEIR CONTRIBUTIONS TO SKULL RECORDED EVOKED POTENTIALS. G.V. Simpson and R.T. Knight. University of California, Dept. of Neurology, V.A. Med. Ctr., Martinez, CA 94553

Auditory averaged evoked potentials were recorded epidurally over the auditory cortex and simultaneously from screw electrodes implanted in the skull over frontal cortex (FCx), at the vertex (Vx), at the posterolateral edge of the dorsal skull over visual cortex (PL) and over the auditory cortex (ACx) contralateral to that being recorded with epidural leads. All recordings were referenced to a skull lead over the far frontal sinus. Seventeen Fischer 344 male rats 10-12 months of age were recorded from while under pentobarbitol anesthesia. Following epidural mapping of the cortical evoked response the dura was removed over the area of maximal response and 1ul of a 100 ug/ul solution of GABA (at body temperature) was topically applied followed by washing with Ringer solution for 10 sec. Evoked responses were recorded at the cortical surface before the application of GABA and at 30 sec intervals following.

The primary auditory cortex response recorded epidurally consisted of a biphasic positive-negative potential with the positivity occurring at 14 msec. (P14) and the negativity at 20 msec. (N20) following the click stimulus. The same biphasic response could be recorded from the lateral skull lead (ACx). Epidural mapping of this response revealed a region of maximal amplitudes centered over Krieg's area 41 and corresponding to the projection area of the ventral division of the medial geniculate (H.A. Patterson 1976 dissertation). However, responses of decreased amplitude extended to more rostral areas which overlap the projection area of the medial division of the medial geniculate. The P14 and N20 appear to be locally generated within auditory cortex as evidenced by the sharp decline in amplitude outside the auditory cortex region. Furthermore, topical application of GABA at the location of maximal cortical responses resulted in a reversible decrease in the amplitude of both the P14 and N20 by 80-100%.

Recordings from dorsal skull leads (FCx, Vx, PL) in the anesthetized animal revealed no components following the far-field brainstem evoked potentials (1-6 msec). Whereas, simultaneous recordings from the surface of the auditory cortex and from the ACx skull lead produced a large P14 and N20. This suggests that activity in the auditory cortex does not contribute to the dorsally recorded auditory evoked potentials in rats. This is consistent with the lateral location of auditory cortex in the rat which would suggest a generator with an equivalent dipole orientation orthogonal to the dorsal skull surface.

347.4 THE RAT'S AUDITORY EVOKED ORIENTING RESPONSE DURING WAKING AND SLEEPING. K. Rudolph*. (SPON: B. Mambrito) Dept. of Zoophysiology, Univ. of Tuebingen, Morgenstelle 28, D-7400 Tuebingen, West Germany.

Auditory stimuli of moderate intensity can evoke behavioral (motor) as well as autonomous reactions of an organism. These reactions habituate upon repeated presentation of the same stimulus and are considered as "Orienting Responses" (OR). Most experiments on this subject have been carried out with humans and comparatively few with animals.

In the present study, the rat's OR was measured under carefully controlled conditions to ensure defined states of vigilance. Stimuli were delivered during the awake state (W) and slow wave sleep (SWS). Electrocorticogram (ECoG), Electrocardiogram (ECG) and motility of the unrestrained rat was transmitted via a miniature FM transmitter placed on the animal's head. Additionally, breathing movements were recorded. White noise and 16-kHz tones (40 to 80 dB SPL, 2 sec duration) served as stimuli.

The rat's auditory evoked OR is characterised by: Arousal reactions in the ECoG; A diphasic heart rate response (a deceleration followed by an acceleration); The respiratory OR consists of a respiratory pause and a reduction in breathing amplitude following both tone onset and offset. With the exception of heart rate acceleration, all of these responses show clear habituation during waking and sleeping, contradictory to the hypothesis that "sleep and habituation are incompatible" (JOHNSON, L.C. & LUBIN, A., EEG Clin. Neurophysiol. 22:11-21, 1967).

No longterm habituation was observed: the OR recovered completely within one week.

This work was supported by the Deutsche Forschungsgemeinschaft grant Schn. 138/13-15 and by the SFB 307 of the University of Tuebingen.

347.5 BINAURAL INTERACTIONS IN AUDITORY BRAINSTEM RESPONSES IN CAT AND OTHER SPECIES. L.J. Achor, A.Starr, and K.S. Wrege*. Dept. of Psychology, Baylor Univ., Waco, Texas 76798 and Depts. of Neurology and Psychobiology, Univ. of Calif., Irvine 92717.

Binaural interaction (BI) was examined in scalp recordings of auditory brainstem responses (ABRs) in cats and other mammalian species. Three aspects of binaural interaction were investigated: (1) BI in the absence of interaural time or intensity differences, (2) BI as a function of interaural intensity differences, and (3) BI as a function of interaural time differences. A BI waveform was derived for each value of the independent variable in each of these conditions. The BI waveform was computer-derived by subtracting the binaural ABR from the sum of the appropriate monaural ABR waveforms. The BI waveform was presumed to reflect activity selectively occuring only when both ears were stimulated. As previously found in several different laboratories, BI was present in all of the species tested (rhesus monkey, cat, rabbit, guinea pig, and rat). Six cats formed the main subject group with comparative data obtained from one animal within each of the other species. In each of the species, except the rat, BI was evident as a decrease in the amplitude of the binaural ABR relative to the sum of the monaural ABRs. In all of the species more than one component of the binaural ABR was affected by binaural interaction. In addition there was a similarity in the latency of onset of the binaural interaction and in the duration of maximum effect. In all of the species, except the rat, the BI waveform could be characterized as a positive-negative-positive sequence of components, although in some cases the leading or trailing positive component was very small. For comparison purposes these components were labelled A, B, and C. A peak-to-peak amplitude was obtained between components A and B (A-B) and between B and C (B-C). A comparison of peak-to-peak amplitudes in the BI waveform versus the sum of the monaural ABR waveforms was used as a measure of the amount of binaural interaction. In the cat component A-B of the BI waveform was 44% of the corresponding peak of the sum of the monaural ABRs. For component B-C it was 36%. In the other species these figures ranged from 20% to 62%. In the first part of the study with no interaural differences, the percentage of BI was found to remain fairly constant as the intensity of the stimulus to the two ears was decreased. Over a 50 dB range in the cat the value for component A-B ranged from 43-47% and for component B-C 35-44%. In the second part of the study BI was found to vary as a function of the interaural intensity difference. In the cat components A-B and B-C steadily decreased in amplitude as the interaural intensity difference increased. Both decreased by approximately 50% when the intensity difference reached 40dB. The analysis in the other species was inconclusive. In the third part of the study BI was found to vary as a function of the interaural time difference. In the cat the percentage of BI decreased systematically from 44% to 15% over a 4.00 msec range of interaural intensity differences. Similar findings were obtained in the rhesus monkey, guinea pig, and rabbit, however the rates of decrease differed. Although the comparative data are based on a very small number of subjects, the similarity in findings across species for BI waveform morphology and for the effects of interaural time differences suggest that analogous sites may be involved in the generation of the BI components across species.

347.6 ABR CHANGES TO TONAL STIMULI IN THE SENESCENT FISCHER 344 RAT. W. A. Cooper, Jr.*, J. R. Coleman, E. H. Newton* and A. E. Youatt*. (Spon: J. J. Freeman) Departments of Communicative Disorders, Psychology and Physiology, University of South Carolina, Columbia, South Carolina 29208.

One of the consequences of aging is decreased sensitivity to auditory stimuli. These changes are well documented in humans but may be confounded with environmental and genetic factors. The rat has proven to be a useful model in early developmental studies of the auditory system and may provide information regarding the process of aging of the hearing mechanism.

The auditory brainstem response (ABR) to one msec tone pips of 3, 8 and 40 kHz was evaluated in groups of 12 and 25 month-old Fischer 344 rats. Vertex-chin recordings were obtained from animals anesthetized with pentobarbital. We examined age-related changes in threshold (the lowest intensity producing an observable response) and in peak and interpeak latencies.

Stimulation at 8 kHz (in the region of greatest behavioral sensitivity) in young adults produced threshold responses at lower intensities than did higher and lower test frequencies. Thresholds in aged animals increased by 30 dB or more at each frequency. The largest change occurred for the high-frequency probe. Five waves were usually observed within the first 5 msec for animals in the young adult group to stimulation at 90 dB PeSPL. The expected decrease in latency with increase in frequency was uniformly observed for wave I, but this relationship was not as consistent for later waves. In older animals there was a prolonged latency of wave I at each frequency. In contrast to young adults, wave I latency in aging animals does not produce the expected frequency dependent relationship. In older subjects the first four waves show shorter latencies to 8 kHz stimulation than to the other frequencies. In addition, the I-III, I-IV and III-V interpeak latencies increase with age by at least 0.3 msec. Our results suggest that both threshold and latency parameters change in senescent animals. The increase in latency of wave I implies peripheral change while the increased interpeak latencies suggest that central transmission is also altered in older animals. (Supported by NIH Grant AG-1571 and a grant from the Veterans Administration.)

347.7 DOSE-DEPENDENT EFFECTS OF KETAMINE ANESTHESIA ON THE RAT BRAINSTEM AUDITORY EVOKED POTENTIAL. M.W. Church and R. Gritzke.* Research Institute on Alcoholism, Buffalo, NY 14203.

Because ketamine is both an abused substance and a commonly used veterinary anesthetic, its effects on brain and sensory functions are of interest. The present study examined the dose-dependent effects of ketamine anesthesia in the rat, using the brainstem auditory evoked potential as an objective, quantitative measure of this substance's acute effects on brain and sensory electrophysiology.

Subjects were 11 young adult female Long-Evans rats. BAEPs were recorded from skull screw electrodes (stimuli = 70, 90, 110 dBpeSPL, 0.1 msec clicks, 12.5/sec) while each animal was restrained in a fixed position relative to a piezoelectric speaker inside a sound isolation chamber. BAEPs were recorded during a baseline period as well as after saline and ketamine treatments. Ketamine was administered (i.p.) in two serial doses. The first dose (100 mg/kg) was followed 30 min later by a second dose (also 100 mg/kg). Equal volumes of normal saline were also injected serially. An interval of 1-2 weeks occurred between the saline and ketamine treatments, with treatment order counterbalanced. Rectal temperatures were maintained within $\pm$ 0.2°C of baseline to control for possible temperature-related effects.

Ketamine produced prolongations in the latencies of all BAEP components (P1 through P6) that were statistically significant relative to baseline and saline control values. These latency shifts were progressively greater for waves P1 through P4 (shifts in P5 and P6 approximated the P4 shift). For example, following the first injection, P1 and P4 were prolonged by .058 and .141 msec, respectively, (at 90 dBpeSPL). The effect of the second ketamine injection was to nearly double the latency shifts. For example, P1 and P4 were prolonged by .090 and .255 msec, respectively, following the second injection. Ketamine also had significant effects on BAEP amplitudes, causing dose-dependent increases in P1 through P4 amplitudes and decreases in P5 and P6 amplitudes. The ketamine-induced changes were not attributable to changes in core temperature, circadian variation, or auditory sensitivity as measured by BAEP thresholds.

Ketamine is sometimes used in animal BAEP studies as a form of chemical restraint. While the prevailing view has been that ketamine does not alter the BAEP, only two prior studies have partially investigated this issue. The results of the present study, combined with those from several recent studies investigating various other drugs (e.g., alcohol, pentobarbital, isoflurene, lidocaine), are challenging the belief that the BAEP is resistent to the effects of anesthetics, sedatives, and hypnotics and suggest that the BAEP is useful in characterizing the CNS and sensory effects of these agents.

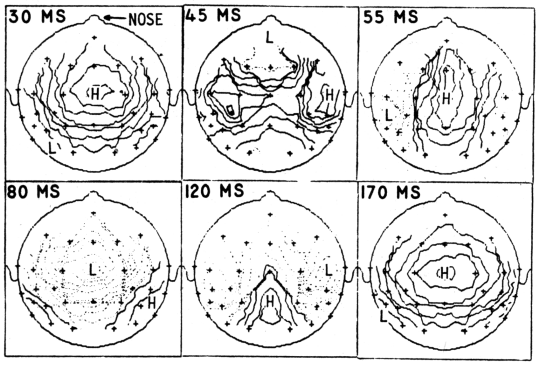
347.8 COMPARISON OF THE HUMAN NORMAL MONAURAL AVERAGED BRAINSTEM AUDITORY EVOKED RESPONSES IN THE HYPERBARIC 6 ATA AIR ENVIRONMENT TO SURFACE CONTROL. S. J. Whidden, T. Thorn*, T. Borresen*, K. Van Meter*, JESM Baromedical Research Institute, New Orleans, Louisiana 70114, U.S.A.

Auditory brainstem evoked responses were recorded with conventional EEG disk electrodes placed on the skin at the vertex (Cz) and earlobes (A1, A2), using Grass EEG paste at the surface, at 165 FSW (6 ATA), at 40 FSW (2 ATA) and back at the surface. Differential amplification (Cz to A1 or A2) of 1,000,000 ($1x10^6$) times was accomplished by two amplifier links in series. The amplifier band-pass was between 100 hertz and 3 kilohertz to achieve resolution of the high frequency components of the auditory brainstem response. The acoustic transient clicks were generated by passing square wave pulses 0.10 milliseconds in duration through an attenuator for regulation of intensity and amplification. The click rate used in these studies was 11/sec. and the intensity of the signal was noted in reference measurement to be 80 dB. The computer time base was triggered by each click for a total of 2,048 clicks. The rate of 11 clicks per second required only about 10 minutes to obtain one set of average monaural responses. The click generating and amplifier equipment used next to the chamber was a Tracor Analytic 3000 evoked potential system. Amplitude and latency data were obtained from three individual experiments. Latency was measured when there was a sharp rise and fall. When the wave forms were broad, as occurred with low intensity waves, latency was defined as the bisection point of lines drawn over the ascending or descending slopes. The component's amplitude was measured from the positive trough to the subsequent negative peak. In these clinical tests using monaural signals, recordings were always made between the vertex and the earlobe ipsilateral to stimulation. The surface results indicate that there were 5 wave forms and the mean latency in milliseconds were Wave I, 1.8 $\pm$ 0.8; Wave II, 2.69 $\pm$ 0.12; Wave III, 3.49 $\pm$ 0.15: Wave IV, 4.92 $\pm$ 0.11; Wave V, 5.8 $\pm$ 0.11. The data suggest that the mean latency does not vary from measurements made on the surface to those made in a hyperbaric 6 ATA air environment. The surface amplitudes measured in microvolts were: Wave I, 0.2 $\pm$ 0.1; Wave II, 0.16 $\pm$ 0.1; Wave III, 0.25 $\pm$ 0.9; Wave IV, 0.12 $\pm$ 0.08; Wave V, 0.34 $\pm$ 0.12. These data suggest that brainstem auditory evoked potentials may be used as a viable technique for evaluating brainstem function.

347.9 SCALP TOPOGRAPHY OF HUMAN AUDITORY EVOKED POTENTIALS. S. Satya-Murti*, A.T. Cacace* and J.R. Wolpaw (SPON: A.J. Popp). Depts Neurol, Surg & Anat, Alb Med Coll, and Wadsworth Labs, NYS Dept Health, Albany, NY 12208.

Auditory stimulation begins a complex series of CNS events reflected in voltage gradients at the scalp. Early potentials have been intensively studied. Later activity has been less well defined. Recent studies indicate that later AEPs reflect activity in several cortical areas, including primary and secondary auditory cortices. We recorded AEPs to binaural and monaural clicks (128 trials, 66dB SL, 3.3s ISI) from 32 locations referred to a noncephalic reference in 58 volunteers ages 20-79, and generated isovoltage topographic maps from 20-250ms post-stimulation.

Prominent features were: a vertex 30ms positive (pos) peak (pk); 45ms temporal (temp) pos pks; a vertex 50-55ms pos pk, accompanied by temp negative (neg) pks; the vertex 80-85ms N1 pk, accompanied by the temp Ta pos pks of the T complex; the 120ms temp Tb neg pks of the T complex; and the vertex 170ms P2 pos pk. The figures illustrate these features. Solid contour lines indicate pos voltage, dotted indicate neg. "H" is the highest point, "L" is the lowest. While binaural and monaural maps had similar features, striking and complex hemispheric asymmetries were evident (Cacace et al, this vol). (Supported by NIH NS19891.)



NEURAL PLASTICITY IN ADULT ANIMALS V

348.1 PLASTICITY OF CHOLINERGIC MARKERS IN MOTOR NEURONS FOLLOWING AXOTOMY. J. Rosenfeld and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2182.

Neurochemical studies following lesions of peripheral nerves have documented changes in choline acetyltransferase (ChAT), acetylcholinesterase (AChE), and acetylcholine (ACh) within motor axons. Relatively few studies, however, have focused on alterations occurring in transmitter markers within perikarya of motor neurons. The present study addresses this issue. In rats, the sciatic nerve was crushed at the level of the lumbar plexus. Using a monoclonal antibody directed against ChAT in immunocytochemical methods, we observed changes in perikaryal immunoreactivity at various times after axotomy. Recently developed computer imaging allowed us to quantitate differences in cell size and immunostaining intensity at the cellular level. These results were correlated with changes in ChAT enzymatic activity and cholinergic receptor binding (measured by in vitro receptor autoradiography). At three days following axotomy, levels of ChAT immunoreactivity were reduced in motor neurons ipsilateral to the lesion. Between 3-6 days following axotomy, ChAT enzymatic activity in the ipsilateral ventral horn was reduced. By six days, the difference in staining intensity, compared with contralateral motor neurons, was more prominent, and the reduction in immunoreactivity was greatest 14 days following axotomy. By ten weeks, ChAT immunoreactivity in the most affected motor neurons returned to normal, and ChAT activity in the ipsilateral ventral horn was not significantly different from the contralateral ventral horn. Changes in receptor binding were dependent on the receptor subtype and on the ligand used to visualize binding sites. Three days following axotomy, high-affinity cholinergic receptor (M2) binding, visualized with [^{3}H] N-methyl scopolamine and carbachol, was significantly reduced in the ipsilateral ventral horn. The binding remained lower than control values through 14 days. High-affinity M1 cholinergic receptor binding, visualized with [^{3}H] pirenzepine, was not affected as severely, but some reduction of binding was observed by 14 days following the lesion. By ten weeks following axotomy, preliminary observations suggest that receptor binding returns to normal. Peripheral axotomy is the simplest model of neuronal injury and provides a direct means for studying changes in transmitter markers resulting from axonal injury. Several more complex degenerative diseases, e.g., amyotrophic lateral sclerosis and Alzheimer's disease, involve abnormalities of cholinergic neurons. Studies of simpler systems, e.g., axotomy of spinal motor neurons, may provide an advantageous model for understanding some of the processes underlying degeneration and regeneration of cholinergic neurons.

348.2 RECOVERY OF RAT HIPPOCAMPAL MONOAMINERGIC & CHOLINERGIC MARKERS FOLLOWING DORSAL INPUT LESIONS. U. E. Gasser* and A. R. Dravid (Spon. M. Karobath). Preclinical Res. Sandoz Ltd., Basel, Switzerland.

We recently reported that in 8 weeks after partial lesions of fimbria in adult rats, marked but incomplete homotypic recovery of hippocampal cholinergic system occurs (Brain Res. 324:119, 1984). Spared axons in the dorsal pathway are essential for this recovery, and sympathetic invasion of the hippocampus does not compete with it (Brain. Res. 367: 368, 1986). In view of aminergic degeneration after such lesions, we examined recovery of cholinergic enzymes (ACh), and uptake of noradrenaline (NA) and serotonin (5-HT) in septal (S), medial, and temporal (T) hippocampal regions in the same rat after partial (medial, lateral) or total lesion of the dorsal pathway.

The results indicate that: (1) The % distribution of NA,5-HT and ACH fibers in medial & lateral (M:L) parts of dorsal input was: 80:20, 60:40, and 50:50, respectively. (2) Major terminal fields of M & L fibers were S & T hippocampal fields, respectively. However, this pattern was least marked for 5-HT, pronounced for ACh and exclusive for NA. (3) Recovery after M or L lesion: ACh: complete at 8 wks in S & T but incomplete in medial region even at 6 Mo. NA: complete at 8 wks after L-lesion, partial after M-lesion even at 6 Mo. 5-HT: negligible after either lesion. (4) Recovery after total lesion: ACH: only in T region, apparent at 8 wks, improvement at 6 Mo but still incomplete. NA: complete in medial & T regions at 6 Mo, marked but incomplete in S region at 8 wks with no improvement at 6 Mo. 5-HT: none. (5) Uni- vs. bilateral lesions reveal contralateral contribution to recovery of NA. (6) Expts. $\pm$ SCG indicate contribution of central and peripheral fibers towards recovery of NA. (7) Spared dorsal or intact ventral fibers contribute to recovery of ACh and NA systems depending on lesion-type and hippocampal region. (8) The 5-HT system exhibits poor recovery although intact fibers are present.

The functional relevance of postlesion homotypic reinnervation has been previously indicated. The present model is thus suitable to search for means to enhance such reconstruction and hence the possible recovery of function after brain injury.

348.3 LESION-INDUCED DOPAMINERGIC SYNAPSE PLASTICITY IS ALTERED IN THE OLFACTORY TUBERCLE OF ADULT RAT EXPOSED TO ETHANOL IN UTERO. Z. Gottesfeld, R.B. Lingham*, C.J. Garcia* and R.B. Chronister*. Dept. of Neurobiol. & Anat. and Dept. of Pharmacol., Univ. of Texas Med. Sch. Houston, TX 77025; Dept. of Anat., Univ. of S. Alabama, Mobile, AL 36688.

This work was designed to examine the lesion-induced plasticity of the dopaminergic (DA) synapse in the olfactory tubercles (OT) of adult rat exposed to ethanol in utero.

The experimental protocol consisted of three groups of weight-matched, timed-pregnant rats (n=15/group) receiving one of the following diet plans: (1) normal diet ad lib, (2) liquid diet containing 35% ethanol-derived calories on days 5-21 of gestation and (3) isocaloric diet (pair-fed). At birth, all pups were cross-fostered to normal dams and weaned at 22 days of age. After maturity, part of the offspring in each group were subject to bilateral olfactory bulb lesions and the remaining litter-mates were sham-operated (n=15/group). To allow for DA axon sprouting in the OT, all offspring were sacrificed 3 weeks post-operative (Gilad and Reis, Brain Res. 160:17, 1979). Biochemical and immunocytochemical techniques were used to examine components of the DA synapse in the partially deafferented OT. The presynaptic compartment of the DA innervation was identified by measuring tyrosine hydroxylase (TH) activity and amount, as well as fluorescence intensity of TH-immunoreactive axon terminals; this was quantitatively measured through a 35μ x 35μ window by computer-controlled microspectrofluorometer. The postsynaptic site was characterized by the activity of DA-sensitive adenylate cyclase (AC) (Krieger, Brain Res. 183:383, 1980; Lingham and Gottesfeld, J. Neurosci., in press).

The results indicate that (1) the lesion-induced increase in TH activity and number of molecules at 3 weeks post-operative is suppressed by prenatal exposure to ethanol; (2) the fluorescence intensity of TH-immunoreactive terminals in the control animals tends to be greater on the denervated left OT in comparison to the contralateral intact side; this difference is not apparent, however, in the ethanol-exposed rats; and (3) the lesion-induced increased activity of GTP-, NaF- and forskolin-stimulated AC is suppressed in the OT of prenatal alcoholics. However, the GTP- and GTP/DA-stimulated AC activity is not significantly affected.

It is concluded that consumption of ethanol during gestation suppresses lesion-induced plasticity of DA synaptic elements in the OT. These may have detrimental consequences on functional recovery following brain injury in the adult offspring.

Supported by USPHS grant AA06158 (ZG)

348.4 GLUCOCORTICOIDS AFFECT THE CONCENTRATION OF THE ASTROCYTE PROTEIN, GLIAL FIBRILLARY ACIDIC PROTEIN, IN THE INTACT AND TRIMETHYLTIN-DAMAGED CENTRAL NERVOUS SYSTEM. J.P. O'Callaghan and D.B. Miller. Neurotoxicology and Experimental Biology Divisions, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Damage to the CNS results in complex cellular responses, one of which is proliferation and hypertrophy of astrocytes. We have demonstrated that astrocyte reactivity can be characterized and quantified by immunohistochemistry and radioimmunoassay (RIA) of the astrocyte protein, glial fibrillary acidic protein (GFAP). Recent evidence suggests that the immune system may play a role in astrocytic response to injury (Billingsley et al. J. Immunopharmacol. 5:95, 1982). Adrenal glucocorticoids also may participate in this response either by suppressing immune function or by increasing metabolic vulnerability in steroid receptor enriched regions of the CNS (e.g. hippocampus: Sapolsky, J. Neurosci: 5:1228, 1985). To explore these possibilities we examined the effects of exogenous corticosterone (CORT) and adrenalectomy (ADX) on glial response to CNS injury. Trimethyltin (TMT), a neurotoxicant that preferentially damages neurons in limbic structures, was used to produce consistent destruction of hippocampal pyramidal neurons. RIA of GFAP was used to monitor astrocytic response, cytopathological effects of TMT were verified by light microscopy, and immune suppression was detected by alterations in thymus and spleen weights.

Male LE rats were treated daily with CORT (10 mg/rat) for 12 days or were adrenalectomized. Data were collected 4 weeks after initiating CORT treatment or ADX. CORT increased GFAP by 60% and 28% in hippocampus (HIP) and frontal cortex (FC), respectively; and ADX increased GFAP by 28% and 29% in HIP and FC, respectively. CORT suppressed thymus and spleen weights, whereas ADX increased the weights of these tissues. Neither condition caused overt neuronal damage to the HIP. Because excess CORT preferentially increased GFAP in a CORT-receptor enriched region, this effect may be mediated through a glucocorticoid-regulated mechanism. Because ADX also increased GFAP, but not in a region-selective manner, the adrenal gland may exert an inhibitory influence on GFAP that is not mediated through glucocorticoid action on the CNS.

When TMT (8.0 mg/kg,i.v.) was administered one week following the initiation of CORT treatment or ADX, GFAP was increased in HIP and FC in control, CORT-treated and ADX subjects. However, the increase in HIP and FC GFAP due to TMT was less in CORT-treated rats and greater in ADX rats. The extent of TMT-induced damage to HIP neurons was unaffected by CORT but was enhanced by ADX. Because CORT attenuated and ADX exacerbated the effects TMT on GFAP in both steroid-sensitive (HIP) and steroid-insensitive (FC) regions, it is unlikely that the neurotoxic effects of TMT are mediated through glucocorticoid-regulated processes in the CNS.

348.5 GLUCURONIC ACID IN EPENDYMINS: GLYCOPROTEINS OF GOLDFISH AND HUMAN CSF. V.E. Shashoua, F.B. Jungalwala, P.F. Daniel, D.K.H. Chou, and M.E. Moore*. McLean Hospital, Harvard Medical School, Belmont, MA 02178 and E.K. Shriver Center, Waltham, MA 02254

Monoclonal antibody HNK-1 recognizes the sulfated glucuronyl glycolipids (SGGL) present in the embryonic brain of rodent and human and also in adult peripheral nervous system of various species. The structure of the major SGGL component is 3-sulfated glucuronylparagloboside (Chou et al., BBRC 128:383, 1985). HNK-1 also binds to the carbohydrate portion of myelin-associated glycoprotein (MAG) and to several neural cell adhesion molecules, such as N-CAM, L1, L2 and J1 antigens. SGGL is absent in adult mammalian brain. We find, however, that adult goldfish brain and extracellular fluid (ECF) contain SGGL in the tetrasialoganglioside fraction extracted from the tissue and ECF. Immunoblots of thin-layer chromatograms clearly demonstrate the presence of the glycolipid.

In additional experiments, Western blots indicate that a number of glycoproteins present in the goldfish brain and ECF are HNK-1 positive. The most highly HNK-1 reactive compnents were the β and γ ependymins of the ECF. These glycoproteins have been implicated in synaptic changes associated with the process of learning and the modification of neural circuits (Shashoua, Cell. Molec. Neurobiol. 5:183, 1985). Treatment of the ECF glycoproteins with endoglycosidase F completely abolished their reactivity with HNK-1 antibody, suggesting that a common carbohydrate epitope may exist in these molecules. HNK-1-reactive glycoproteins were also found in mouse brain and ECF as well as in CSF of adult human brain.

The carbohydrate moiety of ependymin was found to be an N-linked glycan. Gas chromatographic analysis indicates its 10% carbohydrate content is composed of the following sugars: fucose, mannose, galactose, N-acetylglucosamine, glucuronic acid and N-acetylneuraminic acid. Mass spectrometry confirmed the presence of glucuronic acid in the molecule.

(Supported by NIA grant No. AG05675.)

348.6 MORTALITY AFTER ISCHEMIA IN GERBILS REDUCED BY GANGLIOSIDE TREATMENT. S.E. Karpiak, Y.S. Li* and S.P. Mahadik. Div. Neuroscience, NYS Psychiatric Inst., Depts. Psychiatry, and Biochem. & Molecular Biophys., Coll. of Phys. & Surg., Columbia U., NY, NY 10032.

We have found that GM1 ganglioside administration reduces cerebral edema following open-head injury (1). Although ganglioside treatment of animals with various CNS injuries results in long-term functional recovery that may be due to facilitated neuronal regeneration, it is becoming increasingly evident that gangliosides also exert acute effects on CNS injury processes. Focusing on the acute effects, we are studying the effectiveness of gangliosides in reducing CNS damage due to ischemia. Ischemia was induced in male Mongolian gerbils (80gr: N=295) by unilateral ligation of the right common carotid artery. Since most gerbils have an incomplete circle of Willis, with little or no communicating vascular system between the hemispheres, the ischemia is largely lateralized to one cerebral hemisphere in the majority of animals, allowing for comparisons between the occluded and non-occluded sides of the brain. The extent of ischemia induced by this procedure is substantial enough to result in approximately 50% mortality. We found that 48hrs after ligation, control gerbils (saline injected) showed 48.6% mortality, whereas gerbils treated (20mg/kg/day: FIDIA Research Labs) with either GM1 or AGF2 (an internal ester derivative of GM1) had mortalities of 26.6% and 26.1% respectively. Ganglioside treatment thus resulted in a 48% decrease ($p<0.001$) in mortality. By comparing occluded with non-occluded sides of the brain (cortex & hippocampus) we found a loss of membrane Na,K-ATPase activity due to the ischemia in control animals. No differences were found between the hemispheres of ganglioside treated gerbils with ischemia. We hypothesize that gangliosides may be "protecting" membrane function as indicated by ATPase analyses. Gangliosides might be reducing the extent of local CNS damage at the time of injury (i.e. reduced cell loss, fiber degeneration, membrane failure). By limiting the extent of acute CNS tissue damage, conditions may be optimized for subsequent CNS regrowth and functional recovery.

[Supported in part by NSF[BNS8500296].

1. Karpiak and Mahadik. J. Neurosci Res 12:484-492[1984].

348.7 THE NMDA RECEPTOR ANTAGONIST APV BLOCKS LONG-TERM POTENTIATION (LTP), BUT NOT NOREPINEPHRINE-INDUCED LONG-LASTING POTENTIATION, IN RAT DENTATE GYRUS. J.M. Sarvey and G. Decker*. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

LTP is a long-lasting increase in the amplitude of the population spike which can be induced in hippocampus or dentate gyrus by a brief train of high-frequency afferent stimulation. We have previously shown that, in dentate, bath application of norepinephrine also induces a potentiation that persists for hours after wash-out of norepinephrine (Stanton & Sarvey, Brain Res 361:276, 1985). To further compare these two types of potentiation, we examined their susceptibilities to APV, which is an N-methyl-D-aspartate (NMDA) excitatory amino acid receptor antagonist that has been reported to block LTP in field CA1.

Population spikes were recorded in the granule cell layer of dentate gyrus of rat hippocampal slices. A high-frequency train (100Hz X 2sec) to the perforant path reliably induced LTP, measured 30 min after the train. (-)-Norepinephrine (50μM), bath-applied for 30 min, potentiated the population spike during norepinephrine application and after 30 min wash with drug-free solution. Potentiation was defined as a significant increase in spike amplitude ($P<0.05$). In LTP experiments, D-(-)-APV was bath-applied for 15 min before, and washed out immediately after, the high-frequency train. In norepinephrine experiments, exposure to APV began 15 min before and continued during and for 10 min after norepinephrine application.

D-(-)-APV (50 or 100μM) reversibly depressed the population spike 10 to 35%. Nevertheless, these concentrations of APV had no effect on norepinephrine-induced potentiation. APV did, however, completely block LTP.

We conclude that (1) NMDA receptors are involved in normal synaptic transmission in the dentate, and (2) activation of NMDA receptors is required for induction of LTP, but not NELLP, in the dentate. Therefore, it would appear that either (1) the mechanisms underlying LTP and NELLP are different, or (2) the final mechanism of LTP and NELLP is the same, but norepinephrine initiates potentiation by acting at a later, APV-insensitive, step in the sequence of events required for potentiation. (Supported by USUHS Grant No. C07514.)

348.8 SYSTEMIC INJECTIONS OF GM1 GANGLIOSIDES AND d-AMPHETAMINE REDUCE SPATIAL LEARNING DEFICITS CAUSED BY BILATERAL DAMAGE TO THE CAUDATE NUCLEUS. G.L. Dunbar*, M. DeAngelis*, S. Hecht*, S. Merbaum*, and D. G. Stein. (SPON: L. Kennedy). Brain Research Laboratory, Clark University, Worcester, MA 01610 USA.

Previous work in our laboratory (Sabel, B. A. et. al. *Science*, 225:340) demonstrated how GM1 treatments could reduce spatial reversal learning deficits in rats with bilateral lesions of the caudate nucleus (CN). The present study replicated and expanded these findings by analyzing the time-course of behavioral recovery and directly comparing the efficacy of GM1 ganglioside- and d-amphetamine-induced recovery of function.

Three groups of male albino rats were given bilateral CN lesions and received daily IP injections for 14 days, beginning immediately after surgery: group L (n=8) was given saline, group LA (n=9) was given 2 mg/kg d-amphetamine, and group LG (n=9) was given 30 mg/kg GM1 gangliosides. A fourth group, group C (n=8), received sham surgery and daily IP injections of saline.

Using essentially the same testing parameters as in our previous study (Sabel, B. A. et. al. *Science*, 225:340), each group was tested on a series of spatial reversals for 30 days. Analysis of the total number of reversals indicated that LG animals made significantly more reversals than LC animals. They performed at a level which was statistically indistinguishable from the sham-operated controls. The amphetamine treated animals performed at an intermediate level, with the total number of reversals being statistically indistinguishable from either group LC or group C. Time-course analyses indicated a significant ganglioside effect as early as the first week of testing, whereas amphetamine treated animals showed significant impairment relative to the sham-operated controls during the first week. However, the LA animals showed significant improvement when compared to the LC group during the second week of testing.

These data can be taken to suggest that perhaps two or more different mechanisms may underlie ganglioside and amphetamine-induced functional recovery. Gangliosides may operate by 'rescuing' neurons that would ordinarily die as a result of the injury. These rescued neurons (as well as remaining, intact cells) may then be stimulated by the amphetamine treatments to release more dopamine in the zone of injury. We are currently beginning to examine this hypothesis in more detail.

We are grateful for the support of Fidia Research Laboratories, Abano Terme, Italy in the conduct of this research.

348.9 SOCIAL ENVIRONMENT MODULATES THE DEVELOPMENT OF A FOREBRAIN PEPTIDERGIC NUCLEUS IN THE CICHLID FISH HAPLOCHROMIS BURTONI. M.R. Davis*, and R.D. Fernald (SPON: R. Lane). Institute of Neuroscience, Univ. of Oregon, Eugene, OR. 97403.

For many vertebrate species, social rank is known to affect both gonadal maturation and onset of reproductive behavior. In the African cichlid fish, H. burtoni, young adult males (age: 14 weeks) who are placed in social environments free from the presence of larger, threatening conspecifics ("group A") display precocious attainment of territoriality, social dominance, and reproductive competence. In contrast, male siblings who are forced to share tanks with larger, intimidating conspecifics ("group B") show a delay in development of adult body coloration, social dominance, and ability to spawn. Fish in group A show precocious maturation of testes, with gonadosomatic indices (GSI=(testes wt/body wt x 100)) of about 0.74, while group B males have GSIs of from 0.2 to 0.4.

In this animal, social environment also modulates cell growth and the expression of LHRH-like immunoreactivity (irLHRH) in a forebrain nucleus, the anterior magnocellular preoptic nucleus (aMPn). This result is of interest because the irLHRH forebrain axis, a phylogenetically ancient system found in most vertebrate classes, is already implicated in some aspects of reproductive competence. The rostral irLHRH projections, extending centripetally from the nucleus of the terminal nerve, through the aMPn, and into the hypothalamus (Ht), are implicated in the transduction of sex-pheromone perception in goldfish. In addition, forebrain magnocellular neurons are known to play a role in the modulation of pituitary output of luteinizing hormone (mammals) or gonadotropin (fishes). The aMPn may be one of the nuclei involved in this control. Pituitary hormones in turn modulate the differential maturation of testes described above.

Three criteria are used to assess the extent of aMPn maturation: number of cells exceeding 15 micrometers (um.) in soma size, number of cells showing a multipolar pattern of proximal neurites, and outgrowth of projections to the Ht.

Group B animals at age 6½ months (N=3) show about 15% mature neurons, as scored by pattern of proximal neurites, and soma size greater than 15 um.. In precocious (group A) fish of comparable age and size (N=3), however, large multipolar neurons make up about 30% of the aMPn cell population. Correlating with this difference in cell size and morphology is an apparent doubling of aMPn projections to the Ht in precociously maturing males.

At age 5 months (N=2 animals for each group), group A males show 20% and 30% mature aMPn neurons, and rudimentary fibre staining, while group B fish show 0% to 7% mature cells, and no stained fibres. Supported by NIH Training Grant 07257 and NIH EY05051-02.

348.10 HOUSING COMPLEXITY AFFECTS BODY AND ADRENAL WEIGHT OF ADULT RATS WITHIN 10 DAYS EXPOSURE C.S.Wallace,* J.E.Black, H.M.Hwang & W.T.Greenough School of Medicine, Depts of Psychology & Anatomical Sciences, Neural & Behavioral Biology Program, University of Illinois, Urbana, IL 61801 (SPON: P.V.Johnston)

Geller, et al. (J Neurochem, 12, 1965) and Uphouse & Brown (Dev Psychobiol, 14, 1981) have both reported that young rats reared in complex environments (EC) had lighter adrenals and livers than rats reared in isolation (IC). In order to determine if these trends hold for adult rats, body and endocrine organ weights were obtained in conjunction with a study of eighty-two male 90-day-old rats. They were housed in either a complex environment (EC), a large cage with with toys and other rats; a social condition (SC), paired with another rat in an empty cage; or an isolated condition (IC), housed individually in an empty cage. After 10 days, 24 rats were perfused in preparation for a TEM examination of brain tissue. Weights for total body, adrenals, and liver were also obtained. After 30 days exposure, another 26 rats were similarly perfused and weighed. The remaining rats from EC and IC were then assigned to four groups: EC-EC, in EC for 60 days; EC-IC, in EC for 30 days and then in IC for 30 days; IC-IC, in IC for 60 days; and IC-EC, in IC for 30 days and then in EC for 30 days. These "reversal" animals were similarly perfused and weighed at the end of the 60 days. Group differences were analyzed separately for the 10-day, 30-day, and 60-day conditions. The 10-day, 30-day, and 60-day animals each had main effects of housing complexity for change in body weight, adrenal weight, and adrenal/body ratio, but no significant effects were found for liver weight or liver/body ratio.

The present findings of heavier adrenals and no change in liver weight among adult EC rats is in sharp contrast to the changes observed in young EC rats, suggesting that a substantial developmental change occurs in these endocrine organs' sensitivity to experience. In addition, the change in adrenal weight occurs in less than 10 days exposure in adult rats, and the change appears to have persisted for 30 days in the EC rats reversed to the IC condition. Since the peripheral endocrine effects of a complex environment are opposite in development and adulthood, whereas the effects on cerebral cortical neuronal morphology are similar, it seems unlikely that endocrine changes are responsible for these brain changes.

MEAN ± S.E. ADRENAL WEIGHT (mg)

Exposure	EC	SC	IC	EC-IC	IC-EC
10 days	31±1	26±1	24±1	-	-
30 days	30±1	24±1	22±1	-	-
60 days	28±1	-	23±2	27±1	29±2

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348.11 MULTIVARIATE ANALYSES OF MORPHOLOGICAL MEASUREMENTS CAN DISTINGUISH AMONG RATS REARED IN COMPLEX, SOCIAL OR INDIVIDUAL ENVIRONMENTS. Anita M. Sirevaag and William T. Greenough. Depts. Psych. & Anat. Sci. & Neural & Behav. Biol. Prog., Univ. Illinois, Urbana-Champaign, IL 61820.

Studies of occipital cortex have compared morphological measures from rats reared in complex environments (EC) with social (SC) or individual environments (IC). Univariate analyses had shown that EC had a lower density of larger neurons with more axon and dendrite/neuron and more synapses/ neuron and that those synapses were larger, had more postsynaptic thickening (PST) and larger presynaptic boutons than IC. EC had a greater volume fraction (Vv) of glia and capillaries and fewer neurons, synapses and glia per capillary Vv and larger capillaries that were closer together. EC also had fewer neurons and synapses/glia Vv. On most variables SC had intermediate values. Multivariate analyses of morphological variables allow us to distinguish among groups and indicate relationships not evident in univariate analysis. Stepwise discriminant analysis presented 8 variables that accounted for 45% of the variance among environments ($p<.01$). Canonical discriminant analysis determined 2 canonical variates (linear combinations of the variables) that reliably assigned animals into groups ($p<.01$). The 1st incorporated synapse and capillary related measures, the 2nd synapse/neuron and glial measures. Factor analysis identified 3 factors that accounted for 46% of variance across groups. The 1st factor incorporated size of capillaries and glial measures, the 2nd synaptic structure and Vv of axon, the 3rd synapses/neuron, synapses/ capillary Vv, density of neurons and capillaries and distance between capillaries. ANOVA showed all environments differed from each other on factor 1, EC differed from SC and IC on factor 2, IC differed from EC and SC on factor 3 ($p<.01$).

Stepwise Discrim. Analysis		Canonical Discrim. Analysis	
variable	% variance	variate 1	variate 2
Vv capillaries	39	PST length	synapses/neruon
Vv astrocytes	24	syn. contact	size & Vv astro.
synapses/neuron	10	Vv capillaries	size & Vv oligo.
synapse cleft width	9	capillary density	
mean size of astro.	8	inter cap. distance	
capillary density	7	syn./vol. of cap.	

Factor Analysis

Factor1	Factor 2	Factor 3
Vv & size astro.	PST length	syn./vol. capillary
Vv & size oligo.	syn. contact len.	inter cap. distance
syn./vol. astro.	presyn. bouton size	synapses/neuron
syn./vol. oligo.	Vv axon	neuronal density
capillary diameter	Vv capillaries	capillary density

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348.12 PERSISTENCE OF VISUAL CORTEX DENDRITIC ALTERATIONS INDUCED BY POSTWEANING EXPOSURE TO A "SUPERENRICHED" ENVIRONMENT IN RATS. G. S. Withers, J. E. Camel* and W. T. Greenough. Depts. Psychol. and Anat. Sci. and Neur. & Behav. Biology Prog.. Univ. Illinois, Urbana-Champaign, IL 61820.

Quantitative light and electron microscopic investigations of rats reared in complex (EC) and isolated (IC) environments have indicated that there are both more dendritic material and more synapses per neuron in the visual cortex of the EC rats. The present study investigates the relative permanence of the dendritic changes in rats reared for 30 days post-weaning in a "superenriched" environment (Kuenzle and Knüsel, Physiol. Behav.. 1974) then subsequently housed for 30 days in individual cages. The "superenriched" environment (SEC) consisted of two large cages filled with toys connected by a 4-level clear Plexiglas maze complex with interchangeable barriers. The rats were required to solve increasingly difficult maze problems for access to food and water. Four groups of weaning age (23-25 d) male Long-Evans hooded rats, using a split-litter design, were run: SEC - 30 days in the "superenriched" environment; IC - 30 days in individual cages; SEC/IC - 30 days in the "superenriched" environment followed by 30 days in individual cages; IC/IC - 60 days in individual cages. We quantified dendritic fields of layer IV aspinous nonpyramidal neurons and basilar branches of layer III pyramidal cells in area 17 of the visual cortex, by counting the number of intersections between dendrites and a series of spheres at 10 micron intervals around the soma, and by measuring the length and number of branches. For overall dendritic field size measures (total ring intersections, total dendritic length, total number of branches) in both cell populations there were substantial main effects of initial environment (EC > IC), lesser effects of age of sacrifice and no interactions. Thus the general result is for the effects of initial exposure to the "superenriched" environment to persist through a subsequent period of individual housing. However, for some measures interactions were present, indicating less than total persistence of the early experience effects.

	SEC	IC	SEC/IC	IC/IC
NONPYRAMIDAL CELLS				
Ring Int's/Neur.	81.1	77.1	79.9	64.9
Total Branches	24.2	23.3	22.4	20.6
Total Length (μm)	953.8	905.3	931.0	771.9
PYRAMIDAL CELLS				
Ring Int's/Neur.	126.3	106.9	115.1	105.7
Total Branches	39.0	37.6	38.7	34.6
Total Length (μm)	1435.4	1242.5	1301.6	1193.8

Supported by NIMH 35321 and the Retirement Research Foundation.

348.13 SYNAPTIC PLASTICITY IN ADULT RAT OCCIPITAL CORTEX FOLLOWING SHORT-TERM, LONG-TERM, AND REVERSAL OF, DIFFERENTIAL HOUSING ENVIRONMENT COMPLEXITY. B.-M. Hwang & W. T. Greenough Neural & Behavioral Biology Program, and Departments of Psychology and Anatomical Science, University of Illinois, Champaign IL 61820

Animals exposed to a complex environment, such as a large wire cage filled with various toys, learn mazes more rapidly than rats reared in laboratory cages. Relative to rats reared individually or socially in standard cages, the occipital cortex of these animals is heavier and thicker with more dendritic material and more synapses per neuron as well as larger synapses in some layers. Synaptic studies have been performed only on young rats, i.e., those reared differently for 30 days after weaning at 23-25 days of age, and it is of interest to determine whether equivalent effects occur in mature animals. If they occur in adult brain, it is of interest to determine how rapidly they can be detected and how long they are maintained in the absence of differential housing. To answer these questions, we examined synaptic alterations in occipital cortex of adult hooded rats at various stages of differential housing. 120 day old Long Evans hooded rats were assigned to three different housing conditions: 33 grouped in large toy-filled cages with a daily change of toy arrangement and opportunity for play experience in an "open-field" also with a daily variation in toy arrangement for one hour per day (environmental complexity, EC), 16 in pairs (social condition, SC), 33 in individual cages (IC). After 10 days, one-fourth of the EC and IC animals and half of the SC animals were used to examine the short-term effect of differential housing. After 30 days, another fourth of the ECs and ICs and the remaining SCs were used to examine the long-term effect. Half of the remaining ECs and ICs were reversed in housing conditions, i.e., from EC to IC or from IC to EC. Another half of the ECs and ICs remained in the originally assigned conditions. After another 30 days, these animals were used to examine the persistence or reversibility of the effects of differential housing. It was found that ECs had more synapses in layer IV of occipital cortex than SCs and ICs even after just 10 days of housing. The increase in synapse numbers due to 30 days of differential housing was largely maintained for at least 30 days following removal from the EC condition. Detailed analysis of other changes in synaptic structure, such as synaptic size, curvature, and distribution of polyribosomal aggregates associated with dendritic spines, which is thought to be a possible indicant of synaptogenesis, is in progress.

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348.14 EFFECTS OF ENVIRONMENTAL COMPLEXITY AND FEEDING REGIMEN ON BRAIN MORPHOLOGY AND BEHAVIOR IN MICE. E. Bresnahan, N. Muth*, M. Morucci*, J. Freeman*, AND D. Ingram*. Essex Comm. College, Baltimore MD 21237 and Gerontology Research Center, NIA, Baltimore MD 21224

Measures of cerebral cortical area and exploratory activity were conducted on 3-4 mo old male C57BL/6J mice obtained from a study designed to assess behavioral, anatomical, and physiological effects of differential environmental treatments (housing condition and diet) across the lifespan. At 5 wk of age, 256 mice were introduced to one of four housing conditions: enriched (E), social (S), conventional (C), or isolated (I). Within each housing condition, groups were assigned to one of two diets: ad libitum (AL) or diet restriction by intermittent feeding 3 days/wk (RES). For both E & S conditions, 32 mice were free to move throughout 3 large interconnected cages (44x28x17 cm). For E groups, objects offering sensory, motor, and learning stimulation were systematically changed 3 times/wk. C groups were housed 4 per standard-sized plastic cage (28x17x13 cm); whereas, I groups were housed 1 per cage. Area measurements, taken from thionin-stained posterior cortical sections, were analyzed by ANOVA. Results indicated significant main effects of environment ($p's < 0.01$) and diet ($p's < 0.05$) with little interaction. Area measurements in the somatosensory/auditory/visual cortical regions for E were 5-10% higher than those observed in other conditions. In several regions, area measures for AL were 3-6% higher than RES. These morphological measurements are consistent with the pattern of body weight growth in that RES mice weighed less than AL mice. Environment had little effect on body weight. Regarding behavioral effects as measured by ambulation in an open-field for 7 min, only the environmental conditions, not diet, had a significant effect with E mice the most active. Thus, current assessment of these data indicate that the environmental treatment replicated past findings suggesting acceleration of cortical development; whereas, dietary restriction could retard cortical development. Further assessment of the additive and interactive effects of these treatments across the lifespan will be conducted.

348.15 PERIPHERAL INJURY INDUCES MOTOR CORTEX REORGANIZATION IN ADULT RATS S. Suner*, J. P. Donoghue, B. L. Schlaggar* and J. N. Sanes (SPON: V.A. Jennings). Center for Neural Science, Brown University, Providence, RI and Human Motor Control Section, NINCDS, Bethesda, MD.

Peripheral nerve injury in neonatal animals alters the somatotopic organization of MI (Donoghue and Sanes, in press). The present studies were designed to determine (1) the extent of MI reorganization in adults and (2) the effect of motor nerve lesion on MI somatotopy.

In one group of adult rats, the right forelimb (FL) was amputated at the shoulder joint. After a survival period of at least 1 month, the organization of MI was mapped during ketamine anesthesia using intracortical microstimulation (ICMS) techniques. Microelectrode penetrations were spaced 100-500 μm apart throughout the presumptive MI FL and adjacent areas. Current intensities up to 60 μA were delivered 1.8 mm below the pial surface. Control maps were obtained from normal adult rats.

In normal rats the size and location of the MI FL representation were similar to those described in previous studies. ICMS at less than 35 μA most commonly evoked wrist extension or elbow flexion in spatially separate rostral and caudal zones of MI. Proximal FL movements were observed only by increasing levels of ICMS at some distal FL sites and were typically found in the caudal zone. In all amputated rats, ICMS in the caudal region of the presumptive FL representation produced discrete movements about the shoulder or scapula at lower thresholds than in controls. Large zones where ICMS was ineffective were not evident. The MI region in which ICMS produced proximal movements overlapped the normal caudal FL representation, but was smaller than the usual FL area. Within MI, the remaining portions of the presumptive FL zone contained representations of the vibrissa and neck, which are not normally represented in this area.

Amputation removes peripheral afferent and efferent pathways of MI; thus it is unclear whether damage to a motor nerve alone is sufficient to induce MI reorganization. In a separate group of rats, the branches of the VIIth nerve that innervate the vibrissa were transected, eliminating the motor connection to the vibrissa without interrupting the sensory pathways. After 8-35 days, MI was mapped. At threshold currents comparable to those in the normal rat, ICMS In the presumptive vibrissa region evoked movements of the distal forelimb, neck, eye and facial muscles surrounding the eye. The MI region representing the eye and facial muscles around the eye more than doubled in size after VIIth nerve transection. Additionally, ICMS in experimental rats evoked eye or periorbital movements in conjunction with movements of the forelimb, trunk or hindlimb (15 sites in 4 rats), but not in normal animals.

From these data we conclude that peripheral injury induces reorganization of the MI somatotopy in adult mammals. Futhermore, transection of a motor nerve is sufficient to change MI output organization. These data suggest a general principle of modifiability of cortical output pathways. (Supported by NIH grant #NS22517 and ONR contract #N000081-K-0136.)

348.16 EFFECTS OF ASSISTANCE, PRACTICE AND LEARNING RATE ON RECOVERY FROM SENSORIMOTOR CORTEX LESIONS IN RATS. J.Stephens (SPON: J.J.O'Neil). Dept of Phys. Therapy, Moss Rehabilitation Hospital Philadelphia, PA 19141.

Many animal models are currently under development to study the processes and mechanisms of recovery from brain injury. The rat model is particularly attractive because its neurochemistry and pharmacology have been widely studied and experiments can be done with multiple animals relatively quickly. This study has been done to test whether or not several variables relevant to human rehabilitative physical therapy can be studied in the rat.

Male Sprague-Dawley rats (250-300g) were kept water deprived, ½ hour/day, and individually housed for at least 4 days before being trained to run an elevated beam. Training consisted of 12 trials on a 1½" beam and 24 trials on a 1" beam. Animals were then given a right sensorimotor cortex ablation. Post-lesion, animals were given trials on the 1" beam on days 1, 2, 3 then every other day until day 17. Post-lesion animals were divided into 4 groups: I) 2 trials, no assistance; II) 6 trials, no assistance; III) 2trials, assistance; IV) 6 trials, assistance. Beam running was analysed from video tape for time to cross the beam (R) and accuracy of step placement (Q). Before the beam running task each day, animals were tested for two minutes on a modified open field.

Open field activity (total squares crossed) was depressed for 3 days post lesion but there were no significant differences between groups either during training or recovery. Recovery in the beam running task showed several main effects. Time (R) showed differences between groups based on level of assistance, $F(1,16)=8.15$, and level of practice, $F(1,16)=4.93$ with a significant interaction of assistance over time, $F(4,64)=2.6$. Foot placement (Q) showed no significant differences between groups but significant interactions of assistance over time, $F(9,144)=2.56$ and practice over time, $F(9,144)= 1.99$. (Q/R) showed a difference between groups based on practice, $F(1,16)=4.61$ and a significant interaction of assistance x practice over time, $F(9,144)=2.45$. Across all groups there was a linear relation between time to reach a training criterion of (R) $<$ 2 sec. and time to reach a recovery criterion of (R) $<$ 3 sec. For groups with 2 trials/day $r=.65$ ($n=16$). For groups with 6 trials/day, $r=.63$ ($n=12$). There was also a trend, not significant, toward lower number of trials to criterion in beam running and higher activity scores on the open field.

These results suggest that recovery from sensorimotor cortex injury is facilitated by higher levels of assistance and practice during the recovery period and associated with higher rates of learning and exploratory behavior during the training period.

348.17 Behavioral Consequences of Photochemically Induced Cortical Whisker Barrel-Field Infarction. B.E. Hurwitz*,W.D. Dietrich,P.M. McCabe,B.D. Watson*and N. Schneiderman. (SPON:M.Gellman). Depts. of Psych. and Neurol., Univ. of Miami, Coral Gables, FL 33124.

Electrolytic ablation of the cortical whisker barrel-fields and removal of the vibrissae have been reported to induce disruption of several complex behaviors such as locomotion and discrimination of surfaces. The ablative lesions in these studies, however, extended into other somatosensory regions. We have used a photochemical reaction in vivo to induce thrombosis leading to focal cerebral infarction (CI) in the primary cortical whisker barrel-fields of rats. This procedure has been found to produce the main pathological features of human stroke (cf. Watson, B.D. et al., Annals Neurol., 17:497, 1985).

The present study was undertaken to determine in adult rats whether a behavioral task in the dark requiring vibrissal palpation would be disrupted after sham, unilateral or bilateral photochemical barrel-field infarction. Food-deprived adult male rats (N=14) were trained to cross a gap between two elevated platforms. During the baseline sessions, the distance between the platforms was systematically increased every 3-5 trials and otherwise was randomly varied between trials. Usually 50-80 trials were given until a distance was established that the animal would not cross. In addition, on each trial the latency to cross to the other platform was measured. Post-CI testing was then performed over the following 50 days. The bilateral or unilateral CI groups did not differ from sham controls in percentage of trials crossed prior to CI. However, from the fourth day after CI, the bilateral CI rats displayed up to a 30% decrement in the percentage of trials crossed at distances crossed before infarction. This deficit persisted throughout the 50-day testing period. Conversely, unilateral and sham animals did not display any deficit, but in fact a 15-20% increase was observed in the percentage of trials crossed. Furthermore, the bilateral CI rats exhibited a 14 sec increase in the latency to cross; whereas the sham and unilateral rats respectively displayed a 17 and 21 sec reduction in the latency to cross.

The results demonstrated that as a consequence of bilateral barrel-field infarction, rats crossed shorter distances and hesitated longer before crossing than they did prior to infarction, presumably resulting from a vibrissal deficit in the ability to locate the surface of the other platform. However, rats with unilateral CI were able to compensate for the lack of right barrel-fields apparently by using the intact vibrissae circuit and performed as well as sham controls. Supported by AHA (FA505f) and NINCDS (NS18749) research grants.

348.18 **CHANGES IN CELL DENSITY OF GAMMA-AMINOBUTYRIC ACID (GABA)-IMMUNOREACTIVE CELLS IN THE RAT KINDLING MODEL OF EPILEPSY.** **W.Kamphuis', W.J.Wadman*, R.M.Buijs[1]* and F.H.Lopes da Silva*. (SPON: K.S.WONG). University of Amsterdam; Dept of General Zoology. Kruislaan 320, 1098 SM Amsterdam, Netherlands. [1]Netherlands Institute for Brain Research. Meibergdreef 33, 1105 AZ Amsterdam, Netherlands.**

Daily repeated electrical tetanic stimulation of limbic or other brain regions results in progressive epileptiform activity of increasing severity, culminating in generalized seizures. This phenomenon, referred to as "kindling", is persistent for a long period after induction and may be a permanent change. A preliminary study of changes in the fieldpotentials, elicitated by stimulation of Schaffer collateral/commissural fibers in CA1 region, shows that paired-pulse inhibition decreases in the course of kindling. This suggests an impairment of local inhibitory functions.

GABA is considered to be the major transmitter that mediates inhibitory processes in the hippocampus and it is generally accepted that an impairment of inhibitory synaptic transmission can lead to the occurrence of convulsive disorders. Whether the increase in seizure susceptibility in the kindling model of epilepsy is related to a decrease in GABA-ergic functions is not yet clear. Therefore we studied the distribution of GABA-immunoreactivity in two groups of kindled rats and in control animals using antibodies against GABA.

Rats were implanted with electrodes to allow daily tetanization of the Schaffer collateral/commissural fibers of the dorsal hippocampus and to record the elicited afterdischarge. After at least six generalized convulsions the kindling stimulations were ended. One group (I) of rats was fixed 24 h. after the last convulsion. A second group (II) had a rest period of 24 days followed by a short second stimulation period; resulting in three generalized seizures. The rats of this group were also fixed 24 h. after the last convulsion. Numerical cell densities (CD) of GABA-immunoreactive cellbodies were determined in the CA1 regions at the ipsilateral,stimulated side and at the homotopic contralateral side.

In group I mean CD at the ipsilateral side showed no significant difference compared to the control group, although the density of the immunolabelling was slightly decreased. At the contralateral side a significant increase of 22% was found. In group II however mean CD was significantly decreased at the ipsilateral side by 35%. For the contralateral side no difference was found.

These results indicate that kindling is paralleled by a long-term decrease in GABA-immunoreactivity. The increase in CD found at the contralateral side in group I suggests the possibility of adaptation of inhibitory mechanisms.

348.19 LOW INTENSITY 60-HZ MAGNETIC FIELDS AND SEIZURE DEVELOPMENT: EFFECT OF MAGNETIC FIELD PRE-EXPOSURE ON ELECTRICAL KINDLING IN RATS. K.-P. Ossenkopp, D.P. Cain, and S. Smith*. Department of Psychology, University of Western Ontario, London, Canada N6A 5C2.

Numerous reports of biological effects of magnetic fields have prompted concern about possible adverse effects of natural and man made sources of low intensity magnetic fields. Of special interest is the extremely low frequency band in the range of 50-60 Hz since this is the frequency of alternating currents used as sources of electrical energy by man. We previously reported that acute (1 h) exposure to low intensity 60-Hz magnetic fields reduced the incidence of lethal pentylenetetrazol-induced seizures in rats. In the present experiment we investigated the effects of acute pre-exposure to 60-Hz fields on the development of electrical kindling in rats.

The 60-Hz magnetic fields (1 gauss, linearly polarized) were produced by two large (100 cm diameter) Helmholtz coils. Bipolar electrodes were implanted in the basolateral amygdala of adult male hooded rats. Half of the animals were given daily 1 hour exposures to the 60-Hz magnetic field followed immediately by low level electrical brain stimulation (electrical kindling procedure) until electrographic and behavioral seizures developed. The other animals were given daily 1 hour exposures to a sham-magnetic field condition and also kindled. Measurement of the number of afterdischarges (AD) required to produce a generalized seizure indicated no significant difference between the sham and magnetic field exposed groups (means of 12.7 and 15.6, respectively). Durations of AD as a function of stimulation trials revealed a trend for shorter AD durations in the 60-Hz exposed group ($p = .084$). When each rat had developed a generalized seizure the field exposure condition on the following day was switched for the two groups and another seizure elicited. Measurements of AD durations following the cross-over design resulted in a significant ($p = .028$) suppression of AD durations when the rats were pre-exposed to the 60-Hz magnetic field. Thus, exposure to the low intensity magnetic fields resulted in suppression of some of the seizure inducing effects of electrical kindling in rats.

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348.20 KINDLING IN THE HILUS OF THE DENTATE GYRUS AND ITS EFFECTS ON FIELD POTENTIALS. G.M. Grace*, R.W. Skelton and M.E. Corcoran. Dept. of Psychology, Univ. of Victoria, Victoria, B.C. V8W 2Y2.

Despite the large number of studies investigating neural plasticity in the dentate gyrus (DG), there is surprisingly little information regarding the susceptibility of this region to kindling. The purpose of the present study was to characterize hilar kindling electrographically and behaviorally. Changes in synaptic excitability of the dentate granule cells were also monitored by recording field potentials evoked by stimulation of the perforant path (PP) and measuring the amplitudes of the population spikes.

Once daily for 60 days, male hooded rats received one sec of high-frequency stimulation in the left hilus, at an intensity sufficient to evoke afterdischarge (AD). On alternate days, evoked potentials were recorded from the hilus following stimulation of the PP with single 0.1 msec pulses at six current intensities. Field potentials were also monitored in control rats during the same period.

Despite the 60-day stimulation period, most rats developed only partial (stage-1 or stage-2) seizures (Partial group), although a few rats did develop generalized stage-5 seizures (Generalized group). Kindling was very unstable in that most rats displayed no AD on some stimulation days or regressed from a higher behavioral stage to a lower one. Some development in electrographic complexity was observed, but growth in AD duration was observed only in the Generalized group. Hilar stimulation resulted in an initial increase in population spike amplitudes, followed by a decrease to below control levels. By 60 days, amplitudes had partially recovered. The Generalized group showed a greater decrease in population spike amplitudes than the Partial group.

The two major findings in the present study are: First, hilar kindling possesses several characteristics that set it apart from typical limbic kindling, including low rate of kindling, marked instability of the seizures, and little or no growth of AD duration. Second, kindling in the hilus did not result in permanent potentiation of the PP-DG population spike. Rather, after an initial increase in population spike amplitudes, there was a large and lasting decrease.

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348.21 REPEATED I.V. NALOXONE INDUCES DOSE-RESPONSE KINDLING EFFECT AND SENSORY PRECIPITATION OF GENERALIZED SEIZURES IN CATS. F.Pellicer,* J.M. Calvo,* L. Rocha,* R. Fernández,* and A. Fernández-Guardiola. Inst. Mex. Psiquiatría and Fac. Psicología UNAM. Camino Xochimilco 101, cp 14370 México D.F.

There is a general agreement on the convulsant effects of high doses of naloxone in several animal species. (Snyder, E.W. et al., J. Pharmacol. Exp. Ther., 217:299, 1981). In a model of sensory precipitation of seizures (Papio Papio) naloxone reverses the photo mioclonic response depression induced by the ICV administration of synthetic endogenous opioids (Meldrum, B.S. et al. Brain Res., 170: 333, 1979). Also naloxone enhances post-tetanic potentiation and prevents habituation of sural nerve polysynaptic ventral root responses in the spinal cord (Fernández-Guardiola, A. et al. EEG and Clin. Neurophysiol. Suppl. 36: 257, 1982; Pellicer, F., Neurosci. Abs. 142.7, 1984).

As part of an ongoing investigation of the possible role of endogenous opioids in epileptogenesis, 21 cats (Encephale isolé) curarized and artificially ventilated were used. A group of animals (12) received naloxone-alone (2, 4, and 8 mg/Kg, I.V.) with a 15 min. interval. The other group received naloxone on the same schedule and dose and intermittent photic stimulation (IPS) after each administration. The electrical activity of both temporal lobe amygdalae, visual cortex and sensory-motor cortex was continuously monitored, evoked visual potentials were averaged and a power spectral analysis (PSA) was performed on line (data sampled at 320 point/sec, frequency range, 0-20 Hz). 2, 4 and 8 mg/Kg naloxone doses produced a noticeable enhancement of evoked potentials (EP) amplitude in the visual cortex during electrographic paroxismal activity. Also the EP progressively propagated, first to both amygdalae and after to the sensory-motor cortex. In this stage electrographic clonic-tonic generalized seizures appeared. The naloxone-alone treated group also displayed increasing paroxisms and generalized seizures with increased repetitive naloxone doses. The PSA results show that sensory precipitation of seizures occurs when the sensory-motor cortex is invaded by activity from other regions of different sensory input, as the visual or the limbic afferents. These progressive effects on epileptogenesis are kindling-like and could be due to enkephalinergic disinhibition or to GABA antagonism induced by naloxone, or to both mechanisms, as has been proposed by Hydén (Cupello, A. and Hydén., J. Neurosci. Res., 6: 579, 1981).

348.22 NONLINEAR SYSTEMS ANALYSIS OF KINDLED RABBIT HIPPOCAMPUS. G.B. Robinson, R.J. Sclabassi and T.W. Berger. Departments of Psychology, Psychiatry and Neurological Surgery, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Kindling refers to a progressive and relatively permanent increase in both the cellular and behavioral response to repeated epileptogenic electrical stimulation. In the dentate gyrus of the hippocampal formation, kindling increases the magnitude of inhibition, which could reduce the probability or severity of subsequent seizures by decreasing excitatory input to CA3, an area that is extremely epileptogenic. As a first step in the examination of this hypothesis, we used nonlinear systems analysis procedures to examine the development and duration of kindling-induced changes in perforant path-dentate synaptic transmission in the rabbit.

Kindling stimulation (2 s, 60 Hz train of 1.0 ms impulses) was applied daily to the perforant path until 3 consecutive stage 5 seizures were elicited. Prior to, during the development of, and following the last full motor seizure a random stimulus train (4064 impulses drawn from a Poisson distribution, λ=2 Hz) was applied to the perforant path and the amplitude of the dentate population spike in response to each impulse was measured. Cross-correlation techniques were used to compute first, second and third order kernels.

Kindling decreased the peak amplitude of the first order kernel, possibly reflecting an increased duration and magnitude of stimulation-evoked inhibitory effects. However, the peak of the first order kernel also shifted so that the majority of post-kindling responses occurred at a shorter latency. Second order kernels values approached zero (i.e., the response became linear) following the first afterdischarge, and as kindling progressed all second order interactions became inhibitory. Third order kernels also were altered by kindling. Before kindling, third order values were mainly inhibitory, i.e., the two preceding intervals resulted in a smaller population spike than would be predicted from the first and second order kernels. After kindling, however, third order values were mainly positive, i.e., the population spikes were larger than predicted. The kindling-induced changes in first, second and third order kernels were slow to develop and persisted for up to 20 weeks following the last motor seizure. Supported by The Whitaker Foundation, NIMH (MH00343) and a NSERC Postdoctoral Fellowship.

349.1 THE SURVIVAL OF INTRACEREBRAL XENOGRAFTS. K. Rao*, R.D. Lund, H.W. Kunz*° and T.J. Gill III*°, (SPON: W.Cameron), Dept. of Neurobiology, Anatomy and Cell Science, Center for Neuroscience, and °Dept. of Pathology, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

We have found that embryonic neural tissue survives for at least one year after transplantation to neonatal rat brains, but is rejected rapidly if placed in adult hosts.

We have also studied the effect of recipient age on the rejection process by transplanting dissociated embryonic cortical tissue from CD1 mice into neonatal Sprague-Dawley rats of different ages. We found that transplants survived at least 6 weeks when placed in 8 day old recipients but signs of rejection were evident within 1 week after transplantation in 12,16 and 20 day old recipients.

We have also examined whether rejection of such xenografts in the neonatal rat brain can be induced by placing CD1 mouse skin on the flank of recipients. We first grafted mouse retinae (E13-14) to the midbrain of neonatal Sprague-Dawley rats. Then, one month later, skin from adult CD1 mice was grafted onto the flank of some of these recipients. The rest of the recipients, which constituted the control group, received skin autografts. All the animals which had received skin xenografts rejected them. These were then sacrificed at 4 day intervals and frozen sections of the brain were stained and examined.

These sections, when compared to those of the control animals, showed a massive infiltration of lymphocytes and a progressive loss of tissue identity. A robust projection of axons from the transplant to the host tectum that was initially seen, degenerated as the rejection response progressed.

Thus, mouse xenografts placed in rat brains, before the recipients become immunologically competent, integrate well with the host neuropil, but are still susceptible to rejection if appropriate immunogens are presented outside the CNS.

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349.2 OBSERVATIONS ON THE BLOOD-NERVE BARRIER (BNB) OF SURVIVING NERVE ALLOGRAFTS IN RATS. Y. Kadota,* S. Wakai,* N.A. Azzam and A.A. Zalewski. Lab. of Neurochemistry, NINCDS, NIH, Bethesda, MD 20892 and Dept. of Anatomy (N.A.A.), Georgetown University, Washington, D.C. 20007.

Peroneal nerve grafts, 2 cm long, were taken from adult ACI rats and sutured to the cut ends of the peroneal nerve of immunologically deficient nude rats or normal Fischer rats that were immunosuppressed with cyclosporin-A (Cy-A). The purpose of this study was to investigate whether there were any acute or chronic changes in the BNB of a surviving nerve allograft that might affect host axonal regeneration through it. The permeability of the BNB was studied by using horseradish peroxidase (HRP) as a tracer, which was injected intravenously into a vein distant from the graft. At various postoperative intervals, the BNB was viewed by light and electron microscopy after the nerves were subjected to a histochemical reaction that reveals peroxidase activity. In normal nerves, the reaction product for HRP was seen in the lumen of endoneurial blood vessels which are known to be non-fenestrated and therefore impermeable to HRP. In contrast, the tracer leaked from fenestrated epineurial vessels, but its movement into the endoneurium was restricted by the inner layers of the perneurial sheath cells. As expected, all nerve allografts survived in nude rats or normal rats treated with Cy-A. In these allografts, the endoneurial blood vessels became markedly permeable to HRP at 1-4 weeks. Thereafter, a gradual decline in permeability occurred from 4-16 weeks, which was followed by recovery (i.e. impermeability) at 17-24 weeks. HRP passed from vessels through endothelial junctions that were tight in normal nerve. It is interesting that at all times in the allografts, as in normal nerve, the perineurium remained impermeable to HRP that leaked out of epineurial vessels. The permeability changes that occurred in the endoneurial vasculature did not inhibit host nerve fiber regeneration through the grafts, since longer-term grafts contained numerous remyelinated axons. Similar results were obtained with nerve autografts. We conclude that the vascular endoneurial permeability that occurs in surviving nerve allografts without (i.e., in nude rats) or with immunosuppression (i.e., in Cy-A treated rats) does not hinder host axonal regeneration through them or the ability of these fibers to reinnervate muscles of the peroneal nerve.

349.3 THIRD VENTRICULAR TRANSPLANT OF FETAL NEURAL TISSUE PREVENTS THE SEX-SPECIFIC SYSTOLIC HYPOTENSION PRODUCED BY NEONATAL MSG IN FEMALE RATS. R.W. Clough, P.A. Aravich*, C.D. Sladek and J.R. Sladek, Jr. Depts. of Neurobiology and Anatomy and Neurology, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

Monosodium glutamate (MSG), a neurotoxin to developing circumventricular organs, hypothalamic arcuate tissue, and portions of the medulla oblongata, produces several adenohypophyseal related endocrinopathies following neonatal exposure. Our previous studies have investigated the effects of neonatal MSG on neurohypophyseal function and blood pressure profiles in male and female rats. Concentrations of vasopressin in several brain areas and in serum during basal conditions are unaltered in MSG-treated animals. Basal water intake and urine output are unchanged and MSG-treated rats are able to concentrate urine effectively in the presence of water deprivation. Plasma osmolality and serum hematocrit are also not different in MSG-treated compared to control rats. These data indicate that the neurohypophyseal vasopressinergic system is resistent to the neurotoxic effects of neonatal MSG. In contrary, neonatal MSG-treated female rats exhibit a significant degree of systolic hypotension as adults ($p < 0.05$), but fail to show alteration in heart rates (Clough et al., Br. Res. Bull., in press). The present study investigates whether dopamine containing mediobasal hypothalamic (DA-MBH) transplants into the third ventricule of the hypothalamus (near the MSG-lesioned area) will prevent the development of systolic hypotension in MSG-treated female rats. At 28 days of age, MSG-treated (4 mg/g bw, days 1, 3, 5, 7, 9 postnatal) or control-treated rats were anesthesized and prepared for stereotaxic surgery. Cortical or DA-MBH transplants were taken from 16 day postconception fetal rats and placed into the third ventricle. Systolic blood pressures were recorded at approximately 3 months of age by tail cuff pleythsmography. Results are indicated in the following table:

GROUP (N)	SYSTOLIC BLOOD PRESSURE
Controls (10)	129.8 + (mm HG)
MSG (6)	107.0 + 6 *($p < 0.05$)
MSG-Hypothalamic transplants (12)	125.4 + 6
MSG-Cortical transplants (6)	124.6 + 3

MSG-treated female rats exhibit consistent systolic hypotension as adults. We have demonstrated that the development of systolic hypotension in MSG-treated female rats is effectively prevented by stereotaxic placement of neural transplants. Furthermore, these data indicate that both DA-MBH and cortical tissue are effective in this regard. Histological examination of the transplants is in progress.

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349.4 **Experimental Cerebral Ischemia, Tissue Damage, and Neuronal Transplantation.** S. D. Farber[1], S. H. Murphy[1], P.G. Wells[2]*, R. P. Vieric[2]*, J. Wells[2], and W.C. Low[1], [1]Dept. of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN 46223, and [2]Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

The 4 vessel occlusion (4 VO) model of ischemic cerebral vascular accident (CVA) developed by Pulsinelli and Brierley (Stroke, 10:267-272, 1979) was utilized to reliably produce infarcts of the hippocampus and striatum in adult male Wistar rats. Experiments were designed to: 1) characterize the histological and behavioral effects of CVA; 2) and examine the feasibility of using labeled neural transplants in age-matched unoperated controls and in this model of brain ischemia in order to identify the transplanted cells among those of the host.

Surgical preparation for stoke induction included anesthetizing animals, permanently occluding paravertebral arteries and loosly placing atraumatic clasps around each common carotid artery without disrupting blood flow. Animals were allowed to recover overnight from the surgery. After recovery, on day 1 post-surgery, carotid clasps were then tightened in the unanethetized rodent producing 4 VO of 30 minutes duration followed by removal of the clasps. Sham operated controls received anesthesia with exposure of the paravertebral and carotid arteries. All animals were tested for sensory orientation (adapted from Marshall and Teitelbaum, J. Comp. PhysiologyPsy., 86:375-395, 1974) and locomotor activity 14 days after CVA. Animals were subsequently killed and brains were processed for histological analysis.

Results of sensory tests demonstrated significant differences between CVA and sham animals in the areas of orientation to light touch and pressure, and in static equilibrium response. CVA animals showed marked reduction in orienting to both protopathic and epicritic stimuli and were not able to make postural adjustments to lateral displacement applied to the shoulders and hips. Smaller differences were noted during inverted suspension, visual tracking, righting and grasp reflexes with CVA animals demonstrating weaker or delayed reactions compared to controls. Histological analysis of CVA rodent brains revealed cell loss and marked atrophy of the CA1 pyramidal cell region of the hippocampus and dorsolateral striatum while sham controls demonstrated a normal cellular profile. These histological abnormalities correlated with observed behavioral alterations of sensory neglect and dysphagia and perseveration.

For transplantation studies, horseradish peroxidase (HRP) labeled cell suspension from embryonic rat hippocampus was implanted into the CA1 region of the hippocampal formation of age-matched unoperated controls. One week post-injection, animals were killed, brains were removed and processed for histological examination. HRP labeled pyramidal-like cells were found in clusters within the injection site and were clearly distinguishable from hippocampal pyramidal cells of the host. The incorporation of identifiable transplanted nerve cells into tissue damaged by brain ischemia may provide a means of reconstructing neuronal circuitries leading to a restoration of function.

349.5 GLIAL RESPONSE TO FETAL BRAIN CELLS TRANSPLANTED INTO PREVIOUSLY LESIONED HIPPOCAMPUS OF THE ADULT RAT.
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Transplantation of fetal raphe tissue into adult hippocampus has been shown to result in the formation of a glia scar (Azmitia and Whitaker, Neurosci., 38:145, 1983). In order to promote more complete integration of transplant and host tissues, we have attempted to limit the amount of scaring. From preliminary studies it appears that there are fewer glia fibrillary acidic protein (GFA) positive cells surrounding the second serial lesion than those surrounding the first serial lesion or the unilateral and bilateral lesions (Shemer et al., Abstract. Neurosci. Soc., 1984). Therefore, we decided to examine the glial response to fetal brain cells transplanted into the hippocampus of adult rats mechanically lesioned previously. We compared the glia responses in four experimental groups: 1) lesion alone 2) raphe transplant alone 3) lesion followed two weeks later by ipsilateral transplant, and 4) lesion followed two weeks later by contralateral transplant.

Minced mesencephalic raphe tissue from fetal rat (13-14 days of gestation) was injected through a glass pipette into hippocampus of Sprague-Dawley rats weighing 220-250 grams (coordinates: 4.5 mm anterior and 1.5 mm lateral to lambda and 3.7 mm below skull). A hypodermic needle was used to lesion the hippocampus using the coordinates 2.5 mm lateral to the transplant. Chlorapent (200 mg/kg) was administered as anesthetic. The animals were perfused two weeks after the transplant with 4% paraformaldehyde and 0.05% $MgSO_4$ in 0.1 M phosphate buffer (pH 7.2). The brains were sectioned for immunocytochemistry on a vibratome at 50 um. Primary antibody against GFA was used at 1/1000 dilution for 18 hours. The antibody was visualized with the peroxidase-antiperoxidase procedure using diaminobenzidine as substrate. In the lesion alone group, a glial response to the lesioned area was clearly seen. In the raphe transplant alone group we observed a marked glial scar surrounding the transplant. We observed clear evidence of less GFA positive cells surrounding the area of the transplant of serially lesioned brains, compared to those brains that received the transplant alone. We also report no noticeable differences in the glia response whether the serial lesion is carried out ipsilaterally or contralaterally.

It is interesting that both ipsilateral and contralateral pre-lesioning diminished the glial response to raphe transplant. It may be worth noting that the mechanical lesion resulted in reactive glial cells within the corpus callosum as it stretches from one side of the brain to the other. These results support the idea that glial migration plays an important role in the scar formation and may use established fiber pathways as a guide.

349.6 RELATIONSHIP OF ASTROCYTES AND TANYCYTES TO GONADOTROPIN RELEASING HORMONE (GnRH) AXONS GROWING FROM THIRD VENTRICULAR GRAFTS INTO HOST HYPOTHALAMUS. R. Silverman, M.J. Gibson+ and A.J. Silverman. Depts. of Anatony & Cell Biol, Columbia Univ, N.Y. and +Dept. Medicine, Div. Endocrinology, Mt Sinai Sch Med, N.Y. NY. 10029

Genetically hypogonadal (hpg) mice lack the neurohormone, GnRH and have, therefore, an infantile reproductive tract. Transplantation of normal fetal or neonatal preoptic area (POA) containing GnRH neurons into the III ventricle of hpg mice results in restoration of gonadal function. Varicose GnRH axons exit the transplant, arch laterally and ventrally through the arcuate nucleus to converge on the median eminence (ME), the normal neurosecretory terminal. Because these fibers grow toward their target in a directed and consistent way, it is of interest to identify those factor(s) which might mediate outgrowth, guidance or target recognition. To determine if glial architecture plays a role in these processes, we studied the distribution of astrocytes and tanycytes using GFA and vimentin immunoreactivity as markers, respectively. GnRH cells and processes were also identified immunocytochemically. In normal animals, some tanycytes radiate laterally from the ventricular wall arching ventrally towards the ME. Others form parallel arrays in the external zone of the ME. GnRH fibers and varicosities are seen interdigitating between the tancytes. Whether GnRH axons emerging from the transplants follow these processes is now under study. In both normal and hpg mice, the periventricular zone is rich in GFA positive elements. At rostral levels, long thin GFA processes arch ventrally toward the pial surface. In the ME, the internal zone is heavily invested with GFA elements. Both short term (5 day) and long term (>60 day) transplants have dense networks of astrocytes, far greater than that observed in the normal POA. GnRH neurons are situated within basketlike pockets of these astrocytic processes and GnRH fibers can be seen passing out of the glial mass, traversing the ventricular wall between ependymal cells. In the hypothalamic parenchyma into which these fibers grow there is also a substantial number of reactive astrocytic. GnRH fibers appear to grow close to their surfaces. Despite this increase in reactive astrocytes both in the hypothalamus and in the graft, there is no apparent barrier to fiber outgrowth from graft to host. The astrocytic response cannot, therefore, be considered a 'scarring' response. Instead glia may provide a needed scaffolding on which fibers can grow. Supported by USPHS NS 20335 and a Brookdale Fellowship (RS).

349.7 MONOAMINE NEURON SYSTEMS IN THE BRAIN OF THE SKATE. C.B. Jaeger and C. Nicholson. Dept. Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016.

It has been suggested that neurons which sythesize monoamine transmitters are organized primarily within and arround the central brain core (Nieuwenhuys, Chemoarchitecture of the Brain, Springer, '85) and this may represent a fundamental construction plan of the nervous system throughout the vertebrate phylum. Since little is known about the distribution of catecholamine neurons in cartilaginous fishes we studied this in the skate, Raja erinacea. Specific antisera to tyrosine hydroxylase (TH) were used for immunocytochemical identification of catecholaminergic neurons, which are known to contain this enzyme. In addition, serotonin (5HT) was localized in adjacent sections, to observe possible overlap of the two different monoaminergic neuron systems. Mappings were made in forebrain and brainstem rostral to the exiting fibers of of the third nerve.

Numerous dispersed TH positive cell groups were found in the region studied. Rostrally, a diffuse group of small TH-immunoreactive cells was seen in nucleus interstitialis commissurae anterioris and in nucleus 0. In the diencephalon six groups of catecholamine neurons were identified by their association with medial and laterally migrated.cell masses (Smeets et al: The CNS of Cartilaginous Fishes, Springer '83). A rostroventral group was localized in medial portions of the preoptic and suprachiasmatic nuclei. A dorsal midline cluster occurred ventral to the habenular nuclei. The largest midline group extended dorsal to nucleus medius hypothalami to the periventricular gray. This group segregated bilaterally into aggregates in the paracentral core, and at its dorsal aspect two laterally migrating TH-positive neuron clusters were evident. The mesencephalon contained two contiguous groups. Rostrally, a group of larger TH containing cells was localized in the lateroventral region of the mesencephalic tegmentum. Medial to it occurred the largest midbrain group, which distributed within nucleus interpeduncularis, pars dorsalis, and extended from the caudal diencephalon up to the level of the third nerve. At this level this latter group was adjacent to 5HT containing neurons which had a more caudal extend. Little overlap existed between 5HT and TH containing neuron groups. We conclude that the monoaminergic neurons of the skate are concentrated in a central core structure. In addition a substantial number of catecholamine containing neurons are localized laterally in aggregates that are contiguous with the central core. Parts of this study were undertaken at the Mount Desert Island Biological Laboratory. Supported by grants NS-19699 (C.J.) and NS-13742 (C.N.) from NINCDS.

349.8 IMPLANTS OF PC12 CELLS INTO RATS WITH UNILATERAL NIGROSTRIATAL LESIONS. G. Bing[1], M.F.D. Notter[1], C. Kellogg[2], and D.M. Gash[1]. Departments of Neurobiology and Anatomy[1] and Psychology[2], University of Rochester, Rochester, NY 14642.

The rat pheochromocytoma cell line PC12 has the capacity to synthesize, store, and release high levels of dopamine. PC12 cells can be induced to cease proliferation by mitomycin C, and/or nerve growth factor (NGF) treatment. We have found that treatment with NGF alone dramatically increases the levels of dopamine, norepinephrine, and serotonin in cultured PC12 cells as shown by high performance liquid chromatography.

	Undifferentiated PC-12 Cells	NGF-Differentiated PC-12 Cells
Dopamine	48.00 ng/mg protein	4328.60 ng/mg protein
DOPAC	0.705	11.41
Norepinephrine	5.851	30.06
Serotonin	2.600	40.51

In search of alternative sources of donor tissue for grafting purposes instead of embryonic brain, we have investigated PC12 cell implantation into rats with unilateral 6-OHDA nigro-striatal lesions. Both immunosuppressed and non-immunosuppressed rats have served as tissue recipients. Specific pathogen free young adult male Long Evans rats (Charles River Labs) have been used for the studies. Unilateral nigrostriatal lesions were produced by stereotaxically injecting 12 µg of 6-OHDA into the substantia nigra. Immunosuppression was induced by orally administered Cyclosporine A. All animals were tested for rotational preference with i.p. administration of D-amphetamine (5 mg/kg body weight) on day 7, 10, 13, 17, and 20 following transplantation.

PC12 cells were labelled with H^3-thymidine and then induced to differentiate by treatment with nerve growth factor plus mitomycin C, or by nerve growth factor alone. Differentiated PC12 cells were implanted into four different sites (40,000 cells/site) into the denervated caudate nucleus. Survivability of PC12 cells was determined by specific tyrosine hydroxylase immunoreactivity and H^3-thymidine autoradiography. Studies to date have found that grafted PC12 cells can survive and maintain an amitotic state for up to 2 weeks. Immunosuppression with cyclosporine A resulted in an apparently lower degree of lymphocyte penetration when compared to the animals in non-immunosuppressed groups as revealed by Nissl stain; this treatment may have also enhanced the viability of the transplanted cells. The rotational tests indicate that PC12 cells implanted into both dorsal and lateral caudate may diminish and partially reverse motor dysfunction brought about by unilateral 6-OHDA lesions.

Supported by NIH grant NS15109. We also wish to thank George Rusyniak, Robert Babkowski and John Lory for their assistance with this study.

349.9 **TRANSPLANTATION OF B16/C3 MELANOMA CELLS TO THE RAT AND MOUSE BRAIN.** W.J. Freed, R.W. Hargraves*#, U. Patel-Vaidya, H.E. Spoor*, A. Adinolfi##, J.D. Laskin###, H.M. Geller###. (SPON: S.M. Stine). Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032; #Department of Neurosurgery, Naval Hospital, Bethesda, Maryland; ##Department of Anatomy, UCLA, Los Angeles, California and ###UMDNJ-R.W Johnson Medical School, Piscataway, New Jersey.

B16/C3 melanoma cells are capable of production of both catecholamines and melanin in tissue culture (Laskin et al., J. Cellular Physiol. 113:481, 1982). The properties of these cells after transplantation into the brain were investigated.

Cells were transplanted by stereotaxic injection into the caudate-putamen of rat hosts with unilateral lesions of the substantia nigra. Normal Swiss-Webster and C57Bl mice received cell grafts by freehand injection into the head of the caudate-putamen under Chloropent anesthesia. Each animal received 10,000-15,000 cultured B16/C3 cells. Animals were examined after one day to several months.

In rat hosts, numerous cells positive for catecholamine fluorescence were seen at the injection site after one day. Many of these cells had processes. After one week, catecholaminergic cells were more numerous but were more rounded and found in clumps, as if undergoing division. After 2-3 weeks, melanin-containing cells were observed, and outnumbered the catecholaminergic cells in most animals. Some extracellular melanin was also present. No cells containing both melanin and catecholamines could be found. At six weeks and thereafter, few or no catecholamine-containing cells were found, and numerous round densely-pigmented cells were observed. Although pigmented cells were seen at the injection site for at least several months, their viablity at longer periods after transplantation has not been confirmed.

In mouse hosts, the B16/C3 cell grafts grew rapidly, leading to death of the hosts within seven to 28 days. Numerous pigmented cells were observed histologically. These cells had spread out from the injection site, invading surrounding areas of the brain and also forming metasteses in other parts of the brain and body. Growth of the tumor was similar in both mouse strains. The tumor did not appear to be circumscribed as was the case in rat host animals.

Thus, B16/C3 cells produce catecholamines for short periods of time after transplantation into the brain of rat host animals, but after 2-3 weeks catecholamine production ceases and only melanin is produced. Growth of the transplanted cells was restricted in rats, but in mice the tumor grew rapidly leading to death of the host. These data suggest that immunological mechanisms are involved in the restriction of B16/C3 melanoma growth in rat hosts. Additional electron microscopic studies and results of attempts to recover viable cells from the brain after transplantation will also be presented.

349.10 A MULTISYSTEM ANALYSIS OF DIFFERENTIATED NEUROBLASTOMA CELLS AS A SOURCE OF DONOR TISSUE FOR TRANSPLANTATION. J.H. Kordower, M.F.D. Notter, H.H. Yeh, and D.M. Gash. Dept. of Neurobiol. & Anatomy, Univ. of Rochester Sch. Med., Rochester N.Y. 14642.

The present series of experiments aimed to evaluate differentiated neuroblastoma cells as a potential source of cells for neural transplantation. In the first experiment, LA-N-2 cells, were differentiated with mitomycin C/Brdu treatment and placed in an *in vitro* coculture system with muscle cells. Intracellular recording from the muscle was made in the presence or absence of the LA-N-2 cells. The LA-N-2 cells innervated the muscle cells as early as 3h following coculture. Synaptic potentials were only observed when one or more LA-N-2 cells could be localized adjacent to, or on, the muscle cell being sampled thus demonstrating that differentiated neuroblastoma cells and muscle can form functional synapses.

In the second experiment, four groups of rats received electrolytic medial septal (MS) lesions. Seven days later, groups 1 and 2 received transplants of undifferentiated C1300 or LA-N-2 cells respectively into the dorsal hippocampus. Groups 3 and 4 received similar transplants of cells that were differentiated with mitomycin C/Brdu. The differentiated cells had been pretreated with ^{3}-H thymidine for autoradiographic (ARG) visualization. All rats were sacrifice 7 days after transplantation. The undifferentiated cells did not migrate significantly and displayed continued proliferation. They often completely encircled host blood vessels with up to 7 layers of cells. In contrast, following differentiation, both the C1300 and LA-N-2 cells migrated away from the injection site, remained amitotic and were visualized in the parenchyma of the host hippocampus and subependymal regions of the host.

In the third experiment, MS lesioned rats received intrahippocampal transplants of differentiated C1300 or LA-N-2 cells and were sacrificed 7, 30, and 120 days later. At 7 days post-transplant, numerous ARG labelled cells of both type were discerned in the host parenchyma, around blood vessels, and in the subependymal region. At 30 and 120 days, fewer cells were seen. The C1300 cells were predominantly found in parenchyma while the LA-N-2 cells were primarily in the subependymal region. There was no evidence for these cells reverting back to a tumous state nor any evidence of a significant immune response.

In the fourth experiment, the effects of transplants upon cognitive dysfunction were evaluated. Groups 1-3 received MS lesions followed 7 days later by differentiated C1300, LA-N-2, or sham transplants respectively. Group 4 received a sham lesion and sham transplant (all n=8). Three months later they were tested on a delayed non-matching to sample test in a T-maze. The MS lesion-sham grafted animals were impaired relative to sham lesioned-sham implanted animals ($p<.01$). In contrast, rats that received either the C1300 or LA-N-2 grafts displayed an enhanced performance relative to lesioned-sham transplanted animals ($p<.05$).

These studies indicate that differentiated neuroblastoma cells may be a viable source of donor tissue for transplantation.

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349.11 PURIFICATION OF MOUSE MUSCLE GPI-1B AND GPI-1C FOR ANTIBODY PRODUCTION. H. J. Li*, M. G. Wang*, T. G. Goodwin* and P. K. Law. Depts. of Neurol. and Physiol./Biophys., University of Tennessee Memphis, Memphis, TN 38163 USA

Through genetic complementation mechanisms, implantation of cultured normal myoblasts significantly improved the structure and function of dystrophic mouse muscles. Genotype markers GPI-1B and GPI-1C were used to determine the proportion of host and donor cells in muscles receiving implants. In the process to produce antibodies to these isozymes for immunocytochemical localization, we have developed a procedure to purify these isozymes using column chromatography and preparative agarose gel electrophoresis.

About 50g of mouse skeletal muscle was homogenized in 100 ml of buffer A (10 mM triethanolamine, 1 mM EDTA, 0.1% 2-mercaptoethanol, pH 11). The mixture was centrifuged at 27,000 xg for 30 minutes. The supernatent was concentrated with Amicon ultrafiltration (XM50) and dialyzed against buffer A with the pH now adjusted to 7.5. The dialyzed solution was re-centrifuged at 27,000 xg for 30 minutes. The supernatent was then loaded on the phosphate cellulose column (2.5 x 60 cm), and eluted with buffer B (25 mM triethanolamine HCl, 0.1% 2-mercaptoethanol, pH8). The fraction containing GPI was collected and concentrated to about 10 ml. It was further purified with Sephacryl S-200 column (3.0 x 90 cm) by elution with 10 mM Tris HCl buffer at pH 8.2 containing 1 mM EDTA and 0.1% 2-mercaptoethanol. All steps were carried out at 0-4°C. The procedure above significantly increased the GPI concentration from crude muscle extract. Further purification involved preparative gel electrophoresis which was conducted, anode to cathode, on slab gels (184 x 97 x 2 mm) of 1% (w/v) agarose and 5% (w/v) sucrose in Tris-barbital buffer, pH 8.6, at 4°C for 5 hours with field strength of 1.5 V/cm. The position of GPI in the gel was confirmed with GPI - specific stain reported previously. Staining with Coomassie Blue did not reveal any protein adjacent to the GPI band. GPI isolated by this method was found to be of high purity and high activity.

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349.12 SURVIVAL AND INTEGRATION OF FETAL HYPOTHALAMIC CELLS FOLLOWING FLOW CYTOMETRY AND TRANSPLANTATION INTO THE RAT CENTRAL NERVOUS SYSTEM. J.J. Lopez-Lozano, D.M. Gash, J.F. Leary and M.F.D. Notter. Depts. of Neurobiol. and Anat. and Pathology, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Our research group is developing procedures for fluorescence activated cell sorting of relatively pure populations of embryonic or neonatal neurons in order to study the behavior of those populations after being transplanted. This report examines the influence of flow cytometry and cell sorting techniques on a population of fetal anterior hypothalamus sorted cells transplanted into the hypothalamus of adult Long Evans rats. Timed pregnant female Long Evans rats were injected with ^{3}H-thymidine at 12-14 days post coitus (dpc). Seventeen dpc rat fetuses were decapitated and the anterior hypothamus, free of meninges, was quickly dissected out, minced, and placed in a cold Ca-Mg free buffer. Tissue was dispersed by a trypsin-DNase procedure with 90% viability and minimum debris. A single suspension of cells (1×10^{6} cells/mm^{3}) flowing at a rate of 1000 cells/sec was analyzed by multiparameter flow cytometry and was sorted on two parameters according to specific windows. The recovered suspension of anterior hypothalamic cells was then incubated in 0.03% fast blue. After washing away the excess dye. The cells were implanted into the posterior hypothalamus, in close contact to the median eminence, of previously neurohypophysectomized adult rats. At time periods ranging from 1 day to 4 weeks, animals were intracardially perfused with appropriate fixative. The study of the structural organization of the implanted sorted cells was made by means of; fluorescence microscopy, immunocytochemistry, and autoradiography. We have found that fetal neurons and glial cells, including vasopressin neurons, survive these procedures, migrate, and become structurally integrated into the host brain. The results of this study raise the possibility that flow cytometry can be used to sort specific populations of cells to provide a source of donor tissue for transplantation.

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349.13 UTILIZATION OF CULTURED BRAIN CELLULAR AGGREGATES FOR NEURAL TRANSPLANTATION. Jorge A. Colombo and Santiago Molina*. Dept. of Anatomy, College of Med., Univ. of South Florida, Tampa, FL 33612.

In order to manipulate experimentally the biological conditions of neural cellular pools before transplantation (for the purpose of labelling cells, incorporate precursors, modify the ratio between neurons and glia, modify their viability or growth, etc.), it seems necessary to develop an adequate set of procedures for 'in vitro' culture and transfer of such material. While various types of 'in vitro' cultures could be utilized, the formation of cellular aggregates (Moscona 1961) offers the possibility of growing cells in culture conditions where cells remain differentiating and viable, as well as physically readily available. Transplantation of dopaminergic cells after being cultured in cell monolayers has been recently reported while this work was in progress (Brudin et al., 1985).

Fetal rat brains (E 15-17) were subjected to mechanical dissociation and sieving procedures, after which they were seeded (1.1 x 10^6 viable cells/ml) either in DMEM-serum supplemented or in a defined (PC-1, Ventrex Labs.) medium, with a penicillin/streptomycin mixture, in 35 mm polylysine coated multiple plastic wells. Cultures were subjected to continuous rotation (75 rpm) while incubated at 37°C, 95% humidity and 8% CO2 for periods of up to two weeks. The addition of fluorescent latex microspheres (Tracer Technology) resulted in a non-homogeneous distribution of the label. For the most part, cells that incorporated the label remained isolated or formed small clumps, some of which were seggregated within the cellular aggregates. No differences could be observed in short term cultures between those fed with undefined or defined media. The cellular aggregates were collected by means of a microcapillary glass tube and stereotaxically transferred to the thalamus or hippocampus of pentobarbital anesthetized adult rats. After survival periods of 7 or 40 days animals were perfused with 4% paraformaldehyde under P.B. anesthesia. The fluorescent beads, incorporated to the transplanted material while cultured 'in vitro', were identified in an inverted microscope equipped with filters for rhodamine. Alternate sections stained for Nissl substance showed a distinct population composed of round cells with dark nuclei and other larger cells with pale nuclei. This cellular mass overlapped with cells showing intense fluorescence. Present results indicate the feasibility and practical convenience of culturing neural cells in aggregate forms before their transfer to a host brain.

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349.14 TRANSPLANTS SUPPORT THE GROWTH OF CORTICOSPINAL (CST) AXONS ACROSS THE SITE OF SPINAL CORD LESIONS IN NEWBORN RATS. D.I. LaPasha* and B.S. Bregman (SPON. M. Murray) University of Maryland School of Medicine, Baltimore, MD 21201

After spinal cord lesions at birth, immature corticospinal axons are able to take an aberrant route around the site of the lesion, through adjacent undamaged neural tissue to reach normal target areas. These late-growing undamaged axons are unable to cross the glial scar at the lesion site, however. We are using neural tissue transplants of fetal spinal cord tissue into spinal cord lesions in newborn rats in order to determine whether the transplants can provide a terrain for axonal elongation that is more favorable than that encountered in the glial scar at the lesion site.

Newborn rats (1 day postnatal) received spinal cord lesions ("over-hemisection" or transection) at a mid-thoracic level. Transplants of fetal spinal cord tissue from fetuses 14 days in gestation were stripped of meninges and dorsal root ganglia and placed into the lesion site. Littermates with spinal cord lesion but not receiving a transplant served as controls. All animals survived at least 8 weeks. Horseradish peroxidase or horseradish peroxidase-wheat germ agglutinin (HRP-WGA) was injected into the sensorimotor cortex for anterograde neuroanatomical tracing. After 48 hours survival, animals were perfused intracardially and the tissue reacted for histochemical demonstration of HRP.

In animals with lesions and transplants, CST axons grow into and project extensively throughout the transplant tissue. At spinal cord levels caudal to the lesion and transplant, the pattern of innervation is bilaterally symmetrical and extends as far as the lumbosacral cord. Thus, the immature transplanted tissue is able to support the elongation of the developing corticospinal axons. The axons are able to cross from host to transplant and are not impeded by the presence of a glial scar.

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349.15 DEVELOPMENT OF INTRASPINAL TRANSPLANTS OF DISSOCIATED RAT FETAL SPINAL CORD. J. D. Houle and P. J. Reier Depts. of Neurological Surgery and Neuroscience, Univ. of Florida College of Medicine, Gainesville, FL. 32610.

Anatomical reconstruction of the injured adult rat spinal cord has been obtained following the transplantation of fetal rat spinal cord (FSC) either immediately after injury or following a post-lesion delay of up to 7 weeks. In past experiments, whole segments of donor tissue were introduced into extensive resection cavities. It has not been explored, however, whether injections of dissociated cell suspensions can offer a more practical strategy toward repair of large zones of necrosis and cavitation. This consideration especially applies to chronic lesions in which minimal manipulation of preserved host tissue prior to transplantation would be desired.

This issue has been addressed by the present experiments in which hemi-resection cavities were made at lumbar levels of the adult rat spinal cord. Embryonic day 14 (E14) FSC was dissected into small fragments which were dissociated either by trypsinization or mechanical disruption. The resulting cell suspensions were pelleted by centrifugation and introduced into the lesion cavity either immediately after injury or after a 6-7 week post-injury delay. Transplant recipients were sacrificed 30-120 days later and tissues were prepared for study by light or electron microscopy or immunocytochemistry for GFAP or serotonin.

The lesion site in approximately 50% of recipient animals was filled by a cohesive mass of differentiated CNS tissue that was directly apposed to both gray and white matter regions of the host spinal cord without an observable intervening glial scar. In the best cases, silver-stains indicated that some degree of axonal interaction was established between host and donor tissues. In the remaining animals a loose matrix of neuronal elements interspersed with glial and mesenchymal cell components, was formed.

While survival and growth of transplanted dissociated embryonic CNS tissues has been achieved in other studies, such grafts have been made into essentially intact regions of the host brain and spinal cord. Our findings demonstrate that intraspinal grafts of FSC in a dissociated form can promote structural repair in extensive lesions of the spinal cord. Because of the modest amount of host tissue manipulation required by this approach, suspension grafting may offer considerable advantages in the experimental treatment of compression-lesioned spinal cords - an issue which we are currently investigating.

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349.16 DEVELOPMENT OF PEPTIDE-CONTAINING NEURONS IN SPINAL CORD ISOLATES. A. Henschen*, M. Goldstein[1], T. Hökfelt, A. Seiger* and L. Olson*. (SPON: L. Moore). Dept. of Histology, Karolinska Institute, 104 01 Stockholm, Sweden, [1]Dept. of Psychiatry, New York Univ. Med. Ctr., New York, NY 10016

The development and distribution of a variety of peptides, transmitters and transmitter-synthesizing enzymes have been analyzed in isolated intraocular cervical spinal cord grafts, and staining patterns have been compared to normal spinal cord. Our earlier studies have indicated that isolated spinal cord grafts have a substantial degree of intrinsic determination. Monoamine-containing nerve fibers are derived from supraspinally located cell groups in the normal spinal cord and consequently no monoamine containing neurons or fibers are found in isolated grafts using the Falck-Hillarp fluorescence method. Using an antiserum against tyrosine hydroxylase, a marker for catecholamine neurons, it was however possible to visualize single, large distinct neurons with non-varicose fibers in some grafts. Staining with an antiserum against calcitonin gene-related peptide (CGRP), a marker for motoneurons and primary sensory afferents in the normal spinal cord, revealed a dense terminal network and many large cell bodies similar to motoneurons. No CGRP fibers seemed to leave the isolated grafts. A rich network of somatostatin-positive terminals was found with a very high density in a peripheral zone. A large number of medium-sized somatostatin-positive neurons were found throughout the grafts. This is in contrast to normal spinal cord where positive neurons are difficult to visualize and mainly found in the dorsal horn of the mature cord. Enkephalin-immunoreactive fibers were found throughout all grafts. A distinct zone with a dense fiber network was often found peripherally, resembling the dense enkephalin network present in substantia gelatinosa. No enkephalin-positive cell bodies could be visualized. All grafts had a moderate to rich neuropeptide Y (NPY) terminal network. NPY-positive cells were also found. In normal spinal cord NPY is found mainly in the substantia gelatinosa with a sparse terminal network in the ventral horn. The distribution of subtance P-immunoreactivity was similar to that of enkephalin. Dense areas similar to substantia gelatinosa were found peripherally, although fibers were found throughout the grafts. As for enkephalin, no cell bodies could be seen. The above results confirm our earlier findings that the fetal spinal cord carries with it a considerable degree of intrinsic determination regulating its normal development. Development in total isolation from the rest of the central nervous system does however lead to disturbances in the expression of several of the peptides, possibly suggesting a retarded maturation of the grafts perhaps due to lack of normal connections supracervically or with target tissues. The interactions of spinal cord grafts with cografts of other neural tissues in oculo are currently under study.

349.17 **TRANSPLANTATION OF CEREBELLAR GRAFTS INTO ADULT 'PURKINJE CELL DEGENERATION' (pcd) AND WEAVER MUTANT MICE.** B. Ghetti, L.C. Triarhou and W.C. Low. Depts of Pathology and Physiology & Biophysics, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

'Purkinje cell degeneration' (pcd) and weaver (wv) mutant mice are respectively characterized by the selective loss of Purkinje and granule cells. The aim of this study was to test the ability of genetically normal cerebellar tissue to survive and differentiate in the mutant host environment after the degeneration of Purkinje or granule cells had taken place. Grafts were obtained from normal mouse embryos at gestational day 15 and implanted into the cerebellomedullary cistern of 15 adult pcd and 12 adult wv mutants. At 27-39 days post-transplantation, the recipients were perfused and their brains were processed for light and electron microscopy. Grafts placed into both pcd and wv recipients exhibited a layered cellular organization reminiscent of the normal cerebellar cortex. Molecular, Purkinje, and granule cell layers were identified. Grafted Purkinje cells displayed characteristic cytological features, such as hypolemmal cisternae in association with mitochondria in the perikaryon, and lamellar structures in their axons. Occasionally, stubby somatic thorns were observed in Purkinje cells. The cytological features of granule cell somata in the grafts appeared similar to those of mature granule cells. Electron microscopic examination of the molecular layer of cerebella transplanted into pcd mutant hosts revealed the presence of small axons the caliber of parallel fibers, which were not oriented in a parallel fashion; the majority of their axon terminals were presynaptic to profiles which were identified as dendritic spines. Overall, there was a relative paucity of dendrites and of parallel fiber-like axons. In some of the preparations, clusters of large neurons were tentatively identified as deep nuclear neurons; synapses upon the initial segment of dendrites and the somata of such neurons were seen. These findings concerning the survival of grafted normal Purkinje and granule cells within the mutant environments point to the possibility of repairing altered cerebellar circuitries in genetic conditions by replacing missing nerve cell populations. (Supported by PHS grant RO1-NS14426 to B.G., SO7-RR5371 and RO3-AG5575 to W.C.L., and by grant 85-174 from Eli Lilly and Co.).

349.18 CYTOARCHITECTURAL ORGANIZATION OF CEREBELLAR EXPLANTS TRANSPLANTED TO ADULT RAT HOSTS. E.B. Ezerman Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

It has recently been demonstrated that it is possible to successfully transplant to the rat brain CNS tissue from various regions which has previously been grown in explant culture (Lindsay & Raisman, Neurosci. 12 (1984) 513-530; Ezerman & Kromer, Soc. Neurosci Abst., 11 (1985) 253). The rat cerebellum has been used as a model for numerous studies of cerebellar development, organization and cytodifferentiation in explant culture and, more recently, using the intracephalic transplant paradigm. As there are certain features of both the culture and transplant paradigms that are useful in developmental studies, we asked whether early fetal cerebellar tissue could be grown in culture for several days, subsequently be transplanted to a cavity in the CNS and would manifest the typical cytoarchitectonic organization seen in cerebellar transplants.

E13 cerebellar primordia were initially placed in explant culture on collagen for two or four days. At 2 days post explantation, some large neurons are still proliferating; by 4 days neurons have begun to extend neurites onto the substrate. The explants were excised from the culture dish with or with out the collagen subtratum and placed in previously made intracephalic cavities. Histological analysis one month post transplantation showed that normal cytoarchitectural organization, including the trilaminar cortical arrays (molecular layer, Purkinje cell layer, internal granular layer), is present in both the one and 4 day explant transplants. The orientation of the cortical arrays within the explant transplants and with relation to the host brain and pial-vascular bed is similar to that seen in transplants which have not been in explant culture. External germinal cells (EGL) are still present lining pial invaginations or around blood vessels as is also seen in reaggregate transplants. Thus, a brief sojourn in explant culture has no apparent effect on the organization of E13 cerebellar explant transplants. On the other hand, postnatal cerebellar tissue, which grows very well in explant culture, develops poorly in the intracephalic cavity preparation, even after prior explantation. Early postnatal cerebellar vermis tissue was explanted to culture for several days at which time there was glial and neuritic outgrowth with numerous granule cells visible. Transplantation of these explants yielded very little surviving tissue showing poor cytoarchitectural organization. Information will be presented concerning the survival and cytoarchitectural organization of fetal cerebellar primordia grown continuously in culture for 8-12 days (equivalent to postnatal days 0-4) prior to transplantation into an adult rat host.

METABOLISM OF BIOGENIC AMINES

350.1 A RE-EVALUATION OF SOME OF THE MECHANISMS THAT REGULATE STRIATAL DOPAMINE DYNAMICS. C. Slimovitch*, and J.W. Commissiong (SPON. T. Sourkes), Dept. of Physiol., McGill Univ., 3655 Drummond Street, Montreal, P.Q., Canada H3G 1Y6.

Among the hypotheses that guide our thinking about striatal dopamine dynamics are: 1) after blockade of the postsynaptic dopamine receptor (eg. by neuroleptics) negative feedback control operates to activate the dopaminergic cell body in the substantia nigra; 2) levels of DOPAC and HVA in the striatum are an index of dopaminergic neuronal activity; 3) dopamine release in the striatum is dependent mainly on orthodromic action potentials in the neurons. Experiments were done using female Sprague-Dawley rats, 225-250 g. The left nigrostriatal tract (L-NST) was stimulated electrically (400 μA; 15 Hz., 20 min) or chemically blocked (TTX 300 μM) or completely severed, using a knife cut technique. Experiments were done after acute (1-10 hrs) or chronic (6 days) interruption of the L-NST. The test dose of haloperidol (HAL) used was 1.0 mg/Kg i.p. Dopamine (DA), DOPAC and HVA were analyzed by gas chromatography-mass spectrometry. The main findings are: 1) Isolated dopaminergic nerve terminals retain the capacity to regulate the synthesis and metabolism of DA for up to 24 hours after the transection. 2) Under conditions of intense circling induced by lesion of the L-NST, at 4 hrs post-transection, DOPAC increased from 28 to 87, and HVA from 6 to 53 nmol/g on the left side, with no significant change of either metabolite on the right side. 3) After the injection of TTX into the L-NST, the increases in DOPAC and HVA following the injection of HAL were the same in both the left and right striata. 4) After the knife cut of the L-NST, DOPAC and HVA in the left striatum were still significantly increased ($p < 0.01$) vs the vehicle-injected control, after HAL. 5) Increases in DOPAC and HVA in the striatum are not an invariant finding after electrical stimulation of the L-NST or after chloral hydrate anesthesia (> 400 mg/Kg i.p.). When the animals are deeply anesthetized and stress is kept to a minimum, the increases in DOPAC and HVA are not statistically significant. The following conclusions can be drawn from the above observations. 1) The negative feedback hypothesis for the control of the firing of dopaminergic cells may not always be valid. 2) There must evidently be a massive heteroreceptor-mediated control of striatal dopaminergic nerve terminal dynamics (result No. 2). 3) Under certain conditions, reuptake mechanisms would appear to be adequate to match transmitter supply to demand, and transmitter catabolism is kept to a minimum during the release/reuptake process. 4) HAL apparently has a significant direct presynaptic effect in increasing the synthesis and metabolism of DA in the nerve terminal. This effect may be independent of postsynaptic DA receptor blockade (results 3 and 4). These results will be useful in guiding our future thinking about the regulation of striatal DA dynamics.

350.2 EVIDENCE THAT INTRANEURONAL METABOLISM OF NEWLY SYNTHESIZED DOPAMINE IN THE MEDIAN EMINENCE REFLECTS THE ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS

K.J.Lookingland, H.D.Jarry* and K.E.Moore, Dept. Pharmacology and Toxicology, Michigan State Univ., E. Lansing, MI 48824

Dopamine (DA) released from tuberoinfundibular (TI) DA neurons terminating in the median eminence (ME) is transported via the hypophysial portal blood to the anterior pituitary gland where it acts to inhibit prolactin secretion. TIDA neurons have a low affinity reuptake system for released amine and, most likely, dihydroxyphenylacetic acid (DOPAC) measured in the ME predominantly reflects the intraneuronal metabolism by mitochondrial monoamine oxidase (MAO) of newly synthesized DA rather than metabolism of DA that has been released and recaptured by these neurons. Since procedures that alter the activity of TIDA neurons also alter the rate of DA synthesis, changes in TIDA activity may be reflected by corresponding changes in DA metabolism. Accordingly, the purpose of the present study was to examine the effects of increasing or decreasing the activity of TIDA neurons on DOPAC concentrations in the ME.

Blockade of DA synthesis with α-methyltyrosine (250 mg/kg; i.p.) produced a similar decrease in DOPAC concentrations in control and nomifensine-treated rats. These results demonstrate that DOPAC in the ME primarily reflects the intraneuronal metabolism of newly synthesized DA in TIDA neurons. Within 5 min following the administration of the MAO inhibitor pargyline (50 mg/kg, i.v.) ME DOPAC concentrations decline to 15% of control indicating that this metabolite has a very high turnover rate and is rapidly removed from the ME. Probenecid (250 mg/kg, i.p.) produced a time-dependent increase in ME DOPAC concentrations indicating that DOPAC is at least partially transported out of the ME via an acid transport system.

Prolactin (10 ug/rat; icv; 12 h), which is reported to increase TIDA neuronal activity increased, DOPAC concentrations in the ME of control and probenecid-treated male rats. Restraint stress (30 min), which is reported to decrease TIDA neuronal activity, decreased ME DOPAC concentrations in diestrous female rats. ME DOPAC concentrations in diestrous female rats, whose TIDA neuronal activity is reported to be higher than in the male, were two-fold greater than that measured in male rats. The results from the present study suggest that DOPAC concentrations in the ME can be employed as an index of TIDA neuronal activity. (Supported by USPHS Grant NS09174)

350.3 CORTICAL DOPAMINE AND NOREPINEPHRINE RELEASE IN THE RAT: GC-MS MEASUREMENT OF 3-METHOXYTYRAMINE (3-MT) AND NORMETANEPHRINE (NMN). H.S. Kim, C.A. Altar and P.L. Wood. Neuroscience Research, Research Dept., Pharmaceutical Division, Ciba-Geigy Corp., Summit, N.J. 07901.

The assessment of monoamine release in the cortex has been restricted to push-pull perfusion experiments in the past. We now report a simple cleanup procedure which allows the simultaneous GC-MS measurement of dopamine, 3-MT, norepinephrine and NMN in rat cortical regions. This involves the isolation of both the parent amines and their methylated metabolites using a liquid ion exchanger. The samples are next back-extracted into acid, freeze-dried and reacted with pentafluoroproprionic anhydride. Turnover studies can also be performed by monitoring the accumulation of these methylated metabolites after treatment with pargyline. The cortical regions examined were the prefrontal, cingulate and pyriform cortices, which were compared with the striatum and olfactory tubercle.

Steady-state levels of 3-MT and NMN in the cortices were low (<0.2 pmol/mg prot.) but functional, showing a rapid accumulation after pargyline treatment. 3-MT levels were much higher in the striatum (2.2 pmol/mg prot.) and olfactory tubercle (1.7 pmol/mg prot.), however turnover rates were similar to those in the cortex. Although measurable amount of NMN was present in the striatum (0.12 pmol/mg prot.), this pool did not appear to be functional since NMN levels did not increase after pargyline.

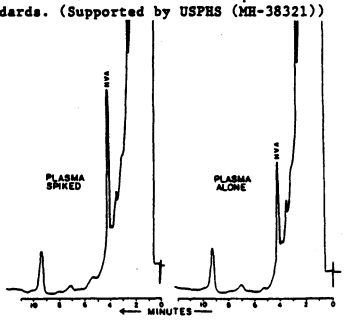
Haloperidol selectively elevated the steady-state levels of 3-MT in the striatum and olfactory tubercle while 3-MT turnover was increased in these areas as well as in the cortical areas. Nomifensine increased the steady-state levels and turnover of both 3-MT and NMN in all regions. In contrast, the alpha-2 agonist, clonidine selectively decreased NMN levels and turnover in the cortex.

In summary, this new assay allows the in vivo evaluation of drug effects on cortical dopamine and norepinephrine release without any surgical manipulations.

350.4 **DETERMINATION OF FREE HOMOVANILLIC ACID (HVA) IN HUMAN PLASMA FILTRATES USING HPLC WITH ELECTROCHEMICAL DETECTION.** **C.J.Drebing*, G.A.Gerhardt, C.Stephen* and R.Freedman (SPON: N.Weiner). Departments of Pharmacology and Psychiatry, U.C.H.S.C., Denver, CO 80262.**

The measurement of free homovanillic acid (HVA) in human plasma has clearly shown some utility as a therapeutic marker for changes in CNS dopaminergic activity with neuroleptic drug treatment. Although many methods exist for the measurement of this substance by GC or LC methods, most of the previous reports entail some type of prior plasma extraction procedure that is often time consuming. We now report a procedure for the rapid determination of HVA in human plasma by filtration followed by direct injection into an isocratic HPLC system with electrochemical detection.

Human blood samples were collected in green top tubes and the plasma was removed by centrifugation; these were stored at -20°C. On the day of the assay, 0.5ml of thawed plasma, either spiked or unspiked, was added to an Amicon Centrifree™ filter. These filter cartridges were centrifuged for 15 minutes in a clinical benchtop centrifuge. Fifty microliters of the resulting filtrate was directly injected into an isocratic HPLC system. The system consisted of a Beckman Model 114 pump, Altex 210 injector, ESA 5100A Coulochem dual-channel electrochemical detector (5011 cell), and a Fisher 5000 dual-pen recorder. All separations were performed on an Altex 4.6mm X 7.5cm Ultrasphere ODS (3 micron particles) reverse phase column. The eluent was a 0.1M citrate-acetate buffer (pH 3.9) containing 10% methanol plus EDTA (0.05g/l) and octane sulfonic acid (0.1g/l). Representative spiked and unspiked chromatograms are seen below. Peak heights and standard addition procedures were employed for quantitation. The method currently involves 2(10 minute) chromatography runs per sample. We will report on the evaluation of this technique as well as the use of internal standards. (Supported by USPHS (MH-38321))



350.5 CHANGES IN THE LEVELS OF 5-S-CYSTENYLDOPAMINE AND RELATED ADDUCTS IN RAT STRIATUM FOLLOWING DIFFERENT PHARMACOLOGICAL TREATMENTS. B. Fornstedt* (SPON:Z. Wiesenfeld-Hallin), E. Rosengren* and Arvid Carlsson*.

The neuronal degeneration in neuromelanin-containing brainstem nuclei in Parkinson's disease (PD) and normal aging results in a decrease of catecholamine levels in the innervated areas. Various hypothesies, involving both genetic and environmental factors, have been presented in order to explain the mechanism behind the apparent vulnerability of catecholaminergic neurons. Our work is based on the fact that catecholamines are readily autoxidized in vitro to reactive, cytotoxic semiquinones and quinones under the generation of toxic oxygen species. Studies on urine, serum, adrenergically innervated organs and other tissues have shown that the quinone form of dopa binds to the SH-groups of cysteine and glutathione to give rise to 5-S-cysteinyldopa and 5-S-glutathionedopa. This prompted us to investigate whether catechols in dopamine-rich brain areas undergo autoxidation in vivo and give rise to the corresponding cysteinyl adducts. We have thus detected 5-S-cysteinyldopamine, 5-S-cysteinyldopa and 5-S-cysteinyldopac in preferentially dopamine-rich brain parts of eight different mammalian species. Man, horse and sheep appear to have the highest and rat the lowest levels of the adducts, with Rhesus monkey, Marmoset monkey, dog and cat forming an intermediate group. Autoxidation of catechols must therefore be considered in addition to the classical metabolic pathways. The possibility that the cysteinyl adducts are merely intermediates in the synthesis of neuromelanin is not supported by the available data. The adducts do occur in the dopaminergic nerve terminal regions in all the species investigated, including the rat whereas neuromelanin is found only in the cell bodies and has not been detected in the rat. In order to obtain further information on the new metabolic pathway for dopamine, we have studied the biochemical events in rat brain following different pharmacological treatments using high pressure liquid chromatography with electrochemical detection. The results from these studies will be presented at the meeting.

350.6 SYNTHESIS OF γ-GLUTAMYLTYRAMINE AND γ-GLUTAMYLOCTOPAMINE IN THE NERVOUS SYSTEM OF LIMULUS POLYPHEMUS. [1]B-A. Battelle, S. C. Edwards*, H. M. Maresch*, and [2]S. K. Pierce*. [1]. C. V. Whitney Laboratory and The Department of Neuroscience, University of Florida, St. Augustine, FL 32086, and [2]. Department of Zoology, University of Maryland, College Park, MD. 20742

We have shown that tissues in the Limulus nervous system which synthesize and store octopamine (OCT), namely Limulus eyes and the cardiac ganglion, also synthesize and store conjugates of OCT and tyramine (TA) that are positively charged at pH 1.9. In addition, we showed that veratridine stimulates the release of the conjugates from these tissues in a Ca^{++}-dependent manner. We have now identified these conjugates as γ-glutamyltyramine (γ-Glu-TA) and γ-glutamyloctopamine (γ-Glu-OCT).

We speculated that the conjugates were peptidoamines. To determine which amino acids the amines might be conjugated to, the conjugates were isolated from acid extracts Limulus lateral eyes and cardiac ganglia, by high voltage paper electrophoresis (HVEPH) followed by acending paper chromatography in butanol, acetic acid and water (1:1:1), and hydrolyzed using mildly acidic conditions. The hydorlysates were then derivitized with OPA and fractionated using a high performance liquid chromatographic system for the separation and detection of OPA-derivitized amino acids. A large peak eluting with a retention time identical to that of standard glutamic acid (Glu) was present in the hydrolyzed samples and was absent in unhydrolyzed control samples. This result suggested that the amines were conjugated to Glu. Consistent with this hypothesis are the observations that radiolabeled conjugates, isolated from ventral eyes as described above after incubating the eyes in vitro with radiolabeled TA, co-migrated with standard γ-Glu-Oct and γ-Glu-TA in reverse phase high performance liquid chromatography. Furthermore, radiolabeled Glu is incorporated into a substance in the lateral eye that co-migrates with γ-Glu-TA on HVEPH followed by ascending chromatography.

The functions of γ-Glu-TA and γ-Glu-OCT in Limulus are not yet known. They may be amine inactivation products. Consistent with this hypothesis is our observation that the blood vessel sheath which surrounds the nervous system in Limulus also synthesizes -Glu-TA from TA. However, the observation that depolarization stimulates the release of γ-Glu-TA and γ-Glu OCT from Limulus eyes and the cardiac ganglion, but not the blood vessel sheath, raises the possibility that these molecules may also serve as intercellular messengers in the nervous system. (Supported by the Whitehall Foundation, Palm Beach, FL. and NIH Grant EY05724. γ -Glu-TA and γ-Glu OCT were obtained through the NIMH Chemical Synthesis Program).

350.7 PARTIAL PURIFICATION OF ENDOGENOUS MONOAMINE OXIDASE INHIBITORS FROM BABOON CEREBROSPINAL FLUID. L. Isaac, R. Schoenbeck* and B. L. Currie* (Spon: K. R. Unna). Dept. of Pharmacol., Col. of Medicine and Dept. of Med. Chem. and Pharmacog, Col. of Pharmacy, Univ. of Ill. at Chicago, 60680.

Previous work demonstrated the existence of endogenous monoamine oxidase inhibitors (EMAOI) in feline CSF and rat brain (Isaac et al. Neurosci. Lett. In Press, 1986). Because electroconvulsive shock resulted in an elevation in the level of EMAOI activity in feline CSF and rat brain we directed our attentions to its purification. We began isolation procedures of EMAOI from baboon CSF because of the quantities of CSF available, its low protein content and because EMAOI activity is in a soluble form only.

CSF was collected from baboons and pooled to volumes of 20 to 40 mls, dialyzed against water (6 L) for 24 hrs and lyophilized. The residue was dissolved in 0.5 M acetic acid/acetonitrile (3:1) and applied to a preparative reverse phase column for high performance liquid chromatography. The effluent was monitored at 214 nm and was obtained by a step gradient of acetonitrile (25%, 40%, 55% and 70%) in 0.5 M aqueous acetic acid. EMAOI activity was assayed against MAO derived from rat brain using ^{14}C-substrates, 5-hydroxytryptamine and phenethylamine, to assess MAO A and MAO B inhibitory activity, respectively.

Three fractions within the 25% step were collected, two each in the 40% and 55% step and one in the 70% step. Based on an average of at least 20% inhibition of MAO A or MAO B (n=3) we found EMAOI activity against MAO A in the 25% and 55% step and MAO B inhibitory activity in steps 40% and 55%. These data demonstrate the presence of EMAOI activity in baboon CSF and partial separation of EMAOI activities for MAO A and MAO B. Because of the wide separation of MAO A inhibitor activity in the 25% and 55% fractions, it is likely that these EMAOI activities result from different substances.

350.8 THE TYPE OF MONOAMINE OXIDASE ISOZYME ASSOCIATED WITH THE METABOLISM OF SEROTONIN IN SEROTONERGIC NERVE TERMINALS. G.M. Williams, L.M. Brown, J.B. Amedro*, A.J. Azzaro, and D.J. Smith, Depts. of Anesthes., Neurol., and Pharmacol./Toxicol., WVU Med. Ctr., Morgantown, WV 26506

Immunocytochemical analysis indicates that the Type B monoamine oxidase (MAO) isozyme is present within rat serotonergic neurons (PNAS. 79:6385, 1982). On the other hand, serotonin (5HT) appears to be preferentially metabolized by Type A MAO in rat brain (Biochem. Pharmacol. 17:1285, 1968). In this study a superfused synaptosomal preparation of rat spinal cord tissue was used to determine the type of MAO responsible for the deamination of 5HT in serotonergic nerve terminals. In this preparation, serotonergic nerve terminals are selectively labelled and 5HT metabolism occurs exclusively in the cytoplasm of these terminals (J. Neurochem. 45:1886, 1985). The ability of selective and non-selective MAO inhibitors to decrease 5HIAA efflux was examined to evaluate the role of each MAO isozyme in the deamination of 5HT.

Control 5HIAA effluxes were approximately 810 DPM and declined to approximately 530 DPM (a 35% reduction) during a 40 minute superfusion. This decline represents the loss of both 5HIAA formed during the labelling procedure and 5HIAA formed throughout superfusion. During the same period, treatment with phenelzine (10^{-5}M), a non-selective MAO inhibitor, reduced 5HIAA efflux by 83%. Since phenelzine at this concentration should completely inhibit MAO A and MAO B activity, it is assumed that the remaining 17% of 5HIAA efflux is due to the metabolite formed during the labelling procedure. Clorgyline (10^{-7}M), a selective MAO A inhibitor, induced a decline in 5HIAA of the same magnitude as that observed with phenelzine. In contrast, deprenyl (10^{-7}M), a selective MAO B inhibitor, had no effect.

In order to permit an increased presentation of 5HT to mitochondrial MAO, reserpine (10^{-5}M) was used to disrupt vesicular storage and elevate cytoplasmic transmitter concentration. Reserpine induced a dramatic (up to 3.4 X) increase in the efflux of 5HIAA. This increase was completely inhibited by phenelzine and clorgyline but not by deprenyl.

Regardless of the apparent presence of both MAO A and B, these data strongly suggest that only MAO A is responsible for the metabolism of 5HT in serotonergic terminals. Suported by NIH grants GM 30002 & T32 GM07039, the WVU Med. Corp. and a Swiger Fellowship (LMB).

350.9 DIFFERENTIAL BRAIN DISTRIBUTIONS OF HUMAN MONOAMINE OXIDASE TYPES A AND B DETERMINED BY POSITRON EMISSION TOMOGRAPHY USING [^{11}C]CLORGYLINE AND [^{11}C]-L-DEPRENYL. C.D. Arnett, J.S. Fowler*, R.R. MacGregor*, A.P. Wolf*, H. Sachs*, M. Smith*, Chem. Dept., Brookhaven National Laboratory, Upton, NY 11973; C. Halldin* and B. Langström*, Institute of Chemistry, Univ. of Uppsala, Sweden

In an attempt to extend the utility and potential of positron emission tomography (PET) for studying normal and diseased human brain beyond metabolism and neurotransmitter receptor binding studies, we have demonstrated the application of PET to the study of a key enzyme in the metabolic inactivation of many biogenic amines, monoamine oxidase (MAO). MAO exists in two forms, MAO-A and MAO-B, with different substrate, inhibitor, and immunochemical specificities. Clorgyline and L-deprenyl, selective irreversible inhibitors of MAO types A and B respectively, were radiolabeled with carbon-11, a positron emitting nuclide with a half-life of 20.4 min. Studies in mice demonstrated the selectivity of these two radioligands for studying the in vivo distributions of MAO-A and MAO-B activities (MacGregor, R.R., et al., Biochem. Pharmacol. 34:3207, 1985). Double injection PET studies in the baboon, with an intervening dose of unlabeled L-deprenyl, demonstrated a high retention of [^{11}C]-L-deprenyl radioactivity in the brain, with specific distribution to some areas as high as 80 percent of the total distribution (Arnett, C.D., et al., Abstracts, IVth World Congress Biol. Psychiat., 1985). By administering [^{11}C]-D-deprenyl to the same baboon, this distribution was shown to be stereospecific, since the [^{11}C]-D-deprenyl was not retained in those areas (in preparation). [^{11}C]Clorgyline did not exhibit a similar specific retention in the brain of the anesthetized baboon. We have extended these studies to the human by a double injection paradigm of [^{11}C]-L-deprenyl followed 2.5 hours later by [^{11}C]clorgyline. PET scans were obtained serially for up to 105 min after each injection. A time-activity curve for arterialized venous plasma was obtained for up to 110 min after injection and corrected for metabolites. The brain influx constant k_i (Patlak, C.S., JCBFM 3:1, 1983) was measured as an indication of functional enzyme activity in various brain regions for each radioligand. The results indicate qualitatively similar regional distributions for the two radioligands, but the k_i for [^{11}C]-L-deprenyl in the thalamus was higher than that for [^{11}C]clorgyline. This is consistent with higher MAO-B than MAO-A activity found in human thalamus obtained at autopsy (Oreland, L., Mod. Probl. Pharmacopsychiat. 19:246, 1983). Only about 2 percent of the radioactivity was excreted in the urine in the first 2 hours after administration. Carbon-11 labeled CO_2 was found to be present in air exhaled from the subject. This is the first demonstration of the use of PET for mapping enzyme distributions in the human brain. Research supported by USDOE, OHER, NIH NS-15380 and 15638.

350.10 ACUTE VS. CHRONIC EFFECTS OF IMIPRAMINE ON BRAIN p-HYDROXYPHENYLGLYCOL AND ON BRAIN AND PLASMA TYROSINE LEVELS IN RATS. D.J. Edwards, D.A. Sorisio* and M.L. Sedlock*. Dept. Pharmacology-Physiology, Univ. Pittsburgh Sch. Dent. Med., Pittsburgh, PA 15261.

This laboratory previously has shown that one injection of imipramine (IMI) decreases the brain concentration of p-hydroxyphenylglycol (pHPG), a neutral metabolite of octopamine, measured 6 or 24 hours later, but pHPG levels had returned to control levels 24 hours after the last of 14 daily injections (Sedlock et al., Neuropharmacology 24, 705, 1985). We now have examined the effects of IMI 6 hours rather than 24 hours after the last daily injection. Groups of male Sprague-Dawley rats were injected with IMI (20 mg/kg, i.p.) for 14 days (chronic group), with saline for 13 days and IMI on the 14th day (acute group) or with saline for 14 days (control group). The animals were decapitated 6 hours after the last injection. Brain pHPG levels were determined by gas chromatography-mass spectrometry. Brain and plasma levels of tyrosine were assayed by high performance-liquid chromatography with electrochemical detection (HLPC-EC). Brain pHPG was 51% of control ($p<.005$) in the rats given an acute IMI injection and 55% of control ($p<.05$) in the ones treated chronically (controls = 2.40 ± 0.35 ng/g). Plasma tyrosine was significantly decreased to 85% of control ($p<.05$) in the acute treatment group but was unaltered (99% of control) in the chronic group (controls = 15.5 ± 1.0 μg/ml). Brain tyrosine levels were unchanged in both groups. Plasma levels of IMI and its metabolites (determined by HPLC-EC) indicated that chronic treatment led to an accumulation of desmethylimipramine (352 ± 47 vs. 77 ± 13 ng/ml) but no significant accumulation of either IMI (chronic, 30 ± 4 vs. acute, 23 ± 5 ng/ml or of didesmethylimipramine (chronic, 66 ± 13 vs. acute, 49 ± 10 ng/ml). Even though IMI did lower plasma levels of tyrosine, the amino acid precursor of pHPG, this would not appear to explain the reduction in pHPG, since 1) tyrosine was not decreased in the brain; 2) no decrease in plasma tyrosine was seen in the chronically treated rats; and 3) the decreases in tyrosine were much smaller than in pHPG. In our earlier studies, the ability of IMI to lower brain pHPG appeared to show tolerance after repeated treatment; however, the present results suggest that this is not the case. The finding that pHPG is lowered to the same extent in acute and chronic treated animals 6 hours after the last injection (but not at 24 hours) indicates that pHPG levels return to control more rapidly after chronic treatment. Supported by NIMH grant MH-28340.

350.11 EFFECT OF AGE ON REGIONAL BILATERAL SEROTONIN METABOLISM. C.A. Taylor and T.P. Jerussi. Dept. of Pharm. & Tox., Rutgers Univ., P.O. Box 789, Piscataway, NJ 08854 and Anaquest/BOC Health Care Group, 100 Mountain Ave., Murray Hill, NJ 07974.

Regional bilateral serotonin (5-HT) metabolism was investigated in two groups of female Sprague-Dawley rats, 10 and 16 weeks of age. Rats were tested twice for one hour, a week apart, for d-amphetamine (1.0mg/kg, ip)-induced rotation. One week later, the animals which had at least 10 full rotations/hour were decapitated at 0, 5 or 10 minutes following the administration of pargyline (50mg/kg, ip). The concentration of 5-hydroxyindoleacetic acid (5-HIAA) was determined by HPLC-EC in the left and right striata, tubercula olfactorium (TO) and frontal cortices (FC).

There were no significant left-right differences in steady state concentrations of 5-HIAA. However, the 5-HIAA turnover rates in the striata and the TO were lateralized in both age groups. Moreover, the pattern of asymmetry was age dependent. At 10 weeks, the turnover rate of the left striatum was greater than that of the right but the turnover rate of the right TO was greater than the left. In contrast, at 16 weeks, these asymmetries were reversed--the turnover rate of the right striatum was greater than that of the left and the left TO was greater than the right. In the FC, however, only 16 week old rats were lateralized with respect to the 5-HIAA turnover--the right FC was greater than the left.

Bilateral neurochemical asymmetries were also evident with respect to the direction of rotation. At 10 weeks, the contralateral TO had a greater 5-HIAA turnover rate than the ipsilateral TO while the ipsilateral FC had a greater turnover rate than the contralateral FC. At 16 weeks, all three regions were lateralized. The striata and FC had greater turnover rates contralaterally while in the TO the turnover rate as well as the steady state concentration of 5-HIAA were greater ipsilaterally.

It is evident from these data that the brains of 16 week old rats are more lateralized with respect to the metabolism of 5-HT and the pattern and degree of asymmetry are age related. Moreover, these asymmetries are in the opposite direction to those regional bilateral differences reported for dopaminergic metabolism (Taylor and Jerussi, Soc. Neurosci. Abs. 11: 443, 1985).

FEEDING AND DRINKING III

351.1 Some aspects of feeding and drinking behavior in the spiny mouse (Acomys cahirinus). D.A. Czech and S.E. Schrank.* Dept. of Psychology. Marquette Univ., Milwaukee, WI 53233.

We previously observed that the murid rodent, Acomys cahirinus (spiny mouse), does not adjust well to a restricted feeding schedule, seeming unable to ingest adequate amounts of food during limited access. In the present study, we investigated several consummatory-linked behaviors in the adult spiny mouse in order to further characterize feeding and drinking behavior in this species, which is being increasingly used in behavioral studies, particularly in early behavioral development (Birke et al., Behav Neural Biol 43:143, 1985).

In the first experiment, the temporal patterns of feeding and drinking were monitored in animals placed on a 12:12 L/D cycle. Entries into the food hopper and water intake data(drops) were collected in 15-min periods throughout the L/D cycle. Acomys exhibited a very robust circadian drinking rhythm; however, as reported for certain other species of rodents, such as the hamster, gerbil and guinea pig, circadian feeding patterns in the spiny mouse were generally relatively weak or absent. These animals ate frequent small meals throughout the 24-hr period.

In a second experiment, one group of animals was placed on a restricted feeding schedule in which 24-hr periods of ad lib food availability were alternated with 24-hr periods of food deprivation. A comparison group had food available at all times. Unlike hamsters, which fail to engage in compensatory increases in feeding following similar periods of deprivation, spiny mice compensated quite effectively, increasing their intake on days when food was available to approximately 170% of pre-deprivation baseline levels ($p<0.001$, compared to ad lib controls). Comparable compensatory overeating (162% of baseline) has been reported for the rat (Silverman & Zucker. Physiol Behav 17:271, 1976).

In the third experiment, spiny mice were injected IP with three doses of 2-deoxy-D-glucose (2-DG) (250, 500 & 1000 mg/kg) and normal saline vehicle in a within-subject counterbalanced design, and powdered food intake was measured at 1, 2, 6 & 24 hours. Injections were separated by at least 4 days. No dose of 2-DG tested led to significantly greater food consumption when compared to baseline (vehicle injection) intake. On the other hand, intakes were reduced at both 6 & 24 hr at the highest dose, which was significantly different from all other treatments ($p<0.05$ or 0.01). Failure of 2-DG to induce feeding responses has been reported for other species of rodents as well, including hamster and gerbil (e.g., Rowland. Physiol Behav 21:291, 1978). Results are discussed.

351.2 PEPTIDE YY AND NEUROPEPTIDE Y STIMULATE FOOD BUT NOT WATER INTAKE IN AN AVIAN SPECIES. W. J. Kuenzel. Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742.

Two members of the pancreatic polypeptide family, neuropeptide Y (NPY) and petide YY (PYY) have been shown to be potent stimulators of food intake in rats. Experiments were conducted to determine if NPY and PYY are orexigenic agents in an avian species. Broiler chicks that have been genetically selected for rapid growth rate and increased food intake were used as experimental animals. Eighteen chicks were chronically implanted with polyethylene cannulas directed to the lateral ventricles of the brain. For each of the three days, 6 chicks were injected with either physiological saline, NPY or PYY in a split-plot Latin Square design. Three dose levels were administered within each experimental group: 1, 5 and 9 µg. The saline group received 1, 5 or 9 µl. Food intake and time spent feeding, drinking, pecking, walking, standing, sitting, sleeping, and preening were monitored throughout three 60 min periods following intracerebroventricular (ICV) injection of one of the neuropeptides or saline.

Food intake of broilers given 5 µg of NPY or PYY was nearly twice that of the saline controls during the first hour post-injection. Analysis of variance followed by Student-Newman-Keuls test for mean separation showed that all groups treated with NPY or PYY consumed significantly more food and spent significantly more time feeding over the 3 hr. observation period than did controls. No difference, however, was observed in the time spent drinking among the three main treatment groups. The saline controls spent significantly more time standing and walking compared to the two peptide groups. The PYY birds spent the least amount of time standing and the least amount of time preening ($P<0.05$). At the end of each 3 hr. observation period, crops were palpated to determine to what degree they were filled with feed. Those chicks receiving ICV injections of PYY consistently had larger crops which were harder to the touch than those birds which received NPY or saline. Experiments are in progress to determine whether the paraventricular nucleus is a locus within the avian brain that is quite sensitive to the effects of the two neuropeptides and whether it contains endogenous levels of NPY.

It is concluded that NPY and PYY are potent stimulators of food intake in chicks but have little if any effect on water intake.

351.3 **THE DIPSOGENIC POTENCY OF ANGIOTENSIN II (AII) AFTER REMOVAL OF VARIOUS POSTINGESTIONAL INHIBITORY FACTORS**
J.J.Salisbury*, N.E.Rowland and M.J.Fregly*. Depts Psychol & Physiol, Univ Florida, Gainesville FL 32611.

Rats treated with AII drink more isotonic NaCl than water, suggesting that taste facilitates and/or that consequences of water ingestion terminate AII-induced drinking. Further, preloads of water potently suppress AII-induced drinking, but 0.15M NaCl preloads are less effective. Two new experiments dissect these factors.

In the first study, rats fitted with indwelling gastric catheters received AII injections (200 ug/kg, SC) while water or 0.15M NaCl intake was recorded. During experimental sessions, either water or 1.5M NaCl was injected via the gastric catheter during drinking bouts, in a volume of 0.1 ml for each ml water drunk. Thus, the 1.5M injection would be diluted to isotonic in the stomach without the rat tasting NaCl. The mean water intake did not differ among the no injection (6.4 ml), water injection (5.4 ml) and NaCl injection (6.4 ml) conditions. In contrast, the oral intake of 0.15M NaCl was much higher, 29.6 ml. In a parallel study using IV AII infusion (125 ng/min) as the stimulus, the intakes in the corresponding injection conditions were 10.0, 8.4 and 14.4 ml (not sig.).

In the second series of experiments, rats were fitted with a chronic gastric fistula and were tested repeatedly, alternating between open and closed tests. (In the open fistula condition, fluid consumed flowed out with little or no absorption.) Rats drank more

Trial #:	1	2	3	4	5	1	2
Closed fist.	9.3*	10.2	9.3	10.4	33.5#	18#	20#
Open fistula	17.7	18.1	24.0	37.1	58.4#	40#	80#

*Mean water or (#)0.15 NaCl intakes/3h after AII(SC)

water or saline on the first open trial than on closed trials, and intakes continued to rise across trials in the open condition. Sham drinking was also increased after ICV injection (2.5 ng) or IV infusion of AII.

These studies indicate that multiple postingestive factors, including osmodilution, taste, and learned satiation, limit the quantity consumed after an AII stimulus. The present methods allow an "open loop" measure of the AII stimulus to drink [NIH AM31837-03].

351.4 MUSCIMOL-INDUCED FEEDING AND DRINKING: A COMPARISON BETWEEN THE MEDIAN RAPHE AND DORSAL RAPHE NUCLEI.
M.A. Klitenick and D. Wirtshafter, Dept. Psychology, Univ. of IL at Chicago Chicago, IL 60680.

Alterations of food and water intake have been demonstrated following microinjection of a variety of neuroactive substances into discrete regions of the brain. In particular, several workers have reported increased food and water intake following injection of muscimol, a GABA agonist, into the dorsal raphe nucleus (DR). The present experiments were designed to compare the effects upon feeding and drinking of muscimol injected into the DR or the median raphe nucleus (MR). Rats were prepared with chronic indwelling cannula aimed at the DR or MR and food and water intake were measured 2 hrs after intra-raphe muscimol (0, 6.25, 12.5 and 25 ng/0.25 ul) injections.

We found that injection of muscimol into either the MR or DR elicited a significant increase in both food and water intake, but the MR appeared to be a much more sensitive site in terms of both the threshold dose for eliciting ingestive behavior and the maximal magnitude of the effect. For example, food intake was increased by 7.7 g over baseline by injection of the 25 ng dose into the MR, compared to a 2.8 g increase with the DR placements. Furthermore, water intake was increased 11.3 ml over baseline following 25 ng of muscimol injected into the MR compared to a 2.4 ml increase after 25 ng muscimol injected into the DR.

The fact that the dose-response curve is shifted to the right for the DR placement suggests that the DR may not be the active site of the muscimol effect, but rather that the feeding may be due to diffusion ventrally into the MR. It is interesting to note that a similar situation exists with respect to locomotor activity where muscimol injections into the MR produce a much larger increase in activity than that seen after DR injections.

Preliminary data indicate that haloperidol (0.8 mg/kg, s.c.) administered 1 hr prior to an intra-MR injection of muscimol (25 ng/0.25ul) attenuates both food and water intake. In contrast, naloxone (5 mg/kg, s.c.) attenuated water but not food intake following intra-MR muscimol (25 ng/0.25 ul).

Supported by NIH Grant RO1 NS21350

351.5 AN EXAMINATION OF FEEDING AND DRINKING BEHAVIOR FOLLOWING SEROTONIN REUPTAKE BLOCKADE.
K. Gill* and Z. Amit (Sponsor: L. Switzman). Center for Studies in Behavioral Neurobiology, Concordia University Montreal, Canada H3G 1M8.

There is considerable experimental evidence to suggest that Serotonin (5HT) is involved in the regulation of consummatory behavior. Agents which deplete central 5HT have been reported to produce hyperphagia and obesity in rats. On the other hand, Serotonergic agents such as direct acting agonists and reuptake blockers or those which increase release and synthesis reduce the consumption of food and/or fluids including ethanol solutions. This work taken as a whole has suggested that 5HT plays an inhibitory role in feeding; possibly participating in the processes which mediate satiety. In order to investigate this notion, the Serotonin reuptake blocker Zimeldine (Zim) was examined for its effects on the initiation, duration and frequency of food and fluid bouts under free-feeding conditions. 1) The first study examined the effects of Zim on the latency to initiate feeding and eating rate in a paradigm where animals were presented with multiple meal segments consisting of Noyes sucrose reward pellets. Subjects were male Long-Evans rats, individually housed with free access to rat chow and water in the home cage. Animals were trained to eat sucrose pellets during daily 20 min feeding sessions, in specially equipped test chambers containing an automatic food dispensing apparatus. During the daily session, the animals were presented with 17 meal segments each consisting of 2 pellets. Each segment was automatically introduced into the chamber, for 36 sec at 72-sec intervals. Once baseline consumption stabilized the animals were treated with vehicle for 2 consecutive days followed by a test day where they received Zim (20 mg/kg). All injections were given intraperitoneally, 2 hr prior to the feeding session. Latency and duration scores across the 17 meal segments on vehicle vs test day were compared. Zim produced a significant increase in both the latency to initiate feeding as well as the rate of eating. The pattern of changes, induced by the drug across the 17 meal segments, suggested enhanced rate of satiation. 2) A second study examined the effects of Zim on the temporal pattern of food, water and ethanol intake in animals continuously maintained in operant chambers equipped with drinkometer circuits and a food pellet delivery system. Anaylsis of food and fluid bout initiation, duration, frequency and size was carried out. Following stabilization of baseline intake patterns, animals were treated with vehicle for 2 consecutive days followed by a test day where animals received Zim (20 mg/kg) for 2 hr prior to the onset of the dark cycle. Comparisons of baseline vs drug day indicated that Zim produced a strong suppression of food bout size and frequency and a suppression in the fluid most closely associated with post-prandial drinking. These results are discussed in terms of the role of Serotonin in satiety.

351.6 EFFECTS OF VAGOTOMY ON SUCROSE INTAKE IN RATS.
M. VIGORITO and A. SCLAFANI. Department of Psychology, Brooklyn College of CUNY, Brooklyn, NY 11210.

Previous work in our laboratory indicates that subdiaphragmatic vagotomy suppresses the intake of sucrose solution but not sucrose powder (Sclafani and Kramer, 1983). Whether this is due to a nonspecific effect of vagotomy on the postingestive disposition of liquids, or represents a specific alteration in sucrose appetite is uncertain. (Note, in normal rats sucrose promotes greater hyperphagia and obesity when presented in solution rather than powder form.) This question was addressed in two experiments.

Exp. 1 compared the effects of total subdiaphragmatic vagotomy (VAG), sham vagotomy (SHAM), and pyloroplasty (PYLOR) on liquid (20%) and granular sucrose intake (chow and water were available ad lib). The groups did not differ in their absolute or percent intake (24 hr/day) of granular sucrose but the VAG group consumed less sucrose solution than the SHAM and PYLOR groups; the SHAM and PYLOR groups did not differ in sucrose solution intake. When both granular and liquid sucrose were available 24 hr/day the VAG group consumed more granular than liquid sucrose whereas the SHAM and PYLOR groups consumed more liquid than granular sucrose. Thus, vagotomy not only reduces liquid sucrose intake but also reverses the normal preference for liquid over granular sucrose. To the extent that pyloroplasty impairs the gastric emptying of liquids, the finding that the PYLOR and SHAM groups did not differ in their response to liquid and granular sucrose suggests that the affect of vagotomy on liquid sucrose intake is not due solely to gastric dumping.

Exp. 2 compared the effects of total subdiaphragmatic vagotomy (VAG) and selective gastric vagotomy (GAST) on sucrose intake. Both treatments suppressed the intake of liquid (20% or 32%) sucrose but not granular sucrose relative to sham surgery. When 32% sucrose was presented as a solid gel the VAG, GAST, and SHAM groups did not differ in their absolute or percent sucrose intake, but the VAG and SHAM groups consumed less of a 20% sucrose gel than did the SHAM group. The VAG and GAST groups both drank less water than the SHAM group, but only the VAG group maintained below normal weight levels. These results indicate that the effects of total vagotomy on liquid sucrose intake are due primarily to the loss of gastric vagal function, and are associated with impairments in water intake regulation rather than impairments in ingestive motor responses (drinking vs. eating) or in the regulation of energy balance or carbohydrate intake.

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351.7 CONSISTENTLY GREATER DRINKING IN RATS FOUND AFTER LOW DOSES OF NALOXONE. G. A. Olson, M. F. Pignatiello*, A. J. Kastin, and R. D. Olson, Dept. of Psychology, University of New Orleans, New Orleans, LA 70148.

There have been inconsistent outcomes in demonstrations of the effect of low doses of naloxone on drinking behavior in rats, with increased drinking, decreased drinking, or no effect all being reported. It now appears that methodological variables reliably produce each result, and those leading to increased drinking have been identified.

In Experiment 1, doses of either 0.0001, 0.001, or 0.0 mg/kg of naloxone were administered to young (30 day old), mature (90 day old), and old (228 day old) rats. Injectons were made at 8:00 am, and measures of consumption of a 10% sucrose solution were taken at intervals of 30, 60, 120, 240, 360, and 480 min after injection. Among the young animals, dose level produced no differences in drinking. For the mature rats, the smallest dose produced significantly greater drinking than either of the other two doses. The other two doses were not significantly different from one another, although the control group drank the least. Similar findings occurred for the old rats, although between-group differences only approached significance.

A second study confirmed the ability of the 0.0001 mg/kg dose to increase drinking in mature (94-day old) rats, using the same procedures as Experiment 1. Total consumption of the animals that received that dose was significantly greater than that of the controls.

The young rats of the first study were tested again when they reached 60 days of age to determine if maturation of the opiate system might be important to these findings. The original three dose levels were used, and although no significant differences appeared, there was a trend for the larger dose of naloxone to increase drinking. It is hypothesized that the opiate system is still immature at this age, thus requiring a higher dose to produce any effect; it might begin to show responsiveness to naloxone at 60 days, however, demonstrating a change from the 30-day performance, when no response to naloxone was found.

In addition, duration of the testing session is important, since significant differences in consumption did not appear until at least four hours after the injections and were greatest after 8 hours. Thus, session length, small dose level (0.0001 mg/kg), and age or maturity seem to be critical variables for producing increased drinking with naloxone in rats.

351.8 HINDBRAIN COMPONENTS OF AN INHIBITORY SYSTEM INFLUENCING EXTRACELLULAR THIRST

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Ablation of either the area postrema (AP), a circumventricular organ, or ventral lateral region of the lateral parabrachial nucleus (VLLPBN) in the rat produces enhanced drinking in response to systemically administered angiotensin II (ANG II) and isoproterenol. In contrast, animals with either AP or VLLPBN lesions do not overdrink to cellular dehydration induced by systemic administration of hypertonic saline. It has been hypothesized that AP and VLLPBN lesions interrupt information either about circulating ANG II levels, presumably monitored by the AP, or ascending baroreceptors/volume receptor information. Present experiments examine the effect of AP and VLLPBN lesions on drinking to dipsogenic challenges administered intracerebroventricularly (i.c.v.). Rats were given bilateral electrolytic or sham lesions of the VLLPBN; another group of subjects received either thermal cautery or sham thermal cautery of the AP. All rats were implanted with chronic cannula to deliver ANG II into the lateral ventricle. Subjects were then tested for drinking in response to systemically applied angiotension II and centrally administered ANG II (50, 100 ng/2 ul) and carbachol (100, 250 ng/2 ul). As anticipated, both the AP and the VLLPBN lesion rats overdrank with respect to their controls following administration of systemic ANG II. Similarly, both groups of lesioned rats over-responded to centrally applied ANG II 30 min post-injection (VLLPBN: $F(1,20) = 16.26$; $p < .001$); AP: $F(1,14) = 10.33$; $p < .01$). In contrast, drinking to carbachol administered centrally was not significant for either group at 30 min (VLLPBN: $F(1,20 = 3.37$; $p > .05$; AP: $F(1,14) = 4.11$; $p > .05$).

Taken together the results indicate that angiotensin II related challenges are enhanced by either AP or VLLPBN ablation regardless of whether the ANG II is administered systemically or centrally. Furthermore, as in the case of systemic challenges, there is specificity for the overdrinking response to centrally applied dipsogens in that overdrinking was not found in response to the centrally administered cholinergic agent, carbachol, which has been associated with the central mediation of cellular thirst.

351.9 CNS-INDUCED NATRIURESIS WITH CARBACHOL IN INFANT RATS. Stella Y. Chow* and Douglas A. Fitts* (SPON: John B. Simpson), Department of Psychology, University of Washington, Seattle, WA 98195.

Injection of a cholinergic drug, carbachol (CBC), into the cerebral ventricles of adult rats causes natriuresis and drinking. Because drinking to CBC does not appear until postnatal day 4, we examined the development of CBC-induced natriuresis in rat pups 0 to 4 days of age. Two pups per litter received injections of 2.12 nmol CBC, and 2 littermates received an equal, 1.0-ul volume of saline vehicle in the lateral ventricles, with 2 to 4 litters per age group. Urine volume and Na and K excretions were then examined after 1 hr in order to establish the age of onset of the natriuretic response to CBC. No influence of age was found. CBC increased Na concentration to 67.3±29.4 from 17.1±15.7 umol/ml (mean and S.D.) and Na excretion to 71.5±50.1 from 17.7±24.1 umol/100 g compared with the vehicle-injected conditions across all 15 litters. Potassium concentration and excretion were not affected by the injections. Muscarinic receptors mediate the natriuresis in infants as in adults, because the response was completely blocked by isomolar co-injection of atropine sulfate. A positive correlation between Na excretion and urine volume ($r(17)=+.74$) following CBC suggested that the natriuresis also stimulated diuresis ($Y=7.05X-0.54$). Plasma concentrations did not differ 1 hr following CBC or vehicle injections in infants; respective averages were: protein, 2.2±0.2 and 2.3±0.1; Na, 137.0±4.3 and 134.2±5.9; and K, 5.0±0.7 and 5.2±0.4. Thus, it appears that the natriuretic response develops prior to the drinking response to intraventricular injections of CBC.

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351.10 HISTAMINE ELICITS DRINKING WHICH CAN BE INHIBITED BY H_1 AND H_2 ANTAGONISM IN WEANLING RATS. S.M. Specht and L.P. Spear. Department of Psychology and Center for Neurobehavioral Sciences, State University of New York at Binghamton, Binghamton, New York 13901.

Release of endogenous histamine (H) from gastric mucosa has been proposed to play a major role in drinking elicited by eating in adult rats (Kraly & Specht, 1984). Given that antagonism of H_1 and H_2 receptors for histamine, using dexbrompheniramine (DXB) and cimetidine (C), respectively, abolishes drinking elicited by exogenous H in adult rats, these drugs can be used as a pharmacological probe for investigating whether endogenous H plays a role in drinking elicited by eating in adult rats (Kraly, 1983).

In order to begin to assess the feasibility of using such a pharmacological probe to investigate drinking at the time of weaning, drinking in 22-day old weanling Sprague-Dawley-derived rats was measured for a 1-hr period during the dark phase of the light/dark cycle following injection of: 1) 0.0, 2.5, 5.0, 7.5, 10.0, 20.0 or 40.0 mg/kg H s.c. and 2) 20 mg/kg H s.c. preceded by i.p. treatment with 32 mg/kg C, 2 mg/kg DXB or both drugs in combination.

Weanling rats drank in a dose-dependent manner to s.c. H, drinking significantly more than saline controls when given 20.0 and 40.0 mg/kg H (p's < .05). These doses, however, are larger than the lowest dose (i.e., 5 mg/kg H) necessary to significantly increase water intake in similarly tested adult rats. While DXB and C, when given separately, tended to attenuate somewhat the drinking response to H, combined antagonism of H_1 and H_2 receptors abolished drinking in response to H, decreasing water intake to a level not different from baseline.

These results suggest that combined antagonism of H_1 and H_2 receptors for histamine may provide a useful pharamcological probe for investigating drinking behavior in young rats.

351.11 EFFECTS OF INTRACEREBROVENTRICULAR ANTAGONISM OF HISTAMINE RECEPTORS ON DRINKING IN THE RAT. F.S. Kraly, R.L. Arias*, S.D. Geasey*, R. Corneilson*, C.J. Klinko* and J.A. Lasser*. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

Combined antagonism of H_1 and H_2 receptors using i.p. dexbrompheniramine (DXB) and cimetidine (C) abolishes drinking elicited by s.c. histamine and inhibits drinking elicited by eating in the rat (Kraly, Appetite, 6, 153, 1985). To begin evaluating whether these effects of i.p. DXB and C on drinking result from antagonism of brain and/or peripheral histamine receptors, we studied the effects of i.c.v. pulse injections of DXB and C on drinking (in 1-hr tests) in 66 adult male Sprague-Dawley rats equipped with a stainless-steel guide cannula directed toward the third ventricle. Placement of the cannula was verified for each rat behaviorally (drinking in response to 100 ng angiotensin II i.c.v.) and anatomically (India ink i.c.v. at autopsy).

Drinking elicited by s.c. histamine. Combined antagonism i.c.v. of H_1 and H_2 receptors (12.5-50 ug DXB plus 25-100 ug C) inhibited (p's $< .01$) water intake elicited by histamine s.c. (5 mg/kg; twice the ED_{50} for drinking); 50 ug DXB plus 100 ug C inhibited drinking by 98%. The 50 ug DXB alone failed to inhibit intake ($p > .05$), whereas 100 ug C alone abolished ($p < .001$) drinking elicited by s.c. histamine. Inhibition appeared to be relatively selective for drinking elicited by histamine, since 50 ug DXB plus 100 ug C failed ($p > .10$) to inhibit intake after 12-hr water deprivation.

Drinking elicited by eating. Combined antagonism i.c.v. of H_1 and H_2 receptors (dosages as above) failed to significantly (p's $> .05$) inhibit drinking elicited by eating pelleted chow after 24-hr food deprivation. Statistically nonsignificant inhibition of water intake was apparent only when DXB (25-50 ug) plus C (50-100 ug) delayed (p's $< .05$) the initiation of eating. The 50 ug DXB alone failed to inhibit food or water intakes (p's $> .10$), whereas 100 ug C alone inhibited food and water intakes (p's $< .05$) by 48% and 62%, respectively.

These results with i.c.v. DXB and C contrast with earlier results with i.p. DXB and C: First, i.c.v. C (H_2 antagonism alone) is sufficient to abolish drinking elicited by s.c. histamine, whereas i.p. C must be combined with i.p. DXB (i.e., combined H_1 and H_2 antagonism) to abolish drinking elicited by s.c. histamine. Second, i.c.v. C or C plus DXB inhibits food-related drinking only slightly and secondarily to these drugs' effects on eating, whereas i.p. C or C plus DXB can inhibit drinking elicited by eating without inhibiting eating. These findings can be taken to support the hypothesis that primarily peripheral (and not central) H_1 and H_2 receptors together mediate a histaminergic component of drinking elicited by eating in the rat.

351.12 EFFECT OF PERIPHERALLY-ADMINISTERED CAPTOPRIL ON THE DIPSOGENIC AND PRESSOR RESPONSES TO PERIPHERALLY-INFUSED ANGIOTENSIN I. S. K. Thompson and K. E. Moe. Dept. Psychology, Washington State University, Pullman, WA 99164-4830.

Captopril (CAP), an angiotensin converting enzyme (ACE) inhibitor, has been used by investigators to assess the role of angiotensin in water and salt intake. Low doses of peripherally administered CAP have been assumed to block conversion of angiotensin I (ANG I) to angiotensin II (ANG II), as they attenuate the pressor response to peripherally administered ANG I. However, these CAP doses paradoxically enhance water intake elicited by dipsogenic stimuli. One commonly-voiced explanation for this enhancement is the "spillover" hypothesis: the high circulating levels of ANG I that are known to result from CAP's direct and indirect stimulation of renin release cross the blood-brain barrier and are converted in the brain to ANG II, which then acts to increase water intake. An alternative explanation is that while these doses of CAP may block ANG II synthesis in the general circulation (thus blocking the pressor response to ANG I), they may not be high enough to block conversion at the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT). These circumventricular organs contain high levels of ACE, and are not protected by a blood brain barrier. Thus, the increased ANG I that results from CAP treatment could be converted to dipsogenically active ANG II at those sites.

The purpose of this study was to determine whether peripherally administered CAP is blocking conversion of ANG I to ANG II outside of the blood-brainbarrier, by assessing the ability of CAP-treated rats to show a dipsogenic or pressor response to peripheral infusion of ANG I.

Eight rats received ANG I through a brachial arterial catheter (100 pmole/50 ul saline/min) for 20 minutes. Water intake during infusion and for 10 minutes post-infusion was recorded. Water was then replaced with CAP-adulterated water (0.1 mg/ml) for 18 hours, followed by a second ANG I infusion and test.

CAP treatment at this commonly-used doses did not attenuate the dipsogenic response to peripheral infusion of ANG I, indicating that blockade of ACE did not occur at some site(s) involved in this response. Effect of this treatment on the pressor response to peripheral infusion of ANG I will also be reported.

351.13 ROLE OF THE SUBFORNICAL ORGAN (SFO) IN CAPTOPRIL-INDUCED DRINKING. Thunhorst, R.L., D.A. Fitts* & J.B. Simpson. Dept. of Psychology, Univ. of Washington, Seattle 98195.

The angiotensin-converting enzyme (CE) inhibitor captopril (CAP) increases water and NaCl intake in rats, possibly via CNS conversion of angiotensin I to angiotensn II, which then produces these effects. Experiments involving brain lesions determined that the SFO is necessary for CAP-induced water, but not NaCl, intake. Rats with control lesions drank more water, and NaCl when it was available in choice with water, when drinking fluids were treated with CAP (0.1 mg/ml). Rats with SFO lesions failed to increase water intake, but did drink more saline if it was available during CAP treatment. A two-bottle test of water and CAP-treated water demonstrated that the above effects were not attributable to the taste of CAP. Experiments involving brain cannulae are determining if the neurological basis for the effects of SFO lesions on CAP-induced ingestion involves CE known to exist in the SFO. First, CAP administered i.p. (2 mg/kg) produced reliable drinking in rats with septal/ventral fornical commissure control lesions (n=6), and this drinking was reduced and eliminated in rats with partial (n=5) and complete (n=5) SFO lesions, respectively (90 min intakes±SEM were 6.5±0.6, 2.9±0.6, and 0.5±0.2, respectively). Drinking elicited by i.p. CAP (4 mg/kg) was suppressed in a dose-dependent manner by simultaneous application of CAP to the lateral ventricles (90 min intakes following 0, 0.5, 5 and 25 µg/µl CAP i.v.t. were 5.3±1.3, 5.3±1.1, 2.8±1.1, and 1.1±0.7, respectively). CAP applied i.v.t. (25 µg/ml) did not significantly reduce drinking to s.c. 2 M NaCl (3.33 ml/kg) or to s.c. isoproterenol (50 µg/kg); reduction in drinking is specific to thirst aroused by systemic application of CAP and is likely due to inhibition of brain CE necessary for the response. Ongoing experiments are determining the location of the brain CE responsible for the drinking following systemic CAP administration.

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351.14 CENTRAL OXYTOCINERGIC NEURONAL ACTIVITY: ROLE IN THE CONTROL OF SODIUM APPETITE IN RATS. Edward M. Stricker and Joseph G. Verbalis. Depts. of Psychology and Medicine, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

The biological bases of sodium appetite have remained elusive despite the availability of several well-established laboratory models of this drive. Although there is little doubt that angiotensin and aldosterone can act synergetically to elicit sodium appetite in rats, their joint actions cannot explain the sodium appetite of adrenalectomized rats, in which mineralocorticoids are absent, the sodium appetite of rats treated with desoxycorticosterone acetate (DOCA), in which renin secretion is suppressed, or the sodium appetite that persists in rats treated with polyethylene glycol after the induced hypovolemia has been repaired, at which time the secretion of both renin and aldosterone are normal.

The present experiments examined neurohypophyseal activity in these three models of sodium appetite in rats. Basal plasma levels of oxytocin (OT) were found to be low both in sodium-deficient adrenalectomized rats and in intact rats with surplus body sodium resulting from repeated daily treatments with DOCA. In contrast, basal plasma levels of arginine vasopressin (AVP) were not affected. Plasma OT also were at basal levels in colloid-treated rats after the induced plasma volume deficits had been repaired. Such low levels of plasma OT represent the first factor known to accompany sodium appetite in each of these disparate experimental conditions. Additional experiments indicated that sodium appetite in these models could be inhibited by ip administration of either lithium chloride or copper sulfate, agents which stimulate secretion of OT in much larger amounts than AVP in rats. The circulating levels of OT do not appear to be a direct factor in the control of sodium appetite because administration of OT did not inhibit NaCl consumption nor did administration of an OT receptor antagonist enhance NaCl intake. Assuming that OT secretion occurs in parallel with activity of OT-containing neurons in the brain, these results suggest that sodium appetite may be inhibited by activity in paraventricular or supraoptic nuclei, the location of neurons responsible for OT synthesis, and may emerge only when activity in those neurons is suppressed. Our data therefore support the possibility that sodium appetite is controlled both by inhibitory and excitatory central mechanisms, similar to the control of thirst. (Supported by Research Grants MH-25140 and AM-16166 from the National Institutes of Health.)

351.15 SODIUM APPETITE OF FISCHER-344 RATS. E.E. Midkiff,* D.A. Fitts,* J.B. Simpson, & I.L. Bernstein. Department of Psychology, University of Washington, Seattle, WA 98195.

Fischer-344 (F-344) rats fail to prefer sodium chloride (NaCl) solutions at any concentration, and indeed avoid NaCl solutions at concentrations ($\geq$ 0.7%) maximally preferred by other strains. The response of F-344 rats to treatments which substantially elevate NaCl intake of other rat strains was therefore examined. Following 12 days of maintenance on a Na-deficient diet, F-344 rats subsequently drank only slightly more NaCl than controls, and then only in the first 15 min. of access. Injection of furosemide, a diuretic which promotes natriuresis, in doses up to 10 mg/kg was found to elicit a vigorous sodium appetite in Wistar but not F-344 rats. Wistar rats drank 0.3 M NaCl rapidly when presented, and ingested Na in excess of need based on calculations of Na balances over the period of observation. In contrast, although NaCl intake of F-344 rats was elevated relative to their baseline intake, they ingested far less NaCl than Wistar rats did. They also excreted far less Na during the period of NaCl access, and consequently achieved sodium balance at 24 h which was comparable to that of Wistars. Thus, F-344 rats responded to an acute sodium-depletion treatment primarily by retention of Na rather than by overingestion of NaCl as seen in Wistar rats. Adrenalectomy, which prevents the retention of Na at the kidney, blocked the renal response to Na challenges. Adrenalectomy was the only challenge which elicited the robust and prolonged sodium appetite which is typically seen in other strains as a result of Na depletion: F-344 rats markedly increased their intake of 0.15 M NaCl and 0.3 M NaCl in response to adrenalectomy. The results suggest that F-344 rats differ from Wistars in their response to a moderate challenge to sodium regulation, using renal rather than behavioral responses. However, when denied adrenal mechanisms for Na retention, F-344 rats can and do greatly increase Na ingestion.

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351.16 A STUDY OF TASTE PREFERENCES AND POLYDIPSIC DRINKING ELICITED BY SACCHARIN BUT NOT ASPARTAME IN RAT, MOUSE, AND HAMSTER. W.J. Pizzi[1,2], R.R. Holson[1]*, G. Merkel[2]* and B. Gough[1]*.

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The artificial sweetener, saccharin, in a 3% glucose solution induces polydipsic drinking in rats (Valenstein et al., 1967). In an attempt to find highly palatable solutions in which drugs could be administered in a nonstressful manner over a long time period, we provided rats, mice, and hamsters with various concentrations of the artificial sweeteners saccharin and aspartame, with and without 3% glucose. In all three species, there was a clear preference for saccharin solutions as measured against intake of water or 3% glucose intake, or on two-bottle preference tests.

No such preference was found for aspartame. Consumption of solutions containing aspartame was similar to that obtained for the vehicle, whether water or 3% glucose. Male and female rats and female mice could detect the presence of aspartame in solutions, since on two-bottle preference tests they clearly preferred a glucose solution over the same solution with aspartame, in concentrations of 0.125% and 0.5%. Male mice and both male and female hamsters failed to show evidence of an ability to detect the presence of aspartame in solution. We conclude from these data that the rodents tested do not taste aspartame solutions as sweet.

Comparisons between species revealed that hamsters showed much less avidity for saccharin solutions than did mice and rats. Hamsters did not display synergistic polydipsia when presented with saccharin in 3% glucose solutions, as did both mice and rats.

These findings suggest that the strategy of comparing the anatomical and physiological differences between those animals that do or do not taste aspartame as sweet may be useful in uncovering the structural conformation required of molecules to stimulate taste receptors for the sensation of sweet. Further, these data may be of interest to those studying the influence of taste on regulatory behavior, and in the development of methods whereby rodents can be reliably induced to self-administer large volumes of fluid.

351.17 NALOXONE EFFECTS ON SHAM FEEDING OF SUCROSE IN FOOD DEPRIVED RATS. J.A. Stuckey* and D.C. Jimerson. Section on Biomedical Psychiatry, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

Naloxone has been reported to reduce food intake in several species under a variety of situations. It has been suggested that the reduction in food intake produced by naloxone is a result of antagonism of endogenous opiate systems in the CNS that are involved in the monitoring of food palatability and/or reward. Sham feeding of flavored solutions provides a sensitive behavioral measure of these functions. Naloxone has been shown to decrease the sham feeding of sucrose in water deprived rats (Rockwood and Reid, Physiol. Behav.,29:1175, 1982). In order to clarify further the effect of naloxone on palatability and/or reward in relationship to feeding, we examined the effect of naloxone on sham feeding of sucrose by food deprived rats.

Male Sprague-Dawley rats (300-350g) were anesthetized with pentobarbital and implanted with stainless steel gastric cannulas. Following recovery (2 weeks) the rats were adapted to a test schedule. Rats were deprived of food for 17.5 hr. Thirty minutes before the test, the gastric cannulas were opened, the rats' stomachs were gently flushed with 0.15 N NaCl, and drainage tubes were attached. The test period consisted of 30 min access to a sucrose solution. At the end of the test period the cannulas were closed and the rats received solid food for six hours. When adapted to the schedule, the rats were tested with 20% sucrose (w/v), 5% sucrose, then 10% sucrose. Thirty minutes before the test the rats received I.P. injections of naloxone (1 mg/kg and 10 mg/kg) or 0.15 N NaCl as vehicle control. Rats received both doses of naloxone at all sucrose concentrations. All testing with a particular concentration of sucrose was completed before testing at another concentration. Sucrose intakes were measured every three minutes.

During sham feeding tests with 20% sucrose, 30 min intakes (in ml) for an initial group of four animals were 36.2, 29.2, and 15.8 after doses of naloxone of 0, 1.0, and 10.0 mg/kg respectively. Naloxone also reduced sham feeding in a dose related manner with 5% and 10% sucrose concentrations. The administration of naloxone had the same apparent effect as decreasing sucrose concentration. These preliminary data support the hypothesis that naloxone decreases palatability and/or reward value of food.

351.18 CHRONIC NALOXONE AND NALOXONAZINE: EVIDENCE FOR A SPECIFIC OPIOID RECEPTOR SUBTYPE IN MAINTENANCE OF BODY WEIGHT AND FOOD INTAKE. P.E. Mann, R.J. Bodnar, G. Currerí*, E. Lubin, E.F. Hahn and G.W. Pasternak. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367, Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Ctr., and Lab. of Biochemical Endocrinology, Rockefeller Univ. New York, NY 10021.

Acute administration of either naloxone (NAL) or naloxonazine (NAZ) reduces food intake in free-feeding and deprived rats, suggesting involvement of the mu-1 binding site in these effects. However, only NAL reduces feeding induced by glucoprivation or administration of opioid receptor agonists indicating a non mu-1 source of opioid action. The present study evaluated the effects of chronic intravenous administration of vehicle, NAL (10 mg/kg) or NAZ (10 mg/kg) on body weight maintenance and food intake in adult, adolescent and dietary-obese rats. Adult rats (540g) treated daily over 14 days displayed orderly, gradual and significant reductions in body weight following NAL (5%) and NAZ (9%) and also reductions in daily food intake following NAL (13%) and NAZ (23%). Adolescent rats (300g) treated daily over 14 days showed marked and significant retardation of body weight gain with NAL (44g, 32% reduction) gaining significantly less than vehicle (65g) and NAZ (31g, 52% reduction) gaining less than either vehicle or NAL. Food intake was also significantly reduced by NAL (13%) and NAZ (22%). Food intake effects could not account for body weight reduction in adult or adolescent rats. After cessation of NAL and NAZ treatment, both adults and adolescent rats recovered to vehicle values within 3 weeks. In contrast to opioid and mu-1 receptor control of body weight and food intake in adult and adolescent rats, animals made obese by a 6 week exposure to a "supermarket" diet (sweetened evaporated milk, cookies, vegetable shortening) failed to display changes in body weight or milk consumption following 8 days of NAL or NAZ treatment. This contrasts with increased short-term sensitivity to NAL observed in obese rats, and illustrates further that opioid modulation of weight control and intake appears to be dependent upon heterogeneous opioid receptors. (Supported by PSC/CUNY Grant 6-65193, NIH BRSG RR07064 and NIDA Grant DA002615.)

351.19 FOOD RESTRICTION AND DIETARY PROTEIN EFFECTS ON BLOOD AMINO ACIDS AND ON BRAIN TRYPTOPHAN AND TYROSINE LEVELS, CHOLINE ACETYLTRANSFERASE ACTIVITY AND BIOGENIC AMINES INTERACTIONS IN CAT. L. Thibault* and A.G. Roberge. Laboratoire de Neurochimie et de Nutrition, F.S.A.A., Université Laval, Québec, G1K 7P4.

Adult cats were adapted for 37 consecutive days to either isoproteinic-isoglucidic or isoproteinic-hypoglucidid semi-purified casein and soya diets. Serum neutral amino acids content and tryptophan and tyrosine levels in the medulla were measured as well as brain biogenic amines content in 9 different structures using a fluorimetric method. Choline acethyltransferase (ChAT) activity was measured in the brain structures using radiochemical method. When isoproteinic-isoglucidic diets were compared, soya diet showed a lower serum methionine content but higher phenylalanine and tyrosine concentrations than casein diet. Moreover, isoproteinic-hypoglucidic soya diet induced a significant decrease in serum valine and methionine levels when data were compared to isoproteinic-isoglucidic soya diet. On the other hand, to unchanged serum values of free tryptophan and tyrosine : neutral amino acids ratios corresponded a higher tyrosine level in the medulla in isoglucidic and hypoglucidic soya diets than in isoglucidic casein diet. Moreover, an enhancement of 5-HT utilization or its degradation in the limbic system in cats fed hypoglucidic casein diets and in the brainstem structures in cats fed isoglucidic and hypoglucidic soya diets was demonstrated. The lower DA : NA ratio corresponding to increased NA levels in the striatum and medulla in hypoglucidic soya diet suggested an enhanced DA utilization whereas in the isoglucidic soya diet a rather DA mobilization was suggested in the brainstem. The selective decrease in CHAT activity observed in the striatum and mesencephalon in cats fed soya (isoglucidic and hypoglucidic) diets associated with a potentiation of 5-HT and DA metabolism in these structures suggest an interaction between cholinergic and aminergic metabolisms. These results suggest that in cats, the dietary protein composition as well as the carbohydrate : protein ratio in the diet rather than serum neutral amino acids ratios have differently affected the neurochemical parameters. In fact, the hypoglucidic casein diets have favored a faster 5-HT metabolism in the limbic system, the hypoglucidic soya diet, a faster 5-HT metabolism in the motor system and brainstem as well as a faster DA metabolism in the brainstem, thus suggesting possible interactions between neurotransmitters which mimic some functional behaviours observed in anorexia.
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POSTURAL CONTROL

352.1 DUAL MOTOR OUTPUT INTERNEURONS
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It was suggested some time ago from extracellular data that certain premotor interneurons in crustaceans could perhaps influence both abdominal positioning and swimmeret behaviors (Evoy and Kennedy, 1967, J.E.Z. 165:223. Williams and Larimer 1981, J.C.P. 143:27.). Behavioral observations support the notion that the underlying neural basis for the two behaviors may be overlapping or coupled through synapses and common elements. Metachronous beating of the swimmerets usually accompanies the initiation of walking, while abdominal extension generally precedes the initiation of the swimmeret rhythm. In addition, abdominal flexion requires that the swimmerets be in the return stroke position. In order to avoid the ambiguities of extracellular methods, we have re-examined the neural basis of these two behaviors using intracellular recording, stimulation and dye-filling techniques in the abdominal nerve cord of the crayfish Procambarus clarkii. We have recorded simultaneously the first roots (swimmeret innervation) of various abdominal ganglia, from second roots (extensor motor neurons) and from third root (flexion motor neurons) while injecting depolarizing current into impaled interneurons. This procedure was followed by dye-filling and morphological examination.

We have encountered numerous cells which are capable of evoking various swimmeret and positioning behaviors. These cells, however, are not confined to evoking abdominal extension/swimmeret beating nor swimmeret return stroke/abdominal flexion, but other combinations as well. Like the positioning cells examined in earlier studies, these vary greatly in efficacy. Not all cells we encounter influence both the swimmeret and the positioning systems; many affect only one or the other. Most of the cells examined thus far are interganglionic, however, a substantial number are local.

Investigations are underway to determine the neural basis of the dual effects. Dual effects could result from the synaptic recruitment of pure positioning and pure swimmeret neurons or from the direct synapses between the dual output cells, the driver and motor neurons of both systems or from some combination of both. An examination of pairs of interneurons and interneuron/motor neuron pairs for synaptic interaction will be required to resolve this. (This research was supported by Grant NS 05423 from NIH).

352.2 AN IDENTIFIED LOCUST PROPRIOCEPTOR MEDIATES LOAD COMPENSATION: EVIDENCE FROM SELECTIVE SENSORY STIMULATION IN FREELY MOVING ANIMALS. S.N. Zill Dept. of Anatomy, Univ. Colo. Med. Sch., Denver, CO 80262.

Many recent studies have shown that reflex effects of most proprioceptors are not constant but can be modified by the CNS to adjust motor activity only when sensory inputs are behaviorally relevant. These studies imply that it is necessary to examine sensory-motor integration in naturally behaving preparations to evaluate the functions of different receptor modalities. I have studied an identified joint angle receptor, the locust metathoracic femoral chordotonal organ, and have previously found that considerable plasticity occurs in reflex effects it produces in leg motoneurons in restrained preparations (Zill, J. Exp. Biol. 116:463,1985). These reflexes have also been demonstrated to consistently change to tonic compensatory reactions in animals that have been trained to maintain a leg position using a learning paradigm (Zill & Forman, J. Exp. Biol. 107:385, 1983). I here report on a new adaptation of this preparation that permits selective mechanical stimulation of this proprioceptor, mimicking sudden changes in joint angle, in freely moving animals, to directly evaluate its functions in behavior.

Adult locusts (Schistocerca gregaria) are first restrained and a piezoelectric crystal is attached to the proximal femoral segment of the hindleg. A small window is cut in the distal femur and a metal hook is inserted through the window under the main ligament of the organ. This ligament mechanically links the organ to the tibial segment of the leg and is stretched by joint flexion specifically exciting receptors that signal joint position and movement. The protruding end of the hook is then waxed to the crystal. Animals are then released into a walking arena. Electrical stimuli applied to the crystal produce mechanical displacements of the organ that signal 10-15° joint flexions. Reflex responses of the tibial extensor and flexor muscles are monitored myographically.

Excitatory reflex responses are elicited, that counter the apparent joint motion, when stimuli are applied to the organ during spontaneously assumed postures in which activity is present in the slow extensor motoneuron to support the animal. Sustained displacements of the ligament produce tonic increases in extensor firing that are maintained throughout the duration of the stimulus (max. 3 min.). The constancy of these reflexes are in contrast to the variability of responses of restrained preparations but are remarkably similar to reflexes obtained from animals that have been trained to maintain a leg position. These studies have therefore demonstrated that the chordotonal organ mediates load compensatory reactions in actively assumed postures and can aid in setting tonic levels of motoneuron activity. Secondarily, these studies confirm that leg position learning is a useful technique for studying the mechanisms underlying how insects naturally assume active postures (Forman, J. Neurobiol. 15:127, 1984).
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352.3 MOTOR UNIT FIRING IN FREELY MOVING NORMAL AND BASAL GANGLIA LESIONED CATS. C.G.Kukulka, A.G.Russell and K.Kultas-Ilinsky. Physical Therapy Research Labs and the Department of Anatomy. The University of Iowa College of Medicine, Iowa City, IA 52242.

In experiments designed to evaluate posture and movement disturbances following unilateral basal ganglia lesions in the cat, we have been able to isolate and evaluate single motor units. Cats (n=3) performed a self initiated forelimb placement task in which they lifted their left forelimb and pushed an elevated bar press. Vertical reaction forces from all limbs and intramuscular EMG from 7 muscles were continually recorded. Single motor unit spike trains were obtained from 3 muscles: triceps brachii bilaterally and right vastus lateralis. Unilateral kainic acid lesions were made in the entopeduncular nucleus and substantia nigra reticulata.

For the 25 spike trains analyzed to date, 21 contained 33 to 121 interspike intervals (ISIs)(x=69). The remaining 4, all from vastus lateralis, ranged from 13 to 21 ISIs. Mean firing rates ranged from 36-65/s for right triceps (n=12), 43-65/s for left triceps (n=7) and 21-34/s for vastus lateralis (n=6). A majority of the spike trains were generated during quiet standing in which little change in the vertical reaction forces was discernable. We therefore feel these firing rates reflect motor unit behavior under isometric-like conditions.

Three general observations were evident. First, no obvious differences in mean rates were found pre and post lesion. The use of a mean rate may be an inadequate criterion to judge motor unit behavior under the conditions described here. The lack of a clear cut steady state of firing was the general rule and suggests that other characteristics of firing might bettter describe the behavior (i.e. variability of firing, incidence of long ISIs followed by short). These factors are currently being evaluated. Second, the high mean firing rates in triceps for periods of .5 to 3 sec of activity, indicate that, in a freely moving cat, motoneurons fire in the secondary range of the frequency-current relationship used to describe intracellularly stimulated hindlimb motoneurons. Third, single unit identification is complicated in awake behaving animals by inconsistencies in amplitude and wave shape of the action potentials. Additional tests are needed to confirm the uniqueness of a single unit. One such test, presented here, is the examination of the distribution of ISIs for a preponderance of excessively short intervals. A model is presented which describes the information obtained when two units are detected and analyzed as one. A comparison is made between the model and actual spike train data.

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352.4 PREPARATORY POSTURE PATTERNS IN NORMAL AND BASAL GANGLIA LESIONED CATS. A.G.Russell, C.G.Kukulka and K.Kultas-Ilinsky. Physical Therapy Research Labs and the Department of Anatomy, The University of Iowa College of Medicine, Iowa City, IA 52242.

Experiments were designed to evaluate postural disturbances following kainic lesions of the cat basal ganglia. We have been able to document a change in the postural adjustment preceeding a movement in a lesioned cat. Cats (n=5) performed a forelimb placement task consisting of lifting the left forelimb upward towards an elevated bar press, pushing on the bar press, and then returning the limb to its standing support position. Vertical reaction forces from all limbs were measured during standing, prior to, during, and following the movement. Intramuscular EMG was recorded from 7 muscles using chronically implanted fine wire electrodes. Unilateral kainic acid lesions were made in the right entopeduncular and substantia nigra reticulata nuclei.

In 1 cat, it was possible to characterize a change in the postural adjustment prior to movement following lesioning. Changes in the vertical reaction force under the left forelimb was used to characterize the postural adjustment and subsequent movement. Prior to lesioning, the animal maintained a stable distribution of body weight among all 4 limbs. The movement was signaled by an abrupt decrease in the left forelimb force plate. The frequency of occurence for this strategy was 76% (19/25 trials) and 100% (12/12) for 2 days of testing prior to lesioning. Three to 6 days post lesion, the movement consisted of 2 stages. The first was a gradual decrease in the left forelimb reaction force lasting 3-5 sec, followed by the abrupt unloading of the limb. The frequency of occurrence for this altered movement was 82% (23/28 trials) 3 days post lesion, 82% (28/34) 5 days post lesion, and 86% (25/29) 6 days post lesion. By 8 days, a transition appeared with the slow approach to the movement lasting a shorter duration. By 9 days, the movement was similar to that of the non-lesioned cat.

Frequencies of other movement patterns pre and post lesion are presently being analyzed together with the more quantitative data on force onset latency, rate of force change, and percent of body weight transferred during the postural adjustment and movement. For the animal presented here, the localized lesion to the substantia nigra suggests that even a small interruption of the basal ganglia output may produce an alteration in the postural adjustment which occurs prior to a single forelimb movement. The effect of this lesion on the postural adjustment appears short lived, with the animal returning to a prelesion pattern within 9 days post lesion.

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352.5 POSTURAL RESPONSES IN CATS TO MOVEMENTS OF THE SUPPORT SURFACE IN THE HORIZONTAL PLANE - COMPARISON OF LATERAL AND LONGITUDINAL DISPLACEMENTS J.M. Macpherson and L.S. Craig*. Dept. of Anatomy, Queen's Univ., Kingston, Ont. K7L 3N6.

Postural responses in cats to disruptions of stance are centrally generated programs which are triggered by peripheral input. This study is the first of a series designed to investigate the hypothesis that these central programs are organized in terms of groups of muscles, or synergies. These experiments compare the postural responses in freely-standing cats to displacements of the support surface in two directions in the horizontal plane, in order to confirm that the strategies for correction are distinct and characteristic for each direction.

Cats are trained to stand quietly on a platform, with each paw placed on an individual, triaxial force plate. The support surface can be moved in any direction in the horizontal plane using hydraulic cylinders. The platform is computer-operated (DEC PDP11/73) and the data are recorded on-line in individual trials of 500 ms periods and at a rate of 1 kHz. These data consist of 12 forces (3 for each limb), positions of 6 cylinders (sensed by LVDTs), and 8 channels of electromyographic signals (EMG).

In each cat, eight hindlimb muscles are implanted with electrodes consisting of pairs of teflon-coated, multistranded stainless steel wires that are drawn through the muscle bellies and stitched in place. The EMG signals are band-pass filtered (260Hz & 2KHz), full-wave rectified, and finally, filtered through a four-pole low pass digital logic filter (3dB cutoff of 100Hz).

Stance was disrupted by displacing the platform 1.5 cm laterally, or 4.0 cm longitudinally, over a period of 100 ms. The difference in amplitudes reflects the difference in stability of the cat in the two directions.

Lateral movement of the platform to the left induced a sway of the cat to the right, thus loading the right and unloading the left limbs. To correct the sway, the cat exerted a force with a rightward lateral component, using primarily the limbs on the right side. Thus, lateral perturbations evoked responses in abductors of the loaded hindlimb, with little or no activation of adductors of the other hindlimb. By contrast, longitudinal perturbations induced sway in the sagittal plane, and were corrected primarily by bilateral activation of hip flexors or extensors (following posterior or anterior translation, respectively), confirming previous findings (Rushmer et al, Exp. Brain Res. 50:45-61, 1983). Therefore, lateral and longitudinal translations of the support surface evoke two distinct postural strategies to reduce body sway. We may now proceed to study the interactions of the muscle synergies characteristic of each strategy.

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352.6 INTERACTIONS BETWEEN REFLEXIVE AND VOLUNTARY POSTURAL ACTIVITY. C.S. Layne* and L.D. Abraham (SPON: R.P. Farrar). Dept. of Physical Education, Univ. of Texas, Austin, Texas 78712.

Normal standing subjects display a distal-to-proximal pattern of muscle activation as an automatic postural adjustment (APA) to a horizontal displacement of the feet (Nashner, Exp. Br. Res., 30: 1977). Also, postural adjustment patterns (PAP) prior to prime mover activation during an arm raising task have been reported (e.g. Lee, J. Mtr. Beh., 12: 1980). It has been presumed that this lower limb activity minimizes pertubations of the body's center of gravity associated with upper limb movement, similar to the action of responses to displacement of the fact. Thus, this activity has been referred to as "postural", with the prime mover activity being labled "voluntary". In order to characterize better the nature of these patterns, we examined the EMG responses of colleged-aged males during a bilateral reaching task in a simple reaction time (RT) paradigm. In particular, we examined the interaction between voluntarily and reflexly initiated "postural" activity.

Four experimental conditions were employed: (A) unexpected backward horizontal displacements designed to allow us to identify the reflexly initiated activity pattern, (B) voluntary reach to a visual stimulus following a fixed warning interval, (C) same as B with the addition of 3 lb. wrist cuffs, and (D) a combination of A and B with the displacements occurring at selected times relative to the RT stimulus. Surface EMG was used to monitor the activity of the following muscles of the right side: soleus, gastrocremius, biceps femoris, and biceps brachii. Ten trails of each condition were analyzed to identify patterns based on latency and amplitude.

In A, subjects displayed a distal-to-proximal APA pattern, while in B PAPs were observed. The additional weight in C resulted in larger EMG bursts and slightly longer RTs. In D, when the perturbation occurred prior to the onset of the voluntary response, PAP was absent while the APA response remained intact. When the perturbation occurred during the PAP, the APA was not observed. When the perturbation occurred after the PAP, the APA occurred as in A. Thus, it appeared that when perturbations occurred early enough in an RT paradigm, subjects were able to rely on the reflexly initiated response to stablize the body, eliminating the early lower limb activity. However, the onset of the PAP seemed to temporarily inhibit the APA.

352.7 SKILL-RELATED DIFFERENCES IN NEUROMUSCULAR AND KINEMATIC POSTURAL ADJUSTMENTS DURING A BALLET SKILL (GRAND BATTEMENT DEVANT). M. K. Cook* and L.D. Abraham (SPON: W. C. Abraham). Dept. of Physical and Health Education, Univ. of Texas at Austin, Austin, TX 78712.

Studies of postural adjustment patterns (PAP) in humans have typically focused on upper limb movements with postural muscles studied in the lower limbs and back (Bouisset & Zattara, Neurosc. Ltrs., 22:263-270, 1981). In this study, two groups were compared to determine the effects of skill level on EMG and kinematic parameters of PAPs for a free-standing forward leg swing from ballet. Supporting leg EMG patterns of biceps femoris (BF), rectus femoris (RF), tibialis anterior (TA) and gastrocnemius (GAS) were recorded from six skilled (S) and six unskilled (U) subjects. Latencies and standard deviations (SD) of latencies of pre-movement silent periods and the first two bursts of EMG were compared between groups. High speed film records (48 fps) of all subjects were analyzed for timing of the elevation impulse of the raised leg, horizontal displacement of the center of gravity (CG), time to greatest backward lean of the supporting leg, and maximum angular acceleration of the raised leg (AA). Kinematic data were time normalized over the duration of the leg lift.

EMG latencies and kinematic variables were analyzed with two-way ANOVA and SD scores with t-tests. For all kinematic and most EMG measures, no differences were found between groups ($p > 0.05$). Differences were found between groups in the occurrence of pre-movement EMG silent periods (Abraham & Cook, Biomech. X, in press), in the mean latency of the second TA burst (S = 212 msec; U = 113 msec) and in the mean consistency (SD) of the second GAS burst (S = 197 msec; U = 105 msec).

The relative consistency between groups in EMG patterns suggested that the timing of the elevation impulse and the AA of the raised leg determined the overall PAP required to maintain stationary balance as the weight was shifted from two feet to one foot. Variability in the timing of the second bursts of TA and GAS between groups suggested that S and U ballet students may have used different strategies for counterbalancing the torque created by the leg swing. When the CG was allowed to shift forward initially, counteractive forces at the ankle joint were necessary later in the leg swing (S strategy). However, the average timing of the second TA and GAS bursts of the U group occurred about the same time as the maximum AA of the raised leg (peak of elevation impulse). Thus, while the U group appeared to have been reacting to the disturbance created by the leg swing, the S group was controlling balance less reactively.

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352.8 INFLUENCE OF INITIAL STANCE POSITION ON HUMAN POSTURAL RESPONSES. S.P. Moore*, F.B. Horak, and L.M. Nashner. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon 97209

Previously, we reported that latencies and amplitudes of automatic postural muscle responses to surface perturbations could be influenced by expectation of perturbation direction. However, it was also noted that subjects appeared to change their initial stance position when the perturbation was expected. To determine whether changes in initial stance position could account for expectation effects, we studied the influence of initial stance position on automatic postural muscle responses.

Subjects were exposed to 50 forward and 50 backward horizontal support surface translations (6 cm, 240 ms) which were randomly presented in blocks of 10 trials. For each block, subjects assumed one of 5 initial stance positions, each which involved leaning about the ankles: normal, maximum forward, half maximum forward, maximum backward and half maximum backward. Stance position was monitored using a linear potentiometer attached via a light rigid rod to a belt around the subjects' hips. Prior to each trial, subjects leaned to instructed positions using visual feedback provided from an oscilloscope which displayed the potentiometer signal. Surface EMG activity of 6 leg and trunk muscles, forces exerted by each foot on the surface and anteroposterior (AP) sway of the body center of mass were collected for all trials.

Postural responses for subjects in the normal and half-lean positions had very similar EMG onset latencies, patterns and activity levels. Also, little change in muscle activity was seen when subjects leaned maximally in the direction opposite of the perturbation-induced AP sway.

Subjects changed their postural strategies when perturbed while leaning near their forward or backward boundaries of stability. When this maximal leaning was in the same direction as the perturbation-induced AP sway, subjects changed from using ankle torque to exerting both horizontal shear force and ankle torque to move the body center of mass. This was associated with reduced and delayed activation of the stretched distal muscle and with an earlier onset and increased activation of proximal muscles.

Therefore, although initial stance position may change postural muscle activation patterns, these changes are not consistent with local stretch-induced effects. Furthermore, changes in initial stance position cannot explain expectation-related changes in postural muscle response latencies and amplitudes.

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352.9 CONTRIBUTION OF THE VESTIBULOSPINAL SYSTEM TO DEVELOPMENT OF POSTURAL COORDINATION. A. Shumway-Cook, F.B. Horak and F.O. Black, Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon 97209.

An intact vestibular system has been considered critical to development of normal coordination of posture and movement. This study investigated the effects of vestibular deficits on postural muscle response patterns and motor skill proficiency in children.

Tests of vestibular function and postural motor coordination were correlated in 95 children ages 8-12 : 52 normal, 30 hearing impaired and 15 learning disabled (LD). Vestibular function deficits were assessed by testing the vestibulo-ocular reflex and 2) vestibulospinal function (changes in amount of body sway when vision and somatosensory inputs are altered). Based on these results, children were classified as having 1) normal, 2) reduced or absent (4 normal and 20 hearing impaired), or 3) distorted vestibular/sensory organization deficits (15 LD and 3 hearing-impaired). Postural motor coordination was tested by examining body kinematics and the latencies, patterns and integrated areas of surface EMG activity in six trunk and leg muscles in response to platform translations while the children stood on either a normal surface or across a 9 cm wide beam. Motor performance (bilateral coordination, strength, speed and balance) were measured using the Bruininks-Oseretsky Test.

Postural motor coordination (EMG latencies, amplitudes, and patterns) in response to postural perturbations while standing on the normal surface were normal in all children regardless of vestibular deficit. Normal movement strategies on the narrow beam however, were found only in children with normal vestibulospinal function, regardless of vestibulo-ocular function. Children with absent or reduced vestibular function had absent or reduced amplitudes of postural EMG responses to perturbations while standing on the beam but normal latencies and patterns. In contrast, children with distorted vestibular function had abnormal temporal and spatial components of EMG responses to perturbations while standing on the beam, even with practice. Motor proficiency, other than balance scores, were normal in children with reduced or absent vestibular function, whereas all aspects of motor proficiency were below the 8th percentile in children with distorted vestibular function.

Results suggest that reduced or absent vestibulospinal function may reduce or eliminate the motor strategies necessary for balancing on a beam without affecting the coordination of muscle action within available strategies or other aspects of motor proficiency. In contrast, distorted and/or central sensory organization deficits may affect postural coordination and motor proficiency. Supported by NIHR, MRF, and NIH 19222, 133FH30086.

352.10 A MECHANICAL MODEL FOR THE PREDICTION OF UPRIGHT BALANCE STRATEGIES IN HUMANS. J.F.Yang, D.A.Winter, R.P.Wells* Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

Upright standing and walking balance has been studied by many from a neurological and feedback control perspective. Balance is a mechanical task, governed largely by the laws of dynamics, hence mechanical analyses could shed light on movement strategies to meet these mechanical demands. Mechanically feasible compensating strategies to an external disturbance were studied using a simulation model. A planar link segment model consisting of feet, legs, thighs and a trunk was simulated on a digital computer. Ligaments were simulated as non-linear torsional springs and dampers which were activated at the extremes of joint range. Inputs to the model were idealized joint torques at the hips, knees and ankles. Outputs were the body segment kinematics. Mechanical disturbances to the feet and trunk were simulated, and all mechanically possible compensating strategies were identified by an iterative procedure. Each joint torque could take on any value between +140 to -140 N.m. All possible torque combinations were iterated in 10 N.m steps. Balance was considered to have been successfully maintained if all segments were within 5 degrees of the unperturbed position after 100 ms. Only very specific combinations of joint torques were successful in maintaining balance. A solution space (i.e. successful combinations of joint torques) was mapped out for each disturbance. The solution space was dependent on the direction and the location of the disturbance application, and on the initial conditions. The solution space for disturbances to the feet could be compared qualitatively with EMG experimental results (Nashner et al., Exp Brain Res, 36:463, 1979). The model predicted that the strategies reported by others are possible solutions, but other mechanically feasible solutions exist. The feasible solutions formed a continuum, not discrete areas. The model predicted that although each joint torque could take on a wide range of values, the relationship between each of the joint torques must remain fixed to be successful. This fixed relationship was approximately 2:1 for the ankle:knee, and 1.5:1 for the hip:knee. These relationships remained invariant for different disturbances. In summary, the experimental results are in accordance with the model predictions, and the model was able to suggest other feasible movement strategies.

352.11 POSTURAL ADJUSTMENT FOR INTERLIMB COORDINATION IN MAN DURING ARM LOCOMOTION. K. Nakajima* and A.M. Sherwood, Dept. of Clinical Neurophysiology, The Institute for Rehabilitation and Research and the Dept. of Rehabilitation, Baylor College of Medicine, Houston, TX 77030

We examined the coordination of muscle activity of the arms and and legs when healthy subjects walked on their arms while both legs were suspended horizontally behind them. We analyzed the events during initiation and during stabilized arm locomotion from recordings of force plate and electromyographic data.

On initiation of arm walking, we observed an anticipatory postural adjustment which consisted of activation of the ipsilateral triceps brachii and quadriceps, and the contralateral triceps brachii and hamstring before a burst of activity in the ipsilateral biceps brachii of arm swing. This anticipatory activity provides a diagonal support pattern, i.e., the ipsilateral quadriceps and contralateral triceps brachii muscle activity maintain the posture, in essentially the same activity pattern as the subsequent arm locomotion. We consistently observed this diagonal pattern in all subjects without exception.

The force platform data showed that the central command for initiation of arm locomotion produced an initial shift of the center of gravity toward the side of the swing phase movement, prior to the readily apparent shift contralaterally. The anticipatory muscle activity coincided with this initial shift of the center of gravity. Both the anticipatory activity on initiation, and the diagonal support pattern during arm walking were suppressed with body support.

We therefore assumed that this property of anticipatory activity is essentially identical to the postural adjustment which is observed in leg muscles when man asked raise an arm in standing position (Belenky et al., Biofizica, 12:135-141, 1967). We further assumed that the postural adjustment in locomotion is centrally programmed and is utilized on the initiation of locomotion, and that subsequent compensatory and anticipatory movements occurred at the spinal or medullary level.

This work was supported by the Vivian L. Smith Foundation for Restorative Neurology, Houston, TX

352.12 BIOMECHANICAL ANALYSIS OF STANDING POSTURE WITH RAPID ARM MOVEMENTS. L.G. Cohen*, W. Friedli*, M. Hallett, S. Stanhope* and S.R. Simon*. Sec. of Human Motor Control, Medical Neurology Branch, IRP, NINCDS, NIH, Bethesda, MD 20892.

In a previous presentation we showed some biomechanical correlations of postural muscle activity (Friedli et al. Soc. Neurosci. Abst. 9; 66, 1983). We have now performed further studies and theoretical analyses. The execution of a symmetric arm movement generates two main postural problems: maintain the center of gravity of the body over the base of support and maintain the body parts in relation to each other. The purpose of this study is to better understand the nature of these problems as well as the mechanisms used for their solutions. We addressed the first problem by studying the internal and external translational forces which act within and on the body and the second by utilizing a theoretical model to evaluate the magnitude and direction of joint reaction torques.

Normal human volunteers standing on a force plate made bilaterally symmetric fast elbow movements (flexion from 180 to 90 and 90 to 0 degrees; extension from 0 to 90 and 90 to 180 degrees). Vertical and anterior-posterior translational external forces as well as center-of-foot-pressure location measurements were derived from the force plate. Internal translational forces acting on individual body segments were estimated using inverse dynamics calculations. Body motions were obtained using a VICON motion analysis system.

For mechanical reasons stability in the anterior-posterior direction is more volatile than in the vertical direction. The analysis of the external vertical forces indicates that changes in the magnitude of these forces during arm flexion are directly related to the vertical acceleration of the forearm. The rest of the body contributes little to these dynamic changes. The analysis of anterior-posterior forces reveals an external force which is the sum of the anterior-posterior forces produced by the rapid elbow flexion and compensatory reaction accelerations of the various body parts. Hence, the body does indeed compensate in the theoretically more destabilizing direction. Despite significant joint reaction torques, movements of the body parts with respect to each other are only minimal. The muscular forces which are represented by the EMG activity in postural muscles (Friedli et al. J. Neurol. Neurosurg. Psychiat. 47; 611, 1984) are appropriate; for minimizing the effects of joint reaction torques while allowing for necessary compensatory body movements to occur.

The postural activity which the body exhibits involves the simultaneous successful solution of both types of postural problems.

352.13 ALTERED WEIGHT BEARING IN A PATIENT WITH HYPERREFLEXIA: EFFECTS ON CENTER OF PRESSURE AND TENDON JERK REFLEX RESPONSES. L Nosse*, TJ Myers, JF Cusick*, BM Myklebust , Prog. Phys. Ther., Marquette Univ., Depts. of Neurosurg. & Neurol., Med. Coll. of Wis., Sensory-Motor Performance Lab., Zablocki VAMC, Milwaukee, WI, 53295.

Randolph has suggested that a means of modulating postural abnormalities in patients with hyperreflexia is to shift weightbearing off the anterior part of the foot (1). This technique was evaluated in a patient with cerebral palsy and cervical spondylotic myelopathy with hyperreflexia and toe clawing. A pair of "heel-support shoes" was fabricated to limit weight bearing to the calcaneous.

The patient was tested before and after wearing the heel-support shoes for 30 min while standing. For each test condition, 20 Achilles tendon taps were manually applied with a reflex hammer, and surface EMGs were recorded from soleus (SOL) and tibialis anterior (TA) muscles (2). Postural steadiness was assessed during standing on a triangular force platform. The platform measured the center of pressure (the vertical projection of the center of gravity) as well as the simultaneous separate vertical forces under the feet.

SOL reflex responses to Achilles tendon taps were unremarkable and unchanged by wearing the shoes. Abnormal TA responses were evoked by Achilles tendon taps before wearing the shoes. For 100 ms following the tap, TA EMG was randomly evoked at onset latencies which did not correspond to the SOL EMG. The TA amplitude was 4 times larger than SOL. After wearing the shoes, 15 tendon taps evoked no TA EMG. Random TA bursts were only recorded from 5 taps when the SOL response was weak. The TA amplitudes were reduced 75%.

When standing barefoot, the amount of fore-aft sway was more than 3 times the medial-lateral sway. The primary effect of the shoes was to shift the center of pressure posteriorly. The shoes also increased the medial-lateral sway and the frequency of sway.

Possible mechanisms for the short-term change in the TA EMG activity and its relationship to posterior shift of the center of pressure include muscle fatigue and modulation of segmental and suprasegmental excitability levels.

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352.14 SOME VISUAL INFLUENCES ON HUMAN POSTURAL EQUILIBRIUM: BINOCULAR AND MONOCULAR FIXATION. Charles R. Fox. Visual Science Lab., Columbia Univ., New York, N.Y. 10027.

The importance of vision to postural equilibrium has long been known in both clinical and non-clinical populations. A highly structured, stationary visual field attenuates sway relative to sway while the eyes are closed. If the field is moving, the observer is destabilized in a manner related to the visual motion. Traditionally, this visual control of posture has been analyzed in terms of optical and/or retinal phenomena. There has however, been some suggestion that binocular and monocular fixation of identical field conditions has different effects. The current research systematically examines this differential effect during quiet standing.

Three experiments were conducted which measured movement of the body's center-of-gravity while varying field structure, ankle proprioception, and fixation. Field structure was varied from total darkness, to single and multiple LEDs in the dark, to full field structure. These structures provided visual feedback about body sway through self-generated visual field motion. Ankle proprioception was manipulated by using either a standard or tandem Romberg posture. Fixation was manipulated by having the observer fixate a target within the structured fields or a remembered target in a totally dark field with both eyes, or with only the non-dominant eye.

The results indicate that 1) Ankle joint input is an important factor, 2) binocular fixation attenuates sway relative to monocular fixation of identical targets under identical field conditions, 3) this difference also exists in totally dark fields. Taken together, the data indicate that the visual influence on postural equilibrium results from a complex synergy which modulates postural reflexes. A simple optical/retinal explanation is not sufficient.

353.1 ENCODING OF MOVEMENT PARAMETERS BY RUBROMOTONEURONAL CELLS. P.D. Cheney and K.H. Mewes*. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

In previous work, we identified corticomotoneuronal (CM) cells in awake rhesus monkeys by their postspike facilitation (PSF) in spike-triggered averages of rectified emg activity (Fetz and Cheney, J. Neurophysiol. 44:751, 1980). Another major descending system that shares the same target muscles as the CM system is the rubromotoneuronal (RM) system. In this study, we identified RM cells using spike-triggered averaging of rectified emg activity and investigated the functional properties of these cells under conditions similar to those previously used for CM cells. In recordings from 120 red nucleus cells, we identified 21 RM cells, 20 of which were tested under alternating ramp-and-hold wrist movements against different spring-like loads (auxotonic conditions). The patterns of RM cell modulation during ramp-and-hold auxotonic wrist movements fell into 4 categories: 1) phasic-tonic (9 cells), 2) phasic only (7 cells), 3) tonic only (1 cell under both auxotonic and isometric conditions), 4) unmodulated (3 cells). Mean depth of modulation was 73 Hz (range 27-115 Hz) over background firing rate; only 4 cells showed modulation of less than 50 Hz. Of the 17 modulated cells, 15 facilitated extensor muscles and were preferentially related to extension. Only 2 cells facilitated flexors; both were related to wrist flexion. The onset of RM cell activity ranged from 220 ms preceding target muscle emg onset to 100 ms following it. Only 3 cells had onsets coincident with or following target muscle emg onset. The relationship between tonic firing rate and static torque measured during the hold phase of the task was examined for 15 RM cells over a 4 fold or greater range of torques. Of these, 9 showed a consistent positive relation for the agonist direction of movement. However, the mean rate-torque slope was about half the slope for CM cells. The possibility that the phasic discharge might encode the rate of change of torque ($d\tau/dt$) or velocity was examined for 12 RM cells with phasic components of discharge. Both peak and mean phasic discharge were plotted after first subtracting the static discharge associated with the hold phase of the task. Six cells were not consistently related to either $d\tau/dt$ or velocity. The other 6 cells showed a consistent relation to both velocity and $d\tau/dt$ under auxotonic conditions. However, for 4 cells the relation to velocity was better. The phasic component of two of these cells was absent or greatly reduced under isometric conditions, confirming its dependence on velocity. We conclude that the static discharge of some RM cells encodes static torque whereas the phasic discharge encodes either velocity or $d\tau/dt$. Supported by NSF grant BNS-8216608.

353.2 CHARACTERISTICS OF RUBROMOTONEURONAL POSTSPIKE EFFECTS IN EMG ACTIVITY OF WRIST AND HAND MUSCLES IN THE BEHAVING MONKEY. K.H. Mewes* and P.D. Cheney (SPON: Marsha E. Melnick). Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

The motor output effects of one hundred twenty red nucleus cells were tested on six extensor and six flexor forearm muscles in the awake rhesus monkey using spike-triggered averaging of emg activity (Fetz and Cheney, J. Neurophysiol., 44:751, 1980). Twenty-one cells produced postspike facilitation (PSF) of agonist muscles in spike-triggered averages at latencies consistent with a monosynaptic input to their motoneuron pools. They were therefore termed rubromotoneuronal (RM) in analogy to the corticomotoneuronal (CM) cells of the motor cortex. Three classes of RM cells were observed corresponding to postspike effects in antagonist muscles: 1) no effect (pure facilitation of agonist, 7 cells), 2) reciprocal suppression of antagonist (7 cells) and 3) suggestive facilitation and/or suppression (5 cells). Two cells were not tested for effects on antagonists. Only two RM cells facilitated flexor muscles; 19 cells facilitated extensors suggesting a strong preference of RM cells toward controlling extensor muscles. The postspike effects were further examined for distribution across muscles, onset latency and magnitude with methods previously applied in the study of CM cells (Kasser and Cheney, J. Neurophysiol. 53:959, 1985). The mean number of muscles facilitated by RM cells was 3.1 (21 cells) and the mean number suppressed was 1.5 (7 cells). These values closely match those observed with CM cells which were 3.0 muscles/cell for facilitation and 1.3 muscles/cell for suppression. The following table summarizes latencies and magnitudes of PSF and PSS effects in comparison to CM cell data.

	RM cell	CM Cell
PSF onset latency (ms)	5.2±1.2	6.3±1.6
PSS onset latency (ms)	8.4±3.6	9.3±2.3
PSF peak (% above baseline)	5.1±1.9	7.0±6.6
PSS peak (% below baseline)	4.5±2.4	4.1±2.4

The onset latencies for both PSF and PSS effects of RM cells are shorter than those of CM cells. This can be explained by the shorter conduction path for rubrospinal neurons. The magnitude of the rubromotoneuronal PSF effect is smaller suggesting weaker synaptic coupling of RM cells with their target motoneurons. Supported by NSF grant BNS-8216608.

353.3 TRANS-CRANIAL STIMULATION FOR EVOKING MOTOR ACTIVITY IN NORMAL MAN: AN EMG AND SINGLE MOTOR UNIT STUDY. B. Calancie, K.-E. Hagbarth*, M. Nordin* and E.U. Wallin*. Dept. of Clinical Neurophysiology, University Hospital, S-751 85 Uppsala, Sweden.

A recently-introduced device (Digitimer D180) for stimulating cortical regions through electrodes applied to the scalp has allowed us to study the organization of motor output to flexor and extensor muscles of the forearm in normal human subjects.

Electromyographic activity (EMG) was recorded with surface disc electrodes and intramuscular needle electrodes. The subject made an isometric wrist flexion or extension contraction appropriate to cause the repetitive, rhythmic discharge of a single motor unit (SMU). Trans-cranial stimuli over the contralateral motor cortex were applied at random intervals of between 3 and 10 seconds for 20 trials at a particular strength of stimulation. The stimulus strength was then increased or decreased, and 20 stimuli were again applied. Records of motor unit discharge were obtained for stimulus strengths below what appeared to be the excitatory threshold, and then for successively stronger stimuli, until the activity of the particular unit being studied could no longer be discriminated from other unit activity during the early excitatory (E_1) period. Surface EMG was averaged and integrated, and peri-stimulus time histograms of SMU discharge were computed. The mean intervals between successive discharges of a unit following stimulation were calculated, and compared to the mean background discharge rate.

Short-latency excitatory responses to trans-cranial stimulation could be elicited in all subjects studied. Minimum latencies ranged from 12 to 14 msec, and were equal for flexor and extensor muscles. In all cases, a weak background contraction of either the flexor or extensor muscle group facilitated the evoked motor responses from those muscles, such that under appropriate conditions excitatory activity in only one group could be seen. However, such excitatory output to one muscle group was never accompanied by an inhibition of the antagonists.

At stimulus strengths sub-threshold for a short latency E_1 response, tonic single motor unit discharge was often suppressed, although surface EMG showed little or no change. These findings suggest that an inhibitory effect may accompany the early excitatory effect of trans-cranial stimulation, and that the threshold for the former effect is less than that for the latter. Such an organization has important clinical implications in the use of this stimulation technique for the analysis and interpretation of central conduction latencies.

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353.4 ACCELERATION PROFILES OF FAST VOLUNTARY MOVEMENTS. A.W. Wiegner, M.M. Wierzbicka*. Clinical Neurophysiology Lab., Massachusetts General Hospital & Harvard Medical School, Boston, MA 02114.

Fast goal-directed voluntary movements are often associated with three distinct bursts of EMG activity (AG1,ANT,AG2) in antagonistic muscles. This study examines relationships among EMG and kinematic variables and provides experimental evidence for predictions made by a mathematical model of antagonistic muscles acting at the elbow (Wierzbicka et al., Exp Brain Res, in press). Since angular acceleration (ACC) is approximately proportional to net torque, after correcting for visco-elastic components, results could be compared with predictions of the model relating input torques to resulting kinematic variables, such as: (1) to minimize movement time, decelerative torque must equal or exceed accelerative torque; (2) decelerative torque is positively correlated with AG2 for fast movements maintained at a target.

Four normal subjects performed elbow flexions at displacements from 5° to 50° "as rapidly and accurately as possible". Biceps (AG) and triceps (ANT) EMG were recorded with surface electrodes. Velocity and ACC were obtained by differentiation of displacement data. ACC and deceleration (DEC) phases were always found to be highly symmetrical, both in terms of peak value and duration, for fast movements over different distances. AG1 was strongly correlated with displacement, peak velocity, and peak ACC. In two subjects, ANT was not significantly correlated with displacement, peak velocity, or peak DEC. This may reflect difficulty in quantitatively estimating DEC torque by means of EMG. AG2 was correlated with displacement, velocity, and DEC. The duration of the ACC and DEC phases, as well as the delay between time of peak ACC and peak DEC, was essentially constant in all subjects for displacements up to 30°, and for one subject for all movements. As predicted by the model, for flexions with constant delay, movement time (defined from movement onset to peak displacement) was inversely related to DEC/ACC for DEC/ACC ratios <1, and was asymptotic for DEC/ACC >1. That is, movement time is minimized by choosing DEC comparable to or greater than ACC.

The correlations among displacement, velocity, ACC, DEC, AG1, and AG2 indicate that magnitude scaling is the strategy chosen by the central nervous system (CNS) for fast movements to targets at various displacements. The consistent lower limit (approximately 100 ms) on duration of ACC and DEC phases suggests that this may be a physiological lower limit reflecting the minimum time for activation and decay of muscle force, and that the use of briefer torque profiles as a means of further reducing movement time is not an option available to the CNS. Thus, fast movements are controlled in a quasi-optimal way by keeping the amplitude of ACC and DEC approximately equal, and their durations minimal, minimizing movement time.

353.5 CHANGES IN FRACTIONATED SIMPLE REACTION TIME AS A FUNCTION OF DIFFERENT LEVELS OF COCONTRACTION AND INERTIA. J.G. Anson, Faculty of Physical Education and The Neuroscience Centre, University of Otago, Dunedin, NEW ZEALAND.

Cocontraction of the agonist-antagonist synergists prior to the initiation of rapid elbow flexion resulted in a reduction in simple reaction time (SRT) which was confined to the premotor time (PMT) component (Anson, Soc. Neurosci. Abstr., Vol. 10, Part 1, p. 637, 1984). Minimal changes in the motor time (MOT) component (time required for agonist muscle contraction sufficient to initiate movement) were observed. It was thought that the reduction in PMT was due to elevated levels of activity in the motorneuron pools specific to the muscles involved.

To test the hypothesis that PMT is dependent upon central rather than peripheral factors, subjects (n=3) took part in an experiment involving two levels of cocontraction (minimum and maximum) and two levels of inertia (normal and weighted). The task required rapid extension of the index finger in response to an imperative auditory stimulus which was preceded by a randomly varied (500 - 1500 msec) foreperiod, initiated by a visual warning signal. The inertia of the index finger was altered by requiring the subject to wear a weighted (145 gram) sleeve. SRT was measured by an infrared emitting diode and matching photo-transistor. Electromyography (EMG) was used to monitor muscle activity in Extensor Indicis and Extensor Digitorum and to measure PMT.

SRT was shortest in the maximum cocontraction-normal inertia condition (M=160 msec) and longest in the minimal cocontraction-weighted inertia condition (M=187 msec). PMT was altered (a 10% reduction) by cocontraction but not be inertia when each was manipulated independently. MOT was changed little by cocontraction but substantially increased (22.3%) by inertia. When maximum cocontraction and greater inertia were combined, SRT increased slightly (2.8%), PMT decreased (9.3%) and MOT increased (21.5%) considerably. Thus in the greater inertia condition maximum cocontraction resulted in PMT savings similar to those for cocontraction alone. Knowledge and perception of the added inertia did not appear to facilitate recruitment of additional motor units prior to stimulus onset; the added weight did not seem to permit increased levels of preparatory cocontraction which would have enabled simple reaction time to be further reduced.

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353.6 CHANGES IN MOVEMENT-RELATED EMG ACTIVITY WITH AGING IN NORMAL HUMANS. J.D. Cooke, S.H. Brown, D.A. Cunningham* & J.D. Brown. Depts. of Physiology and Clinical Neurological Sciences, Univ. Western Ontario, London, Canada.

A commonly observed change with age is that most movements are made more slowly. The elderly human thus presents some of the aspects of bradykinesia seen in patients with basal ganglia lesions. Patients with cerebellar lesions also perform many movements slowly in order to overcome the dysmetria associated with rapid movement. It has been suggested that the cerebellar dysmetria is related to inappropriate timing of phasic muscle activation during movement.

Experiments were performed on normal humans aged 75 to 87. All subjects lived independently and would be classed as active for their age. None had any known history of neurological disease or lesion. Subjects performed visually guided, step-tracking movements about the right elbow. Movements of different amplitudes were made with the subject being instructed to make the movements at a "comfortable" speed or "as fast as possible". Angular position and velocity of the movements were recorded as well as surface EMGs from the biceps and lateral head of the triceps.

Movement performance varied from subject to subject. All subjects, however, showed a tendency to make movements more slowly than young subjects. This was particularly apparent when subjects made large amplitude movements or when they were required to move "as fast as possible". Large amplitude or fast movements showed considerable dysmetria and a degree of movement decomposition was present. Movement abnormalities were associated with abnormalities in the associated EMG patterns. The initial agonist burst magnitude and duration changed with movement amplitude as seen in younger subjects. However, this initial burst was often followed by a more or less tonic increase in agonist activity lasting throughout the movement. The timing and duration of antagonist activity was abnormal. The antagonist burst could occur inappropriately early and/or could be abnormally prolonged to last throughout the deceleration phase of the movement. Phasic antagonist activity could also occur near the end of movement.

It is suggested that the primary cause of "slowing" with age is a loss of the ability to appropriately control the timing and duration of antagonist muscle activity coupled with decreased control over late phasic agonist activity. As a consequence, the elderly human moves more slowly in order to minimize the dysmetria which would otherwise occur in his movements.

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353.7 FACTORS INFLUENCING AGONIST AND ANTAGONIST MUSCLE ACTIVITY DURING STATIC ISOMETRIC ELBOW FLEXION IN NOVICE SUBJECTS. W.A. Lee, K.H. Lee*. Prog. in Physical Therapy, Northwestern Univ. and Rehab. Inst. of Chicago, Chicago, IL. 60611

The purpose of this study was to examine how different mechanical factors influence agonist (biceps:BI) and antagonist (triceps, long head: TRI) muscle activity during static isometric elbow flexions performed by novices. Based on previous research and pilot data, we chose to evaluate the effects of shoulder angle, force load, and rate of dynamic force production on static isometric BI and TRI EMG activity.

Nine healthy adult volunteers performed 4 blocks of 50 trials, at two elbow flexion force levels (20 and 35% of maximum voluntary contraction) and two shoulder flexion angles (60 and 90 deg). Force targets were presented as step changes in a visual signal. Subjects were told to produce the target force with movement times of 1.5, 1.0, .75, .50, .25 sec and "as fast as possible" (6-7 trials per speed). Feedback about movement time was not given. Subjects' wrist, forearm, trunk and shoulder were stabilized; elbow angle was fixed at 90 degrees. Surface EMGs of BI and TRI were filtered and amplified prior to recording, along with force. Recordings were also made of maximal voluntary contractions (for normalization of EMG records) and rapid reciprocal isometric flexions and extensions (used to estimate TRI to BI crosstalk ratios). EMGs were rectified prior to obtaining mean normalized rectified values at baseline and in 3 sequential 500ms intervals during the static isometric period. Time to peak force and average rate of force production were also obtained. All data were taken from individual trials.

Seven subjects had TRI/BI ratios during the static isometric phase which were significantly higher than crosstalk ratios, showing that TRI was significantly active during the static phase. Neither load nor shoulder angle systematically influenced co-contraction ratios. TRI/BI ratios were correlated positively with time to peak force for 5 of those subjects, primarily because static phase BI activity was higher in trials with more rapid force production. Rate of force production had no effect on static BI or TRI in the other 4 subjects.

Co-contraction during the static isometric phase might reflect automatic efforts to stabilize the elbow or shoulder. If so, then TRI activity and co-contraction should decrease with practice, especially for restricted movements. Alternatively, TRI activity could help maintain the correct direction of flexion force (Gielen et al., 1986). Reasons why rate of force development influenced static BI levels in over half the subjects are unclear. Plausibly, either the mechanical problem of static isometric force production varies with the rate of force production, or components of the dynamic motor command extend into the static phase.

353.8 PATTERNS OF MUSCLE ACTIVITY DURING ISOMETRIC CONTRACTIONS IN SPASTIC HEMIPARESIS. D. Bourbonnais, S. Vanden-Noven, K. M. Carey and W. Z. Rymer. Dept. of Physiology, Northwestern Univ. and the Sensory-Motor Performance Prog., Rehabilitation Inst. of Chicago, Chicago, IL 60611.

Previous intramuscular studies from this laboratory have shown that muscles acting at the elbow are used concurrently to generate force in a quantifiable and consistent manner during isometric contractions (Buchanan et al., Neurosci. Abstr. 10:334, 1984). The purpose of this study is to evaluate the patterns of muscle activity in chronic spastic hemiparesis resulting from stroke or head injury.

Normals (n=5) and hemiparetic subjects (n=5) were seated with the test limb supported at the elbow by a sling and positioned to obtain 60° of flexion, 10° of abduction and 80° of internal rotation at the glenohumeral joint and 90° of flexion at the elbow joint. Using an oscilloscope for visual feedback to display the subject's isometric force generated at the elbow and a window to set the requested force level, the subjects were instructed to reach and maintain (2.5 s duration) a range of force levels (.5 to 4 kg). The system allowed the requested force to be directed sequentially in 8 different directions orthogonal to the forearm axis, covering 360° . The EMG activity of 7 muscles (biceps, triceps, brachialis, brachioradialis, anconeus and the upper trapezius, bilaterally) were simultaneously recorded by surface electrodes.

The EMG activities were consistent with those obtained in previous intramuscular studies (Buchanan et al., 1984). In contrast, the patterns of muscle activity in spastic-paretic muscles showed differences with respect to three major characteristics. First, the spastic-paretic muscles showed activity over a broader range of directions; that is, the muscles were less directionally selective. Second, the direction of the maximum EMG value recorded for some spastic-paretic muscles was rotated 45° to 90° relative to the maximum value of the same muscle from the contralateral side or in normals. Finally, an analysis of the EMG activity of one muscle with respect to the EMG activity of a different muscle revealed changes in muscle activation patterns in the spastic-paretic muscle as compared with patterns from the same muscle on the contralateral side or in normals. Different patterns were particularly evident in the muscle activity from antagonists which showed no relationship in the non spastic-paretic limb but which often co-varied in the spastic-paretic limb.

In conclusion, the present results suggest that in hemiparetic spasticity, the functional disability includes a failure to activate the appropriate combinations of muscles as well as to develop the requisite activity levels between these muscles.

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353.9 COORDINATION OF FOREARM MUSCLES IN ISOMETRIC CONTRACTIONS IN FLEXION-EXTENSION AND SUPINATION-PRONATION DIRECTION. C.C.A.M. Gielen, F.J. van Zuylen*, H.A.H. Jongen*, J.J. Denier van der Gon*. Dept. of Medical and Physiological Physics. State University Utrecht, NL 3584 CC Utrecht, The Netherlands.

Many muscles are involved in simple motor tasks such as flexion or supination of the arm. This redundancy raises the question of how these muscles are activated during these tasks. We have investigated motor-unit activity with fine-wire intramuscular electrodes in 6 arm muscles during isometric contractions in flexion/extension and/or supination/pronation direction. About 150 motor units were recorded in 10 subjects.

It has been found that a motor unit is recruited if a linear combination of torques exerted in flexion/extension (F) or supination/pronation (S) direction exceeds a threshold value (ter Haar Romeny et al., Exp. Neurol. 78, 360, 1982):

$$F/F_t + S/S_t > 1$$

where F_t and S_t refer to the recruitment thresholds for flexion/extension and supination/pronation. For m.biceps, m.triceps, m.brachialis, m.brachioradialis, m.supinator and m.pronator teres it has been found that the population of motor units can be subdivided into several subpopulations. Within a subpopulation there is a fixed recruitment order of motor units. All motor units in a subpopulation have a constant ratio for F_t/S_t. The values of F_t or S_t and also the ration F_t/S_t may change if the isometric contraction changes from flexion direction into extension direction or from supination direction to pronation direction. The recruitment thresholds F_t and S_t depend on the elbow angle. Another observation is that a muscle that contributes to torque in a particular direction may also be activated during isometric contractions in other directions (for example triceps, which acts in extension direction only, is activated during supination in order to compensate for the flexion torque of m.biceps). The results can be described by a theory which is related to the tensor theory as proposed by Pellionisz and Llinas (Neuroscience 5, 1125-1136, 1980). Changes in muscle length are the result of the activity of several muscles. Therefore, in general the ratio between activation and change in muscle length is not the same for all muscles. However, it can be shown that for combined movements in flexion/extension and supination/pronation direction there exist four directions where a constant ratio between the activation and the amount of shortening can be found for all muscles. The basic assumption in the theory is that the muscle activations for a movement or contraction in a particular direction are obtained by a linear combination of the muscle activations in these four principal directions.

353.10 VARIABILITY OF INDIVIDUAL JOINT MOVEMENTS DURING FINGER-THUMB GRASP AS A FUNCTION OF MOVEMENT SPEED AND PRACTICE. W.G. Darling, K.J. Cole, & J.H. Abbs. Speech Motor Control Laboratories. Waisman Center, University of Wisconsin, Madison, WI 53705-2280.

Recent studies have shown that movements of individual body structures during complex actions, such as speech and prehension, are generated with a great deal of trial-to-trial variability. In spite of this variability, the goals for such tasks are always achieved successfully. This apparently paradoxical situation is permitted by trial-to-trial covariability among movements of the individual structures. Studies of targeted movements have shown that variability increases with movement speed and decreases with practice. In the present study the effects of practice and movement speed on variability of the individual joint movements in a finger-thumb grasp task were studied.

Four-joint finger-thumb grasp movements were performed by 6 normal subjects. Finger metacarpophalangeal (MP), proximal interphalangeal (PIP), and the thumb interphalangeal (IP) joint angles were monitored using electrogoniometers. Finger and thumb tip trajectories were measured using an LED photo-diode system. Thumb MP joint angle was calculated from the thumb-tip trajectory and the thumb IP angle. Subjects performed movements of about 100, 200, or 400 msec duration in response to an auditory tone. At each movement duration, 200 consecutive movements were performed with the movement duration order balanced across subjects.

Fast movements (100 msec duration) were made smoothly with individual joints manifesting single bell-shaped velocity profiles. By contrast, slower movements (200 and 400 msec duration) often consisted of 2 or more submovements. These submovements were variable in amplitude and duration and were not simply due to small corrections being made prior to movement termination. Such submovements were observed in both initial and well-practiced slow movements. Changes in movement speed had remarkably little effect on the variability of individual joint movements. With practice, reductions in variability of finger joint movements were usually evident, particularly at the slower movement speeds (200 and 400 msec duration). Indeed, movements of 200 msec or greater duration showed evidence of adjustments during movement which acted to reduce variability as the endpoint was approached. Such adjustments may be based on sensory information regarding the kinematics of the early parts of the movement which could be used in predictive control of the terminal phase. (Supported by NIH grants NS-13274 and HD-03352).

353.11 SYNCHRONIZATION OF MOTOR UNITS IN WRIST EXTENSOR MUSCLES DURING TREMOR. M.M. Wierzbicka*, E.L. Logigian*, A.W. Wiegner, R.R. Young, B.T. Shahani, F. Bruyninckx*. Clin. Neurophysiology Lab., Mass. General Hospital & Harvard Medical School, Boston, MA 02114.

We studied correlation between discharges of simultaneously recorded motor unit potentials (MUPs) in physiological and terbutaline-induced enhanced tremor in 4 normal subjects. Recordings were made during weak and moderate levels of contraction. Two needle electrodes were inserted into the muscle, each positioned to record from a single motor unit (MU). Tremor amplitude was measured with an accelerometer attached to the hand. We recorded 26 pairs of MUPs during physiological tremor (mean displacement 72 μm RMS) and 39 pairs in enhanced tremor (range 88-2051 μm RMS). Cross-correlation histograms of relative firing times of one MU with respect to the other were calculated and a synchronization index (SI) defined.

Six pairs of the 26 recorded during physiological tremor showed relatively small but significant correlation (mean SI=.14, $p<0.05$) between discharge times of the 2 MUs. In all cases, synchronized activity was observed when both MUs fired at nearly the same frequency (difference < 0.7 Hz). Examination of temporal patterns revealed clusters of several almost simultaneous discharges of the 2 MUs separated by periods of asynchronous activity.

In enhanced tremor, there also was a tendency toward synchronous discharges of MUs firing at similar frequencies (7 of 39). In 4 pairs, one or both MUs fired in doublets. We monitored 3 of these pairs during increasing tremor: they fired independently during small tremor, irregular double discharges appeared with increased amplitude of tremor, and frequent double discharges strongly synchronized with tremor beats were seen in large tremor. Strength of synchronization between MUs, as quantified with our index (SI range .09-.79), was significantly correlated with tremor amplitude ($r=.83$, $p<0.001$). The remaining 28 of 39 MUP trains were not statistically correlated; they fired asynchronously.

For MUs firing in doublets, frequency analysis at the dominant tremor frequency showed increased coherence between discharges of 2 MUs as tremor amplitude increased. In contrast, MUs firing at similar rates with only clusters of synchronization showed little or no coherence between their discharges at the tremor frequency.

Perhaps both observed mechanisms of motor unit synchronization (temporal adjustment of firing rates and double discharges) account for grouping of MUPs observed in surface EMG during tremor. Based on our results, we hypothesize that in lower amplitude tremor, irregularly grouped discharges may be a result of temporal synchronization of several MUs firing at similar frequencies; successive groups may consist of discharges from different MUs. Actual synchronization of individual MUs was not commonly observed in modestly enhanced tremor.

353.12 INTERACTION BETWEEN ESSENTIAL TREMOR AND VOLUNTARY MOVEMENTS. R.J. Elble, C. Moody* and C. Higgins*. Southern Illinois Univ. Sch. of Med., Springfield, IL 62708

Five patients with advanced essential tremor were required to insert their right hand, fingers extended, into a plastic manipulandum and ballistically extend or flex the wrist 15 deg. in response to visual and auditory stimuli. The manipulandum was attached directly to the shaft of a computer-controlled torque motor which was programmed to deliver random 50-msec torque pulses to the wrist. Forearm EMG was measured with bipolar skin electrodes. Using a digital computer, 2-second epochs of wrist angle and integrated EMG were sampled, time-locked to the perturbations and signals to move. Successive data epochs were then averaged. The wrist was maintained at a neutral angle during the intervals between ballistic movements and torque perturbations. Muscles controlling the wrist were voluntarily activated to a level which produced passive return of the wrist to neutral following each perturbation. Patients were instructed not to react voluntarily to the perturbations, and the accuracy of the wrist position was not emphasized. After a few practice runs, each patient discovered the minimum level of voluntary forearm muscle activation needed for optimum performance. During periods of relative tremor quiescence, the voluntary wrist movements invariably initiated a run of tremor bursts in the forearm EMG. This tremor was time-locked to the voluntary movement. During periods of active tremor, the ballistic wrist movements seemed to be time-locked to the ongoing tremor rhythm. The movements increased the amplitude of tremor, but there was no resetting of the tremor rhythm. The torque pulse disturbances, by contrast, disrupted the tremor rhythm and produced the usual short-and medium-latency reflex responses. However, the perturbations did not produce time-locked resetting of the tremor rhythm, and during periods of strong tremor, reflex responses were frequently unobtainable. Previous data from our lab suggest that the essential tremor oscillator is a pathologic exaggeration of the normal 8-12 Hz rhythm of physiologic tremor (Neurology 1986;36:225-231). The present data suggest that this oscillator may normally function as a gating mechanism designed to coordinate descending ballistic commands with sensory feedback. (Supported by NINCDS grant RO1 NS20973.)

353.13 SENSORY AND MECHANICAL CONTROL OF CEREBELLAR INTENTION TREMOR IN HUMANS. J. N. Sanes and K.-H. Mauritz. Human Motor Control Section, Medical Neurology Branch, NINCDS, Bethesda, MD 20892 and Neurologische Klinik, Universität Düsseldorf, 4000 Düsseldorf 1, FRG.

Cerebellar dysfunction in humans and non-human primates is often accompanied by an intention tremor of 3-5 Hz. There have been several reports on the role of somesthetic and visual sensation on the genesis and maintenance of this tremor. In this work we studied how variations in mechanical and sensory inputs (visual and somesthetic) and type of muscle contraction (isometric or isotonic) affected intention tremor.

Patients with cerebellar intention tremor were examined. The etiology of cerebellar dysfunction was typically infarcts or degenerative diseases. In the isotonic task, patients performed visually guided flexion-extension movements about the wrist. Patients were prevented from viewing their hand, instead they viewed a video monitor that showed a representation of hand position and a targeted position. The patients were required to maintain alignment between the position and target cursors. To examine the role of visual inputs on cerebellar tremor, movements were performed with or without visual guidance by either removing the target or position cursor. In separate experiments mechanical conditions were varied; these included the amount of constant load, inertial load or viscous load opposing movement. In the mechanical load experiments visual guidance was always available. In the isometric task, patients made visually guided flexion-extension muscle contractions about the wrist. Muscle activity was recorded from wrist flexors and extensors.

Cerebellar tremor was most pronounced when the visual loop was closed. Opening the visual loop, by removing either the target or position cursor, reduced or abolished intention tremor. These changes occurred even when patients performed nearly identical voluntary movements in the visual open or closed loop conditions. As expected, viscous and inertial loads damped cerebellar intention tremor. However, non-linear relationships between tremor reduction and load magnitude were observed, such that the lighter loads had a greater proportionate effect on tremor magnitude than the heavier loads. In many cases constant loads opposing extension yielded more tremor than comparable flexor loads. During isometric contractions cerebellar intention tremor, measured from wrist torque, was abolished or greatly reduced.

These data illustrate the importance of sensory and mechanical factors in the genesis and maintenance of cerebellar intention tremor. The results on loads opposing movement could suggest that cerebellar intention tremor is related to mechanical factors, but not in a simple linear fashion. However, since the tremor is generally absent during isometric contractions, somesthetic afferents, and in particular muscle spindle inputs, appear to be necessary for the genesis of cerebellar intention tremor. Visual guidance is an important parameter in sustaining cerebellar tremor during intentional action. The composite results support the notion that the cerebellum integrates multimodal sensory information used in voluntary movement.

353.14 MOVEMENT DEFICITS AND RECOVERY OF MOVEMENT OF RIGHT HEMISPHERE DAMAGED HUMANS. K. A. BURSON* and C. R. ALMLI (SPON: E. Montgomery). Programs in Neural Science and Occupational Therapy: Departments of Anatomy and Neurobiology, Preventive Medicine, and Psychology: I.W.J. Institute of Rehabilitation, Wash. Univ. Sch. of Med., St. Louis, MO 63110

There has been little quantitative research on the motor deficits and recovery of motor function of stroke patients. The present study quantified movement dysfunction and recovery of function of human patients sustaining right hemispheric stroke involving the middle cerebral artery distribution.

The Stroke and matched Control subjects were filmed while performing a "reaching task" at 3-5 days (2 trials) and 11-13 days (2 trials) poststroke. The films were analyzed for movement, frame by frame, using a quantifiable movement notation system. All subjects were right-handed, could follow simple verbal directions and were not displaying neglect. Movements of the "affected-extremity (left; forearm, upperarm and shoulder)," head and trunk were measured for latency to initiate movement (sec), number of movements, number of body segments moved, movement type (plane, rotatory), movement magnitude (degrees), movement duration (sec), and movement synchronization. In addition, the subjects were tested for use of the left arm in the performance of functional tasks, such as stabilizing a paper to write name and folding a piece of cloth.

During the movement tests at 3-5 (Test 1) and 11-13 (Test 2) days poststroke, the Stroke subjects displayed elongated latencies to move, an increased number of head movements, and asynchronous movement patterns compared to Control subjects. Stroke subjects also performed more poorly than Controls on the functional tasks. On Test 2, the Stroke subjects displayed recovery for number of movements, duration of movements and some aspects of movement type. The Stroke and Control subjects did not differ on either test for shoulder movements, magnitude of movement, or for some types of movement. Significant correlations were found between functional performance scores and the "no-recovery" movement variables, i.e., latencies to move (r=-.88), number of head movements (r=-.75), and movement synchronization scores (r=-.85).

Results show that during the time interval studied, certain aspects of movement showed "no recovery", some showed "recovery" and others were unaffected by the stroke. The characteristics of movement showing "no recovery" were highly correlated with functional performance deficits suggesting that motor planning dysfunction may be related to movement disorders of right hemispheric stroke patients. (Supported by AOTA Grant #1234).

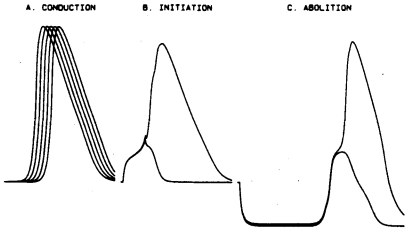
DISORDERS OF MOTOR SYSTEMS AND NEURAL PROSTHESES

354.1 **A COMPUTER MODEL OF ACTION POTENTIAL CONDUCTION, INITIATION AND ABOLITION IN MAMMALIAN MOTOR NERVE.** J.D. Sweeney, D. Durand and J.T. Mortimer. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve Univ., Cleveland, OH 44106.

Electrical stimulation of myelinated motor nerve forms the basis of the field of Functional Neuromuscular Stimulation (FNS). Some FNS applications also require electrically induced abolition of motor action potentials (Soc. Neurosci. Abstr., 9: 1038, 1983). Models of motor nerve function (IEEE Trans. BME, 23: 329, 1976; J. Physiol., 341: 59, 1983) are useful for the design of FNS electrodes and electrical parameters. Such models have to date been based not on data from mammals but instead from frogs. To our knowledge no model has made use of the growing literature (e.g. see Science, 228: 1502, 1985) on the distinct differences between the properties of mammalian and frog myelinated nerves.

We have implemented a compartmental cable description (Neurosci., 6: 823, 1981) of mammalian myelinated motor nerve that uses sodium and leakage channel parameters of rabbit or rat nodes of Ranvier. No nodal potassium channels are included because at mammalian motor nodes of Ranvier such channels are few in number or absent (J. Physiol., 292: 149, 1979; J. Physiol., 298: 171, 1980) . The parameters of the cable have been tuned so that, at 37 degrees C, action potential conduction velocity (V in m/sec) is related to fiber diameter (D in um) by the relation V = 5.7*D. Fig. A shows conduction across five nodes of Ranvier of a 10 um fiber (time is 0.5 msec; action potential amplitude is 90.4 mV).

Initiation and abolition of action potentials have been studied by injecting rectangular "effective stimulating currents" (Biophys. J., 2: 11, 1962) at single nodes of Ranvier. Fig. B depicts responses (sub-threshold and super-threshold) of a 10 um fiber for 100 usec cathodal stimuli. Fig. C shows responses (sub-abolition and super-abolition) for the same cable given anodal stimuli longer than the duration of an approaching action potential. Through such simulations strength-duration curves for action potential initiation and abolition have been derived.



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354.2 FORCE FEEDBACK SIGNAL PROVIDED BY NERVE CUFF RECORDING ELECTRODES. J.A. Hoffer and T. Sinkjaer*. Dept. Clin. Neurosci., Univ. Calgary Fac. Medicine, Calgary, Alberta T2N 4N1, Canada; & Inst. Electronic Systems, Aalborg University Center, DK-9000 Aalborg, Denmark.

In persons with spinal cord injuries, limb use can be partially restored by functional electrical stimulation (FES) of paralyzed muscles or motor nerves. A present limitation of FES is the large variability in the resulting force, which depends on the load, limb position and muscle fatigue. FES could be regulated by feedback if a suitable "force sensor" became available. All artificial sensors to date have met technical or cosmetic limitations. We report here that the required force information: 1) is accurately coded by natural sensors present in the skin and 2) can be easily extracted from the electroneurogram (ENG) recorded from a cutaneous nerve.

The cat footpads served as our model of human glabrous skin. We implanted a 30mm-long recording cuff electrode on the tibial nerve, proximal to the ankle[1]. In walking cats, 75% of the neural traffic recorded from tibial nerve arises from sensory axons[2], mainly from low-threshold glabrous skin mechanoreceptors from the plantar pads, and hair afferents. In this study we applied depilatory cream to reduce activity from hairs. Motor traffic in the tibial nerve was eliminated transiently by a proximal block[3]. Triceps EMG, force, length and temperature were monitored with implanted transducers[3].

Relations among ground contact force, triceps tendon force and tibial nerve ENG were assessed as cats stood unaided on pedestals or walked on a treadmill. One pedestal could be suddenly displaced[4] and contained a vertical force sensor. The tibial ENG (1-10 KHz), after rectification and filtering (20 Hz LP), closely resembled the triceps force simultaneously recorded during walking. In pedestal tasks, the filtered ENG, triceps force and vertical contact force accurately followed each other even for rapid postural adjustments.

In para- or tetraplegics, we propose that nerve cuff recording electrodes, implanted on cutaneous nerves of the hands or feet, will render a feedback signal proportional to skin contact force, suitable for closed-loop control of FES. Since spinal cord injuries generally do not affect the integrity of afferent neurons with cell bodies in dorsal root ganglia at levels above or below the lesion, most glabrous skin mechanoreceptors remain functional. Electrode longevity and the stability of peripheral nerve signals recorded with cuff electrodes have been demonstrated in animal research. For these as well as for cosmetic reasons, a fully implantable nerve cuff "force sensor" is likely to be well accepted by users.

1) Davis et al, J Physiol 285:543, 1978.
2) Gordon et al, J Physiol 303:243, 1980.
3) Hoffer et al, Soc Neurosci Abstr 9:470, 1983 & 10:330, 1984.
4) Sinkjaer and Hoffer, Can J Physiol Pharmac 64:Axxvi, 1986.

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354.3 COORDINATION AND COCONTRACTION IN OPEN AND CLOSED LOOP CONTROL OF GRASP THROUGH FUNCTIONAL NEUROMUSCULAR STIMULATION. P.E. Crago, P.H. Peckham*, K. Kilgore*, K. Tills*, and G.B. Thrope*. Depts. Biomed. Eng. and Orthopaedics, Case Western Reserve Univ. and V.A. Medical Center, Cleveland, OH 44106.

Control of grasp-release has been elicited in the C5 or C6 tetraplegic human by electrical activation of paralyzed muscles. Two grasp patterns are achieved; lateral prehension and palmar prehension. Control of the position of the digits and the grasp strength is regulated by a single proportional command signal which the patient volitionally generates by a physical movement.

The muscles activated are peripherally innervated but without CNS control. Intramuscular electrodes are placed percutaneously and remain implanted for extended periods (years in many cases). The muscles activated are Flex. poll. brev., Flex. poll. long., Abd. poll., Add. poll., Ext. poll. long., Ext. poll. brev., Flex. dig. sup., Flex. dig. prof., and Ext. dig. comm.. Four or five muscles are activated for each grasp.

Muscle force is graded by recruitment modulation, achieved by varying the stimulus pulse width (PW) at a constant stimulus period and current amplitude. There is a nonlinear muscle and time dependent relationship between PW and the force or joint position produced by stimulated muscles. For open-loop coordinated activation of all the muscles involved in a grasp pattern, the single command signal modulates the PW to each channel according to a predefined piecewise straight-line approximation (PW map) set up specifically for that muscle and electrode. The piecewise PW maps are determined by the inverse of the recruitment characteristics for each channel. For closed-loop control, linearization of the input/output properties is achieved by virtue of the feedback control rather than by PW mapping. The PW mapping scheme is used to coordinate muscles that are outside the feedback loop with those that are inside.

Cocontraction of antagonists is employed to eliminate deadbands in the transition between extension and flexion, and to increase stiffness at low net forces as an aid in the rejection of external disturbances.

For closed loop control, a combination of force and position feedback (stiffness regulator) provides regulation of grasp under a wide range of loading conditions, lets the load properties control the relative weighting of the two feedback parameters, and allows a single input command to control both position and force.

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354.4 A SPIRAL NERVE CUFF ELECTRODE FOR USE IN FUNCTIONAL ELECTRICAL STIMULATION. G.G. Naples*, J.T. Mortimer, U. Roessman*, J.D. Sweeney, T.J. Crish* and A. Scheiner*. (SPON: G. Perry). Applied Neural Control Laboratory, Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Nerve cuff electrodes have been used with several neuroprosthetic devices including systems designed to stabilize the knee in lower extremity paralysis (Brindley, G.S., et al., Paraplegia, 16: 428, 1978), correct foot-drop (Waters, R.L., et al., J. Bone Joint Surg., 57A: 1047, 1975), relieve intractable pain (Picaza, J.A., et al., Appl. Neurophysiol., 40: 223, 1977), and aid respiration (Kim, J.H., et al., J. Thor. Cardiovasc. Surg., 72: 602, 1976). Mechanically-induced nerve damage is a major concern whenever nerve cuffs are used. Cuff diameter is usually recommended to exceed nerve diameter by at least 40% to minimize such damage. Surgical trauma can also cause nerve damage.

A new easily-implanted nerve cuff electrode designed to minimize mechanically-induced neural damage has been developed. Traditional nerve cuffs usually consist of one or more components sutured together to form a closed cylinder around a nerve. The new spiral nerve cuff consists of electrodes contained within a self-curling sheath of silicone rubber. The cuff is held in an unrolled state adjacent to a nerve and controllably permitted to curl around it. This simpler installation procedure minimizes surgical trauma to the nerve. The spiral cuff is not strictly a fixed cylinder since it can be loosened or tightened by pressure variations inside and outside the cuff. Experiments have been performed to evaluate whether the cuff's capacity to respond to internal pressure caused by swelling would permit the safe implantation of tighter cuffs than was previously recommended.

Eighteen cuffs of various diameters were implanted aseptically on nerves of three anesthetized cats. Cuff diameter was chosen to evaluate four different Cuff/Nerve diameter Ratios (CNR): 0.9, 1.0, 1.2 and 1.4. The cats were maintained for seven months after which the nerves were prepared for microscopic examination.

Observations made during the maintenance period failed to reveal any gross motor or sensory defects which would reflect neural damage. Preliminary findings indicate that while some nerves implanted with tighter cuffs exhibited perineurial thickening, increased endoneurial connective tissue and a marked decrease in axon density, others survived with little or no damage. Damage can likely be correlated with several factors in addition to CNR, such as cuff stiffness, implant site characteristics, and nerve size. Studies in progress will identify the most likely combination of factors. The effects of electrical stimulation on peripheral nerve are also being studied.

This work was partially supported by VA Grant No. V541P-1341, NSF Grant No. PFR 80-17190 and NIH Grant No. NO1-NS-3-2344.

354.5 INTRAMUSCULAR ELECTRICAL STIMULATION: TISSUE DAMAGE. A. Scheiner* and J.T. Mortimer (SPON: R. Riso). Dept. of Biomedical Engineering, Case Western Reserve U., Cleveland, OH. 44106

Electrical activation of skeletal muscle can be acheived with the use of IM (intramuscular) electrodes. Electrodes, of the type described by Caldwell and Reswick (Caldwell, C.W. Ph. D. thesis, Case Western Reserve U. 1970) are of the type most commonly used today. Stainless steel is the popular construction material because of its availability, and good mechanical and electrochemical properties. Coiled wire electrodes have been evaluated and found to be safe under passive conditions (Caldwell et al, IEEE Trans. Biomed. Eng. 22:429-432, 1975). A study using balanced biphasic and cathodic monophasic stimulation has also been reported (Mortimer et al, Ann. Biomed. Eng. 8:235-244, 1980). Their investigation showed that balanced biphasic stimulation (up to a charge density of 2.0 uCoulombs/mm^2/phase) and cathodic monophasic stimulation (up to a charge density of 0.2 uCoulombs/mm^2/phase) showed little muscle damage. Studies have shown (Lan, N., M.S. thesis, Case Western Reserve U., 1985) that severe corrosion of stainless steel electrodes can occur using balanced biphasic stimulation at amplitudes normally used during IM stimulation. McHardy et al (Ann. Biomed. Eng. 5:144-149, 1977) conducted tests in vitro which indicate that the "safe" charge limit (the charge density per phase at which corrosion first becomes serious) can be at least tripled by the use of imbalanced pulses.

Our studies have investigated the tissue reaction of feline muscle using imbalanced biphasic and cathodic monophasic IM stimulation. A comparison was made between the amount of damage to tissue near coiled wire electrodes in the inactive region of the electrode where the metal is coated with an insulator and the active region where bare metal is exposed to the tissue medium. The results show that the magnitude of net DC current and the type of current waveform (biphasic vs. monophasic) are predominate factors in determining muscle damage. For biphasic stimulation, damage in muscle stimulated with less than 20uA/mm^2 net DC current is not statistically different from control. At all current levels tested, monophasic stimulation produced greater tissue damage than biphasic stimulation at the same net DC current. Coil electrodes were imbedded in an agar medium containing Ringer's solution and pH indicator. The results of the in vitro study showed that pH changes for monophasic stimulation are greater than for biphasic stimulation of the same net DC current. This suggests that tissue damage is related to pH changes near the stimulating electrode.

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354.6 CVA AFFECTS MOTOR UNIT ACTIVITY IN BOTH PARETIC AND NONPARETIC HUMAN FOREARM MUSCLES. S.S. Fitts, Ph.D.*, M.C. Hammond, M.D.*, P.B. Nutter, M.D.*& G.H. Kraft, M.D.* (SPON: J.C. Slimp, Ph.D.) Dept. Rehab. Med., Univ. of Wash., Seattle, WA. 98195.

Stable stroke patients' weakness and slowness of movement may be related to their reported inability to maintain a steady motor unit discharge. We investigated whether or not the flexion synergy pattern seen in upper extremity hemiparesis corresponds to greater deficit in motor unit activity of extensor than of flexor muscles.

Dual channel needle EMG recordings were made from flexor carpi radialis and extensor carpi radialis longus of 7 paretic, 7 non-paretic, and 7 normal control arms. Patients ($\bar{X}$ age 69) had moderate to mild upper extremity paresis. Twenty-four isometric contractions were recorded for each wrist, including 12 flexion and 12 extension trials in a balanced random order. Contractions of 3, 6, or 9 sec were signaled by buzzers, after random 2- to 4-sec warning periods. EMG activity was sampled seven times per msec, and motor unit events exceeding 750μV baseline-to-peak amplitude were counted. Interpotential intervals were recorded for subsequent analysis of sustained activity vs gaps longer than 100 msec. Rates were computed as events per sec during sustained activity.

Agonist firing rates were slower in paretic than in control and nonparetic muscles ($\bar{X}$'s=24.5, 41.6 & 45.3, respectively; $p < .01$), with no difference between flexors and extensors. Paretic agonists also had less sustained activity than controls ($\bar{X}$'s=0.956 & 2.450 sec, $p < .01$), with the combination resulting in fewer total events ($\bar{X}$'s=40 & 113, $p < .01$). Nonparetic agonists, while firing at a normal rate, had more sustained activity ($\bar{X}$=3.542 sec) and more events ($\bar{X}$=189) than controls. In antagonist muscles, firing rates, number of events, and total time of sustained activity were not significantly affected by condition (F's < 1).

The reduced time of sustained activity in paretic agonists confirms previous qualitative descriptions of a spluttering phasic pattern with irregular bursts and gaps. Periods of inactivity and a slower rate when active both reflect impaired motor unit recruitment in paretic agonists, although the present data cannot distinguish between reduced activity in equivalent numbers of motor units and unchanged activity in fewer units. The number of events and total sustained activity of paretic extensors differ from controls more than flexors; while not statistically significant, this observation is consistent with the clinically challenging flexor synergy pattern. Finally, because activity was increased in nonparetic agonists, the "unaffected" side is not an appropriate control for studies of hemiparesis.

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354.7 The Influence of Antagonistic Cocontraction on Labial Movements in Parkinson's Disease. Michael P. Caligiuri*and James H. Abbs (Spon. Michael E. Baker) Speech Motor Control Labs. University of Wisconsin, Madison WI 53706

An increase in background EMG and the presence of antagonistic cocontraction typically coincides with rigidity and reduced movements in Parkinson's Disease (PD). Yet, the literature is equivocal with respect to the notion that bradykinesia is due to the "failure to relax" of the antagonist muscles. Furthermore, it has been argued that antagonistic cocontraction may have differential effects on slow versus fast movements. The goal of the present study was to determine the extent to which antagonistic cocontraction contributes to reduced labial movements for speech in PD. It was hypothesized that PD subjects who exhibit increased activity of the antagonist muscle during movement onset would manifest reduced displacement amplitude and velocity and increased movement time compared with PD subjects who exhibit normal antagonistic muscle activity.

Lower lip elevation movements were recorded along with EMG activity from m. orbicularis oris and m. depressor labii inferior during repetitive syllable production. Eleven PD and nine normal age-matched control subjects were studied. The syllable /va/ was produced at two speaking rates: normal and fast. PD subjects were grouped according to the presence or absence of antagonistic cocontraction (DLI) during lower lip elevation into rigid and non-rigid subgroups, respectively.

The results indicated that all PD subjects exhibited reduced displacement amplitudes and velocities compared with the normal controls. In addition, for the normal speaking rate, rigid PD subjects exhibited significantly lower displacement amplitudes than the non-rigid PD subjects, while peak instantaneous velocities did not differ for the two PD subgroups. For the fast speaking rate, analyses of labial kinematics did not distinguish the two subgroups. Analyses of labial movement times did not reveal significant differences among the three subject groups.

On the basis of the above findings, it may be hypothesized that for rapid labial movements, antagonistic cocontraction does not adversely influence movement integrity. On the other hand, for slower movements, antagonistic cocontraction may contribute to a further reduction of displacement amplitude but not velocity. Thus, labial movements may be associated with an underlying sensorimotor control deficit which is exacerbated by antagonistic cocontraction for movement tasks demanding slow precise execution.

354.8 PERSISTENT SPEECH DYSPRAXIA: BRAIN LESION SITES AND SPEECH TIMING DEFICITS. C.L. Ludlow, N.P. Connor*, S.E. Sedory* and A.M. Salazar*. Human Motor Control Section, MNB-IRP, NINCDS, Building 10, Room 5N226, Bethesda, MD 20892, and Vietnam Head Injury Study, Walter Reed Army Medical Center, Washington, DC 20307-5001.

The purpose of this study was to determine the brain structures involved in speech dyspraxia and to quantify disruptions in speech timing. Nine patients were identified with speech dyspraxia (SD) 15 years following penetrating head injury. Six had some expressive language deficits while 3 had no history or signs of aphasia. Brain lesion sites from CT scans were compared with those of head injured patients (HIP) without speech/language deficits, but with similar brain lesion volumes. All SD patients had left cortical involvement of the primary motor strip (area 4) with extension to areas 6, 8 and 9, and deep extension involving the insula, superior longitudinal fasciculus, frontal corona radiata and anterior corpus callosum. None of the HIP controls had this combination of structures involved in the left hemisphere.

The SD patients with some residual language impairment (N=6), and the SD patients without a history of aphasia (N=3) were compared to non-head injured controls (NC) on experimental speech timing tasks which assessed; 1) execution time for syllables, words, phrases, pauses and sentences; 2) changes in execution with speech rate change; 3) changes in execution time with speech stress change; and 4) speech syllable repetition rate. Different speech timing impairments were found in the 2 subtypes of SD. SD with language impairment was associated with slow execution time within words and syllables, no pauses between phrases and reduced percent change in execution time with increased speech rate. Those without language impairment had decelerating rates of syllable repetition, normal word and phrase execution times, and longer sentence execution times than normal due to increased pause lengths between phrases. All SD patients had normal relative timing ratios indicating normal planning of speech timing. Therefore, only a few selective deficits in speech execution occurred with extensive lesions to the primary motor cortex and connecting pathways in the left hemisphere.

354.9 ANALYSIS OF WRIST MOVEMENT SPEED IN CLOSED HEAD INJURED SUBJECTS WITH SPASTICITY AND NON-NEUROLOGICALLY INVOLVED SUBJECTS. R. L. Segal and E. Yousseff.* Div. of Physical Therapy, Dept. of Rehab. Med., Emory Univ. Sch. of Med. Atlanta, GA 30322.

The present study is part of larger study comparing human normative data (non-CHI; n=14) on the control of wrist flexion and extension to data obtained from human subjects with upper extremity spasticity secondary to closed head injury (CHI; n=6). All subjects signed informed consent materials approved by the University Human Investigations Committee. The data offered in this presentation pertains to wrist movement velocity.

Each subject was asked to perform slow (approximately 30°/sec) and fast (as possible) flexion-extension movements of 30° and 60° with the forearm in neutral position. Movements were performed while sitting and under 2 conditions: 1) forearm secured to table with elbow flexed to 90° (supported position); and 2) active shoulder flexion to 90° and full active elbow extension (unsupported position). CHI subjects used their involved extremity while non-CHI subjects used their dominant extremity. Movement was monitored by a wrist electrogoniometer and recorded on FM tape. A computer program assisted in the calculation of the speed of the entire movement and the speed of 10° movement intervals. The average of 3 trials for 16 movements was used during analysis.

There are many statistically significant differences between the CHI and non-CHI groups (one-way ANOVA, $p. < 0.05$). The major differences are as follows: 1) Non-CHI subjects have higher overall speeds during fast movements (7 of 8 movements); 2) Speed differences during fast movements are greater in the unsupported position (Non-CHI being faster except in last 10°); 3) During several slow 60° movements there are no differences in the speed of the overall movements, but significant differences in 10° intervals of movement are noted. Thus, although slow movement overall speeds appear similar, CHI and non-CHI subjects probably achieve the speeds by different strategies; 4) The only time CHI interval velocities are statistically greater, than non-CHI is at the end of two fast 60° movements.

The present findings will be integrated with electromyographic data to develop treatment protocols to improve upper extremity function of CHI individuals with spasticity. For example, speed differences between supported and unsupported positions reinforce the need to incorporate several positions in treating the upper extremity.

Funded by the Foundation for Physical Therapy.

354.10 BEHAVIORAL EFFECTS OF CONTINOUS INTRASTRIATAL DOPAMINE INFUSIONS IN ANIMALS WITH AND WITHOUT UNILATERAL LESIONS OF THE SUBSTANTIA NIGRA. R.W. Hargraves*#and W.J. Freed. #Department of Neurosurgery, Naval Hospital, Bethesda, Maryland and Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

We have examined the effects of the chronic administration of dopamine directly into the striatum of rats with and without unilateral lesions of the substantia nigra (SN). Rats received unilateral lesions of the SN by stereotaxic administration of 6-hydroxydopamine hydrobromide. After three weeks the animals were tested for apomorphine induced rotational activity (.1 mg/kg s.c.), and general activity. Alzet osmotic mini-pumps (model 2002) were filled with solutions containing dopamine in various concentrations and 0.4% ascorbate, implanted subcutaneously and connected to a 25 gauge cannula which was implanted directly into the striatum. The system delivered the solutions at a rate of .5 μ l/hr for two weeks.

In normal animals which received dopamine infusions without prior lesions of the SN, there was no spontaneous rotational activity, nor was there any rotational preference after apomorphine injections (.1 mg/kg s.c.). At higher dopamine concentrations (10 μ g/hr), there was an increase in stereotyped behavior including an increase in grooming and cage exploration, but no rotational effects.

In animals with prior SN lesions there was initially a strong spontaneous rotational behavior contralateral to the infusion site with infusions of dopamine greater than 5 μg/hr. These spontaneous rotations were, however, no longer evident after the intital 48 hr. of infusion. At a dose of .5 μg/hr there was initially a slight increase in stereotyped behavior such as cage exploration and grooming. This activity also disappeared after 48 hr. of infusion. General activity levels were not significantly different from those observed prior to infusion. These infusions produced a significant decrease in apomorphine-induced rotational behavior. At a dose of .5 μ g/hr (n=10), the mean ($\pm$ SEM) reduction in rotation was 79 $\pm$ 3.1%. These reductions were evident throughout the two-week duration of the infusions. Control animals which received NaCl/ascorbic acid infusions showed no change from their pre-implantation rotational behavior status. When the infusions were terminated all animals returned to their pre-implantation levels of rotational behavior.

Spread of the infused dopamine was determined by adding tritium labelled dopamine to the pumps in tracer quantities. Radioactivity was highly concentrated at the infusion site and decreased rapidly with increased distance from the point source, being minimally detectable beyond 6 mm.

In this model system, continous infusion of a neurotransmitter into an area of a known deficiency corrected a behavioral abnormality caused by the absence of the neurotransmitter, without the reestablishment of synaptic connections. This or similar methods may eventually prove to be effective in the treatment of nigro-striatal degenerative diseases.

354.11 PRESENCE OF DOPAMINE AND RELEASE OF TRANSMITTER ARE NECESSARY FOR MPTP INDUCED DECREASE IN SYNAPTIC TRANSMISSION. J. A. Wilson, J. S. Wilson, & F. F. Weight Dept. Physiology, Creighton Univ. Omaha, NE 68178, Dept. of Anatomy, Howard Univ. Washington, DC 20059, & Lab. of Preclinical Studies, Nat. Inst. Alcohol Abuse & Alcoholism, Rockville, MD 20852.

The specific neurotoxin, MPTP, causes a Parkinson's disease-like syndrome in man, monkeys, mice, and dogs. At present it is well established that the MPTP must be oxidized and that the dopamine uptake system must be functional for these toxic effects to occur. However, other aspects of the MPTP induced toxicity remain subject to question.

The mechanism by which MPTP has its toxic effect is still unclear. It has been suggested that the second oxidation product, MPP+, is the toxic agent; however, direct application of this agent to brain slices does not produce a non-reversible decrease in cortico-striate transmission. The first oxidation product, MPDP+, has also been suggested as potentially being the toxin due to its extreme reactivity. MPDP+ may react with dopamine and produce toxic quinones which can kill the nigral neurons. If this mechanism is to be considered, the presence of dopamine in the preparation should be necessary for a non-reversible decrease in synaptic transmission. To determine whether or not dopamine is necessary for MPTP's effect of decreasing cortico-striate synaptic transmission, we used a mouse brain slice preparation which we have previously characterized (Wilson et. al. BRAIN RES. 368:357, 1986). MPTP was bath applied to slices from animals which had been pretreated with reserpine as is known to only decrease the dopamine levels in the brain. The amplitude of the synaptic responses to cortical stimulation was monitored. The amplitude of the field potential was found to equal control value within 1 hour from the start of washing. We were next interested to know if there had to be a release of transmitter for the non-reversible decrease in synaptic transmission. Using low calcium / high magnesium artificial CSF, synaptic transmission was reversibly blocked. MPTP, 300 uM, was added to this solution for 20 minutes. The superfusate was changed back to the original ACSF and synaptic transmission returned to its control value within one hour. We interpret these results to indicate that the presence of dopamine and release of transmitter are necessary for the MPTP induced non-reversible decrease in synaptic transmission.

354.12 DISTURBANCES IN THE NEURONAL DISCHARGE OF AREA 4 IN MPTP-TREATED MONKEYS. CENTRAL AND PERIPHERAL HYPOTHESIS. Doris DOUDET, Christian GROSS, Françoise GARANX, and Bernard BIOULAC, Laboratoire de Neurophysiologie, Groupe Motricité, Université de Bordeaux II, UA 1200 CNRS, 146 rue Léo Saignat, 33076 BORDEAUX CEDEX (FRANCE).

Some reports showed that the systemic administration of 1-Methyl-4-Phenyl, 1, 2, 3, 6-Tetrahydropyridine (MPTP) in monkeys induces a parkinsonian-like syndrome with hypertonia, hypokinesia, and a degeneration of the dopaminergic neurons of the substantia nigra (SN) as observed in parkinsons'disease. Using the MPTP primate model the aim of the present study was to evaluate these possible influences Respective influence of the ascending and descending pathways originating from the SN. **Methods** : Extracellular unit activity of motor cortex was recorded in two rhesus monkeys trained to perform a rapid flexion or extension of the contralateral forearm in response to an auditory cue. Movement parameters and EMG activity were recorded under the same conditions. Degeneration of the SN was obtained by systemic administration of MPTP. (total dose : 3,5 to 5 mg/kg). **Result** : In normal animals, area 4 neurons alter their discharge frequency before the onset of displacement. The static discharge rate is low. The majority of neurons (50 %) tested during movement in both directions showed a reciprocal pattern of activity for the two directions of movement, a small percentage (18 %) exhibited a change for only one direction and the latter 32 % displayed a similar change for both directions of movement. Movement related responses of area 4 neurons were markedly affected in the lesioned monkeys. The duration of the changes of neuronal firing both preceding and following the onset of displacement was increased. The other hand, the cellular reaction time was not significantly altered. Spontaneous firing of precentral neurons increased slightly but not significantly and the dynamic discharge rate was about the same after nigral lesion. However, only 18 % of cortical neurons still presented a reciprocal organization. The percentage of unidirectional neurons increased, while the bidirectional one remained the same. The slowed movements were associated with a generalized depression in the rate of rise and the amplitude of EMG activity in the agonist muscle with a usual coactivation of the antagonist. **Discussion** : Two explanations are proposed to explained the motor cortex dysfunction : (a) the SN could act as a "time-marker" on area 4 activity by setting bounds to the end of the modification of firing ; (b) the marked increase in muscle tone (parkinsonian hypertonia) could interfere as a peripheral load inducing a load-compensation"-like phenomenon on area 4 cells as is observed in the normal situation. The possible neuronal basis for the observed motor impairments is discussed. A different involvement of descending corticospinal and brainstem pathways is proposed in the genesis of the deficits seen in Parkinson's disease..

354.13 COORDINATION BETWEEN POSTURE AND MOVEMENT IN PARKINSONIANS ANALYZED THROUGH A BIMANUAL TASK. J. Massion*, F. Viallet, R. Massarino*, R. Khalil*. Service de Neurologie, CHU La Timone, 13005 MARSEILLE and Laboratoire de Neurosciences Fonctionnelles, CNRS, 31, ch. Joseph Aiguier, 13402 MARSEILLE CEDEX 9, France.

Voluntary movements are accompanied by an adjustment of posture which minimizes the imbalance caused by the movement. When the forearm held in a horizontal position is loaded with a weight, voluntary unloading performed by the other hand is accompanied by an anticipatory decrease in the EMG activity of the flexor muscles of the "postural" arm. The upward arm rotation which should result from unloading is thus markedly reduced (HUGON, M., MASSION, J., WIESENDANGER, M.,Pflügers Arch., 393: 292-296, 1982; DUFOSSE, M., HUGON, M., MASSION, J.,Exp. Brain Res., 60: 330-334, 1985). Imposed unloading, which was brought about by the experimenter's lifting the weight (1 kg), and voluntary unloading, which was performed by the subject's other hand in response to a tone burst, were analyzed in 14 Parkinsonian patients and in an age-matched group of control subjects.

The variables recorded were reaction time (time interval between the tone and the beginning of unloading, RT) and movement time (duration of the force changes measured by a force platform on the forearm, MT). Elbow angle changes were also measured with a potentiometer. The EMG activity from biceps, triceps and brachioradialis of the postural arm and that from the biceps of the active arm were recorded. The Parkinsonian patients showed an increase in both RT and MT. An impairment of the coordination was indicated by an increase in amplitude of the elbow rotation after voluntary unloading. Moreover, in Parkinsonian patients, the decrease in EMG activity in the flexor muscles of the postural arm was markedly reduced during voluntary unloading. This disorder of postural command was often accompanied by a lack of anticipatory EMG changes. Comparison between treated and non-treated patients showed that DA agonists led to the recovery of both RT and MT but did not improve postural coordination. The coordination was more severely impaired when the voluntary movement was performed by the non dominant hand. Several hypothesis are discussed concerning the mechanism underlying the impaired coordination between posture and movement, such as a defect in force control of the voluntary arm, a defect in phasic postural control, and a specific disorder of the internal circuits responsible for the coordination.

354.14 ALPHA-TOCOPHEROL IN TARDIVE DYSKINESIA: A PRELIMINARY STUDY. J.B. Lohr*, J.L. Cadet*, M.A. Lohr*, E. Wasli*, E. Aposteles* and D.V. Jeste. Neuropsychiatry Branch, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Persistent tardive dyskinesia (TD) following long-term neuroleptic treatment is a growing problem for which there is no satisfactory treatment. It is possible that some cases of TD result from neuronal damage due to excess free radical production that may occur during catecholamine metabolism (Cadet, Lohr and Jeste, Trends Neurosci 9:107, 1986). We therefore decided to assess the efficacy of alpha-tocopherol (vitamin E), a potent free radical scavenging agent, on the clinical signs of TD.

Methods: 10 patients with the diagnosis of either chronic schizophrenia or schizoaffective disorder (by DSM-III criteria) and persistent TD for at least 2 years completed the study. TD was diagnosed by two independent raters using specific criteria. Patients received alpha-tocopherol and matched placebo in a randomized cross-over design. The dose of alpha-tocopherol was raised from 400 I.U. p.o. q.d. to 400 I.U. p.o. t.i.d. over a 2 week period and maintained at that level for an additional 2 weeks. Abnormal movements were rated using a modified version of the Abnormal Involuntary Movement Scale (AIMS) and a modified Simpson-Angus Scale for Parkinsonism (SAS) by two independent raters "blind" to medication status. Psychopathology was rated on the Brief Psychiatric Rating Scale (BPRS).

Results: Patients demonstrated a significant overall reduction in AIMS score after treatment with alpha-tocopherol ($p<0.01$), but not after placebo. The mean reduction in the AIMS score with alpha-tocopherol was 48%, with 4 patients showing greater than 50% reduction in their dyskinesia. There were no significant changes in SAS or BPRS, although there was a trend for a decrease in BPRS score.

Discussion: Our preliminary findings are consistent with the possibility that alpha-tocopherol is beneficial in the treatment of some patients with TD, but further studies are necessary to establish the efficacy of this agent.

354.15 EFFECT OF GAMMA-HYDROXYBUTYRATE ON NOCTURNAL MYOCLONUS. L. Scrima, E. Thomas*, F. Johnson* & F.C. Hiller*. Sleep Disorders Center - 594, Univ. of Arkansas for Medical Sciences, Little Rock AR 72205

Nocturnal myoclonus (NM) is a sleep disorder in which phasic muscle activity is associated with frequent arousals, causing fragmented sleep and chronic partial sleep deprivation, leading to complaints of insomnia and excessive daytime sleepiness. No treatment has been demonstrated to be efficacious for treating NM. Gamma-hydrodroxybutyrate (GHB) is a metabolite of GABA, is present in the CNS of humans and animals, and is known to inhibit muscle tone (Vickers, M. Int. Anaesthesiol. Clin. Neurophysio. 22: 558-562, 1967). Moreover, GHB promotes natural-like sleep without suppressing REM or delta sleep at low doses of 25mg/kg, has about a 2 hour half-life and suppresses the H-reflex response (Muyard, J. & Laborit, H. In: Psychopharmacology Series II 2: 13399-1376, 1977). These properties of GHB are suggestive of possible therapeutic effectiveness for patients with NM. Indeed, GHB has been reported to increasingly suppress Muscimol induced myoclonus in mice as GHB dose was increased (50, 100, 200 mg/kg) (Menon, E. Neurology 32: 434-437, 1982).

Two patients, ages 37 & 47, diagnosed as having severe NM at our Sleep Disorders Center, participated in this study. Each patient signed an informed consent form approved by the Food and Drug Administration (IND 19,911). Each patient was prescribed 25mg/kg GHB at bedtime and 25 mg/kg of GHB 3 hours later, taken on one evening while being monitored in the sleep laboratory. The following sleep parameters were monitored and recorded during both the diagnostic (Dx) and the GHB sleep studies: sleep stages with EEG, EOG & triangularis-electromyogram (EMG), ECG, respiration and myoclonic activity with bilateral anterior tibialis EMG.

A comparison of the NM events and NM arousals from the Dx and the GHB sleep recordings indicate that GHB decreased the number of arousals per hour of sleep (NM Index) in both patients by about half (see Table below). GHB at this dosage did not reduce the number of myoclonic events per hour of sleep. A higher dose of GHB may also reduce the number of myoclonic events, as it did in mice. Further research is needed to assess nightly use of GHB at this and larger doses to evaluate the potential therapeutic benefit of GHB for NM patients' excessive sleepiness and/or insomnia. Since 25 mg/kg of GHB did not suppress the number of NM events but did decrease the number of arousals, it appears that GHB raised the arousal threshold rather than acting on the myoclonus generating neuromechanism.

Patient	NM Event Index		NM Arousal Index	
	Dx	GHB	Dx	GHB
1	77	72	23	12
2	71	132	24	14

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354.16 MORPHOMETRIC CHANGES OF THE POSTJUNCTIONAL FOLDS IN DYSTROPHIC MICE. C. Belhumeur*, J.P. Tremblay, L. Grégoire* and R. Sasseville*, (SPON: F. Garcin), Laval University, Quebec, Canada, G1K 7P4.

We have studied the structure of the motor end plates in the gastrocnemius muscle of 21 normal (C57BL/6J +/+), 6 heterozygous dystrophic (C57BL/6J $dy^{2J}/+$) and 22 dystrophic (C57BL/6J dy^{2J}/dy^{2J}) mice aged between 2 and 3 months. The muscles were fixed with 5% glutaraldehyde in 0.1M cacodylate buffer. The Karnovsky and Roots method (J. Histochem. Cytochem. 12, 219, 1964) was used to reveal the cholinesterase activity. Small pieces of tissue containing NMJs were embedded in epon. Twelve to eighteen NMJ profiles were photographed in EM for each animal. The following parameters were measured: length of synaptic cleft, number of secondary postjunctional folds, the total length of postjunctional membrane (i.e. primary synaptic gutter and every visible secondary folds) and the depth of postjunctional folds. The length of the synaptic cleft is significantly increased by 22% in the dystrophic animals (4.62 ± 2.65 µm) relative to the normal animals (3.77 ± 2.07 µm) but not relative to the heterozygous animals (4.62 ± 2.65 µm). The linear density of the secondary postjunctional folds was calculated for each profile by dividing their number by the length of synaptic cleft. In normal animals, this density is 2.02 ± 0.80 folds/µm (298 profiles). The density is significantly reduced ($p = .001$) in the dystrophic mice to 1.37 ± 0.75 folds/µm (388 profiles). Intermediate values between the normal and dystrophic densities are obtained in the heterozygous animals (1.76 ± 0.61, 83 profiles). This may represent a weak manifestation of the dystrophic gene in these carriers. The length of the postsynaptic membrane was normalized by dividing it by the length of the synaptic cleft. This normalized length of postjunctional membrane is significantly reduced by 45% in the dystrophic animals (3.38 ± 2.12 µm/µm) relative to the normal (6.14 ± 2.46 µm/µm) and by 41% relative to the heterozygous animals (5.75 ± 2.14 µm/µm). The depth of the secondary folds is also significantly reduced by 26% in the dystrophic animals (0.51 ± 0.27 µm) relative to the normal (0.69 ± 0.21 µm) and by 35% relative to the heterozygous animals (0.78 ± 0.19 µm). These morphometric changes of the postjunctional membrane are observed on NMJs whose nerve terminals were not significantly modified by the dystrophic process and were sometimes observed on apparently normal muscle fibers. Morphometric changes of the postsynaptic membranes are thus early manifestations of the dystrophic gene.

NEURAL BASIS OF BEHAVIOR: AGING I

355.1 AGE AND MANIA. J.R. Falk* and R.C. Young. (SPON: C.A. Shamoian). Washington University School of Medicine, St. Louis, Mo., and Cornell University Medical College, White Plains, N.Y. 10605.

Investigation of the interaction of aging with the presentation and response to treatment in affective disorders has focussed on depressive syndromes. Despite suggestions that manic symptoms may differ in the elderly and that some symptoms may be slow to resolve, prospective quantitative investigation of these issues is needed.

Patients admitted to an acute care psychiatric hospital who met the diagnostic criteria for mania of Feighner et al (1972) were studied. Forty (n=40) subjects ranged in age from 17 to 66 years. They were predominantly white and female. Subjects were interviewed on two occasions, each time by two clinicians trained in the use of the Mania Rating Scale (MRS; Young et al, 1978), and independent ratings were averaged. The MRS is an eleven-item instrument with which high interrater reliability can be achieved, and for which validity and sensitivity have been demonstrated.

Soon after admission to hospital there were negative third-order partial correlations between age and MRS scores on the "activity-energy" ($r= -.30$, $p<.10$), "sexual interest" ($r= -.42$, $p<.05$), "language-thought disorder" ($r= -.39$, $p<.05$), and "mental content" ($r= -.35$, $p<.05$) items, controlling for race, sex, and number of hospitalizations. There also were positive third-order partial correlations with item scores for "irritability" ($r= .44$, $p<.02$) and "speech-rate and amount" ($r= .34$, $p<.10$).

Two weeks later, during combined treatment with neuroleptics and lithium carbonate, there were positive third-order partial correlations between age and scores on the "mood" ($r= .35$, $p<.05$), "activity-energy" ($r= .28$, $p<.10$), "speech-rate and amount" ($r= 0.42$, $p<.05$), "language-thought disorder" ($r= 0.34$, $p<.10$), "disruptive-aggressive behavior" ($r= 0.33$, $p<.10$), and "insight" ($r= 0.52$, $p<.01$) items, and between age and total MRS scores ($r= .46$, $p<.02$). Age was positively related to maximum serum lithium level ($r=0.28$, $p<.10$) but was not related to average neuroleptic dose. Older patients tended to be hospitalized longer ($r= 0.33$, $p<.10$).

These preliminary data suggest effects of age on symptom pattern and response to treatment in mania. Further investigation is warranted.

355.2 VALIDATION OF THE CORNELL SCALE FOR DEPRESSION IN DEMENTIA. D. Deliyannides*, G. S. Alexopoulos*, R. C. Abrams*, R. C. Young and C. A. Shamoian. (SPON: K. Halmi). The New York Hospital-Cornell Medical Center, White Plains, New York 10605.

Simultaneous development of depression and dementia is frequent in geriatric patients. Depression rating scales rely on responses from patients, and therefore their value is compromised when used in demented persons. The recently developed Cornell scale can quantify depression using a brief contact with the demented patients and an interview with their caretakers. The Cornell scale has high interrater reliability and sensitivity in demented and non-demented geriatric patients. We now report preliminary data of a validity study of this instrument.

The subjects were psychiatrically hospitalized geriatric patients who met Research Diagnostic Criteria for major depression. They were assessed during the first week after admission and during the week prior to discharge. Depression was rated with the Cornell scale and the 24-item Hamilton Depression Rating Scale (HDRS). Cognitive dysfunction was rated with the Minimental State Examination (MMS). Cornell scale scores were significantly lower on discharge compared to those obtained on admission (paired t-test 4.65, $P < 0.002$) (criterion validity). There was no significant correlation between Cornell scale scores on admission and on discharge ($r_s = 0.35$, $P < 0.35$). Concurrent validity was examined by comparing Cornell scores with HDRS scores. Cornell scale scores correlated significantly with the HDRS scores both on admission ($r_s = 0.82$, $P < 0.007$) and on discharge ($r_s = 0.69$, $P < 0.04$). Change in Cornell scale scores from admission to discharge correlated significantly with change in HDRS scores ($r_s = 0.83$, $P < 0.006$). Change in Cornell scale scores did not appear to be influenced by cognitive dysfunction measured by the MMS ($r_s = 0.27$, $P < 0.48$). The data support the validity of the Cornell scale for rating depression in geriatric patients. This scale may be useful in the investigation of subjects with and without dementia and in psychopharmacologic studies.

355.3 PRE-MORBID NEUROPSYCHOLOGICAL RISK-FACTORS IN FAMILIAL ALZHEIMER'S DISEASE. G.D. Miner[1], L.A. Miner *[1], D.A. Stryker*[2], and R.C. Burgus*[3]. [1]Familial Alzheimer's Research Foundation, P.O. Box 701674, Tulsa, OK 74170-1674; [2]Eastern Nazarene College, Graduate Division, Quincy, MA 02170; and [3]City of Faith Medical and Research Center, Tulsa, OK 74170.

Several familial Alzheimer's Disease families appearing to fit a dominant gene mode of inheritance model and with homogeneous age-of-onset in the late forties/early fifties have been studied over the past few years with a battery of neuropsychological tests in an attempt to detect early "pre-disease" risk factors. Prior to this study, anecdotal evidence from these families had indicated that the members who developed the illness had been identified by surviving members as "distinctive" even at an age as early as late teens or twenties. Analysis of six of the many tests included in this battery show that the at-risk group (i.e. offspring of an affected member) segregate into two groups (a bimodal curve), whereas the control subjects fall into only one group. A subject with the lowest value in the at-risk group was clinically diagnosed with Alzheimer's Disease two years after being tested, lending credence to the hypothesis that those subjects that fall into the lower score grouping have a greater chance of being part of the 50% who are destined to develop the disease, if the genetic model is true. Refinements in the testing battery are needed to further define a test strategy that will more accurately identify at-risk members of these familial Alzheimer's Disease families.

355.4 AGING AND VISUAL SELECTIVE ATTENTION TO COLOR AND SPACE: EVENT RELATED POTENTIALS. M.M. Schroeder* and M.R. Harter (Spon:H.G. Vaughan). Department of Psychology, University of North Carolina at Greensboro, Greensboro, N.C. 27412.

Studies of selective attention in normally aging individuals using event related potential (ERP) and reaction time (RT) measures have suggested slowing and reduced selectivity with age, particularly when the location of the relevant stimulus is uncertain. The purpose of this study was to investigate the effects of age on the scalp distribution of ERP indicants of two different types of selective attention: the effects of attending a particular location (spatial selection) and particular feature (feature selection). It was predicted that the older subjects would show topographically distinct slowing and reduced selectivity of task relevant ERP effects, depending on the task.

Healthy normal subjects were recruited for the study. The mean age of the 8 younger and 8 older subjects was 25 yrs. and 70 yrs., respectively. The stimuli consisted of red and green flashes presented either 10 degrees in the left or right visual field. Subjects were instructed to give a finger lift response to one of these four stimulus conditions. ERPs were recorded from occipital, central, and frontal sites following each of the four stimulus conditions. The amplitude and latency of the largest deflection in three time windows of the ERP associated with feature and spatial selection were measured (P100-200, N200-350, and P350-600).

As predicted, the degree of slowing and reduced selectivity of processing of the older as compared to the younger group depended on the task and the scalp location. For the feature selection task, the occipital P350-600 showed an amplitude enhancement following the relevant color for both groups. However, the latency of this enhancement was significantly increased only for the older group. For the spatial selection task, there was an early occipital (P100-200) amplitude enhancement for stimuli in the relevant location for both groups. However, the negativity (N200-350) at central and frontal sites, associated with selection of the relevant location, was only evident for the younger group.

The results support the hypothesis that the older group would exhibit impairment of feature selection associated with ERP changes over posterior cortex, whereas impaired spatial performance would be associated with more anterior ERP effects.

355.5 RATE OF COGNITIVE DECLINE IN PARKINSON'S AND ALZHEIMER'S DISEASES. T.J. Rosen*, J.H. Growdon, and S. Corkin. Department of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139, and Department of Neurology, Mass. General Hospital, Boston, MA 02114. (SPON: B. Dawson).

Although the prevalence and progression of dementia in Parkinson's disease (PD) are uncertain, cognitive impairments can be measured in most patients with this disorder. An instrument sensitive to memory disorders is the memory and information subscale of the Blessed Dementia Scale (BDS), which is commonly administered to patients with Alzheimer's disease (AD). We define BDS subscale scores of 0-2 as normal, and the maximum score is 37. In order to compare the progression of memory impairments in PD and AD, we administered the subscale at least twice to 33 patients with PD and 70 with AD. These patients were examined consecutively in either the memory or movement disorders clinic in a general hospital and were retested at six-month intervals. The AD patients have so far been studied for a mean of 15.7 months and the PD patients for a mean of 13.8 months.

At the initial examination, the mean BDS for the AD patients was 15.88 (SD = 8.38), and the mean BDS for the PD patients was 2.08 (SD = 2.68) ($p < .001$). The BDS scores of AD patients increased at a mean rate of .365 points per month (SD = .420). In contrast, the BDS scores of PD patients were essentially stationary, decreasing at a mean rate of -.022 points per month (SD = .189); the greatest rate of change of any PD patient followed for 6 or more months was .210. The mean rates of change in AD and PD differed at the .001 level of significance.

It was of interest to know whether differences in the rate of cognitive change persisted when analyses compared PD and AD patients who showed memory deficits of the same magnitude. The 6 PD patients who consistently scored 3 or more on the BDS had a mean rate of change equal to -.156, whereas the 51 AD patients who scored between 3 and 22 (the maximum PD score) at the initial visit subsequently had a mean rate of change equal to .383. Thus, the PD and AD groups still differed significantly ($p < .01$).

The uniform failure to observe cognitive deterioration among PD patients suggests that any PD patients who deteriorate at a rate typical of AD may have AD or a related dementing disorder in addition to the characteristic nigral-striatal degeneration of PD. The present data should not, however, be interpreted as implying that the dementias of PD and AD result from lesions in different brain regions.

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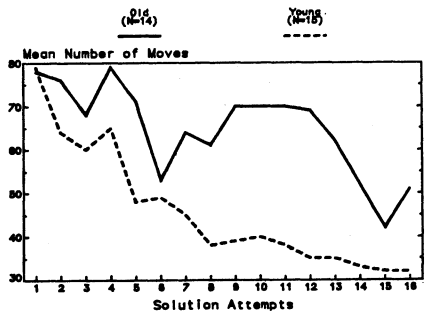
355.6 PERFORMANCE OF HEALTHY ELDERLY AND YOUNG SUBJECTS ON THE TOWER OF HANOI TASK. H.P. Davis, C. Cowles*, K. Sather*, J. Yates*, and M. Winslett*. Department of Psychology, University of Colorado, Colorado Springs, CO 80933.

A distinction between memory for skills and memory for facts or episodes is supported by studies of amnesic patients of various etiologies (Squire & Zola-Morgan, Ann. N.Y. Acad. Sci., 444:137, 1985). These two kinds of memory have been referred to as procedural and declarative memory. Acquisition and retention of procedural skill has been previously examined with the Tower of Hanoi puzzle . In the present study we extend examination of this distinction to healthy elderly individuals over sixty years of age.

Fourteen elderly (Mean age = 71 years; Mean IQ = 109) were matched on the WAIS-R Full Scale IQ with 15 young subjects between the ages of 18 and 30 (Mean age = 24 years; Mean IQ = 107). All subjects were also administered test of frontal lobe function (FAS test, Stroop test, and Wisconsin Card Sort). Subjects received 4 sessions on the Tower of Hanoi with 1 week between sessions and 4 soultion attempts per session. The optimal solution requires 31 moves. The experimenter was located behind the subject and verbal interaction was restricted to statements such as "Please keep trying." Performance of both young and elderly improved ($p<.01$), but the young subjects demonstrated significantly better performance ($p<.01$). Performance of young and elderly are shown below.

TOWER OF HANOI



355.7 DISSOCIATION OF VISUAL CAPACITIES IN ALZHEIMER'S DISEASE. S. Corkin, M.J. Nissen, J.H. Growdon, F.J. Huff, M.M. Keane*, H. Lieberman, A. Gruber*, and J. Rizzo*. Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139, Dept. of Neurology, Mass. General Hospital, Boston, MA 02114, and Dept. of Neuro-opthalmology, Mass. Eye & Ear Institute, Boston, MA 02114.

Vision is not a unitary capacity. In the monkey, a large expanse of posterior cortex is devoted to visual functions, but this area is structurally and functionally heterogeneous. Evidence from animals and humans suggests that striate and prestriate cortex are involved in basic visual functions, whereas an occipito-temporal system and an occipito-parietal system mediate dissociable higher-order visual functions. Because AD is characterized by minimal degeneration in primary visual areas and by maximal degeneration in temporal and parietal cortex, it is reasonable to expect a relative sparing of basic visual processes in AD, coupled with severe deficits on higher-order visual tasks. Nevertheless, our previous measurements of contrast sensitivity functions had shown patients with AD to be impaired at all spatial frequencies relative to control subjects. Thus, we sought to determine the relative contribution of several basic visual functions to the performance of higher-order visual tasks.

The subjects were 34 patients with a clinical diagnosis of AD, who ranged in severity of dementia from very mild to severe, as reflected in Blessed Dementia Scale scores of 2 to 52. Subjects received tests measuring basic visual processes (color vision, critical flicker fusion, stereoacuity, vernier acuity, contrast sensitivity, and flash and pattern masking) and several higher-order visual tests (form discrimination, copying designs, and object identification). Pattern masking was more often impaired than flash masking in AD, and copying designs and object identification were severely compromised; these results confirmed earlier reports. Of the basic visual capacities, color vision, flicker fusion, and vernier acuity were impaired in only a minority of the subjects, whereas stereoacuity and contrast sensitivity were more commonly impaired. These findings suggest that the latter two tasks may be mediated in cortex anterior to the striate and prestriate areas.

A subset of 9 AD subjects also received a detailed neuro-opthalmic examination and electroretinograms. The findings thus obtained were normal, confirming that the deficits observed in certain basic and higher-order visual capacities are central in origin.

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355.8 PERSONALITY DYSFUNCTION AND GERIATRIC DEPRESSION. R.C. Abrams*, G.S. Alexopoulos*, R.C. Young*. (SPON: P.E. Stokes) New York Hospital-Cornell Medical Center, White Plains, NY 10605

The relationship between personality dysfunction and geriatric depression has received little attention despite its clinical and theoretical importance. The Personality Disorder Examination (PDE), a recently developed structured interview for diagnosing DSM III personality disorders, has provided an advance for investigations in this area. Unlike other self-report personality instruments, such as the Eysenck Personality Inventory or the Minnesota Multiphasic Personality Inventory, the PDE is based on standard DSM III clinical criteria. We report a study of personality dysfunction in recovered elderly depressives and normal geriatric subjects using the PDE.

The subjects included 21 elderly individuals, of whom 10 were psychiatric patients with a history of major depression by DSM III criteria (mean age 75.1 $\pm$ 5.8 S.D.) and 11 were volunteers living in the community (mean age 70.3 $\pm$ 6.4). The volunteers had no significant history of psychiatric illness. All subjects were screened for cognitive impairment using the Mini-Mental State Examiniation (mean score 29.3 $\pm$ 1.2 S.D.). The patients did not participate in the study until their symptoms of depression had been adequately treated. To minimize the influence of residual depression on their responses, subjects were required to have scores of 11 or less on the 24-item Hamilton Depression Rating Scale (mean score 1.6 $\pm$ 2.1 S.D. for controls, 7.5 $\pm$ 3.6 S.D. for patients). All subjects were interviewed according to the structured format of the PDE. Because personality by definition describes both current and long-term functioning, subjects were instructed to base their responses on what had been typical of them throughout adult life. None of the subjects met DSM III criteria for personality disorder according to the PDE. The patient group received higher dimensional scores than controls on PDE items related to all Axis II diagnoses ($P < .05$) except antisocial personality disorder. The largest differences ($P < .001$) between the two groups were found for the dependent, schizotypal and avoidant categories.

These preliminary data suggest that elderly patients with a history of major depression have more lifetime personality dysfunction than normal elderly individuals, although no specific pattern could be identified. Further studies will determine whether the PDE profile of elderly depressives consists of generalized lifetime dysfunction across all Axis II categories or instead emerges in a particular form.

355.9 GLUCOSE ENHANCEMENT OF MEMORY IN AGED HUMANS. J.L. Hall*, L. Gonder-Fredericks*, J. Vogt* and P.E. Gold (SPON:M. Simon). Dept. Psychology and Dept. Behavioral Medicine and Psychiatry, University of Virginia, Charlottesville, VA 22903.

Age-related impairments in human memory are evident not only in cases of pathology (e.g. Alzheimer's disease) but also in healthy populations. Age-related deficits in storing and remembering new information have also been observed in a wide range of other animal species, ranging from aplysia to rodents and primates.

Findings in animal studies point consistently to the view that hormonal responses to training regulate the storage of new information. In particular, epinephrine enhances memory in a wide range of conditions, including normal aging in rodents. Recently, we obtained evidence suggesting that the effects of epinephrine on memory may be the result of its hyperglycemic actions. Like epinephrine, glucose enhances memory in an inverted-U, dose-dependent manner. Additionally, posttraining plasma epinephrine and glucose levels predict later retention. Therefore, we investigated the effects of glucose on memory in aged humans.

Subjects were volunteers, aged 57-70, tested over 3 consecutive days. Each morning, the subjects entered the experiment in a fasting state. On the first day, subjects were given a basic mental competency test, after which they received a glucose (50 g/subject) or saccharin (2.0 g) flavored fruit drink. The subjects then received a series of memory tests (modified from Wechsler memory scale) including a paired-associate test with 24 hr recall. On the second morning, after testing retention of the previous day's paired associate items, the procedure was repeated except each subject received the alternate beverage and a different version of the same memory tests (counterbalanced design--each subject served as his/her own control). The last day of testing consisted of a final retention test and debriefing.

Glucose, but not saccharin, ingestion resulted in a significant increase (67 $\pm$ 6.2 mg/dl) in circulating glucose levels. The most reliable glucose effects on memory were obtained with 24 hr recall of paired associates. When trained under the glucose condition, subjects remembered significantly more word pairs than when trained in the saccharin condition.

These findings support the view that plasma glucose can regulate memory and, further, that animal models of aging can directly predict pharmacological treatments effective in humans. Determining therapeutic pharmacological treatments for memory impairments during aging, and perhaps other disorders, will require additional information about the mechanisms by which glucose enhances memory. (Supported by NIMH 31141 and ONR NOOO14-85-KO472).

355.10 FURTHER BEHAVIORAL EVIDENCE OF WIDESPREAD CEREBRAL DYSFUNCTION IN AGED RHESUS MONKEYS (MACACA MULATTA). S.K. Presty*, L.S. Watermeier*, L.C. Cork*, D.L. Price, R.G. Struble, L.C. Walker, J. Bachevalier† and M. Mishkin† (SPON: M.R. DeLong). The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2182; †National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20205.

Previous studies have shown that aged monkeys were impaired, relative to young adults, on delayed nonmatching-to-sample with trial-unique objects (DNMS), a task that assesses object recognition memory (Presty, S.K. et al., Soc. Neurosci. Abstr., 10:774, 1984). This memory ability is known to be dependent on the occipitotemporal visual system as well as on limbic structures in the temporal lobe. To examine the same series of monkeys on a different form of memory, animals were trained on spatial delayed response (DR), a task known to be dependent on the integrity of the prefrontal cortex and head of the caudate nucleus. Eighteen monkeys were divided into four age groups with 4-6 monkeys in each group: Group I, 4-7 years; Group II, 15-18 years; Group III, 20-25 years; and Group IV, 27-31 years. Animals were trained by the direct method, i.e., to retrieve a food reward they saw concealed in one of two wells, first at "0-second" delay without screen (Phase 1), then at "0-second" delay with screen (Phase 2), and finally at "5-second" delay with screen (Phase 3). Criterion in each phase was set at 27 correct responses in a daily session of 30 trials with a training limit of 1,000 trials in each phase. In Phase 3, animals were advanced by the titration method from 1- to 5-second delays in steps of one second. Significant differences among groups were found only in Phase 3. Half of the animals in Groups III and IV failed to attain criterion within the training limit, and the other half required an average of 150 trials compared to an average of 40 trials for all animals in the two younger groups ($p < 0.01$). These results, combined with those obtained earlier on DNMS, indicate that at least two functionally and anatomically distinct neural systems are compromised in our older animals, perhaps due to the development, with increasing age, of plaques (Struble, R.G. et al., Brain Res. 361:267-275, 1985) and other abnormalities. Although damage to ventromedial prefrontal cortex is known to produce impairments in both DNMS and DR, it is unlikely that pathology in this region alone accounts for age-related deficits, since there was no correlation between performance on DNMS and DR within the two impaired groups. The latter finding suggests not only that multiple neural systems are vulnerable to increasing age but also that the distribution of neuropathology among these systems varies from animal to animal. In the future, we plan to examine this hypothesis directly.

355.11 EFFECT OF AGE UPON MEASURES OF CENTRAL AND PERIPHERAL SEROTONERGIC FUNCTION IN HUMAN SUBJECTS. P.A. McBride*, J Kream*, G. Anderson*, J.J. Mann.Lab. of Psychopharmacology, Cornell Univ.Med. Coll., New York, NY 10021 and Yale Child Study Center, New Haven, CT 06510.

Effect of age upon central and peripheral serotonergic function in normal subjects was assessed by multiple measures, including assay of serotonin receptor binding indices in postmortem brain tissue and on the blood platelet, administration of a neuroendocrine challenge test, and assay of whole blood serotonin content. Scatchard analysis of [^{3}H]spiroperidol binding to 5-HT$_2$ receptors in human frontal cortex revealed a decrease in Bmax with aging ($r=-0.61, n=16, p\leq 0.02$), but no change in K_D. Binding of [^{3}H]5-HT to 5-HT$_1$ receptors in frontal cortex was not significantly correlated with age ($r=-0.08, n=31$, NS).

Central serotonergic responsiveness was assessed by the fenfluramine challenge test. Plasma prolactin and cortisol levels were measured before and hourly for five hours following oral administration of a 60mg dose of fenfluramine versus placebo. A significant negative correlation was found between age and the amount of prolactin released in response to fenfluramine challenge, in excess of levels following placebo administration ($r=-0.84$, $n=17, p\leq 0.001$). Effect of age upon fenfluramine-induced cortisol release was obscured in a mixed-gender group by the tendency of females to release less cortisol in response to challenge than age-matched males. A trend toward an inverse relationship between age and cortisol release was noted when data were analyzed by gender (males: $r=-0.61, n=11$, NS; females: $r=-0.73, n=7$, NS). Plasma levels of fenfluramine and norfenfluramine were not significantly correlated with age.

In order to assess a potential systemic effect of age upon 5-HT$_2$ receptor function, saturation studies of [^{125}I]I-LSD binding to 5-HT$_2$ receptors on the human platelet membrane are in progress. In male subjects, a trend toward a negative correlation between age and Bmax has been observed ($r=-0.44, n=12$, NS). Studies of the relationship between age and whole blood serotonin content in normal subjects have also been undertaken. An inverse relationship between age and whole blood serotonin content was observed in psychiatric patients with affective and personality disorders ($r=-0.49, n=26, p\leq 0.02$).

These data suggest a systemic decrease in 5-HT$_2$ receptor number with age, accompanied by a decline in central serotonergic responsiveness which might be secondary to decreased density of postsynaptic serotonin receptors and/or availability of releasable serotonin from the presynaptic neuron. Interactions between effects of age and gender require further assessment. Supported by PHS Grant #MH 40210.

355.12 REGIONAL CEREBRAL PERFUSION AND RELATIVE TISSUE WEIGHT IN AGING. G. Smith and I. Prohovnik*. Brain Imaging Division Dept. of Psychiatry, Columbia University College of Physicians and Surgeons New York, NY 10032

The existing literature concerning cerebral perfusion in normal aging has demonstrated perfusion declines, without reference to such issues as the relation of atrophic changes to metabolic function and the differential involvement of grey and white matter structures. The focus of the current study is to examine regional cerebral blood flow (rCBF) in young as compared with aged normal subjects to confirm previous reports of flow reductions and to extend these findings to indices of relative grey matter weight and white matter clearance.

Twelve young subjects (twelve males, mean age 25.6 2.4, PCO2=45.1) were compared to eight aged subjects (seven females, one male, mean age 64.3 9.2, PCO2=38.7). All subjects were carefully screened for neurological and psychiatric abnormalities. rCBF was measured by the 133-Xe inhalation technique, with sixteen detectors over each cerebral hemisphere. The data were analysed in terms of the M2 model (two artifact, two unknowns). The data are presented in terms of four parameters: ISI (Initial Slope Index), which represents clearance from grey and white matter structures, dominated by grey matter flow; fg, a measure of pure grey matter flow; k2, a measure of white matter clearance and wg, the relative weight of perfused grey matter. The rCBF studies were conducted with the subjects' eyes closed and ears unoccluded.

A hyperfrontal pattern of regional flows was found, as expected, in both groups. Perfusion was significantly reduced (both ISI and fg) overall in the older subjects (10-15%) and this diminution was more accentuated prefrontally. There were no differences in white matter clearance. The relative weight of the grey matter(wg) was significantly and diffusely reduced in the older sample (9-12%).

These data indicate that the normal aging process is characterised by decreased grey matter flow and weight, but not by declines in white matter flow. The effects observed are probably not due to sex differences since females manifest higher flows until approximately age 60, when flow levels converge with that of males (Meyer, 1985). The diffuse flow reduction in the aged normals is in contrast with the focal findings in Alzheimer's Disease, in which degeneration is seen in temporal-parietal regions. Future research will be undertaken to assess the deficits seen in Alzheimer's Disease relative to normal aging, utilizing rCBF as an in vivo index of perfusion and atrophic processes.

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NEURAL BASIS OF BEHAVIOR: AGING II

356.1 RELIABILITY IN BRAINS AND GENES: MATHEMATICAL MODEL SUGGESTING SPARE CAPACITY OR REDUNDANCY FACTOR OF TWO. R. B. Glassman. Dept. of Psychol., Lake Forest Col., Lake Forest, IL 60045.

Behavioral recovery from brain damage (Finger & Stein, Academic Press, 1982) sometimes suggests spare capacity. This is particularly so in dramatic human cases, normal in spite of missing as much as half the brain, described in Aaron Smith's papers--yet there is also evidence of accelerated behavioral deterioration with aging in some recovered patients (Smith, in Almli & Finger, Academic, 1984). Perhaps natural selection yields spare capacity to protect against high cumulative probability, in long-lived organisms, of a neural subsystem malfunction which could cause a fatal behavioral error.

Let q_i be the probability of malfunction in a single operation of any subsystem, i; n_i is the number of copies of subsystem i (including the original), and m_i is the number of times one or another copy of each subsystem i must operate successfully in a lifetime. Then the brain's overall reliability (R), or the probability of living long enough to reproduce, is

$$R = \prod_{i=1}^{s} (1 - q_i^{n_i})^{m_i},$$

where s is the total number of neural subsystems. For simplicity, assume all subsystems have the same q, n, and m; then the formula collapses to

$$R = (1 - q^n)^{sm}.$$

Note that large sm, in a long-lived organism, implies an eventual malfunction, even with extremely reliable subsystems. The burden of head size suggests natural selection against large n, and selection instead for small q. However, for $q \ll 0.5$ a vast improvement in reliability can then occur if n increases from 1 to 2. The implication is that brains are twice as large as short-term survival would require.

With genes, natural selection might similarly work to limit the size of cell nuclei but then exploit high reliability of each gene by backing each up with a single similar allele. Thus, Bernstein, Byerly, Hopf & Michod (J. theor. Biol, 1984) argue plausibly for their minority position regarding origins of diploidy and sexual reproduction.

With both brains and genes there is a puzzle as to how the normal rather than the abnormally functioning information-bearing subsystem is usually preferentially chosen by the whole. As Bernstein, et al. suggest, perhaps this is effected by partial, complementary information in the most closely related interacting subsystems, as each functional cycle takes place.

356.2 HIPPOCAMPAL CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY AND MAZE PERFORMANCE IN "OLD" AND YOUNG RATS. S.H. Murphy, S.D. Farber, N. Postlewaite*, T. Paine*, Y. Kaseda*, J.R. Hofstetter, J.A. Richter, and W.C. Low. Depts. of Physiology and Biophysics, Pharmacology and Biochemistry, and the Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

Recent behavioral studies of aged rats have revealed a subpopulation that is severely impaired in their performance in a Morris water maze. Neural transplants from embryonic septal nuclei containing cholinergic neurons to the hippocampal formation of aged animals in this affected sub-group were able to improve their performance significantly (Gage et al., Science 225:533-536, 1984). These observations suggest that poor performance in the water maze may be the result of deficits in cholinergic function within the hippocampal formation. To test this hypothesis, we determined ChAT activity in dorsal and ventral hippocampi in "old" retired breeders eight months of age, and in young rats two months of age. Twenty four retired breeders and twelve young rats were first tested in a Morris water maze to determine the time required to locate a submerged platform within the maze and escape from the water. Latency measurements of the old rats revealed a bimodal distribution and were used to divide the old rats into two groups, designated as old "impaired" and old "non-impaired." Old impaired rats required 29.7 $\pm$ 2.5 (mean $\pm$ SEM) seconds to locate the platform in comparison to 12.7 $\pm$ 1.1 seconds for old non-impaired rats and 13.4 $\pm$ 2.2 seconds for the group of young animals. After behavioral testing, the animals were decapitated and the hippocampi were dissected from each animal and analyzed for ChAT activity. In the old impaired group, ChAT was 29.9 $\pm$ 1.9 pmol/ug protein/h in the dorsal hippocampi, and 38.6 $\pm$ 1.9 pmol/ug protein/h in the ventral hippocampi. In the old non-impaired group, ChAT activity was 29.4 $\pm$ 2.4 pmol/ug protein/h in the dorsal hippocampi, and 36.9 $\pm$ 2.2 pmol/ug protein/h in the ventral hippocampi. In the group of young rats, ChAT activity was 28.2 $\pm$ 1.4 pmol/ug protein/h in the dorsal hippocampi, and 35.2 $\pm$ 1.7 pmol/ug protein/h in the ventral hippocampi. A two-way analysis of variance was performed to examine the variance among the three groups of rats as well as the variance between dorsal and ventral hippocampi. This revealed no significant difference among the ChAT activities of the three groups of animals ($F=0.88$; $df=2,66$; $p > 0.25$). These results suggest that the impairment in water maze performance as displayed by a sub-group of eight month old rats may not be a result of cholinergic dysfunction as determined by measures of choline acetyltransferase activity.

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356.3 **INCREASED CHROMOSOME 21q22.1 MARKER IN ALZHEIMER'S DISEASE AND DOWN'S SYNDROME.** F.P. Zemlan, O.J. Thienhaus* and H.B. Bosmann*. Lab. of Geriatric Research, Univ. of Cincinnati College of Medicine, Cinti., Ohio 45267.

Several lines of evidence indicate a strong genetic relationship between Alzheimer's disease (AD) and Down's syndrome (trisomy 21). The causal etiology of Down's syndrome is related to the presence of an extra copy of the q22 region of chromosome 21. Gene products coded on locus 21q22 include the enzyme superoxide dismutase (SOD-1) which is elevated about 50% in fibroblast cell lines of Down's patients. The present study determined whether the activity of SOD-1, a genetic marker of the 21q22.1 region was elevated in fibroblast cell lines deprived from Alzheimer's and Down's patients when compared to euploid cell lines from normal controls.

As sample freezing decreased SOD-1 activity in AD cell lines but not normal cell lines all assays employed nonfrozen samples trypsinized within the previous six hours. A significant ($p<0.001$) 54% increase in SOD-1 activity was observed in Down's cell lines (N=3) when compared to normal euploid cell lines (N=20; 10.4 SOD U/10^6 cells). An average 53% increase in SOD-1 activity was observed in five cell lines derived from familial AD patients when compared to normal controls ($p<0.01$). Although SOD-1 activity in all AD cell lines was greater than the enzyme activity in the normal cell lines appreciable variation among the AD patients was observed. For example, an 83% increase was found in the cell line derived from an AD patient in which an autosomal dominant form of inheritance was identified. An 81% increase was found in an AD proband with two affected siblings. A 56% increase was observed in an autopsy confirmed AD proband with at least two first degree relatives who were demented. At the other extreme, an 11% increase was found in a proband where neuropathological data was unavailable for the proband or father (only affected relative).

The present data indicating that familial AD and Down's patients demonstrate increased activity of an enzyme coded on chromosome region 21q22.1 provides additional support for a genetic similarity between these two disorders.

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356.4 **ALZHEIMER'S DISEASE CHOLINERGIC DEFICIT: HUMAN GROWTH HORMONE RESPONSE TO TENSILON.** O.J. Thienhaus, F.P. Zemlan, D. Bienenfeld*, H.B. Bosmann*. Division of Geriatrics and Department of Psychiatry, Univ. of Cincinnati College of Medicine, Cinti., OH 45267.

It is well established that Alzheimer's disease (AD) is associated with degeneration of cholinergic neurons in several CNS regions. Recent neuropathological data in Alzheimer's patients indicates the presence of neurofibrillary tangles in numerous hypothalamic regions associated with regulation of pituitary hormone release. These hypothalamic regions, which receive a strong cholinergic projection, are located immediately caudal to the basal forebrain cholinergic cell bodies which degenerate in Alzheimer's patients. It is not known whether the cholinergic projection to hypothalamic regions controlling pituitary growth hormone release degenerates in Alzheimer's patients. The present study explores this hypothesis. Specifically, the present study examined whether Alzheimer's patients demonstrate a blunted growth hormone (GH) response to a presynaptic cholinergic challenge. Three baseline blood samples were taken prior to administration of the cholinesterase inhibitor, tensilon (0.15 mg/kg), and five samples taken during the 80 minutes following drug administration. A peak GH response of 14 ± 5 ng/ml was observed in 8 age-matched non-demented control patients, while a significantly blunted GH response of 2 ± 1 ng/ml was observed in 14 Alzheimer's patients ($p<0.04$). Positive correlations between the patient's memory performance ($r^2=0.22$, $p<0.03$) and Reisburg Stage of AD ($r^2=0.24$, $p<0.02$) with the GH response indicated that more severely affected AD patients demonstrated the most blunted GH responses to tensilon administration. The implications of the present data for assessing cholinergic deficits in Alzheimer's patients will be discussed.

356.5 NEUROPATHOLOGY OF THE OLFACTORY SYSTEM IN ALZHEIMER'S DISEASE AND NORMAL AGING. P.D. Fleming and J. Rogers. Departments of Neurology and Pathology, University of Massachusetts Medical School, Worcester, MA 01605.

The ability to detect and distinguish different odors has been found to decline with age, a deficit exacerbated in Alzheimer's disease (AD). The present study describes possible neuropathologic correlates of olfactory dysfunction in AD and normal aging. Olfactory bulbs and tracts were dissected intact from 20 autopsied patients with histologically confirmed AD, 20 age-matched control patients, and 5 younger patients. After paraffin embedding, the tissue was sectioned sagittally then stained by Bielschowsky, Bodian, and hematoxylin and eosin methods. All AD specimens (20/20) contained intraneuronal neurofibrillary tangles with maximum densities ranging from 1-27 ($\bar{X}$ = 8.25) tangles per high power field (HPF) (400X). In contrast, 11/20 aged-matched controls contained tangles ranging from 1-9 ($\bar{X}$ = 1.45) tangles/HPF. Neuritic (senile) plaques had a mean maximum density of 3.05 plaques/HPF in AD. In contrast only one control case contained a neuritic plaque. Plaques were concentrated in the bulb, especially in the anterior olfactory nucleus. Tangles were found in the bulb as well as in neurons along the tract. A striking finding was the extraordinarily high density of polyglucosan bodies (corpora amylacea) in AD and age-matched control cases compared to the younger patients as shown in a previous study (Fleming, 1986). Whereas polyglucosan bodies were sparse and isolated in the bulb, they occurred in dense clusters in the tract. Polyglucosan bodies were often associated with deviations in the paths of adjacent myelinated nerve fibers, with apparent compression and rupture of axons. AD pathology is also observed in primary olfactory cortex and other projection areas of the olfactory bulb.

We conclude that neuropathologic changes typical of Alzheimer's disease are abundant in the human olfactory bulb and tract, and may contribute to the hyposmia associated with Alzheimer's disease.

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356.6 **DIFFERENCES IN THE RESTING ECoG OF YOUNG AND AGED RATS INDICATE AGE-RELATED CHANGES IN VIGILANCE.** T.A. Emrey*, J.J. Inserra and R.R. Notvest (SPON: J. Maran). Dept. of Pharm., Ayerst Lab. Res., Inc., Princeton, NJ 08543-9990.

The electrocorticogram (ECoG) was used to assess age-related changes in cerebral function of conscious male Fisher (F-344) rats. Young (3-month old; n=26) and aged (26-month old; n=18) rats were anesthetized and implanted with electrodes one week prior to recording of brain waves. The recording session included five minutes of artifact-free ECoG. The ECoG was quantified by power-frequency analysis: the parameters included total power (TP, 1-30 Hz) and the power of four specific frequency bands (delta, 1-4 Hz; theta, 4-8 Hz; alpha, 8-13 Hz; and beta, 13-30 Hz). Differences were evaluated by a t-test.

Age-related differences in the ECoG were observed for all parameters (Table 1). Specifically, the aged group had a significantly lower TP, which reflected lower power in each of the four frequency bands (*$p<0.01$, ** $p<0.001$).

TABLE 1 MEAN ABSOLUTE POWER IN MICROVOLTS (± SD)

Group	TP	delta	theta	alpha	beta
Young	98 (11)	24 (3)	36 (5)	19 (3)	18 (2)
Aged	73 (8)**	20 (2)**	25 (4)**	12 (1)**	15 (1)**

The relative power of each frequency band (% of TP) was used to evaluate the distribution of power in the spectrum (Table 2). Aged rats showed a greater distribution of power in the delta and beta frequency bands and a reduction in the alpha and theta frequency bands.

TABLE 2 MEAN RELATIVE POWER (± SD)

Group	delta	theta	alpha	beta
Young	24 (2)	37 (2)	20 (1)	19 (1)
Aged	27 (2)**	35 (3)*	17 (1)**	21 (1)**

The relative increase in delta and decrease in alpha indicated a lower state of vigilance in aged rats.

356.7 PERFORMANCE OF AMNESIC AND DEMENTED PATIENTS ON EPISODIC AND SEMANTIC MEMORY TASKS. N. Butters, E. Granholm,* D. Salmon,* J. Grant and J. Wolfe.* Psychology Service and Depts. of Psychiatry and Neurosciences, VA Medical Center and Univ. of Cal. Sch. of Med., San Diego, CA 92161.

Episodic (recall of short passages) and semantic (letter and category fluency) memory tasks were administered to 13 Alzheimer's Disease (early stages), 12 Huntington's Disease (HD), 9 alcoholic Korsakoff's Syndrome (AK) and 26 intact control (13 young, 13 elderly) subjects. The three patient groups were matched for overall severity of dementia with a dementia rating scale. Although all three patient groups were severely (and equally) impaired on recall of four passages, only the Alzheimer and AK patients emitted numerous prior-story intrusion errors. That is, more than 20% of the Alzheimer and AK patients' total recall of the meaningful short stories represented information from previously presented passages.

On the fluency tasks, the performance of the mild Alzheimer patients were distinguishable from that of the other two patient groups. The HD and AK patients demonstrated severe and moderate deficits respectively, on both fluency tasks, whereas the mild Alzheimer patients were impaired only on the category fluency task (i.e., names of animals). As with the episodic memory test, the Alzheimer and AK patients made more perseverative errors (i.e., repetition of words) than did the HD patients on letter fluency (i.e., words beginning with F, A and S).

These findings suggest that Alzheimer and HD patients' impairments on episodic and semantic memory tasks reflect different underlying processes. The performance of Alzheimer patients is affected by their language dysfunction and an increased sensitivity to proactive interference; the deficits of the HD patients appear due to a general retrieval problem. Similarities in the error patterns (i.e., perseveration errors) of Alzheimer and AK patients are consistent with the notion that the two disorders may share some common neuropathological (i.e., basal forebrain) and neurochemical (i.e., cholinergic) dysfunctions.

This study was supported by funds from the Medical Research Service of the VA, NIAAA grant AA-00187, and by NIA grant AG-05131.

356.8 MAINTENANCE OF GOOD SPATIAL MEMORY CORRELATES WITH THE PRESERVATION OF HIPPOCAMPAL PERFORATED SYNAPSES IN AGED RATS. Y. Geinisman, L. de Toledo-Morrell* and F. Morrell. Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611 and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60612.

Spatial working memory, which crucially depends on the structural integrity of the hippocampal formation and its afferent connections, is impaired in the majority, but not all, aged rats (de Toledo-Morrell et al., Behav. Neurosci., 98:902, 1984). This study was designed to verify whether aged animals that do not exhibit the spatial memory deficit are the ones in which the hippocampal synaptic connectivity remains preserved with advancing chronological age. Young adult rats (5 mo. old) with good spatial memory, aged rats (27 mo. old) with impaired spatial memory and equally aged rats with intact spatial memory were compared. Spatial memory was tested in an 8-arm radial maze. Rats were trained to a stringent criterion of 3 consecutive trials without errors or to a maximum of 30 trials. After the behavioral testing, rats were perfused with aldehyde fixatives. The tissue was taken from the hippocampal formation at the same septo-temporal level in each animal and routinely processed for electron microscopy. The central segment (in the medio-lateral direction) of the dorsal blade of the dentate gyrus was examined. Synapses were studied in the middle of the dentate molecular layer. The numerical density (N_V) of synapses and granule cells per unit volume was estimated from serial sections by the method of Cruz-Orive as applied for synapse morphometry by Verwer and De Groot (Progr. Brain Res., 55:195, 1982). Synaptic N_V (in μm^{-3}) divided by neuronal N_V (in μm^{-3}) gave an estimate of the average number of synapses associated with one granule cell. The most important results were obtained when axospinous synapses were divided into perforated and nonperforated ones according to the appearance of their postsynaptic density. A significant decrease in the number of perforated synapses per neuron was found in memory-impaired aged rats as compared to either young adults or aged animals without memory deficits; the latter two groups did not differ from each other with respect to this measure. The number of nonperforated synapses per neuron was diminished in memory-deficient aged rats relative to young adults, but not relative to memory-intact aged rats. However, it was only the loss of perforated synapses that correlated with the degree of spatial memory impairment as measured either by the number of trials necessary to reach the behavioral criterion or by the choice accuracy score for the last 5 days of training. It appears, therefore, that a preserved complement of hippocampal perforated synapses is crucial for the maintenance of good spatial memory during aging.

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356.9 CLASSICAL CONDITIONING OF RABBITS 2½ TO 4 YEARS OLD USING MOSSY FIBER STIMULATION AS A CS. D. S. Woodruff-Pak[1], J. E. Steinmetz[2], & R. F. Thompson[2], Dept. of Psychology, Temple U., Philadelphia, PA 19122[1] & Dept. of Psychology, Stanford U., Stanford, CA 94305[2]

A fundamental issue in age-related deterioration in memory formation concerns whether it is due to impairment of sensory receptive capabilities or central storage mechanisms. Age differences in peripheral portions of the CS pathway could account for the observed age differences in conditioning in rabbits. In this study we bypassed peripheral portions of the CS pathway and input the CS more centrally by electrical microstimulation of mossy fibers from the dorsolateral pontine nucleus (DLPN).

Six female and one male New Zealand white rabbits ranging in age from 30 months to 44 months (mean age of 36 months) were anesthetized and implanted bilaterally with bipolar stimulating electrodes in the DLPN or mossy fibers. After two days of recovery from surgery they were adapted in the running chamber for one hour. Training began the following day and consisted of 108 trials per day in which stimulation near the DLPN (CS, 200 Hz, 350 msec, 30-60 uA) was paired with a coterminating corneal airpuff as the UCS (2.1 N/cm^2, 100 msec). Rabbits were trained with a tone CS to determine if the mossy fiber stimulation CS would transfer to an auditory CS.

Results indicated that older rabbits could be trained with DLPN stimulation as the CS, but they take many more trials to criterion than 3-month-old rabbits. The mean number of trials to learning criterion was 529.57. This is over five times as long as the mean number of trials to criterion taken by young animals. Although the range of ages of the older rabbits in this study was limited to just over one year, the correlation between trials to criterion and age (in months) was high ($r = .67$; $p < .05$). All but one of the older rabbits attained criterion to the tone CS within 100 trials indicating that some transfer from the DLPN stimulation CS occurred.

The present study demonstrated that stimulation of the DLPN mossy fibers can be an effective CS for training an eyelid response in rabbits older than 2½ years. However, acquisition is five times slower than in 3-month-old rabbits. These results indicate that the age differences in eyelid classical conditioning which have been observed in rabbits cannot be entirely attributed to age differences in the peripheral CS pathway. Changes in the nervous system occurring more centrally in the cerebellum appear to be more important for age differences in learning.

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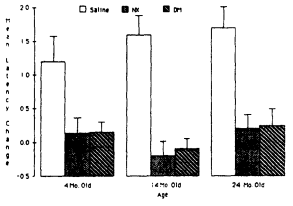
356.10 **AGE-DEPENDENT DECLINES IN REFLEXIVE AND MOTOR CAPACITIES: SIMILARITIES BETWEEN SENESCENT C57BL/6NNIA AND YOUNG AUTOIMMUNE MICE.** M.J. Forster, M.D. Popper*, S.K. Paul*, K.C. Retz and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

We have previously shown a relationship between the formation of brain-reactive antibodies (BRA) and declines of learning abilities in senescent C57BL/6 mice (Lal and Forster, Drug Dev. Res. 7: 195-208, 1986) and in chronologically younger mice of genetically autoimmune strains (Forster et al., Soc. Neurosci. Abstr. 11:722, 1985). A similar relationship exists with respect to age-related abnormalities in pharmacologic sensitivities of these strains (Forster et al., Life Sci., 38:1433-1439, 1986), suggesting that BRA are a cause or manifestation of functional CNS alterations in aging and autoimmune disease. The potential similarities between aging- and autoimmune-related behavioral alterations were investigated in the present study, by comparing age-dependent patterns of decline in reflexive and motor abilities of C57BL/6 and autoimmune New Zealand Black (NZB) mice. These abilities were expected to show specific patterns of non-uniform decline during aging in C57BL/6 mice, and it was predicted that a similar pattern would be seen in young to mature NZB mice. C57BL/6Nnia mice (aged 1.5, 3, 6, 12, 24 or 28 mo.) and NZB/BINJ mice (1.5, 3, 6, or 12 mo.) were tested for simple reflexive capacities (negative geotaxis, vibrissae-elicited forelimb placing, midair righting) and capacities involving integrated reflex patterns and sensorimotor coordination (rotating cylinder and swimming tests). Although geotactic responses of NZB mice became slower with advancing age, all of the simple reflexes could be elicited at all ages tested in both strains. The maximum speed at which mice could remain on a rotating cylinder showed a marked decrease with age in both strains, the decrease occurring at younger ages in NZB mice. Deficient performance by the young NZB mice and aged C57BL/6 mice was partially alleviated with daily practice. The forelimb inhibitory and head position components of the swimming reflex (Shapiro et al. Science, 168:148-151, 1970) showed heterochronic changes with age in C57BL/6 mice. At 1, 3 and 6 months, C57BL/6 mice showed inhibition of forelimb movements during swimming, whereas progressively more forelimb movements were seen with increasing age thereafter. Swimming ability (as indicated by head position) was impaired only in the 28-month-old C57BL/6 mice. NZB mice showed nearly identical age-dependent changes in swimming, but during a period between 3 and 12 months. Overall, the results indicate that young to mature autoimmune NZB mice show senescence-like, non-uniform declines in reflexive and motor capacities. These findings suggest that immune abnormalities are closely associated with behavioral indicators of aging.

356.11 THE ANALGESIA PRODUCED BY FOOD DEPRIVATION IN 4-MONTH OLD, 14-MONTH OLD, AND 24-MONTH OLD RATS. R. J. Hamm and J. S. Knisely. Dept. of Psychology, Virginia Commonwealth University, Richmond, VA 23284.

The analgesia produced by 24 hr of food deprivation was examined in 4-mo, 14-mo, and 24-mo old rats. To assess opioid and hormonal involvement in food deprivation induced analgesia, different groups of rats from each age group were injected with naltrexone (7 mg/kg), dexamethasone (0.4 mg/kg), or equivolume saline. A 3(Age) x 3(Drug) x 2(Time) analysis of variance of the tail-flick latencies before and after 24 hr of food deprivation indicated that food deprivation produced an equivalent increase in tail-flick latency in each saline-treated age group. In the naltrexone- and dexamethasone-treated groups, food deprivation did not produce an analgesic response. In addition, naltrexone and dexamethasone were equally potent in blocking food deprivation analgesia in each age group. See Figure.



These results demonstrate that food deprivation activates an endogenous opioid-mediated analgesic system that involves hormonal factors and that this system does not decline in function with increasing age. The absence of an age-associated decline in the analgesia produced by food deprivation is in agreement with the developing body of data on the age-dependent alterations in the hormonal system. The lack of an age-related decline in this hormonally mediated analgesic system is in contrast to the reported decline in two forms of CNS-mediated analgesic responses (J. Geront.,40,268-274; Soc. Neurosci.Abstr., 11,731). Thus, not all endogenous analgesic systems are affected equally by the aging process.

356.12 AGE-RELATED LOSS OF DENDRITIC SPINES IN RAT HIPPOCAMPUS: EFFECT OF PHOSPHATIDYLSERINE ADMINISTRATION. M.G. NUNZI, F. MILAN*, D. GUIDOLIN*, G. CALDERINI and G. TOFFANO. Fidia Neurobiological Labs., 35031 Abano Terme (PD), ITALY.

Disfunction of the hippocampal system has been involved in cognitive disturbances which develop with age in both humans and animals.

Phosphatidylserine (BC-PS), a pharmacologically active component of brain cortical phospholipids, has been shown to ameliorate retention of passive and active avoidance behaviour in old rats (Drago F. et al., Neurobiol. Aging, 2:209-213, 1981; Corwin J. et al., Neurobiol. Aging, 6:11-13, 1985; Aporti F. et al., Neurobiol. Aging, in press).

The present study was undertaken to investigate age-associated alterations in dendritic spine density of pyramidal neurons in rat hippocampus and to examine in advanced age possible effects of BC-PS administration on spine populations. Seven male Sprague-Dawley rats for each of the following groups were perfused for microscopy: 4 month-old rats, 27 month-old rats and 27 month-old rats which received BC-PS suspended in drinking water (total daily dose 50 mg/Kg) and administered from the age of 4 months to the day of sacrifice. The dorsal hippocampus was processed for Golgi staining and 4 pyramidal cells per animal were selected from sector CA1. By a computerized interactive analysis system (IBAS, Kontron, Zeiss) spine density was measured on 2-5 segments per dendritic order for each cell. In old-untreated animals spine density showed, with respect to young animals, a significant decrease in both apical and basal dendrites ($P<0.01$ for all dendritic orders; one-way Anova), with a mean loss of 12,11% in the basal dendrites and of 9.77% in the apical ones. In BC-PS treated rats values of spine density, in both basal and apical dendrites, were not statistically different when compared to those of young animals while they were significantly different when compared to those of the old-untreated rats. This correlates well with the capability of BC-PS in completely preventing the deficit in active avoidance learning in aged rats.

These findings indicate that a selective loss of afferent fibers to pyramidal cells may occur in the aged hippocampus. Moreover, they also suggest that a long-term BC-PS treatment may have a role in the maintenance of the morpho-functional integrity of the hippocampal circuitry.

356.13 ALZHEIMER'S DISEASE FIBROBLASTS ACCUMULATE MORE CYCLIC AMP IN RESPONSE TO B-ADRENERGIC STIMULATION VS NORMAL FIBROBLASTS. J.D. McSwigan* (SPON: F. Seil) V.A. Medical Center (Portland Division) and Dept. of Medical Psychology, Oregon Health Sciences University, Portland, OR 97201.

Recent studies have shown that fibroblasts from patients with Down's syndrome have a much greater accumulation of cyclic AMP in response to beta- adrenergic stimulation than normal fibroblasts. Autopsy studies have shown that virtually all Down's syndrome patients who reach the age of 40 have the same degree of neurofibrillary tangles and neuritic plaques as that found in Alzheimer's patients. Therefore, it was possible that fibroblasts from Alzheimer's patients would show the same increased cyclic AMP accumulation as that found in Down's syndrome. To test this hypothesis, skin fibroblasts from seven Alzheimer's (ALZ) patients and 5 normals (NSF) were exposed to 1 μM of the beta-adrenergic agonist isoproterenol. Three experiments each performed in triplicate indicate that Alzheimer's fibroblasts are more responsive than normal fibroblasts to beta-adrenergic stimulation. ($t = 2.67$, $p < .05$) $\bar{X}_{ALZ} = 941 \pm 196$; $\bar{X}_{NSF} = 494 \pm 159$ p mol cAMP/mg /10 min. This increased response did not extend to stimulation with PGE_1.

It is possible that this in vitro test may be useful as a premortem diagnostic procedure to detect Alzheimer's disease. This would be valuable as at present the diagnosis relies on the exclusion of other causes of dementia. Currently fibroblasts from individuals with other causes of dementia are being tested. In addition, this is another piece of evidence relating Down's syndrome and Alzheimer's disease.

357 SYMPOSIUM. NEURONAL ACETYLCHOLINE RECEPTORS. Darwin K. Berg, Univ. of Calif., San Diego (Chairperson); Lorna W. Role, Columbia Univ. Coll. Phys. & Surg; James W. Patrick, Salk Institute; Lawrence M. Marshall, Univ. of North Carolina Sch. of Med.

Despite the wealth of information available for nicotinic acetylcholine receptors (AChRs) in muscle, little has been learned about nicotinic AChRs on neurons until recently. Early studies identified a membrane component on neurons that bound alpha-bungarotoxin and cholinergic ligands, and supported the interpretation that the component represented the functional nicotinic AChR on neurons. More recent results have challenged this view and have identified other membrane components as likely neuronal AChRs on vertebrate neurons. Work with DNA probes and with monoclonal antibodies have raised the possibility that several homologous but distinct AChRs may be expressed in the vertebrate nervous system. The symposium will present recent advances in identifying neuronal AChRs and in examining their structure, function, distribution, and regulation.

The first speaker, Lorna W. Role, will initially review the physiology, pharmacology, and single channel properties of nicotinic AChRs on neurons, and will compare them where possible with muscle AChRs. Peptide modulation of receptor function will also be discussed, and new results will be presented on the regulation of neuronal AChRs by cellular interactions in culture. The second speaker, James W. Patrick, will describe the isolation and characterization of cDNA clones for muscle and neuronal AChRs. Studies on the control of gene expression will be presented, and current progress will be summarized examining the properties and function of translation products coded by the neuronal AChR clones. The third speaker, Darwin K. Berg, will review recent studies with monoclonal antibodies and alpha-neurotoxins that identify a neuronal AChR protein. New results will be presented on the subunit composition, cellular distribution, function, and regulation of the receptor. The fourth speaker, Lawrence M. Marshall, will describe studies on the regulation of neuronal AChRs by presynaptic input. Influences on the number and distribution of receptors will be discussed, and evidence will be presented indicating that the type of axon can determine the single channel properties of the receptor.

Current results indicate that in some respects the structure and regulation of neuronal AChRs reflect principles elucidated for muscle AChRs. In other respects, however, neuronal AChRs are clearly distinct and follow interesting regulatory mechanisms apparently unique to neurons.

358 WORKSHOP. NEUROGLIA: FUNCTION, DEVELOPMENT, AND DIVERSITY. J.S. Edwards & P.V. Sarthy, Univ. of Washington (chairpersons); R.H. Miller*, Case Western Reserve Univ; M.R. Meyer*, Univ. of Washington; J. Silver, Case Western Reserve Univ.; K.J. Muller, Univ. Miami Sch. Med.; N. Cowan*, New York Univ. Med. Sch.; E.A. Newman, Eye Research Institute; R. Orkand, Univ. of Puerto Rico.

Levels of knowledge of the diversity of neuroglia and their various functions are unequally distributed through the major animal groups. Regulatory and developmental roles are known for vertebrate glia. Annelid neuroglia are implicated in regeneration. In arthropods on the other hand, even basics of classification are open to question. Our objective in proposing this workshop is to highlight growth points in physiological and developmental areas of research from three major phyla, as a vehicle for mutual broadening of perspectives.

R. Miller will discuss cell lineages of type I and II astrocytes and oligodendrocytes during development of the rat optic nerve. The use of immunological probes to study glial cell diversity and development in the nervous system of the cricket will be presented by M. Meyer. J. Silver's talk will deal with the morphological and molecular factors of the glial environment that may contribute guidance information to developing axons. K. Muller will describe his work on the developing and regenerating leech nervous system which shows that glia may play a role in the appropriate growth and channeling of axons. N. Cowan will talk about the organization of the mouse GFAP gene and its expression and regulation in the developing mouse brain. E. Newman will present his experiments on compartmentation of K^+ conductance in Muller cells of the salamander retina and will also present evidence for the presence of four types of voltage-gated ion channels in those cells. R. Orkand will discuss the role of glial cells in maintaining ion homeostasis during photostimulation of the honeybee drone retina.

PHYSIOLOGICAL EFFECTS OF PEPTIDES III

359.1 THE MOLECULAR MECHANISMS OF CRF AND SOMATOSTATIN REGULATION OF CALCIUM MOBILIZATION IN PITUITARY TUMOR CELLS. T. Reisine and S. Guild*. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, 19104 and Dept. of Pharmacology, Univ. of Glasglow, Glasglow.

CRF is the most potent and effective natural stimulant of adrenocorticotropin (ACTH) release. To study the molecular mechanisms by which CRF evokes ACTH secretion, we have used a tumor cell line of the mouse anterior pituitary, AtT-20. Activation of cAMP-dependent protein kinase is essential for CRF to stimulate ACTH release, since insertion of the inhibitor (PKI) of this enzyme into AtT-20 cells, using a liposome technique, blocks the ACTH secretory response to CRF. Calcium acts as a trigger for the stimulation of hormone secretion and CRF raises cytosolic calcium levels (measured using Quin 2) in AtT-20 cells in order to elicite ACTH release. The mechanism by which CRF increases calcium mobilization also involves cAMP-dependent protein kinase. Insertion of PKI into AtT-20 cells prevents CRF and forskolin, an activator of adenylate cyclase, from elevating intracellular calcium levels. In contrast, the ability of K+, a membrane depolarizing agent to raise cytosolic calcium levels is not affected by the PKI treatment. The increase in calcium mobilization initiated by CRF could be due to the phosphorylation of calcium channels or other ionic conductance channels involved in regulating free cytosolic calcium content. Interestingly, another peptide, somatostatin (SRIF) regulates calcium mobilization to inhibit ACTH release. SRIF lowers cytosolic calcium content of AtT-20 cells and prevents forskolin from increasing calcium levels. Inhibition of forskolin's effect is due to a blockade of adenylate cyclase activity. A GTP inhibitory protein (Ni) couples SRIF receptors to adenylate cyclase. This was indicated from studies employing pertussis toxin, an agent which inactivates Ni. Pertussis toxin reduces the effect of SRIF, itself, in lowering cytosolic calcium levels. Since SRIF does not diminish basal cAMP levels or prevent 8-bromo-cAMP from raising calcium levels, it is possible that SRIF receptors couple to an N protein not linked to adenylate cyclase but associated to either ionic conductance channels or other cellular mechanisms responsible for maintaining cytosolic calcium concentrations. Pertussis toxin treatment also diminishes the affinity of SRIF receptors for SRIF. This is probably due to an uncoupling of Ni from the SRIF receptor. Ni therefore acts to both link SRIF receptors to effector systems as well as to regulate the sensitivity of SRIF receptors. Thus, CRF appears to act through a single second messenger, cAMP, to initiate a cascade of cellular events to release ACTH whereas SRIF acts via multiple mechanisms to inhibit hormone secretion. How SRIF receptors couple to multiple second messenger systems is a question which remains to be answered.
Supported by NIH grant GM34782

SUBSTANCE P(SP)-INDUCED DESENSITIZATION IN CANINE CEPHALIC ARTERIES IS NOT MIMICKED BY NEUROKININ A (NKA) AND NOT MEDIATED BY ENCHANCED PHOSPHATIDYLINOSITOL (PI) METABOLISM. M.A. Moskowitz, C.Kuo*, S.E. Leeman, M.E. Jessen*. Neurology Neurosurgery, Massachusetts General Hospital, Boston, MA. 02114; Dept Physiology, Univ Mass, Worcester, MA.

Substance P and neurokinin A were measured by radioimmunoassay in canine cephalic blood vessels and tested for their vasoactivity in vitro. Levels of iSP were approximately 2-3 times greater than those of iNKA in common carotid, basilar and middle cerebral arteries. Both SP and NKA relaxed precontracted segments of common carotid and basilar arteries in a concentration dependent manner with an EC_{50} (common carotid artery) of 8.9×10^{-11} M and 7×10^{-10} M, respectively. Relaxation was endothelial dependent for both substances and not blocked or enhanced by pretreatment with indomethacin, propranalol, lithium chloride or atropine. SP caused significant desensitization to subsequent additions of itself or to NKA but not to sodium nitroprusside, calcitonin gene-related peptide or bradykinin. This suggests that the mechanism of desensitization points more to events surrounding the agonist-receptor interaction than to a secondary effect common to metabolic and physiologic processes which relax vascular smooth muscle directly or via the endothelium. The development of SP-induced desensitization required 10 min exposure and was attenuated but not abolished by increasing the number of washes between the two SP applications. The two basic N-terminal amino acids (Arg, Lys) probably mediated the development of desensitization since tachyphylaxis did not develop to multiple additions of NKA, physalaemin, eledoisin. These results suggest the importance of both the N- and C-terminal sequences for tachykinin effects on vascular tissues. Finally neither SP nor NKA released 3H-inositol phosphates from phospholipid membranes of canine carotid segments after preincubation with 3H-inositol. Taken together, these results suggest that more than one endothelial receptor mechanism mediates vascular responses to the tachykinins and that these responses are probably not coupled to increases in PI turnover.

359.3 **NEUROPEPTIDE Y MICROINJECTION INTO THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS PRODUCES INCREASES IN BLOOD PRESSURE.** D.I. Diz, C.B. Wolf,* K.L. Barnes and C.M. Ferrario. Department of Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44106.

Neuropeptide Y (NPY) immunoreactive neurons and fibers are present throughout the rat hypothalamus (Chronwall et al., Neuroscience 15: 1159-1181, 1985). Moreover, specific NPY binding sites have been shown in synaptosomal preparations from the brain of rats (Saria et al., Eur. J Pharmacol. 107: 105-107, 1985). Since intraventricular injections of NPY produce a variety of autonomic effects including changes in blood pressure (Fuxe et al., Acta Physiol. Scand. 118: 189-192, 1983), a more precise characterization of the function of this novel neuropeptide on the regulation of blood pressure is warranted. We report here the hemodynamic effects of discrete administration of NPY into various hypothalamic nuclei.

Either NPY (12 pmol) or phosphate buffered saline vehicle (pH 7.4) was microinjected in a volume of 100-300 nl via double-barreled glass micropipettes (O.D. 20-70 μm) while mean and pulsatile arterial pressure and heart rate were monitored. The baseline mean arterial pressure and heart rate in 26 chloralose anesthetized rats were 83 ± 5 mm Hg and 324 ± 15 beats/min, respectively. In seven rats, injections of NPY into the ventromedial nucleus of the hypothalamus produced a 9 ± 3 mmHg ($p < 0.02$) increase in pressure lasting for approximately 20 minutes; heart rate did not change. In contrast, injections of the peptide into other sites including the anterior (n = 10), paraventricular (n = 8), dorsomedial (n = 8) and dorsal (n = 8) nuclei of the hypothalamus or the zona incerta (n = 6) evoked no noticeable changes in either pressure or heart rate.

We conclude that while the widespread distribution of NPY-like immunoreactivity suggests a general involvement of this peptide in the hypothalamic control of arterial pressure, our observations indicate that the pressor activity of NPY is restricted to the ventromedial nucleus. The significance of the pressor actions of NPY described here and the effector pathways responsible for these effects remain to be established. However, that these pressor effects are a component of the other known autonomic and behavioral actions of NPY is an interesting possibility that should be investigated further. (Supported by NHLBI grant HL-6835).

359.4 MICROINJECTION OF NEUROPEPTIDE Y (NPY) OR HISTAMINE INTO THE POSTERIOR HYPOTHALAMIC NUCLEUS ELICITS A PRESSOR RESPONSE IN THE RAT. J.R. Martin*, M.C. Beinfeld* and T.C. Westfall (SPON: M.A. Walz). Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

High levels of NPY and histamine are known to exist in the mammalian hypothalamus. Although the precise physiological role of histamine in the hypothalamus is unknown, evidence exists which suggests a neurotransmitter function. Less information is available concerning the physiological function of hypothalamic NPY but a role of either a neurotransmitter or neuromodulator seems possible. The presence of these two substances in the hypothalamus raises the question as to whether or not they play a role in influencing autonomic functions such as blood pressure (BP) regulation. Stimulation of several nuclei in the hypothalamus has been shown to alter BP. For instance, stimulation of the posterior hypothalamic nucleus (PHN) elicits a pressor response while stimulation of the anterior hypothalamic nucleus elicits a depressor response. Since intracisternal or intracerebroventricular injections of NPY or histamine have been shown to influence BP, the present study was undertaken to determine if microinjections of NPY or histamine into the PHN can alter BP.

Male Sprague-Dawley rats (250-300 g) were anesthetized with 1.6 g/kg of urethane. The femoral artery of a rat was then cannulated for the purpose of measuring BP after which the head of the rat was fixed in a stereotaxic apparatus. A 26 gauge guide cannula was then lowered through a burr hole drilled in the skull such that the guide cannula was aimed at the PHN. After allowing the BP to stabilize over a 30 min period, a 32 gauge injection cannula was placed inside and lowered through the guide cannula. The injection cannula extended 2 mm beyond the guide cannula and was used for the administration of NPY, histamine, or various receptor antagonists directly into the PHN. Not more than 2 microinjections were made per rat. Cannula placement was histologically verified following each experiment. Microinjection of NPY or histamine elicited dose-dependent pressor responses which began immediately upon injection. To determine if the pressor response elicited by these agents was due to an activation of the sympathetic nervous system, hexamethonium (10 mg/kg, i.v.) was administered 10 min prior to NPY or histamine. Various receptor antagonists were microinjected into the PHN prior to NPY or histamine in order to further pharmacologically assess these pressor responses. The results of this study show that NPY or histamine administered into the PHN can elicit an increase in BP. These findings suggest that NPY and histamine play roles in the central regulation of BP.

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359.5 CENTRAL NERVOUS SYSTEM ACTION OF NEUROPEPTIDES TO INFLUENCE GASTRIC EMPTYING IN RATS. Y. Taché, M. Maeda Hagiwara* and C.M. Turkelson*. Center for Ulcer Research and Education, VA Medical Center, Department of Medicine, UCLA, Los Angeles, CA 90073 and Harry S. Truman Memorial Veterans Hospital, Columbia, MO 65201.

Peptides including thyrotropin-releasing factor (TRH), corticotropin-releasing factor (CRF), bombesin, calcitonin and calcitonin gene-related peptide (CGRP) act within the brain to alter gastric secretory function. The central nervous system (CNS) action of these peptides to influence gastric emptying was evaluated. Rats fasted for 24 h were injected intracisternally under light ether anesthesia with peptides or saline and 5 min later received per oral intubation 1.5 ml of a liquid meal consisting of a solution of 50 mg of phenol red in 100 ml aqueous methylcellulose (1.5%). Animals were sacrificed at 10-120 min after the meal for time course studies and 20 min after the meal for other experiments. Gastric emptying was determined as described by Scarpignato et al., Arch. Int. Pharmacodyn., 246, 286, 1980). In saline-treated rats, the percentage of gastric emptying 20 min after a meal was 58.4 ± 5.4 (n=11). Intracisternal injection of CRF (21-210 pmol), calcitonin (0.2-29 pmol) and CGRP (260 pmol) induced a dose dependent inhibition (0-90%) of gastric emptying. Human growth hormone releasing factor (260 pmol) was inactive whereas TRH (2.8-83 pmol) and the TRH analog RX 77368 (7.8-78 pmol) enhanced the rate of gastric emptying. Except for TRH, peptide action on gastric emptying was lasting 60-120 min. The inhibitory effect of intracisternal CRF was not reversed by naloxone (2.5 mg/kg, iv), adrenalectomy (-1 h) or intravenous injection of rat CRF antiserum which prevented the delay in gastric emptying following intravenous injection of CRF (63 pmol). TRH (0.2-2.8 nmol) injected intravenously has no effect on gastric emptying. The stimulatory effect of intracisternal TRH was prevented by vagotomy and atropine. Intracisternal injection of the analog [N Val^2]-TRH devoid of TSH-releasing activity also enhanced gastric emptying. These results demonstrated that intracisternal CRF induces a centrally mediated inhibition of gastric emptying through pathways unrelated to the release of opioid peptides or adrenal derived substances. Intracisternal TRH acts centrally to stimulate gastric emptying through vagal and cholinergic dependent mechanisms. The role of CRF in mediating stress induced delay of gastric emptying needs to be further evaluated.

359.6 SMALL CARDIOACTIVE PEPTIDE B MODULATES FEEDING MOTONEURONS IN THE TERRESTRIAL SLUG, LIMAX MAXIMUS. S.D. Hess*& D.J. Prior. Physiology Group, School of Biol. Sci., Univ. of Kentucky, Lexington, KY 40506.

Several studies have shown that small cardioactive peptide B (SCP_B) serves as a positive modulator of feeding neurons and musculature in gastropod molluscs. Using the isolated CNS of Limax, Prior, Watson & Hess (Am. Zool.25:16A,1985) have shown that exogenous SCP_B decreases the threshold for the expression of the feeding motor program and increases the burst frequency of the fast salivary burster neuron (FSB). In addition, SCP_B-like immunoreactive material is present in the cerebral and buccal ganglia of Limax.

To further examine the potential role of SCP_B in the regulation of feeding, we have used intracellular recordings to describe the effects of SCP_B on FSB activity. The isolated cerebral and buccal ganglia were continuously superfused with saline in a small volume recording chamber. Exogenous SCP_B ($2X10^{-8}$ to $2X10^{-6}$ M) was superfused over the ganglia during 2 min trials.

In 6 CNS preparations, SCP_B caused a dose dependent depolarization of the FSB. Although the onset of this effect occurred within approximately 1 min, the duration of the repolarization was dose dependent. The maximum amplitude of the depolarization varied from 1.8±0.3 mV (mean±SD,N=7 trials) with $2X10^{-8}$ M SCP_B to 5.6±3.6 mV (N=14 trials) when the preparation was exposed to $2X10^{-6}$ M SCP_B. SCP_B also increased the rate of interburst depolarization (RIBD) in a dose dependent manner. With $2X10^{-6}$ M SCP_B, the mean RIBD increased from a pretrial level of 0.8±0.02 mV/s (±SEM,N=7 trials) to 2.9± 1.4 mV/s, a 262% increase. The peak response occurred 1 min after the start of the wash, and the RIBD decreased linearly to control levels within 5 min. The response to $2X10^{-8}$ M SCP_B was near threshold.

We have been unable to mimic the SCP_B effect on the RIBD via current injection. When the FSB was depolarized to levels which occurred during SCP_B trials, the burst pattern was disrupted. This suggests that SCP_B increases the burst frequency and RIBD of the FSB by modulating pacemaker currents. Following 30 min of superfusion with low Ca^{++}, high Mg^{++} saline, the usual responses to SCP_B were observed. Thus it appears that the FSB may have SCP_B receptors.

These data suggest that an SCP_B-like substance modulates feeding by affecting the activity of identified feeding neurons. Supported by a Whitehall grant to DJP.

359.7 YGGFMRF-NH_2 POTENTIATES NEURO-MUSCULAR TRANSMITTER RELEASE AND EXCITATION-CONTRACTION COUPLING: A POSSIBLE THIRD IDENTIFIED NEUROPEPTIDE IN LOCUST. M. Schiebe, K.H. Voigt* and C. Walther* (Spon: H.C. Kwan). Dept. Appl. Physiol., Univ. Ulm, F.R.G., D7900.

Two neuropeptides have so far been identified which modify the contraction of the slow innervated muscle fibers of the M. extensor tibiae of the locust: 1. AKH, the adipokinetic hormone, which causes contractions (O'Shea, M. et al., J. Neurosci., 4: 521, 1985); and 2. proctolin, which is coreleased with glutamate and causes sustained contractions (Witten, J. et al., Soc. Neurosci. Abst., 11: 327, 1985). As we have reported earlier (Walther, C. and al., Neurosci. Lett., 45: 99, 1984) YGGFMRF-NH_2 increases the transmitter release, the membrane resistance of the slow innervated fibers and augments e-c coupling.

Here we report that a YGGFMRF-NH_2-like peptide is present in the locust Schistocerca gregaria. By using antibodies raised against FMRF-NH_2 we identified the neurohaemal organ (NHO) as the storage and putative release-site of the neuropeptide. Co-chromatography of NHO-extracts and YGGFMRF-NH_2 yielded preliminary evidence on the structural identity of both substances. Blockade of NHO-extract by preincubation of the extract with this antibody reduced the increase of the twitch by 90-95%. Comparison of effects of extracts of 1/3 NHO in 100 ul saline with those of $5*10^{-8}$ M YGGFMRF-NH_2 on the muscle showed no difference in all recorded physiological parameters (CV of e.j.p.s., total membrane conductance, relaxation-rate).

Proctolin at $5*10^{-9}$ M increased the relaxation-rate and caused neither an increase in transmitter release nor an effect on membrane potential.

Octopaminergic projections into the median nerve are not known, and, although 10^{-6} octopamine showed a response pattern similar to $5*10^{-8}$ M YGGFMRF-NH_2, its effects could be blocked by 10^{-5} M phentolamine, whereas effects of YGGFMRF-NH_2 could not be blocked. Extracts of a piece of leg nerve (nerve 5, 10 x the mass of the NHO) caused only marginal increases in contraction amplitude.

We therefore conclude that a third neuropeptide, released from the NHO, is YGGFMRF-NH_2-like and suggest that it be called "Locust Neurohaemal Peptide(s)", LNP.

359.8 HISTOCHEMICAL AND NEUROPHYSIOLOGICAL EVIDENCE FOR A CHOLECYSTOKININ-LIKE PEPTIDE IN THE BUCCAL GANGLION OF NAVANAX, AN OPISTHOBRANCH MOLLUSC. M. B. Zimering*, A. J. Madsen, Jr.*, and R. Elde (SPON: J. Wirtschafter). Hennepin County Medical Center (MBZ) and Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

Navanax is a carnivorous mollusc that uses its large muscular pharynx to swallow live prey whole. The buccal ganglion of Navanax contains identified, feeding-specific neuronal circuitry. For example, expansion motoneurons controlling radial muscles of the pharynx mediate prey capture, whereas circumferential motoneurons controlling circumferential muscles mediate swallowing.

Navanax buccal ganglia were processed according to the indirect immunofluorescence method using rabbit antisera against CCK-8 (or an absorption control) in conjunction with fluorescein-labeled goat anti-rabbit IgG. Discrete nerve cell bodies and varicose fibers within the ganglion displayed intense CCK-immunoreactivity.

In mechanically-desheathed buccal ganglion preparations which remained connected to the pharynx via buccal nerves, sulfated CCK-8 was delivered by pressure injection near the surface of buccal neurons impaled with an intracellular voltage-recording electrode. Physiological concentrations of CCK-8 (100 pg/ml) inhibited spontaneous firing in small and medium expansion motoneurons associated with 10-12 mv hyperpolarizations lasting several seconds. Similar concentrations of CCK-8 had no effect when applied to the surfaces of many circumferential motoneurons. CCK-8 caused hyperpolarizations in a large, previously unidentified neuron in the left lateral hemiganglion in 9 out of 10 preparations. The amplitude of the hyperpolarization was dose-dependent in the 0.1 - 100 pg/ml range of concentrations of CCK-8: 0.1pg/ml caused 2-4 mv hyperpolarization, 2 pg/ml caused 6-8 mv hyperpolarizations and .10 pg/ml caused 8-12 mv hyperpolarizations.

To our knowledge this is the first report that a CCK-like peptide inhibits activity in identified neurons that mediate prey capture (i.e. expansion motoneurons). The distribution of effects of physiological concentrations of locally applied CCK-8 and the occurrence of CCK-8 immunoreactivity in discrete neurons suggests that a CCK-like peptide is a neuromodulator in the Navanax buccal ganglion. The proposal that CCK-like peptides play a role in appetite regulation can now be investigated in a mollusc by direct neurophysiological techniques.

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359.9 CHOLECYSTOKININ ANTAGONISTS POTENTIATE SOME EFFECTS OF BENZODIAZEPINES. A.E.Panerai, L.C.Rovati*, M.Bianchi* and P.Sacerdote* (SPON: F.Clementi). Dept. of Pharmacol., Chemoter. and Medical Toxicol., School of Medicine, Univ. of Milano, 20129 Milano, Italy.

Recently, benzodiazepines have been demonstrated to act as cholecystokinin antagonists, both in periphery, where they inhibit the contractile response of the isolated guinea-pig gallbladder to sulphated cholecystokinin-8 (Kubota,K. et al., Europ.J.Pharmacol., 110: 225, 1985), and in the central nervous system, where they antagonize sulphated cholecystokinin-8 induced activation of rat hippocampal neurons (Bradwejn et al., Nature, 312: 363, 1984).

Our study was conducted with the aim of evaluating whether the cholecystokinin antagonism exerted by benzodiazepines was significant for their pharmacological actions and might be utilized in order to modulate their effects. We investigated whether compounds acting as cholecystokinin receptor antagonists could potentiate benzodiazepines in their pharmacological effects. The cholecystokinin receptor antagonists employed were proglumide and a newly developed more specific and potent analogue: CR 1409 (Makovec et al., Arzneim-Forsch., 35: 1048, 1985)(Rotta Labs., Milano, Italy).

Proglumide and CR 1409 administered prior or concomitantly with diazepam (Roche, Basle, Switzerland) were able to cause a shift to the left in the dose-response curve of diazepam itself in the anticonvulsant activity (pentetrazole induced seizures in mice) and in the muscle-relaxant activity (rotarod test in mice), but not in the spontaneous locomotor activity (Animex meter, rats). When administered alone, proglumide or CR 1409 did not show any intrinsic activity in these tests.

Studies are in progress both in the experimental animal, in behavioural tests, and in humans, in order to confirm in other tests and fully characterize the interaction between benzodiazepines and cholecystokinin antagonists.

Our preliminary results could open new perspectives in the clinical use of benzodiazepines and in the development of new anxiolytic agents devoid of sedative activity.

359.10 LONG-TERM HALOPERIDOL TREATMENT INDUCES A SUPERSENSITIVITY TO CHOLECYSTOKININ BUT NOT TO DOPAMINE IN THE RAT NUCLEUS ACCUMBENS. G. Debonnel* and C. de Montigny. Institut Philippe Pinel de Montréal and Centre de recherche en sciences neurologiques, Université de Montréal, Montréal (Québec), Canada H3C 3J7.

Cholecystokinin (CCK) and dopamine (DA) coexist in neurons of the ventral tegmental area (VTA) projecting to the nucleus accumbens. A 6-hydroxydopamine lesion of the VTA induces a supersensitivity to both CCK and DA (Debonnel and de Montigny, Neurosci. Abst. 11: 27.16, 1985; Hu and Wang, Neurosci. Abst. 11: 217.7, 1985). Long-term haloperidol treatment suppresses the firing activity of VTA dopaminergic neurons (Bunney and Grace, Life Sci. 23: 1715, 1978). The present experiments were carried out to determine the effect of acute or long-term haloperidol treatment on the responsiveness of accumbens neurons to CCK and DA.

Male Sprague-Dawley rats (200-400 g) were treated for three or five weeks with the long-acting haloperidol decanoate (4 mg/kg i.m. once a week). Unitary recordings were obtained one week after the last injection from neurons of the dorso-medial part of the caudal half of the nucleus accumbens, under urethane anesthesia (1.25 g/kg, i.p.). The following solutions were used for microiontophoresis: sulphated octapeptide CCK (1 or 10 M in 200 mM Nacl; pH:5), DA (100 mM; pH:4) and kainate (KA) (1 mM in 400 mM Nacl; pH:8).

Acute haloperidol (1 to 5 mg/kg, i.v.) altered neither the excitatory effect of CCK nor the suppressant effect of DA. Long-term haloperidol induced a marked time-dependent supersensitivity to CCK. After three weeks of treatment, the degree of activation by CCK was about three times that of controls and, after five weeks about five times. The most striking change in haloperidol-treated rats was the markedly prolonged duration of the excitation induced by 50 sec applications of CCK, resulting in a 100-200 fold increase in the total number of spikes generated per nA after five weeks of treatment. In contrast, neither the three-week nor the five-week haloperidol treatments produced any significant increase in the efficacy of DA or KA.

The lack of effect of acute haloperidol on the suppression induced by microiontophoretic applications of DA suggests that, in the nucleus accumbens, this response is mediated by an adrenoceptor other than the D1 or D2 subtypes of dopaminergic receptors identified by the radioligand technique. This is in keeping with the lack of supersensitivity to microiontophoretically-applied DA after long-term haloperidol. The dramatic supersensitivity to CCK could be due to the diminution of the tonic release of CCK consequent to the suppression of the firing of VTA dopaminergic neurons by long-term haloperidol.

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360.1 **SOMATIC MUSCLE AND CUTANEOUS HINDLIMB AFFERENT MODULATION OF MEDULLARY SOLITARY TRACT NEURONS IN THE CAT.** **R.J. Person. Dept. of Physiology and Biophysics, Univ. Okla. Health Sci. Ctr. Oklahoma City, OK 73190.**

Dorsal column hindlimb muscle afferents are known to project bilaterally to nucleus tractus solitarius (NTS) neurons. NTS neurons receiving somatic input were described electrophysiologically in cats acutely prepared under chloralose, succinylcholine paralysis, and artificial ventilation. Recording and stimulating (distal) electrodes were placed on the sural (S) and medial gastrocnemius branch of the peroneal (P) nerves under oil and compound action potentials monitored to apply 200 usec shocks in multiples of Group I fiber threshold (T). P and S stimulation was limited to 1.25-30T. Stimulating electrodes were placed on the contralateral cervical vagus (V). Tungsten microelectrodes (10 Megohm) were directed at the contralateral medial NTS using the obex as reference.

Thirty-seven histologically localized units excited or modulated by P or S stimulation were studied in 10 animals: 75% were spontaneously active to rates of 15 Hz, 70% received convergent P and S excitation; 55% of the units tested for vagal input received convergent P, S, and V excitation. Data for P and S effects were not significantly different: latencies to unit excitation gave a minimum mean afferent fiber conduction velocity (CV) of 26±1.5 (SE) m/s (range 9-45 m/s). Sixty % of P and 36% of S CVs were within the Group II range. Many (35% S-excited, 46% P-excited) spontaneously active units had a subsequent 120±20 msec (range 23-235 msec) period of spike inhibition beginning at a latency of 80±10 msec. P and S excitation and inhibition was produced at stimulation intensities below Group III threshold.

Interactions of P, S, and V input on the NTS units were tested in conditioning-test (C-T) paradigms. Prior P stimulation (down to 1.5T) reduced S and V input to 0-20% of control for up to 200 msec and below 50% for up to 800 msec (S test input) or 200 msec (V test input). In different units prior S stimulation (down to 1.5T) had similar effects or facilitated P input to 160% of control. Preceding V stimulation inhibited P input for 100 msec and facilitated P input to 160% of control 100 to 200 msec post-V stimulus.

These results suggest that Group II cutaneous and muscle afferents are capable of modulating NTS unit activity. The C-T data show potent inhibition of subsequent convergent somatic and visceral sensory input. Since Group III-IV "ergoreceptor" afferents are thought critical to systemic cardiorespiratory exercise adjustments, specific Group II afferents may modulate ergoreceptor input to NTS and subsequent cardiorespiratory adjustments.

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360.2 EFFECT OF FATTY ACID COMPOSITION ON FAT-INDUCED STIMULATION OF SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN THE RAT. M.C. Walgren, J.B. Young* and L. Landsberg*. Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

The addition of fat (as lard) to a lab chow diet stimulates sympathetic nervous system (SNS) activity, as indicated by accelerated norepinephrine turnover (NETR) and by increased urinary norepinephrine (NE) excretion. Although the mechanism(s) governing this SNS response to fat feeding is (are) unknown, the possibility exists that dietary fatty acids may affect SNS activity. To investigate the potential influence of fatty acid composition on fat-induced stimulation of the SNS, NETR was compared in groups of rats fed diets enriched by addition of either of two oils: hydrogenated coconut oil (HCO, Teklad), a highly saturated fat (94%) containing only 2% linoleic acid, and safflower oil (SFO, Teklad), a relatively unsaturated fat containing 75% linoleic acid. In the first experiment [3H]-NE turnover was measured in male rats (150-175g, CD, Charles River) fed a fixed amount of lab chow or an identical chow ration supplemented with either HCO or SFO to increase caloric intake by 100%. After 6d on these diets, fractional and total NETR in heart was increased in both fat-fed groups compared to NETR in hearts of the chow-fed group. Calculated rates for cardiac NETR were 23.4 ng NE/h in chow-fed rats and 44.0 and 40.1 ng NE/h in animals fed the HCO- and SFO-supplemented diets, respectively ($P<0.01$ for differences between either fat-enriched diet and chow control). In a subsequent study, a similar comparison of SNS responses to HCO- and SFO-feeding was performed after 14d. The feeding protocol was modified slightly from that employed in the 6d study. While animals in the two fat-fed groups were fed diets identical to those employed in the previous study, control animals received an amount of chow isocaloric to the chow-fat mixtures. Despite equal caloric intake in all three groups, caloric substitution with SFO, but not HCO, stimulated fractional and total NETR. In heart, NETR was 36.1 ng NE/h in chow-fed rats, 39.4 in HCO-fed and 60.3 in SFO-fed animals ($P<0.01$ compared to either chow- or HCO-fed rats). Similar results were also obtained in interscapular brown adipose tissue. The faster cardiac NETR noted in SFO-fed, compared to HCO-fed, rats was confirmed in a second 14-d study (55.9 ng NE/h in SFO-fed vs 42.3 in HCO-fed rats, $P<0.05$). In summary, while both HCO and SFO stimulate NETR in heart after 6d, the effect of HCO, but not SFO, was not apparent after two weeks of dietary exposure. Since these two oils were consumed in isocaloric amounts and the only difference between the two diets was in the fatty acid constituents of the fat supplements, fatty acid composition may play an important role in the regulation of SNS activity.

360.3 **BILATERAL LESION OF THE C1 AREA OF THE ROSTRAL VENTROLATERAL MEDULLA GLOBALLY IMPAIRS THE CEREBROVASCULAR RESPONSE TO HYPOXIA.** M.D. Underwood, C. Iadecola and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Cerebral hypoxia elicits a potent and widespread increase in regional cerebral blood flow (rCBF), believed to reflect the local vascular response to changes in tissue metabolism. Yet, cerebral ischemia, which also elicits hypoxia, elicits systemic hypertension which is reflex and depends upon stimulation of neurons in a restricted region of the rostral ventrolateral medulla (RVL) (Dampney and Moon, Am. J. Physiol., 239: H349, 1980) corresponding to adrenergic neurons of the C1 group, the C1 area (Ross et al., J. Neurosci., 4: 474, 1984). We therefore investigated whether the C1 area also contributes to the increased rCBF elicited by hypoxia.

Rats were anesthetized (chloralose: 40 mg/kg, s.c.), paralyzed (tubocurarine) and artificially ventilated. Arterial pressure (AP), heart rate and blood gases were continuously monitored and controlled. Lesions were placed electrolytically (500 uA for 30 sec) with microelectrodes. Following bilateral lesion of the RVL, AP was kept in the autoregulated range for rCBF (80-150 mmHg) by the continuous infusion of phenylephrine. Hypoxic-hypoxia or hypercapnia was produced by varying the inspiratory nitrogen/oxygen ratio or by introducing CO_2, respectively. rCBF was measured using the ^{14}C-iodoantipyrine technique, with bilateral dissection of 10 brain regions. Placements of electrodes and/or lesions were localized post-mortem in all animals.

In unlesioned controls rCBF ranged from 50 in corpus callosum to 103 ml/min x 100g in inferior colliculus. Hypoxic-hypoxia (pO_2 = 41 + 0.4 mmHg, n=6) increased rCBF in all structures ($p<.01$) ranging from 207% of control in hypothalamus to 301% of control in occipital cortex. Bilateral lesions of RVL had no effect on rCBF. However, they significantly reduced ($p < 0.01$, n=5) the magnitude of increase in rCBF elicited by hypoxia in all structures. The reduction in response ranged from 50% in corpus callosum to 69% in inferior colliculus. In contrast, hypercarbia (pCO_2 = 63.5 ± 0.9 mmHg, n=5) which increased rCBF ($p < 0.01$) in all structures in intact rats from 220% of control in parietal cortex to 299% of control in superior colliculus was unaffected by bilateral lesion of RVL.

We conclude: (a) that the global vasodilation elicited by hypoxia is in part reflex; (b) the reflex component of the vasodilation elicited by hypoxia depends upon the integrity of neurons and/or processes within the C1 area of the RVL; and (c) the role of C1 area of RVL is specific since it does not mediate the cerebrovasodilation to hypercarbia. Our findings suggest that neurons of the RVL, possibly those of the adrenergic C1 group, may represent a central chemosensor for hypoxia which initiates changes in both the systemic and cerebral circulation.

360.4 FUNCTIONAL IMPLICATIONS OF THYROID HORMONE-PROCESSING MECHANISMS IN RAT BRAIN. M. B. Dratman and F. L. Crutchfield*. Phila. VA Med. Center and Medical College of PA, Philadelphia, PA 19104.

Recent biochemical evidence favors a functional role for iodothyronines in adult brain. Thus, fractional conversion of thyroxine (T4) to triiodothyronine (T3) is more active in brain than in any other known tissue site. Moreover, despite long-term hypo- or hyperthyroidism, T3 homeostasis is maintained in the brain through coordinated biochemical adjustments in both CNS and somatic tissues. Film autoradiographic (ARG) studies after iv ^{125}I-T3 demonstrate that the hormone is saturably processed in discrete neural systems. Over a 48 hr period after uptake into neuronal cell bodies of selected brain nuclei, the hormone is axonally transported and eventually concentrated within synaptic elements of their terminal fields. Evidence for the specificity of the observed ARG reactions has now been derived from similar studies carried out after administration of other ^{125}I-iodothyronines. The more prolonged (72 hr) but similar evolution of radioactive labeling seen after iv ^{125}I-T4 is entirely dependent on its intracerebral conversion to labeled T3, as evidenced by the effects of ipodate, a centrally-acting T4-5'deiodinase inhibitor which does not effect ^{125}I-T3 but markedly impairs ^{125}I-T4 distribution patterns. By contrast, propylthiouracil (PTU), which blocks hepatic but not intracerebral T4-5'deiodinase, produces no qualitative changes in the evolution of brain labeling after iv ^{125}I-T4. To test further the hypothesis that specific brain labeling is based on availability of the active hormone or its products and not simply due to the presence of any compound with an iodothyronine configuration, film ARGs were prepared after iv delivery of the (presumably) inactive T4 product, reverse triiodothyronine (rT3). Marked retardation in the rapid serum disappearance rate of this iodothyronine was achieved by pretreating rats with ipodate or PTU (vs saline). Under all conditions, brain labeling after ^{125}I-rT3 was non-discriminatory; during deiodinase inhibition, concentration of the isotope above background was found only in brain regions with known high rates of vascular perfusion. After ^{125}I-rT3, normally conspicuous and persistent sites of saturable T3 and delayed T4 labeling (hippocampus, cerebellum, thalamus) were hardly differentiated from other diffusely-labeled structures in the brain. CONCLUSIONS: The hormonal activity of different iodothyronines has been correlated with their binding and metabolism within responsive tissues. ARG studies now reveal strong correlations between generally-accepted in vivo activity ratings of the iodothyronines and their distribution within discrete neural systems in the CNS. The biological activity of an iodothyronine may reflect in part, its ability to be differentially processed and presumably to act within the brain. Supported by VA Medical Research Service and NSF grant #BNS 8210354.

360.5 INCREASED SENSITIVITY OF CENTRAL ALPHA-2 ADRENOCEPTORS IN CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS (SHR) ON HIGH SODIUM INTAKE. J.P. Koepke, S. Jones* and G.F. DiBona*. Dept. Int. Med., Univ. Ia. Col. Med. & VA Med. Ctr., Iowa City, IA 52242

In conscious SHR, the increased renal sympathetic nerve activity (RSNA), and decreased urine flow rate (V) and urinary sodium excretion ($U_{Na}V$) responses to environmental stress are enhanced by a high sodium intake (Hypertension 7:357-363, 1985). In addition, the RSNA, V, and $U_{Na}V$ responses to environmental stress are abolished by intracerebroventricular (icv) injection of alpha-2 adrenoceptor agonists (Hypertension 8:133-141, 1986). In the present study, we examined whether high sodium intake (3-4 wks of 1% NaCl for drinking) in SHR alters the sensitivity of central alpha-2 adrenoceptors. The effects of cumulative icv injections of an alpha-2 adrenoceptor agonist (clonidine 1, 5 and 25 µg; 0.9% NaCl vehicle 1 µl) on RSNA, V, and $U_{Na}V$ were examined in conscious SHR. Clonidine injections occurred 10 min before experimental periods; each experimental period was 10 min duration. Data are mean±SE; RSNA, integrator resets/min; V, µl/min/g KW; $U_{Na}V$, µEq/min/g KW; mean arterial pressure, MAP, mmHg; *p <0.05 compared to control.

	CONTROL	CLONIDINE µg icv		
		1	5	25
SHR - Normal Sodium (n=7)				
RSNA	6.7±1.4	6.1±1.0	3.6±0.4*	1.4±0.2*
V	24.7±2.9	29.7±4.0	43.8±5.0*	72.4±6.6*
$U_{Na}V$	2.9±0.4	3.2±0.5	5.4±0.8*	12.0±1.2*
MAP	157±4	158±3	148±5*	150±5*
SHR - High Sodium (n=7)				
RSNA	6.8±1.0	4.7±0.8*	2.4±0.5*	0.9±0.4*
V	21.6±2.9	39.6±5.9*	56.8±5.9*	73.8±10.5*
$U_{Na}V$	3.2±0.6	5.9±0.9*	7.9±1.2*	13.7±1.3*
MAP	165±7	166±7	157±7*	160±10

At each dose of clonidine, RSNA was lower ($p < 0.01$) and V and $U_{Na}V$ were higher (except for V at 25 µg; $p < 0.01$) in SHR on high than on normal sodium intake. The ED_{50} (µg) for RSNA (% control), V and $U_{Na}V$ (% maximum response) were lower ($p < 0.05$) in SHR on high than on normal sodium intake:

	SHR - Normal Sodium	SHR - High Sodium
RSNA	7.8±1.5	3.0±0.5
V	4.1±1.4	1.7±1.0
$U_{Na}V$	6.7±1.2	4.7±1.4

Conclusion: Clonidine icv decreases RSNA and increases V and $U_{Na}V$ to a greater degree in conscious SHR on high than on normal sodium intake. High sodium intake increases the sensitivity of central alpha-2 adrenoceptors in conscious SHR.

360.6 SPECIFIC RESPONSE OF PLASMA ANF TO A HYPERTONIC NACL STIMULUS. M. Morris* and A. Rauch* (SPON:M. CALLAHAN) Bowman Gray Sch Medicine of Wake Forest University, Winston-Salem, NC 27103

Atrial natriuretic factor (ANF) is responsive to changes in volume status. However, little is known about osmotic control of ANF secretion. This study was performed to determine whether ANF is affected by changes in plasma osmolality induced by hypertonic saline or mannitol. Male Sprague Dawley rats were prepared with chronic jugular catheters. Hypertonic saline (HS; 18%), hypertonic mannitol (HM; 25%) or isotonic saline (0.9%) was injected (0.18 ml/100gm) over a 1.5 min period into conscious rats. The animals were sacrificed by decapitation at 0, 5, 15, 30 and 60 min. The blood was collected on ice in EDTA for the measurement of plasma sodium, hematocrit and ANF. The plasma was extracted with Sep-Pak reverse phase columns prior to radioimmunoassay.

Effect of iv HS or HM on plasma ANF (pg/ml) (* = $p < .01$)

Time-mins after injection	0	5	15	30	60
Hypertonic Saline (n = 6)	50.6 ±2.1	131.8* ±22.6	77.3* ±9.7	45.9 ±6.2	45.7 ±4.9
Hypertonic Mannitol (n = 6)	82.8± 18.0	51.2± 9.4	59.9± 20.1	60.0± 14.7	

IV HS produced a significant increase in ANF secretion, while HM had no effect. The NaCl effect was transient peaking at 5 mins and returning to control levels by 30 mins. The injection of isotonic saline did not change plasma ANF levels. This lack of effect of either saline or HM demonstrates that the HS response was not related to a volume or osmotic change. Plasma sodium was increased 13 mEq/l by the HS injection. Hematocrit was not altered by any of the treatments.

These results provide evidence for a specific stimulation of ANF secretion by an increase in plasma sodium and sugggest that this may be an important factor in the control of ANF release.

360.7 PUSH-PULL PERFUSION MONITORING OF PROSTAGLANDIN E_2 PRODUCTION IN THE CAT HYPOTHALAMUS DURING FEVER. S.P. Sirko*, I. Bishai*, F. Coceani. Research Institute, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada.

It is generally assumed that blood-borne pyrogens elicit fever by promoting the formation of prostaglandin E_2 (PGE_2) in the anterior hypothalamic-preoptic area (AH/POA). However, no direct information is available on the site of entry of pyrogens into the CNS, nor has accelerated formation of PGE_2 been verified in the AH/POA of the febrile animal.

In this investigation, we have addressed the question of the central action of pyrogens by monitoring PGE_2 release from discrete AH/POA sites with a push-pull perfusion procedure. Experiments were conducted in conscious cats before and during fever to intravenous administration of endotoxin (bolus) or leucocytic pyrogen (LP) (infusion). Perfusate was collected over four consecutive 1-hour periods and PGE_2 content was ascertained by radioimmunoassay. In the absence of fever, PGE_2 release was stable from the second through the fourth hour of perfusion (hour 2, 0.106 ± 0.032 pg/min; hour 3, 0.104 ± 0.036 pg/min; hour 4, 0.112 ± 0.046 pg/min). Endotoxin and LP produced fever of comparable magnitude that persisted throughout the collection period. LP administration significantly elevated mean PGE_2 production (i.e. from 0.114 ± 0.021 pg/min in hour 2 to 0.232 ± 0.054 and 0.252 ± 0.057 pg/min in hours 3 and 4, respectively; n=12). Endotoxin also increased PGE_2 synthesis during fever but this increase became significant only in the last hour (i.e. from a basal value of 0.128 ± 0.028 pg/min to 0.169 ± 0.037 and 0.266 ± 0.074 pg/min during hours 3 and 4, respectively; n=7).

In conclusion, exogenous and endogenous pyrogens stimulate PGE_2 synthesis in the AH/POA. This finding is consistent with an intermediary role of PGE_2 in the genesis of fever.

(Supported by the Medical Research Council of Canada).

360.8 PRANDIAL INSULIN RELEASE AFTER CELIAC VAGOTOMY. J.E. Cox. Dept. Psychology, Univ. Alabama Birmingham, Birmingham, AL 35294.

Vagal innervation of the pancreas participates in the control of insulin release, especially of the early, cephalic-phase response to food intake. Though the celiac branches of the vagus have been classically defined as the principal route for this innervation, the importance of the celiac vagus in the rat has been disputed (Berthoud, H.R. et al., J. Autonomic Nerv. Syst., 7: 97, 1983). To address this issue, I examined the effect of celiac vagotomy on basal and food-stimulated plasma insulin.

Adult, female Sprague-Dawley rats underwent two rounds of surgery, the first round for transection of both celiac branches (C group; N=17) or sham surgery (S; N=17) and the second round for implantation of chronic, silastic catheters in the inferior vena cava. For testing, rats were food deprived for 5-6 hr and then attached to a remote blood collection system. Blood samples were taken 5 min prior to and 2, 4, 8, and 15 min after initiation of a 5 min meal of 60% sweetened-condensed milk. Plasma insulin and glucose concentrations were subsequently determined by radioimmunoassay and the hexokinase method, respectively.

Mean milk intakes in the C (4.4 ± 0.2 ml) and S (4.6 ± 0.2 ml) groups were not different. Means for plasma insulin (uU/ml) are shown in the following table. An asterisk indicates a significant difference between groups ($P < .05$).

	basal	2 min	4 min	8 min	15 min
S	22.5 *	39.4 *	49.2 *	84.9	137.0 *
C	15.2	25.3	31.2	66.5	189.6

Celiac vagotomy resulted in decreased basal insulin, and values remained significantly depressed for 4 min after meal initiation. The change in insulin concentration from the basal to the 2 min point was reduced by 40% ($P < .05$) in the C group. These results suggest that celiac fibers contribute to the tonic maintenance of insulin secretion and contribute substantially to cephalic phase insulin release. Because, however, plasma insulin did increase significantly ($P < .05$) in the first 2 min of the meal, these results also suggest the contribution of other vagal branches to cephalic responsiveness.

In contrast to reduced levels at the early time points in the C group, plasma insulin increased very rapidly from 8 to 15 min to produce postprandial hyperinsulinemia. This increase may have been secondary to rapidly increasing plasma glucose; over the same period, glucose increased by 25% in the celiac group while remaining stable in controls ($P < .05$). These data are consistent with the notion that early insulin release serves as a buffer against radical increases in plasma glucose following a meal. (Supported by NIH grant AM31805).

360.9 CONVERGENCE OF SUBDIAPHRAGMATIC VAGAL AFFERENTS WITHIN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT.
W.B. Laughton, L.A. Campfield and D.O. Nelson. Department of Physiology, Northwestern University Med. School, Chicago, IL 60611

Numerous neuroanatomical and physiological studies indicate that vagal afferent fibers originating from subdiaphragmatic viscera terminate in the caudal and medial portions of the nucleus of the solitary tract (NTS). While it is known that gustatory and hepatic vagal afferent inputs are relayed to non-overlapping portions of the NTS, the interactions between afferent inputs originating from different structures within the abdomen are not well understood. Convergence of information from different visceral modalities (eg. chemo-, osmo-, and mechanoreceptor, etc.) within the NTS has also not been examined. This study reports preliminary results of electrophysiological studies of subdiaphragmatic vagal afferent inputs to the NTS.

Single and multiunit activity in the NTS was recorded through one barrel of a glass micropipette in chloralose-urethane anesthetized male rats. Vagal afferent fibers were electrically stimulated through bipolar cuff electrodes positioned on the hepatic, anterior gastric, and accessory coeliac branches of the subdiaphragmatic vagus. Chicago Sky blue dye was iontophoricially ejected at selected sites to permit histological reconstruction of the recording site.

Evoked activity was readily observed in the NTS following stimulation of each of the three subdiaphragmatic branches of the nerve. Neuronal excitation was observed in the caudal medial portion of the NTS along the wall of the fourth ventricle, from approximately 500μm caudal to 1000μm rostral to the calamus scriptorius. Activation latencies ranged from 70 to 130 msec, corresponding to conduction velocities of 0.6 to 1.0 meters/sec, characteristic of non-myelinated fibers. Convergent excitatory input to single units was observed following stimulation of the anterior gastric and hepatic branches of the vagus, or following activity of the accessory coeliac and hepatic branches.

These data indicate that information arising from the liver, stomach, and the sensory field of the accessory coeliac branch of the vagus can influence the activity of single neurons in the NTS. The relationship of these peripheral branches of the vagus to the physiological information (eg., gastric distention, hepatic portal chemosensation, etc.) they carry is currently under investigation.

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360.10 DUAL PEPTIDERGIC CONTROL OF GASTRIC MOTILITY R.C. Rogers and G.E. Hermann Dept. Physiology, University of Nevada School of Medicine, Reno, Nevada 89557

Electrical stimulation of the peripheral cut end of the cervical vagus nerve, as well as stimulation of the dorsal motor nucleus of the vagus (DMN), will produce both increases and decreases in gastric motility. It has been postulated that this paradoxical result is due to the existence of excitatory cholinergic neurons and inhibitory peptidergic neurons together in the DMN. Thus, gross electrical stimulation of either the DMN or their axons in the vagus produce mixed effects on gastric smooth muscle.

It has been known for more than 100 years that the central nervous system is capable of independently exciting OR inhibiting gastric motility; the "mixed" pattern seen with electrical activation of vagal neurons is an artifact. However, since vagal efferent outflow is the principal extrinsic regulator of gastric motility, it is clear that the CNS must possess some means to activate either excitatory or inhibitory vagal outflows to the stomach.

Both oxytocin and TRH have been implicated in the descending hypothalamic control of gastric acid secretion. Thus, it is reasonable to expect that they might also play a role in the regulation of gastric motility. To date, we have observed that:

a) a wide range of unilateral DMN microstimulation parameters produce an increase in peak gastric contraction amplitude superimposed on a reduction in baseline "tone"

b) PVN microstimulation (i.e., activating the likely source of TRH and oxytocin projections to the DMN) evokes much the same motility pattern

c) TRH (2nL X 0.002M = 4 picomoles) injected into the DMN dramatically increase the frequency and amplitude of gastric contractions without a change in baseline

d) similar amounts of oxytocin injected into the DMN produce reductions in tone with no increase in frequency or amplitude of gastric contractions

e) an oxytocin antagonist injected into the DMN in picomolar quantities greatly increases gastric motility in response to PVN microstimulation

On the basis of these preliminary results, we predict the existence of an excitatory TRH and an inhibitory oxytocin pathway from the PVN to the DMN which is responsible for the central control of gastric motility.

Supported by an NIADDK grant (AM35346) and an American Heart Association, Nevada Affiliate grant to RCR.

AUDITORY SYSTEM II

361.1 RESPONSES OF CAT COCHLEAR NUCLEUS NEURONS TO HUMAN SPEECH SOUNDS AND CAT VOCALIZATIONS.
D.O. Kim and W.S. Rhode*, Dept. Neurophysiology, University of Wisconsin, Madison, WI 53706

Responses of primary auditory nerve fibers to basic human speech sounds have been relatively well documented. In contrast, much less is known about single neuronal responses to speech stimuli at the level of the cochlear nucleus. We have conducted single unit studies of neurons in the posteroventral and dorsal cochlear nuclei (PVCN and DCN) of anesthetized cats under stimulations with human speech sounds and conspecific vocalizations. The objective of this study is to elucidate neural signal processing mechanisms that operate on complex acoustic signals. The sample of human speech sounds included several synthetic and natural vowels and natural syllables [ra] and [la]. The sample of cat vocalizations included an estrous call of a female cat, a vocal "dialog" of a mother cat and a kitten, and a stress call of a kitten. For each unit in our experiments, we determined the characteristic frequency (CF), response area, spontaneous rate, pattern of response to pure tones at CF in addition to obtaining responses to speech sounds and vocalizations. Acoustic stimuli were presented by a Digital Stimulus System. Occurrence times of each spike in 20 μsec resolution were stored on a computer disk for display of various histograms during the experiments as well as for subsequent analyses.

We have found that the onset-chopper (Oc) neurons (Rhode, Oertel & Smith, 1983, J. Comp. Neurol. 213, p448) in the PVCN show precise timing of spike discharges synchronized to the voice pitch period; we have called this phenomenon a "pitch-period" following response (Kim, Rhode & Greenberg, 1986, Auditory Frequency Selectivity Symposium). The precision of the pitch-period following response of the Oc neurons was on the order of 0.2 msec in dealing with the pitch period of about 8 msec. Our observations of the pitch-period following response suggest that the Oc neurons may play an important role in perception of the pitch of complex periodic signals, e.g., in perception of speech intonation and musical melody. We also confirmed the very large dynamic range (about 80 dB) of the Oc neurons observed previously with pure tones (Rhode et al., 1983) and extended the observations to conditions of complex stimuli. A possible mechanism producing both a precise pitch-period following response and a large dynamic range of the Oc neurons is one where the spike discharge of an Oc neuron requires simultaneous arrival of spikes on multiple nerve endings of primary afferents converging on the Oc neuron.

Some of the neurons in the DCN and PVCN exhibit complex response behaviors in terms of nonmonotonic rate-intensity behaviors or complex excitatory and inhibitory regions in the frequency-intensity plane of pure tone stimulus. Our study attempts to relate complex responses of cochlear nucleus neurons obtained with human speech sounds and cat vocalizations to each neuron's response obtained with pure tones as well as to spectral and temporal patterns of the complex acoustic stimuli.

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361.2 FINE STRUCTURE OF TONOTOPIC ORGANIZATION IN CAT INFERIOR COLLICULUS.
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The central nucleus of the inferior colliculus (ICC) of the cat contains a highly ordered tonotopic organization (Merzenich,M.M. and Reid,M.D., Brain Res. 77:397-415, 1974). The main tonotopic gradient lies in the dorsoventral dimension of the nucleus across histologically defined cellular laminae. The orientation of the cellular laminae appears to coincide with the orientation of physiologically definable 'frequency-band laminae' that contain neurons with similar characteristic frequencies (CFs).

It had been previously shown (Schreiner,C.E. and Langner,G., Soc. Neurosci. Abstr. Vol.10, Part 1, p.395, 1984) that some response properties of neurons other than the characteristic frequency are represented in an orderly spatial fashion within 'frequency-band laminae'. Those include spatially ordered re - presentations of the 'best modulation frequency' of amplitude modulated tones, of the onset latency of tone bursts and of the sharpness (Q-10dB) of frequency tuning curves. The questions addressed in the present study are: a) Is there a fine structure of the CF-distribution within physiologically defined 'frequency-band laminae'? and b) Does the distribution of CFs relate to the distribution of other functional parameters in the neural laminae?

Experiments were conducted in barbituate-anesthetized cats. Multi- and single unit responses were recorded and the CF of each recording site was determined with manual and/or automated analysis procedures. Up to 30 locations in each 'frequency-band lamina' between 2 and 7 kHz were obtained. The CF in each dorso-ventral electrode penetration increased in steps of a few hundred Hertz each, consistent with a laminar organization of the ICC. From the depth profile of the electrode locations and the encountered CFs, 'frequency-band laminae' were constructed. Within all sufficiently sampled laminae, a CF gradient was seen with CFs shifting upwards from rostromedial to caudolateral. The encountered CF-range corresponded to 10-14% of the average CF within a given lamina. This frequency range is of a magnitude comparable to psychophysical critical bands. This finding supports the hypothesis that 'frequency-band lamina' in the ICC are a neural substrate that is related to critical bands.

Within a lamina, the distribution of CFs and 'best modulation frequencies' appeared to be largely independent.

Supported by the Coleman Fund, Hearing Research Inc. and a Heisenberg Stipendium (to G.L.).

361.3 PROCESSING OF TEMPORAL CUES FOR THE LOCALIZATION OF HIGH FREQUENCY SOUNDS: NEURONAL RESPONSES IN THE INFERIOR COLLICULUS OF UNANESTHETIZED RABBITS. R. Batra, S. Kuwada, T.R. Stanford*. Dept. Anatomy, Univ. of Conn. Health Center, Farmington, CT 06032.

Low-frequency sounds are localized using interaural phase disparities generated by the difference in the arrival time of sound at the two ears. The maximum frequency at which temporal cues are used is limited by the inability of mammalian auditory neurons to discharge synchronously to frequencies higher than 3-5 kHz. However, neurons can discharge synchronously to the low-frequency envelopes of sounds with a high carrier frequency. Neurons tuned to high frequencies and sensitive to interaural time differences in the envelope have been reported in the anesthetized cat. Here we report properties of similar neurons in the inferior colliculus (IC) of the unanesthetized rabbit.

During recording the rabbit was restrained in a stocking and its head immobilized by clamping a bar fixed to its skull. Calibrated sinusoidally amplitude modulated (AM) tones were delivered dichotically. Interaural time sensitivity was assessed by presenting stimuli with the same carrier frequency but with modulation frequencies that differed by 1 Hz at the two ears. This stimulus resulted in a periodic variation of the interaural difference in modulation phase (IDMP). Neurons sensitive to interaural time differences should respond to such a stimulus with a corresponding periodic variation in their discharge.

Cells in the IC of the rabbit which are tuned to high frequencies can synchronize to contralateral AM tones, periodic variation of the IDMP, or both. Peak synchrony to contralateral AM tones rarely occurs above 200 Hz although some synchrony can occasionally be detected as high as 900 Hz. By contrast, cells which are sensitive to the IDMP often show peak synchronization to the IDMP at frequencies above 400 Hz, and synchrony is observed to 900 Hz. The discharge to our binaural stimulus sometimes synchronized to the contra and ipsilateral modulation frequencies as well. In these cases, peak synchrony to these modulations generally occurred at a frequency less than that at which synchrony to the IDMP was maximal. Moreover, it was possible to predict the interaural phase difference at which the cell would discharge maximally from the phases of the contra and ipsilateral responses.

In sum, neurons in the IC which are tuned to high frequencies can process temporal information by discharging synchronously to the AM envelope and to the IDMP. Peak synchrony to the modulation of the envelope can occur at higher frequencies than in the IC of the anesthetized rat, and synchrony to the IDMP can occur at slightly higher frequencies than in the anesthetized cat. These differences may be due to the species studied or to effects of anesthesia. Supported by NIH grant NS18027 to S. Kuwada.

361.4 INTERAURAL PHASE SENSITIVITY OF LOW-FREQUENCY NEURONS IN THE INFERIOR COLLICULUS OF THE UNANESTHETIZED RABBIT. T.R. Stanford*, S. Kuwada and R. Batra. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

A cue for the localization of low frequency sounds is the difference in their arrival times at the two ears. This creates an interaural phase disparity (IPD). If the IPD is systematically changed, the discharge of some cells varies in synchrony with the IPD. When the responses at several frequencies are plotted on a common time axis, some cells show a common relative amplitude at a particular or characteristic delay (CD). The CD is thought to reflect the difference in the arrival time of the monaural inputs at the binaural cell. We have examined the phase sensitivity of cells in the inferior colliculus using the unanesthetized rabbit.

The rabbit was restrained in a stocking and immobilized by clamping to a bar fixed to its skull. Lidocaine was applied to the dura and an electrode was lowered through a small hole in the skull. Acoustic stimuli were delivered through fitted earmolds.

For each unit, interaural phase sensitivity was assessed at several frequencies with a binaural beat stimulus, which was created by delivering tones that differed by 1 Hz to the two ears. This resulted in a IPD that varied at a rate of 1 Hz. The CD and the characteristic phase (CP) were calculated from the slope and the phase intercept, respectively, of a plot of response phase vs. frequency. The discharges to changes in IPD for all effective frequencies were plotted as a function of the equivalent interaural time difference. At each frequency, one cycle on either side of zero was plotted to represent both ipsi and contralateral stimulus delays and were averaged to yield a composite curve.

For most cells the CD and the composite peak occurred at ipsilateral delays suggesting that these cells would be most sensitive to sounds in the contralateral field. Furthermore, most CDs and composite peaks were distributed within the estimated physiological range ($\pm$275 μs). These findings differ from those in the anesthetized cat where the CDs are centered about zero μs with a nearly equal distribution among ipsi and contralateral delays. Furthermore, in the cat, more neurons have CDs that are beyond the limit of the predicted physiological range.

Most cells had a CP near zero phase indicating that these cells fired maximally when the inputs from the two ears were in phase. This suggests that the binaural neuron acts as a coincidence detector. In contrast, the CPs in the anesthetized cat are uniformly distributed.

In sum, while the sensitivity to IPD of cells in the unanesthetized rabbit is qualitatively similar to that seen in the anesthetized cat, the distributions of CD and CP differ. These differences may reflect species differences or the effects of anesthesia. Supported by NIH grant NS 18027 to S. Kuwada.

361.5 SOUND LOCALIZATION AFTER LESIONS OF THE CAT SUPERIOR COLLICULUS. T.T. Allard* and W.M. Jenkins, Coleman Laboratory, UCSF Medical Center, San Francisco, CA 94143.

Permanent and profound deficits in sound localization are produced by unilateral lesions of the ascending auditory pathway in cat, from the lateral lemniscus to auditory koniocortex (e.g., Jenkins and Masterton, *J.Neurophys. 47*: 987-1016, 1982); these localization deficits are restricted to sounds originating in the auditory hemifield opposite the lesion. Similarly, recent physiological evidence has shown that the auditory inputs to the cat superior colliculus are largely segregated by hemifield (Middlebrooks and Knudson, *J.Neurosci 4*: 2621-2634, 1984); these physiological data constitute the first strong evidence that external space is encoded topographically in the mammalian central nervous system. The question addressed in the present study was whether the integrity of this extra-lemniscal pathway is essential to sound localization.

Cats with unilateral lesions of the superior colliculus were tested both pre-operatively and post-operatively on the ability to localize 35-msec bursts of white noise. Each noise burst was presented randomly from one of seven speakers distributed symmetrically across midline along the horizontal azimuth. Cats with unilateral lesions in primary auditory cortex (A 1) show a severe contralateral deficit on this test (Jenkins and Merzenich, *J.Neurophys. 52*: 819-847, 1984). Cats with superior colliculus lesions showed no deficit on the 7-speaker sound localization test. Overall, mean performance was normal and better than 85% correct for 20 consecutive 42-trial sessions. A comparison of errors in the hemifields contralateral and ipsilateral to the lesions showed no systematic asymmetries.

These results indicate that the auditory representation of external space in the cat superior colliculus serves a distinct and segregated function from the mainline lemniscal pathway. Intact collicular function is unnecessary for accurate sound localization in the horizontal azimuth.

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361.6 AUDITORY SPATIAL TUNING DERIVED FROM SENSITIVITY TO INTERAURAL INTENSITY DIFFERENCES. J.C. Middlebrooks. Department of Neurobiology, Stanford University, Stanford, CA 94305.

Several recent studies have indicated that sensitivity for interaural intensity differences (IID's) must contribute to the spatial tuning of auditory units in the superior colliculus. The current study correlates the spatial tuning of units with their sensitivity to IID's and their frequency response areas. The responses of single units were recorded from the superior colliculus in anesthetized cats. Broad band stimuli were presented from a movable sound source in a free field, and the resulting IID's were manipulated by acutely plugging one or the other ear or by changing the sound source location. Frequency response areas were measured using a free field tone source.

The responses of the majority of units were increased by manipulations which increased the IID favoring the contralateral ear, such as shifting the sound source contralaterally from the midline or plugging the ipsilateral ear. Thus, the responses of those units appeared to be monotonically related to stimulus IID. The group of units with monotonic IID sensitivity included all of the units with spatial receptive fields extending behind the cat ("hemifield" receptive fields) and about a third of the units with spatial receptive fields located entirely in front of the cat ("frontal" receptive fields). The remainder of units exhibited responses which were non-monotonically related to IID: responses decreased whenever the IID was decreased or increased away from an optimum value either by changing the sound source location or by plugging either ear. Some of those units appeared to respond best to IID's near the physiological maximum, while other units required nearly equal intensities at the two ears.

The spatial tuning of units correlated in general with their frequency tuning. Of the units which responded to tones, units which were tuned frontally exhibited frequency response areas limited to high frequencies, while more peripherally tuned units responded to the lowest tested frequencies.

These results confirm previous suggestions that units which differ in their spatial tuning also differ in their sensitivity to stimulus IID and in the frequency bands from which they derive their most salient spatial cues. Several lines of evidence now lead to a simple model in which the spatial tuning of units in the superior colliculus can be accounted for in large part by selectivity for IID's within particular bands of frequency.

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361.7 THE ROLE OF STIMULUS ONSET IN SENSITIVITY TO INTERAURAL TIME DIFFERENCES IN THE BARN OWL. H. Wagner and M. Konishi. Division of Biology 216-76, California Institute of Technology, Pasadena, California 91125.

Interaural time differences (ITD) can provide information about the azimuth of a sound source. A sound signal contains ITDs in its envelope, especially at the on- and offset of the sound, and in the continuing or "ongoing" waveform of the carrier. Many neurons in the auditory midbrain of the barn owl are tuned to ITDs. Moiseff and Konishi (J. Neurosci. 1: 40, 1981) showed that the information contained in the ongoing waveform (ongoing ITD) was sufficient to explain the selectivity of these neurons to ITDs. It was not clear, however, whether the ITD at the onset (onset ITD) also plays a role.

We tested the importance of onset ITD for neurons in the inferior colliculus. The response to dichotic stimulation with correlated and uncorrelated noises were compared. In the uncorrelated paradigm the signal to the two ears did not contain an ongoing ITD. The onset ITD was shifted within the barn owl's physiological ITD-range. The onset amplitude varied randomly. The selectivity for a particular ITD was tested with a Rayleigh test. The period of the unit's best frequency provided the testing range.

Extracellular recordings were made from 21 single units. To stimulation with both paradigms the units increased their spike rate over the spontaneous activity, even if they were not monaurally excitable. For each ITD, the units' responses were typically characterized by a transient (5-15 ms) increase in spike rate after stimulus onset, followed by a less pronounced sustained response that lasted throughout the duration of the stimulus. The sustained response of all units to stimulation with a correlated noise was highly significantly ($p<0.001$) tuned to ITDs. The transient response of 15 units in the correlated paradigm was also highly significantly tuned. By contrast, only 1 unit was tuned to the onset ITD in the uncorrelated paradigm and the sustained response of 8 units was also tuned to ITDs. However, in 6 of these 8 cases the response maximum was near the same ITD that yielded the response minimum in the correlated noise paradigm. Since the correlated stimulus contains a time cue every period of the unit's best frequency, in some recordings the number of cycles in the correlated noise was made equal to the number of repetitions in the uncorrelated paradigm. Then 5 out of 8 neurons still showed tuning to ongoing ITDs.

These results suggest that onset ITDs do not play a role in the manifestation of ITD sensitivity in the barn owl's nervous system.

361.8 SENSITIVITY OF CELLS IN CAT PRIMARY AUDITORY CORTEX (AI) TO INTERAURAL INTENSITY DIFFERENCES (IID'S) AS A FUNCTION OF FREQUENCY. J. R. Mendelson and M.S. Cynader, Department of Psychology & Physiology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

When a cell demonstrates a sensitivity to an IID it is often thought to be involved in coding the azimuthal position of a sound source. Because the sound shadowing properties of the head cause higher frequency components to be attenuated more than lower frequencies in the ear furthest from the source, the size of the resulting IID is necessarily dependent upon the spectral content of the stimulus. Thus, selectivity for a given IID implies a different azimuthal location depending on the frequency of the stimulus. We sought to determine whether units in AI showed different IID preferences depending on stimulus frequency (IID-frequency nonseparability) or whether this frequency compensation process is best ascribed to later processing stages.

Initially, the CF (the frequency to which the cell responded best) and tuning profile were determined for each cell. Then, the unit's response to varied IID's (from -30 dB to +30 dB in the ipsilateral ear) was recorded at a variety of stimulus frequencies (which were always the same in both ears). All IID-by-frequency conditions were presented in a randomized fashion under computer control.

The majority of cells(61%) responded well to stimuli which, under free-field conditions, would originate throughout an entire hemifield. These cells, like those observed in the free-field continued to respond regardless of how large the IID became. A second group of cells were classified as circumscribed since they, like their free-field counterparts had clearly demarcated borders dictated by both frequency and IID.

Forty percent of all neurons displayed IID-frequency separability (i.e., the response to different IID conditions was independent of the stimulus frequency). The remaining 60% exhibited responses to IID's which were at least partially dependent on the frequency of the stimulus (i.e., 60% exhibited some IID-frequency nonseparability). The form of this nonseparability, however, varied from cell to cell and was, in general, not consistent with the notion of a neural mechanism capable of specifying the exact azimuthal location of a stimulus. Instead, the responses appeared to be suggestive of a mechanism which may provide information on the general, rather than the specific, location of a sound source.

361.9 FUNCTIONAL DEVELOPMENT OF AUDITORY CORTEX OF THE CAT. J.F. Brugge*, R.A. Reale* and G. Wilson* (SPON: C.N. Woolsey). Dept. Neurophysiology and Waisman Center, Univ. Wisconsin, Madison, WI 53705

The auditory system of the newborn kitten is structurally and functionally underdeveloped. We recently completed a study of monaural and binaural response properties of single neurons in the primary auditory cortex (A1) of kittens ranging in age from 8 to 44 days. Kittens were anesthetized with ketamine and sodium pentobarbital and a recording chamber was cemented to the skull over the exposed middle ectosylvian area. Action potentials were recorded by tungsten microelectrodes advancedby a Davies microdrive which hydraulically sealed the oil-filled chamber. Tone bursts were delivered under computer control to the two ears using a sealed and calibrated sound system. Many of the monaural and binaural response properties that characterize AI neurons in the adult cat were exhibited by AI neurons in kittens within the first 2-3 postnatal weeks, before thresholds and discharge latency reach adult values. Most neurons were excited by monaural contralateral ear stimulation; ipsilateral stimulation alone was either excitatory or noneffective. Nonmonotonic spike count vs intensity functions that are associated with circumscribed excitatory response areas were recorded by 6 days of age. For other cells, spike count was a monotonic function of stimulus level with the shape of the function not demonstrably different from that commonly recorded in adult A1. Binaural interactions were robust. Many neurons excited by stimulation of the contralateral ear were inhibited by simultaneous stimulation of the ipsilateral ear. The shapes of functions relating spike count to interaural intensity difference were indistinguishable from those exhibited by adult AI neurons. Other neonatal neurons required binaural inputs in order to discharge or were excited by stimulation of either ear alone with simultaneous binaural stimulation serving to facilitate a monaural response. In still other cells, spike count was a cyclic function of interaural phase delay at low stimulus frequencies. We conclude that some of the major mechanisms responsible for the shape of response areas and the sensitivity to interaural intensity and time differences begin operating at a very early age, when the auditory pathways are structurally underdeveloped and the inner ear is unable to respond to many environmental sounds. (HD03352, BNS&215777, NS07026)

361.10 INTRINSIC PATCHY CONNECTIONS IN PRIMARY AUDITORY CORTEX OF THE CAT. J.A. MATSUBARA AND D.P. PHILLIPS. Depts. of Anatomy and Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

Local connections within cortical areas link together groups of neurons which appear as patches or clusters of cells after microinjections of wheatgerm agglutinin-HRP or succinyl Con A. The intrinsic patches are now accepted as a universal feature of the anatomical organization of both sensory and non-sensory cortex. In cat visual cortex the intrinsic patches have a inter-patch periodicity of 1 mm and an injection-to-patch distance slightly less than 1 mm. It was previously shown that the local patches in area 18 of the cat link together cells with different preferred orientations (Matsubara et al., 1986).

In an attempt to uncover functional correlates to the local connectivity in other cortical areas we have studied the internal connections in primary auditory cortex of the cat where, as in the visual cortex, physiological properties are arranged in a quasi-orderly fashion. Injections less than 1 mm in overall diameter were made using WGA-HRP (12 nl of 0.5%) and succinyl Con A (12 nl of 1%). In some cases the injection sites were identified physiologically as being in A1. In other cases the injections were placed into the crown of the middle ectosylvian gyrus which is usually the site of A1. After a 24 hr survival period the animals were deeply anaesthetized and perfused. Blocks of auditory cortex were sectioned on a freezing microtome in the coronal or the tangential plane at 50 µm. The WGA-HRP was reacted using tetramethylbenzidine as the chromogen. Succinyl Con A was processed using an antibody against Con A and the biotinylated avidin-peroxidase method (Nance and Matsubara, 1986).

Between three and seven discrete patches of labelled neurons were observed from single injections. Coronal sections revealed that the intrinsic patches were comprised of both pyramidal and non-pyramidal cell types arranged in vertical columns. Labelled cells were found in all cortical layers with an exceptionally dense projection in Layers 3 and 4. Sections cut tangential to the cortical surface revealed a large variation in the nearest-neighbor spacing: inter-patch interval was between 800 µm and 1.5 mm. We also observed a large variety in the shapes of the patches: some were quite round while others were elongated along the rostral-caudal axis. The distribution of patches around the injection site was anisotropic with more patches occurring along the dorsal-ventral cortical axis. Along this axis we found patches as far as 3 mm from the injection site. Since iso-frequency contours run parallel to the d-v axis it is likely that the intrinsic patches interconnect neurons of similar best frequency. The other physiological properties of cells in the interconnected patches are currently being studied. This work was supported by the MRC of Canada, MA-9150 (J.M.).

361.11 PITCH PERCEPTION OF COMPLEX TONES AND HUMAN TEMPORAL-LOBE FUNCTION. Robert J. Zatorre Montreal Neurological Institute and Hospital, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The apparent pitch of a complex tone need not correspond to a component physically present in the signal; listeners typically perceive the pitch corresponding to the fundamental period of vibration, even when no energy is present at the fundamental. Electrophysiological (Goldstein et al, 1971) as well as behavioral studies with animals (Whitfield, 1980) suggest that auditory cortex plays an important role in this phenomenon, but little else is known about the neuroanatomical substrate involved.

In order to clarify this issue in the human case, the present study was undertaken. Sixty patients who had undergone left or right temporal-lobe excisions for the relief of epilepsy were tested as well as normal control subjects. In some cases the excision included all or part of Heschl's gyri (the primary auditory receiving area) whereas in other cases this region was spared. Subjects were required to indicate if the pitch of a pair of complex tones rose or fell. Stimuli were constructed such that the distribution of spectral energy was equivalent within a pair, but the periodicity, or fundamental frequency, differed (e.g. a complex with 600, 900 and 1200 Hz components, corresponding to a fundamental of 300 Hz, versus one with 600, 800, 1000 and 1200 Hz components, corresponding to a fundamental of 200 Hz). A control task was also devised in which complex tones with prominent fundamental frequency components were present.

All subjects included for study were able to perform well on the control task, indicating that auditory cortex lesions do not affect simple pitch perception when the fundamental is present, in agreement with previous studies (Milner, 1962). On the experimental task only those subjects with a right temporal lobectomy in whom Heschl's gyri were excised committed significantly more errors than the normal control group. Patients with left temporal-lobe lesions or with anterior right-temporal lobe excisions sparing Heschl's gyri were unimpaired. These results suggest that Heschl's gyri and surrounding cortex in the right cerebral hemisphere play a crucial role in perception of the pitch of complex tones in humans when the pitch must be assigned on the basis of partial information (i.e., when the fundamental is missing).

361.12 MEASUREMENT OF PHANTOM AUDITORY SENSATION IN RATS. P.J. Jastreboff J.F. Brennan* & C.T. Sasaki. Sect. Otolaryngology, Yale U. Sch. Med., New Haven, CT 06510; Dept. Psychology, UMass, Boston 02125.

Measurement of phantom perception, i.e. sensory perception with no physical correlate, is elusive. Yet, such measurement is critical for developing animal models of human disorders involving phantom perception. Auditory phantom perception, commonly known as subjective tinnitus, is a widespread auditory dysfunction which defies explanation and treatment. In our attempt to create an animal model for tinnitus, induced by salicylate, we developed a behavioral method to address the appropriateness of our approach.

66 adult male hooded rats were divided into 3 control and 8 experimental groups. From onset of the procedure, animals were exposed to white noise (80 dB SPL) around- the-clock. Background white noise offset or 3 kHz, 80 dB SPL tone superimposed on noise offset, were used as the conditioned stimulus (CS), presented four times for 30 sec (2.5 min) during a 35 min training session. Following initial acclimation exposure to the CS, animals received a session of Pavlovian suppression training; subsequent sessions were extinction tests. Chronic injections of sodium salicylate (300 mg/kg s.c. between acclimation and Pavlovian training day or between Pavlovian training and extinction day were repeated 2 h before each following session. In 2 groups, salicylate was substituted by around-the-clock tone (7 kHz, 60 dB SPL) starting before/after Pavlovian training day.

All animals showed no reaction to CS during acclimation, and suppression of licking resulting from Pavlovian conditioning was strong and rapid. However, we observed contrasting differences in extinction, depending on the phase of the procedure when salicylate (or continuous tone) was introduced. Specifically, the extinction procedure was more rapid compared to control in groups receiving salicylate after Pavlovian training but before extinction. Extinction was slower in the group injected with salicylate before Pavlovian training. All differences were statistically significant. Groups with continuous tone, substituted for salicylate injection, were statistically indistinguishable from their corresponding salicylate groups. All results are consistent with the hypothesis that animals experience tinnitus induced by salicylate. Assumptions that the nonspecific action of salicylate on nonauditory physiological functions, as well as increased threshold of auditory perception, were not sufficient to explain the phenomena observed.

Our data offer a method for measuring phantom auditory sensation in animals, thus providing the animal model for tinnitus. Furthermore, the data may hold considerable promise for measuring phantom sensations in other sensory systems. (Supported by NINCDS grants NS 16288, NS 22024, and Deafness Research Foundation Grant, 1986.)

361.13 MAGNETOENCEPHALOGRAPHIC EVIDENCE OF DICHOTIC BEAT PHENOMENA G. Anogianakis*, P.A. Anninos, M. Hoke*, K. Lehnertz*, Ch. Pantev*, B. Lütkenhöner* Dept. of Exp. Audiology, ENT Clinic, Univ. of Münster, FRG (°)

We used a Superconducting QUantum Interference Device (SQUID) to study the influence of dichotic stimuli on the Magnetoencephalogram (MEG) of normal subjects. The experiments were performed in an acoustically and electrically shielded room with ambient magnetic noise level of the order of 50 fT/√Hz. The MEG was recorded 3 mm over the temporal area of the scalp. The subjects were connected to the sound source through plastic tubes and were instructed to relax and keep their eyes closed during the trials (Anninos,P.A. et al., Brain Res. Bull., in press, 1986; Pantev, Ch. et al., Audiology, 25 : 54-61, 1986). The stimulus consisted of two simultaneously delivered pure tones: A 90 Hz tone to the right ear and a second tone to the left ear, set at discrete frequencies in the range between 98 and 118 Hz before each trial. The stimulus intensity (70 dB SPL) was matched for both ears. During each trial the MEG was recorded for 128 consecutive 1sec epochs. Each epoch was sampled at 256 samples/sec and its Power Spectrum estimated using an FFT. The stimulus onset occured at the end of the 64th epoch of each trial.

Our results show a definite shift of the MEG amplitude spectrum to the frequency which coincides with one-half of the difference between the frequencies presented to each ear. The phenomenon is discernible 5-8 sec after stimulus onset. It shows definite time lag dependencies and habituation-properties. Finally, construction of input-output frequency diagrams shows multiple stability and instability points indicating an extremely non-linear phenomenon.

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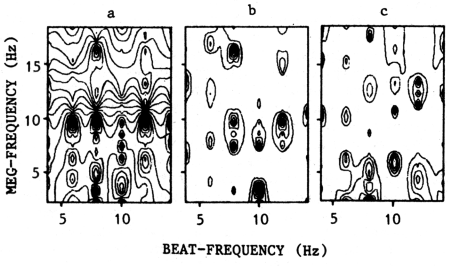

Fig.: I/O frequency diagram (a), stability points (b) and instability points (c),for diagram (a)

362.1 GABAERGIC ELEMENTS IN THE MONKEY NEOSTRIATUM. Pedro Pasik, Tauba Pasik, Gay R. Holstein and Jozsef Hamori*, Depts. of Neurol. & Anat., Mount Sinai Sch. Med., CUNY, New York, NY 10029 and First Dept. Anat., Semmelweis Univ. Med. Sch., Budapest, Hungary.

A direct GABA antibody was used with the PAP procedure to visualize GABA immunoreactive components of the neostriatum in 50 um Vibratome sections of rhesus monkey brains.

Light Microscopy. The caudate nucleus and the putamen are filled with labeled neuronal cell bodies, most of which are of medium size. The immunostain is present in the cytoplasm as well as the nucleus, the nucleolus consistently being negative. Neurons are predominantly oval. Occasionally, emerging dendrites are also visible, giving the cell a multipolar appearance. Very rarely, a large neuron also shows immunoreactivity. The neuropil contains a plexus of fine GABA-positive processes uniformly distributed throughout the striatum. Neuroglial elements are unstained.

Electron Microscopy. Two classes of labeled neurons, both of medium size, may be distinguished, particularly by the presence or absence of deep invaginations of the nuclear envelope. The reaction product in the perikarya is primarily membrane bound, coating the outer surfaces of mitochondria, smooth endoplasmic sacs and large vesicles. Ribosomes and nuclear chromatin appear more electron dense in these neurons than in unlabeled cells. No large cell is found labeled, and there are also many examples of unstained medium size neurons, some of which may be GABAergic elements remaining unreacted because of inconsistencies in antibody penetration. The most frequently observed GABA-positive dendrites have abundant dark mitochondria and, in longitudinal sections, exhibit emerging spines. Labeled isolated spine profiles, probably belonging to this dendritic type, are also seen. Other stained dendrites have strictures and dilations with fewer mitochondria but more microtubules. A third type has smoother contours and is richer in microtubules. Immunoreactive axonal boutons are numerous and of two classes. The predominant ones contain small pleomorphic vesicles; the others have large ovoid vesicles. The reaction product is present in the outer mitochondrial membranes and heavily delineates the walls of synaptic vesicles, which remain with clear centers. Generally, synapses made by labeled boutons are symmetric. The postsynaptic partners are somata, dendrites, dendritic spines or axon initial segments. The most frequent elements are dendritic shafts, either immunoreactive or unstained. Occasionally, the postsynaptic profile is an unlabeled dendritic spine, which also may receive input from an unstained bouton, and rarely a neuronal soma which again may be GABA-positive or negative. No junctions between immunoreactive boutons and stained spines are seen. Finally, immunostained dendrites and spines may be postsynaptic to unlabeled boutons of varied morphology.

The simultaneous demonstration of immunoreactive somata, dendrites and axon terminals by the direct GABA antibody allows a more accurate formulation of striatal inhibitory circuits than previous studies with antisera against the GABA-synthesizing enzyme. Aided by NIH Grants #NS-11631, NS-18657 and EY-01857, and the American Parkinson's Disease Association.

362.2 FINE STRUCTURE OF GABAERGIC CIRCUITS IN MONKEY SUBSTANTIA NIGRA. Gay R. Holstein, Pedro Pasik and Jozsef Hamori*, Depts. of Neurol. & Anat., Mount Sinai Sch. Med., CUNY, New York, NY 10029, and First Dept. Anat., Semmelweis Univ. Med. Sch., Budapest, Hungary.

The substantia nigra (SN) contains the highest levels of gamma-aminobutyric acid (GABA) and glutamic acid decarboxylase in the mammalian brain. These substances are localized primarily within axon terminals of afferents from neostriatum, pallidum and nucleus accumbens septi. Such pathways appear to mediate inhibition. Previously, we reported light microscopic observations on GABA immunoreactive fibers and neurons in SN pars reticulata (SNr) and pars compacta (SNc), as well as the fine structure of labeled axonal boutons (Soc. Neurosci. Abstr., 1985). Using the same GABA antibody, we have conducted further immunocytochemical studies with the PAP technique on the tissue from two adolescent monkeys (1 M. mulatta, 1 M. fascicularis) and one 4-week-old monkey (M. mulatta). No reaction product was visible in control sections, and similar results were obtained from all subjects.

Electron microscopic examination of SNr showed GABA-positive dendrites, recognized by heavy immunoreactivity, particularly associated with microtubules, ribosomes, and outer mitochondrial and cisternal membranes. The mitochondria were usually paler than those contained in labeled axonal boutons. In general, these dendrites were only partially covered with synapsing axon terminals, few of which were also immunostained. The latter endings established clear synaptic contacts, where the postsynaptic membrane was slightly thicker than the presynaptic partner. In addition to GABA-positive dendrites, there were unlabeled dendritic elements with thick pale shafts tightly and fully covered by boutons, many of which were immunoreactive. The synaptic contacts between GABA-immunoreactive axonal and dendritic elements in the SNr most likely represent extrinsic afferent terminations on SNr projections neurons, although some of the axonal endings may be intrinsic and belong to interneurons, or to axon collaterals of projection cells. The unlabeled dendrites resemble those of SNc neurons which are known to penetrate SNr. The GABA-positive boutons synapsing on these dendrites are of the type less frequently observed, i.e. those with larger and lighter mitochondria, and may be derived from SNr neurons, since a similar type of organelle is seen in the immunostained dendritic profiles.

The present study provides direct morphologic evidence for the existence of synapses between GABAergic components of the SNr neuropil in monkeys. This synaptology may represent the basis for the disinhibition of SNc neurons seen following neostriatal or pallidal stimulation in rats. Taken together, the results contribute to the understanding of inhibitory circuits in the primate substantia nigra.

Aided by NIH Grants #NS-11631, NS-18657 and EY-01857, and The American Parkinson's Disease Association.

362.3 IN SITU HYBRIDIZATION OF mRNAs FOR GAD, TYROSINE HYDROXYLASE AND TACHYKININS IN THE BASAL GANGLIA. M.-F. Chesselet (1,2), C. Wuenschell*(3) A.J. Tobin (3) H. U. Affolter*(2). Dept of Pharmacology, The Medical College of Pennsylvania, Philadelphia, PA 19104,(1) Lab. Clin. Sci., NIMH, Bethesda, MD 20205 (2) Dept Biol., UCLA, Los Angeles, CA 90024.(3)

Neurotransmitter-related mRNAs were detected by in situ hybridization in sections of the rat brain using ^{35}S-radiolabelled RNA probes transcribed from cDNAs cloned in the SP6 vector. Cryostat-cut frozen sections(10um) were briefly post-fixed in paraformaldehyde, acetylated and dehydrated, then incubated with .05 to .5 ng ^{35}S-labelled antisense RNA (10^8-10^9 dpm/ug) 3hrs at 50°C. Washes were conducted at 52°C in 50% formamide/2xSSC and overnight in 2xSSC/TritonX-100 at room temperature. The sections were dehydrated, delipidated in xylene and processed for autoradiography using Kodak NTB_3 emulsion. The distribution of mRNAs for glutamic acid decarboxylase (GAD), tachykinins (substance P and K) and tyrosine hydroxylase (TH) was examined in the striatum, pallidum and substantia nigra (SN). A positive signal with antisense RNA for tachykinins was found in large clusters of medium size neurons in the striatum; with antisense RNA for TH, silver grains were found in neurons of the SN pars compacta and adjacent A10 and A8 dopaminergic cell groups. In contrast to these discrete distributions, dense clusters of silver grains were observed with the GAD antisense RNA over most large neurons in SN pars reticulata, globus pallidus, ventral pallidum and entopeduncular nucleus. In the striatum, three types of medium-to-large neurons were observed with the GAD antisense RNA: few very densely labelled cells, numerous cells with a number of grains at the limit of specific detection, and unlabelled cells. These results show a good correlation with immunohistochemical studies, suggesting that the corresponding genes are only expressed in neuronal subpopulations and that neurotransmitter specification in these systems occurs at the transcriptional rather than translational level. In addition, these results suggest that the well documented differences in the levels of GAD and substance P immunoreactivity in subpopulations of striatal neurons are related to differences in the levels of expression of the corresponding genes rather than stability or transport of the gene products. Finally, no evidence was found for a significant transport of specific mRNAs to nerve terminals.

Funded by the Hereditary Disease Foundation and the Commonwealth of PA (MFC).

362.4 SYNAPSE-RELATED ANTIBODY IMMUNOSTAINING IN THE DEVELOPING CAT STRIATUM: A LIGHT AND ELECTRON MICROSCOPIC STUDY. H. Newman-Gage* & A.M. Graybiel (SPON: F.O. Schmitt) Brain and Cognitive Sciences, MIT, Cambridge, MA, 02139

The dopamine-containing innervation of the developing striatum is organized into clusters of afferent fibers known as dopamine (DA) islands. The DA islands are darkly stained with histochemical procedures revealing tyrosine hydroxylase (TH) and acetylcholinesterase (AChE). During maturation this islandic pattern changes to the more homogeneous TH distribution and to the compartmental distribution of AChE-pale striosomes typical of the adult. We report here on the development of striatal immunoreactivity, in the cat, to two new antibodies that are closely associated with synaptic terminals (SV48 and 6G9), and on the relation of these immunoreactivities to TH and AChE staining during maturation. SV48 (W. Matthew) recognizes synaptic vesicles. 6G9 (M. Kennedy) is directed against a Ca^{+2}/calmodulin-dependent protein kinase known to be a major component of postsynaptic densities and to have synapsin I as a primary substrate.

During the last third of gestation and the first postnatal week, both SV48 and 6G9 were distinctly localized in circumscribed zones aligned with the TH- and AChE-rich DA islands found in serial sections. With maturation, the distribution of SV48 and 6G9 immunoreactive sites changed so that by postnatal day 42 and into adulthood, they, like TH immunoreactivity, had a relatively homogeneous distribution. These observations suggest that the appearance and subsequent maturation of synapses in DA islands may precede that of synapses in the surrounding matrix.

TH-positive reaction product in DA islands was found primarily in symmetrical, round-vesicle terminals, in preterminal axons, and in growth cones. A few asymmetrical terminals and individual axons within fasicles were also positive. In the matrix, fewer TH-positive fibers and occasional synapses were found, but the majority of axons and synapses were unlabeled. SV48 reaction product was associated only with vesicles. It was found primarily in round-vesicle terminals (which frequently formed symmetrical synapses) and in growth cones. Many terminals appeared to be lightly labeled or unlabeled. SV48 was rarely associated with pleomorphic vesicles. Labeled terminals were found apposed to spine heads, dendrites, and growth cones. 6G9 has not yet been studied ultrastructurally.

These experiments demonstrate: 1) the co-distribution during striatal development of immunostaining for two synapse-related antibodies and TH-positive DA islands, and 2) labeling by SV48 of terminals that are ultrastructurally similar to TH-positive terminals. We suggest on this basis that the DA islands may be early loci of synaptogenesis within the striatum.

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362.5 INNERVATION OF STRIOSOMES AND EXTRASTRIOSOMAL MATRIX BY DIFFERENT SUBDIVISIONS OF THE MIDBRAIN A8-A9-A10 DOPAMINE-CONTAINING CELL COMPLEX. J. Jimenez-Castellanos* and A.M.Graybiel,Whitaker College, Mass. Institute of Technology, Cambridge, MA 02139

The dopamine-containing innervation of the developing striatum has been divided into two broad categories: the early-forming islandic innervation and the later-maturing diffuse innervation (Olson et al '72). The islandic system is known to innervate regions that are the forerunners of striosomes. To identify the cells of origin of these two major systems at maturity, we injected anterograde tracer into the substantia nigra pars compacta (SNpc, cell group A9)in the cat and found (Jimenez-Castellanos and Graybiel '85)that injections centered in a medial and caudal part of the SNpc preferentially labeled nigrostriatal fibers innervating striosomes (identified in adjoining sections processed for acetylcholinesterase). We have extended this study by placing injections of ^{3}H-labeled amino acids into different parts of cell groups A8, A9 and A10 in 10 cats (and HRP-WGA into A10 in 1 cat)so as to analyze the resultant striatal labeling in relation to striosomes and the extrastriosomal matrix. Nigral subdivisions were identified in the same or in standard brains by tyrosine hydroxylase immunohistochemistry.

A discrete innervation of striosomes by a caudomedial cell-dense subdivision of the SNpc was confirmed. The smallest injections in this region labeled dorsal striosomes, with little labeling of the extrastriosomal matrix. Larger injections labeled more of the striosomal system and weakly labeled lateral parts of the matrix. In one case, dorsal striosomes were labeled, but some ventral striosomes were avoided by the labeled fibers.

Injections centered in cell group A8 labeled primarily the extrastriosomal matrix. Such differential labeling of the matrix occurred in cases with little or no detectable labeling of the nucleus accumbens-ventral striatum. Sparse ventral matrix labeling occurred with deposits centered in the ventral tegmental area (A10).

Mixed labeling of striosomes and matrix occurred with deposits centered in the large remaining part of the SNpc and in the pars lateralis of the SN. The heightened labeling of striosomes was primarily in lateral and/or caudal striatal regions. As finger-like extensions of the cell-dense zone of SNpc intrude into rostrolateral parts of the SNpc, these, rather than the more scattered cells of SNpc, might have accounted for part or most of the striosomal labeling.

We conclude that different parts of the A8-A9-A10 dopamine-containing cell complex project to striosomes and to extrastriosomal matrix, and suggest that these differential projections correspond, in part at least,to the islandic and diffuse innervations originally detected by catecholamine histofluorescence.

Supported by NSF BNS8319547 and The Seaver Institute.

362.6 NEURONS CONTAINING TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITY IN THE CAUDATE AND PUTAMEN OF A NONHUMAN PRIMATE. M. Dubach, R. Schmidt, D. M. Bowden, D. Kunkel, R. Martin, D. German, Departments of Psychiatry and Behavioral Sciences and of Neurological Surgery, and Regional Primate Research Center, University of Washington, Seattle, WA, and Departments of Physiology and Psychiatry, University of Texas, Dallas, TX.

Tyrosine hydroxylase (TH) immunohistochemistry labels midbrain dopamine (DA) neurons, nigrostriatal fibers, and neostriatal terminals, but TH-like immunoreactive (THLI) cells in the neostriatum itself have not been previously reported. We present evidence, based on TH peroxidase-antiperoxidase light (LM) and electron (EM) microscopy, that small (8-12μm), ovoid, usually bipolar THLI cells are present in the caudate, putamen, and adjacent white matter. Coronal 50-μm sections contain 75-200 cells. Each cell has a large, deeply invaginated nucleus surrounded by a thin layer of labeled cytoplasm, and gives rise to one or two processes visible by LM. Analysis of several such cells by EM has suggested that the processes are aspiny dendrites. Synapses appear on the cell bodies and proximal dendrites; some of the terminals contacting the cells are themselves THLI-positive, and others are not. Labeling was specific--TH antiserum from two different sources labeled the cells, and non-immune serum control sections contained no labeled cells. The THLI cells were not labeled by dopamine beta-hydroxylase antibody, suggesting that they do not contain norepinephrine or epinephrine. Neostriatal THLI neurons are present in the macaque (M. fascicularis) and the baboon (P. cynocephalus), but we have not found them in the rat.

Treatment of monkeys with the DA neurotoxin MPTP, sufficient to induce parkinsonian symptoms, does not eliminate THLI cells from the neostriatum, suggesting that if they are DA cells they, like DA cells in ventral tegmentum, are relatively insensitive to MPTP.

The existence of THLI cells has been previously reported in orbitofrontal and olfactory cortical areas and adjacent white matter near the nucleus accumbens (Kohler et al., Neurosci. Lett. 37:161, 1983). The present work confirms that finding. The neostriatal cells are similar to the rostrobasal cells, but slightly smaller; they also appear in white matter (corona radiata and internal capsule) adjacent to neostriatal gray. Neostriatal acetylcholinesterase (AChE) cells of similar size and EM/LM appearance have been described (Bolam et al., Neurosci. 12:687, 1984). Because midbrain DA neurons and axons are AChE-positive (Henderson, J. Comp. Neurol. 197:185), neostriatal THLI cells may represent the same population as neostriatal AChE cells.

The role of these neurons in DA-mediated behavior remains to be assessed. Their normal presence in the primate neostriatum should be considered in the histological evaluation of DA-neuron transplants in monkeys. (Supported by USPHS RR00166, NS07144, NS20030 and Dallas Area Parkinsonism Society)

362.7 EFFECTS OF THE WEAVER GENE ON THE ISLANDIC AND DIFFUSE DOPAMINE-CONTAINING INNERVATIONS OF THE STRIATUM: A BIOCHEMICAL AND ANATOMICAL STUDY. S. Roffler-Tarlov and A.M. Graybiel, Depts. Neurology and Anat., Tufts Univ. Sch. Med., Boston, MA 02111 & Whitaker College, M.I.T., Cambridge, MA 02139

The dopamine-containing innervation of the striatum originates in cell groups A8, A9, and A10 of the midbrain. At birth, much of this innervation is organized into macroscopic clusters, known as dopamine islands. Outside these clusters, the dopamine-containing innervation is sparse. With increasing age, the non-islandic or "diffuse" innervation gradually increases, so that eventually the dopamine islands are no longer visible unless pharmacological treatments are given. Anterograde transport experiments have shown that the islandic and diffuse systems persist into adulthood and that they originate, at least in part, in different dopamine-containing cell groups (Jiminez-Castellanos & Graybiel, 1985, 1986). The weaver mutation is known to result in a dramatic reduction of dopamine in the adult caudoputamen (CP) (Schmidt et al., 1982; Roffler-Tarlov and Graybiel, 1984). In the study reported here, we examined the early postnatal development of the dopamine-containing mesostriatal innervation in homozygous weaver and normal mice. We monitored synaptosomal accumulation of ^{3}H dopamine as a measure of the extent of the dopamine-containing innervation, as well as endogenous dopamine in CP of control and weaver mice, at intervals between P7-P33. In parallel, we studied the anatomical pattern formed by the developing nigrostriatal innervation by tyrosine hydroxylase immunohistochemistry.

The biochemical findings demonstrated that synaptosomal uptake of ^{3}H dopamine is already reduced by about 30% in one week-old weaver animals although the dopamine content is nearly normal. During the next three weeks the discrepancy between weaver and control grows as the dopamine content and uptake capacity of the control CP increases at a rate that is not matched in weavers. Thus the biochemical findings suggested that the defect in the mature weaver mouse results from a thwarted development of the nigrostriatal system.

The anatomical findings, by contrast, demonstrated that degenerative events are also involved. The dopamine islands, the main dopamine-containing fibers innervating the caudoputamen at birth, are especially prominent both in weavers and normals in the dorsal caudoputamen, the region of maximum dopamine loss in the adult weaver. This islandic system degenerates during the third postnatal week in the weaver. Thus, in addition to failure of development of the diffuse dopamine system in the weaver, there is degeneration of the early arriving islandic system. Supported by NIH-NS20181, the Seaver Institute, and the American Parkinson Disease Association.

362.8 THE DEVELOPMENTAL AND BIOCHEMICAL BASIS OF DUAL "PATCH" and "MATRIX" NIGROSTRIATAL DOPAMINERGIC SYSTEMS IN THE RAT. C.R. GERFEN. Lab. of Neurophysiology, NIMH, Bethesda, MD 20892

The striatum is a mosaic of two neurochemically and anatomically distinct compartments, the "patches" and the "matrix". This organization provides parallel striatal input-output pathways: the "patches" receive inputs from certain cortical areas, such as the prelimbic cortex, and project to the dopaminergic neurons of the substantia nigra, whereas the "matrix" receives inputs from other cortical areas, such as sensory and motor areas, and projects to the GABAergic neurons of the substantia nigra pars reticulata (Gerfen, C.R., Nature,311:461, 1984). Though segregated in terms of input-output connections, patches interact with surrounding matrix via at least two systems, through local connections of striatal somatostatin interneurons (Gerfen, '84) and through the more indirect but widespread nigrostriatal feedback circuit. This study examines the latter system using PHA-L axonal tract tracing combined with tyrosine hydroxylase immunoreactivity to distinguish dopaminergic fibers (Gerfen and Sawchenko, Br.Res.,343: 144, 1985) and with 3H-Naloxone binding of mu opiate receptors to mark patch-matrix areas. Results show that 1) non-dopaminergic nigrostriatal fibers are restricted to the matrix, 2) dopaminergic neurons in the ventral tegmental area (VTA) and retrorubral area project to ventral and dorsal striatal matrix, respectively, and 3) dopaminergic neurons in the substantia nigra constitute mixed populations that project to both patch and matrix areas. The population of nigrostriatal neurons can be dissociated in several ways. First, the relative degree of PHA-L labeling of inputs to patches and matrix is dependent on the location of dopaminergic neurons injected, i.e. injections of dopaminergic neurons in the ventral pars reticulata label principally inputs to the patches. Second, a brain calcium binding protein (CaBP-28kD) is localized to a subset of dopaminergic neurons, most of those in the VTA and a subset of those in the substantia nigra pars compacta (and few in the pars reticulata), that match in their distribution those PHA-L labeled dopaminergic neurons projecting to the matrix. Third, 6-hydroxydopamine-induced lesions of neonatal striatal dopaminergic afferents result in an adult pattern of dopamine fibers enriched in the matrix and depleted in the patches. In these cases the surviving dopaminergic neurons are those that contain CaBP-28kD and match the distribution of the PHA-L labeled neurons projecting to the matrix. Thus, there exist two sets of dopaminergic neurons in the substantia nigra, 1) those that appear to be contiguous with the VTA and retrorubral dopamine cell groups in that they both express CaBP-28kD and project to the matrix and 2) those that, though intermixed with the first group, are distinguished by their preferential projections to the patches, by their lack of expression of CaBP-28kD and their earlier developmental growth.

362.9 LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF IMMUNOREACTIVE SUBSTANCE P IN THE MONKEY NEOSTRIATUM. M. DiFiglia and N. Aronin. Dept. of Neurology, Mass. General Hosp., Boston, MA, 02114 and Dept. of Medicine, U. Mass. Med. Sch., Worcester, MA, 01605.

There is evidence that substance P is a neurotransmitter in the basal ganglia. We examined the localization of immunoreactive substance P (iSP) in the monkey neostriatum at the light and electron microscopic level. Monkeys (Saimiri sciureus; N=4) were injected bilaterally with 80 ug of colchicine and perfused intracardially for 30 min with buffered 4% paraformaldehyde. Brains were postfixed for 1 hr in 4% paraformaldehyde and 0.1% glutaraldehyde. Vibratome sections 35 μm thick were incubated in a SP monoclonal antisera at a dilution of 1:100 for 48 to 72 hr and processed using the biotin-avidin immunohistochemical method with diaminobenzidine as the chromagen. Controls which included tissue sections incubated either in antisera preabsorbed with 50 μg/ml of SP or in non-immune serum exhibited no SP immunoreactivity. Tissue sections were treated with 1% osmium tetroxide and embedded flat in Epon.

Results showed that iSP was localized to medium-sized caudate neurons about 15 μm in size. Most of the iSP neurons were sparsely distributed within 500 μm of the ventricular surface and showed moderate to dense precipitates of reaction product in their cell bodies and primary dendrites. A dense plexus of iSP terminals was also visible throughout the neostriatum. Ultrathin serial sections through 20 iSP somata showed that the majority of moderately (9 of 14 cells) and darkly labeled (4 of 6) neurons contained a centrally located, unindented nucleus, and a thin rim of cytoplasm which was sparsely populated with organelles. A smaller proportion of the neurons (6 of 20) had similar features with the exception that several shallow enfoldings or a single deep indentation appeared in the nucleus. Immunoreactive SP fibers were mostly unmyelinated, of fine caliber (0.1 μm), and contained numerous swellings filled with vesicles. In caudate neuropil iSP axon terminals were relatively frequent in number (up to 8 boutons per 100 μm^2), showed dense precipitates of reaction product, and formed mostly symmetric synapses. Of 90 boutons identified at the level of their synaptic contacts 83% synapsed with small dendrites, 9% with cell bodies, 6% with spines and 2% with axon initial segments. Results show that in the monkey neostriatum the majority of iSP neurons have morphological features similar to medium-sized spiny cells (type 1) and a smaller proportion of iSP neurons belong to an unidentified group possibly of the medium-sized aspiny type. The relative distribution of synapses formed by iSP axons with various postsynaptic targets in caudate differs from that previously observed for immunoreactive enkephalin, somatostatin, and choline acetyltransferase containing axons and suggests preferential sites for the action of each of these putative transmitters within the caudate nucleus. Supported by NIH grant NS 16367 to MD and AM 01126-04 to NA.

362.10 DUAL LOCALIZATION OF [LEU]-ENKEPHALIN AND CHOLINE ACETYL-TRANSFERASE IN THE RAT BASAL GANGLIA. G.R. Penny, H.T. Chang and S.T. Kitai. Dept. of Anatomy and Neurobiology, University of Tennessee Memphis, The Health Science Center, Memphis, TN 38163

Spiny projection neurons containing the peptide neurotransmitter [leu]- enkephalin (L-ENK) make up almost 50% of the neurons in the neostriatum. These neurons give rise to both local collaterals within the striatum and terminal arborizations in the globus pallidus. Both areas contain populations of cholinergic neurons: those within the striatum are local circuit neurons, but those within the globus pallidus are sources of cholinergic innervation of the cerebral cortex. The purpose of the present study was to determine whether enkephalin-containing neurons form synaptic contacts with cholinergic neurons in either structure.

We used a double labeling method to simultaneously localize, within a single section, L-ENK-immunoreactivity using rabbit antiserum to L-ENK (Immunonuclear) and choline acetyltransferase (ChAT) using rat anti-ChAT antibody (a gift from F. Eckenstein). We used the ABC method and DAB reaction product to label ChAT-immunoreactive neurons and an immunogold method to localize L-ENK. In other experiments we reversed the order, and L-ENK was labeled by DAB and ChAT by immunogold. Sections for light microscopic analysis were silver-intensified. After osmication all material was section-embedded in Spurr's resin.

We find that (1) in the light microscope the DAB reaction product appears brown and homogeneous; the silver-intensified gold is punctate; and the two are clearly distinguishable at both light and electron microscopic levels. (2) The distribution and morphology of the ChAT-immunoreactive neurons and the L-ENK-immunoreactive terminals are consistent with previously published reports. (3) The penetration of the colloidal gold labeling is limited to the outer 2 to 3 um of the section, whereas the ABC method gives labeling thoroughout the thickness of the section. (4) Although the enkephalin- immunoreactive terminals are found near the ChAT-immunoreactive neurons in the globus pallidus, it does not appear that synapses form between the two, suggesting that there is no monosynaptic input from neostriatal enkephalinergic projection neurons onto pallidal cholinergic neurons.

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362.11 Electron microscopic evidence of striatal input to intrapallidal neurons of cholinergic cell group Ch4 in the rat. E.A. Grove and C.A. Ingham*(SPON:W.J.H. Nauta). Dept. of Psychology,MIT,Cambridge, MA, and Dept. of Pharmacology, South Parks Rd., Oxford, UK.

In the rat, many of the cholinergic basal forebrain neurons that innervate the cortex and amygdala (Ch4) lie within the ventral pallidum (VP) and globus pallidus (GP), and thus within reach of a direct projection from the striatum. After injections of the anterograde tracer PHA-L into the medial striatum, lectin-filled axonal varicosities (putative terminals) can be seen in the light microscope directly apposed to the soma and dendrites of GP cholinergic neurons (Grove et al., 1986, Br. Res. 367:379-384). We now report that some such juxtapositions, at least, represent synaptic contacts between striatofugal axons and cholinergic cells.

To optimize combined PHA-L and choline acetyltransferase (ChAT) immunohistochemistry, rats were perfused transcardially with fixative containing 4.0% paraformaldehyde and 1.0% glutaraldehyde; then sections were cut at 50 um on a vibratome, rinsed for 1 hr in Tris buffered saline containing 0.06% Triton-X, and incubated for 60 hr with primary antisera directed against ChAT (Boehringer Mannheim) and PHA-L (Vector). Both types of immunoreactivity were localized using the peroxidase anti-peroxidase procedure with 3,3' diaminobenzidine as the substrate, Viewed in the light microscope, the GP was seen to contain stretches of dark brown stained varicose axons and lighter brown immunoreactive cell bodies and dendrites. In control sections incubated with the ChAT primary antiserum omitted, no immunoreactive cell bodies or dendrites appeared outside the PHA-L injection site. When the PHA-L primary antiserum was omitted instead, the GP was free of varicose axon labelling.

In a double-labelled section through the caudal globus pallidus, a PHA-L-filled axon appeared to contact a ChAT-immunoreactive dendrite with a long varicosity. The region containing this juxtaposition was sectioned on an ultramicrotome and serial sections were examined in the electron microscope. The axonal varicosity represented an axon terminal that made two synaptic contacts with the ChAT-immunoreactive dendrite. These synaptic specializations were symmetric, like most formed by striatofugal axon terminals in the GP.

Other PHA-L-labelled axon terminals were seen in juxtaposition to ChAT-immunoreactive elements in this material, but clear synaptic contacts could not be discerned. Some juxtaposed terminals made synaptic contact instead with adjacent unlabelled elements.

Our present findings complement earlier EM evidence of synaptic input from the nucleus accumbens to cholinergic neurons in the VP (Zaborszky et al., 1984, Soc. Neurosci. Abstr. 10:8), and indicate that some intrapallidal cholinergic neurons, at least, are integrated into the circuitry of the basal ganglia.

Supported by PHS grants 1 R02 NS 19945 and P01 MH 31154.

362.12 STRIOSOMAL MARKERS IN THE STRIATUM IN HUNTINGTON'S DISEASE. L.A.Feigenbaum, A.M.Graybiel, J.P.Vonsattel,* E.P. Richardson, Jr.* and E. D. Bird, Whitaker College, Mass. Institute of Technology, Cambridge, MA 02139

The neuropil of the striatum is characterized by chemically distinct macroscopic compartments related to the spatial arrangement of striatal transmitters and connections. These compartments were first detected in the human brain by acetylcholinesterase (AChE) histochemistry as 0.5-0.8 mm-wide AChE-poor zones in an AChE-rich matrix in the caudate nucleus (CN) and as less sharply defined, and sometimes larger, zones in the putamen. They have since been shown to correspond to patches or annuli of heightened met-enkephalin-like immunoreactivity(mENK-LI). In the study reported here,we sought to learn whether this striosomal architecture survives in the striatum in the course of Huntington's disease(HD), an inherited disorder in which there is massive, progressive cell death in the striatum. We analyzed striatal tissue from 8 brains identified by the Vonsattel and Richardson grading scale (0-4, 4 most degenerated):Grade 2 (G2, n=2), Grade 3 (G3, n=3) and Grade 4 (G4, n=3). One of the G4 brains was characterized as juvenile onset HD. We stained selected sets of sections for AChE, for mENK-LI, and for two other enzymes that we have found to reveal striosomal compartmentalization in the normal human striatum: NADPH diaphorase (NADPHd) and butyrylcholinesterase (BuChE). Other sections were stained for glial fibrillary acidic protein (GFAP) and for Nissl substance. As controls, we analyzed striatal tissue from 9 brains free of known neurological defects.

Each of the 4 stains used to detect striosomes in the normal brains (AChE,mENK-LI,NADPHd and BuChE)revealed continued presence of striosomal architecture in HD brains identified as G2. There were differences in the clarity of the patterning with the different markers. In the CN, striosomes were AChE-poor, NADPHD-poor and matched annuli or patches of mENK-LI, but zones of reduced BuChE staining, which matched striosomes in normal striatum, were not detected. In the putamen, compartments were clear in NADPHd and AChE but detectable only ventrally with the other stains.In the G3 specimens,striosomal architecture was still evident in AChE-stained sections but patterning in mENK-LI was not visible except in the ventral striatum(VS),a zone preferentially spared in HD,least stained for GFAP in our samples, and characterized by vivid patterning in the normal brain. No NADPHd and BuChE sections were available. In G4 striatum, patterning in MENK-LI was absent dorsally and reduced in the VS,and was not visible (1 sample) in NADPHd. In AChE and BuChE sections,however,compartments of high and low staining were present with pronounced abnormal patterns in the putamen. Large numbers of AChE-positive neurons were found in the HD striatum,even in advanced grades and in the juvenile onset case.

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363.1 DOPAMINERGIC, HISTAMINERGIC AND ENKEPHALINERGIC PROJECTIONS FROM THE DI- AND MESENCEPHALON TO THE NUCLEUS RAPHE MAGNUS IN THE RAT. H.W.M.Steinbusch,[a] J.Veening*, J.Bol* and A.H.Mulder*. Dept. Pharmacology, Free University, Amsterdam and [a]Dept. Anatomy and Embryology, Catholic University, Nijmegen, The Netherlands.

Lately it has been shown that the nucleus raphe magnus (nrm) receives a medium to dense innervation of dopamine-immunoreactive (DAi) fibers, which are mainly confined to its lateral parts. In addition some scattered histaminergic (HAi) and enkephalinergic (ENKi) fibers are distributed throughout the nrm. The nrm contains a large number of serotonergic neurons, mainly localized in its bilateral wings. In the present study we have investigated the precise origins of the dopaminergic, histaminergic and enkephalinergic innervation of the nrm. Using TRITC-immunofluorescence, in conjunction with antibodies to DA,HA,HDC and ENK and in combination with injections of the retrograde tracers True Blue (TB) or Granular Blue (GB) we have studied those di- and mesencephalic DA-,HA and ENK-immunoreactive cell groups which have been predicted to give rise to dopaminergic, histaminergic and enkephalinergic projections to the nrm. Following injections of TB or GB (0.1 ul 5%) into the lateral parts of the nrm labeled cells were found mainly ipsilateral in the posterior diencephalon and mammillary bodies. The majority of the dopaminergic neurons was confined to the A13 cell group, while the histaminergic cell bodies giving rise to the descending projection to the nrm were situated in the nucleus caudalis magnocellularis, postmammillaris (pcm). The enkephalinergic neurons which project upon the nrm were distributed in the periaquaductal central gray (PCG). Simultaneous analysis of retrograde labeling and DAi,HAi and ENKi on the same section revealed that approximately 10-20% of the DAi cells in the nuclei hypothalamicus posterior and the periventricular gray matter of the caudal thalamus; about 5% of the HAi cells in the pcm and a few ENKi cells in the PCG project to the nrm.
This study indicates the presence of a dopaminergic projection from the posterior hypothalamus and the area of the substantia griseum centralis, a histaminergic projection from the pcm as well as an enkephalinergic projection from the PCG to the nrm.

363.2 PROJECTIONS FROM HYPOTHALAMUS TO BRAIN STEM AND SPINAL CORD. AN HRP AND AUTORADIOGRAPHICAL TRACING STUDY IN THE CAT. G. Holstege. Dept. of Neuro-anatomy, Med. Fac. Erasmus University, P.O. Box 1738, 3000 Dr Rotterdam, The Netherlands.

Previous studies have demonstrated direct projections from hypothalamus to caudal brain stem and spinal cord. Main targets are the caudal raphe nuclei, the solitary and dorsal vagal complex and the thoraco-lumbar intermedio-lateral cell column. In this study, using HRP and autoradiographical tracing techniques, we tried to define which hypothalamic areas project to which areas in brain stem and spinal cord. The HRP results indicated that the projections to the spinal cord, (including its most caudal levels), were derived from neurons in the paraventricular hypothalamic nucleus (PVN) and from the lateral and posterior hypothalamic areas. The projections to the area of the caudal raphe nuclei were derived from the same areas but also from the anterior hypothalamic area.

The autoradiographical results revealed that neurons in the medial portions of the hypothalamus (anterior as well as posterior) project mainly to medially located brain stem structures (PAG, VTA, dorsal part of the substantia nigra, interpeduncular nuclei except its central nucleus, locus coeruleus, rostral and caudal raphe nuclei with adjoining ventral tegmentum). When the injection site involved the PVN, a distinct bundle of labeled fibers was observed, descending lateral to the pyramidal tract into the lateral funiculus of the spinal cord. As expected, from this bundle, labeled fibers were distributed to the solitary and dorsal vagal complex and to the thoraco-lumbar intermedio-lateral cell column. However there were also projections to the area postrema, laminae I and X, the sacral intermedio-lateral cell group and to the nucleus of Onuf, located in the upper sacral segments and containing motoneurons innervating pelvic floor musculature. The lateral hypothalamic areas (anterior as well as posterior) project mainly to lateral parts of the brain stem tegmentum. These projections resembled those derived from amygdala and BNST.

These results suggest a medio-lateral organization in the hypothalamo-brain stem projections. The medial hypothalamus projects to medially located brain stem structures, which are involved in level setting of spinal cord somatosensory and motor systems. The lateral hypothalamus project to laterally located brain stem areas, which are involved in more specific functions such as respiration, swallowing, vomiting and micturition. The PVN occupies a separate position within this organization.

363.3 VISUALIZATION OF PUTATIVE DOPAMINERGIC NERVE FIBERS IN THE RAT HIPPOCAMPUS AND POSTERIOR CORTICAL AREAS BY THE USE OF TYROSINE HYDROXYLASE/DOPAMINE β-HYDROXYLASE DOUBLE-LABELING IMMUNOHISTOCHEMISTRY. T.Melander[1], T. Hökfelt[1]* and M. Goldstein[2]. (SPON: S. Whittemore) [1]Department of Histology, Karolinska Intitutet, Box 60400, S- 10401 Stockholm and [2]Department of Psychiatry, New York University Medical Center, School of Medicine, 550 1st Avenue, New York, N.Y. 10016

The dopaminergic innervation of the limbic forebrain has been extensively mapped and dopamine containing projections from the mesencephalon to e.g. the amygdala and frontal cortex are well established. However, some controversies still remain concerning the existence of a minor dopaminergic innervation of the hippocampal formation and posterior neocortical areas. In this study we have used an immunohistochemical double-labeling technique where we have visualized tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) immunoreactivities in the same sections, thus identifying putative dopaminergic nerve fibers and varicosities as structures staining for TH but not for DBH.

The majority of the TH-positive hippocampal fibers also contained DBH immunoreactivity, but a distinct population of coarse TH single-labeled (putative dopaminergic) fibers was seen in stratum oriens of the CA1 region and, to a lesser extent, in the medial dentate gyrus. These were concentrated to mid-dorsoventral levels. Putative dopaminergic fibers were also detected in very low amounts in posterior neocortical areas, with a concentration in superficial layers. For correlation two areas known to receive extensive dopaminergic innervation was examined. Thus, in the amygdala TH single-labeled innervation was concentrated to the basolateral and central amygdaloid nuclei, where it was mixed with TH/DBH double-labeled fibers, while the intercalated nucleus lacked DBH, agreeing well with what in known about the dopaminergic and noradrenergic innervation from earlier studies. Also, in the frontal cortex many TH single-labeled fibers were seen.

In general, fibers which only contained TH immunoreactivity were heavier labeled than when they also stained for DBH, indicating higher levels of TH in dopaminergic than in noradrenergic fibers (a fact previously postulated). In most cases the TH single-labeled fibers were of a coarser morphology than TH/DBH double-labeled ones.

363.4 AN ANALYSIS OF THE STRUCTURAL AND CHEMICAL ORGANIZATION OF THE RETROCHIASMATIC NUCLEUS. J. P. Card, R. P. Meade and R. Y. Moore, Central Research and Development Department, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware 19898 & Department of Neurology, SUNY at Stony Brook, Stony Brook, New York.

The retrochiasmatic nucleus (RCN) is a midline hypothalamic cell group which is located caudal to the optic chiasm. In a preliminary study we found that neurons in the RCN share an overlapping dendritic field with neurons in the suprachiasmatic nuclei (SCN). This suggests that the RCN may be involved in the neural control of circadian rhythmicity that has previously been documented for the SCN. In the present investigation we have looked at the structural and immunohistochemical organization of the RCN and its relationship to the SCN.

Golgi impregnated neurons in the RCN are characterized by small (15-25 um widest diameter), spherical or oval perikarya. Two to five sparcely branching dendrites emanate from each neuron. Individual dendrites are studded with spines and apppendages and often extend through the RCN into adjacent areas of the hypothalamus. In addition, numerous dendrites from adjacent hypothalamic nuclei project into the RCN. This relationship is particularly apparent between the RCN and the SCN. Dendrites arising from neurons in each of these areas project extensively into the other's cellular domain. Ultrastructural analysis of the RCN revealed small neurons with scant cytoplasm and a centrally placed, invaginated cell nucleus. The most striking feature, however, was the extremely large number of dendritic profiles that fill the RCN. These dendrites, which are clearly the dominant neuronal profile in the nucleus, give rise to numerous large, pedunculated spines and are postsynaptic to a dense afferent input. Preliminary immunohistochemical observations have characterized the chemical identity of some of these afferents. They include dense terminal arborization of axons containing dopamine-B-hydroxylase, neuropeptide Y, glutamic acid decarboxylase and leu-enkephalin and a moderate plexus of serotoninergic and neurotensin-containing fibers.

These data demonstrate that neurons in the RCN and SCN share interdigitating dendritic fields. Additionally, the dense synaptic input to dendrites and spines within the RCN, along with its strategic location among several major fiber projections, indicates that the RCN integrates information from a variety of different sources which may be significant in the neural modulation of circadian rhythmicity.

363.5 STIMULATION-INDUCED HIPPOCAMPAL GRANULE CELL SEIZURE ACTIVITY CAUSES A PERSISTENT DECREASE OF INHIBITION AND A SELECTIVE LOSS OF SOMATOSTATIN-CONTAINING INTERNEURONS

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Previous work showed that intermittent perforant path (PP) stimulation for 24hr decreased recurrent inhibition (RI) acutely and damaged dentate interneurons and hippocampal pyramidal cells. We concluded that the loss of RI might be the result of damage to GABA-containing basket cells (Sloviter, Brain Res. Bull., 10:675-697, 1983). Using electrophysiological and immunocytochemical methods, we have now determined: 1) if seizure activity causes a persistent decrease in inhibition and; 2) precisely which dentate interneurons are damaged by seizure activity. This was accomplished by evaluating GABA-, CCK-, VIP- and somatostatin (SS)-immunoreactive (IR) neurons in stimulated- vs. unstimulated hippocampi. The results are as follows: 1) granule cell excitability (population spike amplitude) was increased and RI, as measured with the twin-pulse technique, was decreased or absent during the 2 wk after stimulation. PP stimulation caused a selective loss of the SS-positive (GABA-negative) hilar interneurons. Cell counts of stimulated- vs unstimulated hippocampi in each section showed a >90% loss of SS-IR dentate interneurons. Surprisingly, there was no apparent loss of GABA, VIP or CCK-positive cells despite a loss of virtually all adjacent SS-IR neurons. 2-12 wks after stimulation, axons of GABA/CCK-positive basket cells sprouted in the stimulated area dentata and formed an abnormally dense fiber plexus in the inner molecular and granular layers. During this period, RI recovered. The available evidence suggests that dentate inhibitory neurons are excited monosynaptically by dentate granule cells and disynaptically via the SS-IR hilar cells that form the associational/commissural path. This suggests that the seizure-associated loss of inhibition that occurs without a loss of GABA-IR basket cells may be due to the loss of SS-positive, probably excitatory, interneurons whose physiological function may be to activate GABA-IR basket cells (Buzsaki and Eidelberg, 1981). The seizure-induced loss of a basket cell activating system without a loss of basket cells may be relevant to the human epileptic state and may explain the paradoxical and probably compensatory increase in GABA- and GAD-staining observed in our study and in epileptic gerbils (Peterson et al, Brain Res. 1985), respectively. The differential vulnerability of immediately adjacent dentate interneurons is intriguing.

363.6 EFFECT OF ESTRADIOL ON THE AMYGDALA AND VENTROMEDIAL HYPOTHALAMIC NEURONS.

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Estradiol (ES) is concentrated in the medial amygdala (Med-AMG), medial preoptic area, ventromedial hypothalamus (VMH) and arcuate nucleus. ES implated in the Med-AMG and the VMH modulates feeding behavior, gonadotropin release, and ovulation. To investigate the mechanism of the effect of ES inhibition on the Med-AMG neurons and of its excitation on the VMH neurons, intracellular recording was used to monitor neurons in rat coronal brain slices. Two weeks after ovariectomy, a single priming by subcutaneous injection of ES was performed. Two days later preparations were made.

1) 17β-ES 10^{-9} to 10^{-7} M but not 17α-ES rapidly inhibit the excitability of Med-AMG neurons with a dose-response manner. Hyperpolarization within a few min and increased membrane conductance were produced. The reversal potential for ES hyperpolarization shifted by 53 mV by 10 times external K^+ change. The Hill's coefficient estimated from the relationship between the membrane conductance and the ES-dose was approximately 1. This effect persisted after elimination of synaptic input and after suppression of protein synthesis by actinomycin D or cyclohexamide. These results indicated that 17β-ES directly changes the K^+ conductance of the postsynaptic membrane. Significant difference in responding rates between male (responded neurons, 8%) and female 27% was demonstrated. Intracellular horseradish peroxidase staining showed that ES responding neurons were mainly fusiform shape (14 μm diameter) with a few dendrites. ES non-responding neurons were pyramidal shaped (17 μm) and had multidendrites (more than 4) that extended in various directions.

2) One type of neurons in the VMH was depolarized by 17β-ES with a decrease in the membrane conductance. Most of these were glucoreceptor neurons. The reversal potential of the depolarization was approximately -90 mV, close to the K^+ equilibrium potential (E_K). The depolarization was greatly augmented and prolonged by application of phosphodiesterase inhibitor, isobutyl-methylxanthine (BMX) or adenylate cyclase activator, forskolin. These neurons had mostly multipolar dendrites and large soma (diameter, 15 μm). From these evidences, ES depolarization might be mediated through an increase in intracellular C-AMP level. The other type of neurons in the VMH were hyperpolarized with an increase in the membrane conductance. These were not the glucoreceptor neurons. The reversal potential of hyperpolarization was approximately -90 mV, E_K. Neither IBMX or forskolin did affect on this type of neurons.

The Med-AMG and VMH are estrogen sensitive target tissue that are involved in the integration of chemosensory information.

363.7 HIPPOCAMPAL FIELD-POTENTIAL AND NEURONAL RESPONSE FACILITATION AND DEPRESSION DURING PAIRED-PULSE STIMULATION IN HUMANS.

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The population spike evoked by stimulation of perforant path afferents in the angular bundle is modulated by behavioral state at all levels of the trisynaptic excitatory pathway within rodent hippocampus, such that transmission is maximal during slow wave sleep (SWS) and reduced during waking states (AWA) and REM (Winson and Abzug,1978, J. Neurophysiol., 41: 716). Studies in the human hippocampal formation have identified similar state dependent properties of neuronal transmission in this pathway.

Telemetry monitoring of activity from depth electrodes is required in some patients for identification of focal sites of epileptogenesis. In these patients, depth electrodes implanted in amygdala , pes hippocampi and the area of anterior parahippocampal gyrus homologous to the entorhinal cortex are stimulated and recorded in order to study the relative excitability of epileptic and non-epileptic regions. In the present study, paired or single stimulation pulses consisting of biphasic square waves of 100um duration were presented at a maximal rate of 0.1 Hz. Input/output (I/O) curves were obtained using a range of stimulus charge densities ranging from threshold to asymptote of average population spike responses, or a maximum of 50 uC/cm^2/ph. Conditioning and test pulses of equal intensity evoking conditioning responses which were a known percentage (e.g. 50%) of maximal were then used to assess changes in hippocampal formation excitability, by using pulse-pair intervals ranging from 20 to 1600 msec and observing test pulse amplitude. Behavioral state, neocortical EEG, EKG and respiration were monitored along with recordings from limbic sites.

I/O curves made during light sleep and SWS showed significantly increased amplitude when compared to those obtained during alert awake conditions (reading, conversing or watching TV) at all levels of stimulation. Thresholds decreased and response levels which had been asymptotic in the waking state were no longer maximal during SWS, reflecting increased effectiveness of synaptic volleys in evoking action potentials. Test pulse facilitation, which was usually prominent at intervals up to 200 msec during waking, was replaced by depression of the test pulse population spike response amplitude below conditioning response levels. Paired-pulse intervals which showed no interaction during the waking state (i.e. 400-800 msec intervals) showed test response depression during SWS. Single and multiple unit extracellular recordings from the same electrodes showed correlated increases and decreases in firing during the above changes in field potential amplitude, and verified their identification as population spikes.

The neurophysiological and behavioral significance of such state dependent modulation of transmission in the hippocampal formation will be discussed along with possible mechanisms of its origin. Supported by NIH grant NS 02808.

363.8 THE APICAL DENDRITES OF LAYER II COMMISSURAL NEURONS FORM DENDRITIC BUNDLES IN LAYER I OF RETROSPLENIAL CORTEX.

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Several researchers have reported that neurons in the deep layers of the cortex have apical dendrites which tend to cluster together and form dendritic bundles in layers III and IV of the cortex. In the present study we have identified a population of layer II neurons that have dendrites which cluster in layer I of the retrosplenial cortex in the rat. In 15 anesthetized rats, 5-20 nl of Fluoro-Gold or Fast Blue was pressure injected into the retrosplenial cortex via a 10 μm tip glass pipette. Follow a 2-4 day survival, the rats were transcardially perfused, and the brains were removed, sectioned on a freezing microtome at 25 μm and mounted on gelatin coated slides. After coverslipping the sections were inspected using a Leitz A fluorescent filter system. Among the commissural projections, it was clear that the contralateral layer II neuronal cell bodies had a point-to-point relationship with the injection site. Upon further inspection, it was noted that the apical dendrites of these layer II neurons tended to cluster together in bundles of 20-30 dendrites. The clusters had a width of approximately 100 μm, and the 150 μm on either side of the bundle was relatively free of labeled dendrites. Further, the dendritic bundles tapered in layer Ib giving the bundle a hourglass appearance. In this regard it should be noted that the outlying neurons which contributed to the bundle had apical dendrites which ran at up to a 45° angle to join the clusters. The pattern of the dendritic bundles was compared to anterograde autoradiographic labeling from commissural, associational or anterior thalamic injections of [^{3}H]-amino acids. There was a significant correlation between the pattern of the thalamic projections and that of the bundles. In contrast, the associational and commissural projections appeared to display the opposite pattern. These data demonstrate the existence of this previously undescribed pattern of dendritic bundling by commissural neurons and suggest that this pattern may be important in the integration of thalamic and commissural information. This study was supported by NIH grant #HL 34315.

363.9 INTRALIMBIC INHIBITORY DYNAMICS IN HUMANS. M. Isokawa-Akesson, T.L. Babb and C.L. Wilson. Brain Research Institute and Dept. of Neurology, School of Medicine, UCLA, Los Angeles, CA 90024

Human limbic neurons, amygdala(AMYG), anterior hippocampus(AH) and anterior entorhinal cortex(AENT), were examined extracellularly for their firing inhibition by applying local(intrastructural) and adjacent(interstructural) single electrical stimulation. Local stimulation of AMYG, AH and AENT produced a complete absence and/or significant reduction of firing in AMYG(283.4 msec in average duration of 50 stimuli, N=27), AH(191.4, N=35) and AENT(172.4, N=13), respectively. During local stimulation, graded inhibitory responses were observed in all the structures as the stimulus current was increased. The duration of the inhibition ranged from 0-250 msec in AH and 40-400 in AENT. This inhibition was mostly preceded by an initial excitation whose intensity showed a linear relationship to the duration of the following inhibition. The only condition under which a longer inhibition was observed in AH was a period of synchronized firings(412 msec, N=10; Isokawa-Akesson, et al. 1985. Neurosci. Abst. 11:1230) under the same stimulation method. Our data suggest that the inhibition observed in AH and AENT was mainly due to a local recurrent circuit whose activation depends on the degree of population neuronal firings. In AMYG, however, in addition to the excitatory-inhibitory firings based on the recurrent pathways(Takagi and Yamamoto, 1981. Brain Res. 206: 474) similar to those of AH and AENT, a longer duration of inhibition was observed in some neurons when a high current was administered. The duration of this inhibition was 550-680 msec and was not preceded by a proportionally-large excitation, suggesting that the nuclear structure of AMYG with greater cell densities could cause significantly greater inhibition than laminated cortical structures such as AH and AENT.

Adjacent electrical stimulation produced firing inhibition in only limited areas without any graded response. AENT stimulation produced inhibition orthodromically in AH for 246 msec (N=6), and this inhibition was slightly longer than the inhibition produced in AENT by stimulation of AH(192.8 msec, N=10). However, AENT stimulation did not influence AMYG firings. Only AH stimulation produced firing inhibition of 395.9 msec in AMYG, and this inhibition was longer than the locally produced AMYG inhibition (283.4 msec). Hippocampal influences on amygdala firings to induce inhibition have been studied in monkey(Morrison and Poletti, 1980. Brain Res. 192:353), and our findings agree with their reports. On the other hand, AMYG stimulation did not produce any firing inhibition on either AH or AENT, suggesting that AH has a stronger effect on AMYG neurons than vice versa to cause inhibition(sometimes with preceding excitation) through a direct hippocampal-amygdaloid pathways. Supported by NIH Grant NS02808.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS

364.1 SENSORY AFFERENTS IN THE DORSAL HORN OF THE SPINAL CORD CONTAIN ANGIOTENSIN II RECEPTOR BINDING SITES R.C.Speth, R.C.Ritter, E.H.South and S.R.White College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164-6520 and WOI Program in Veterinary Medical Education, Univ. Idaho, Moscow, Idaho

Receptor binding sites for angiotensin II (AII) are present in the dorsal horn of the spinal cord of the rat. These binding sites may be present on secondary afferent neurons, interneurons or axon terminals of afferent nerves which innervate this area. To determine whether afferent terminals of sensory neurons contain AII receptors, a unilateral rhizotomy was performed on a single lumbar root. After a 2 week survival time, the rats were anesthetized with sodium pentobarbital, perfused intracardially with ice-cold 0.9 % saline and the lumbar cord was removed, frozen, sectioned on a cryostat at a thickness of 20 microns in the coronal plane, and thaw mounted onto microscope slides. The sections were incubated with 0.25 nanomolar 125-I-Sarcosine-1,Isoleucine-8-angiotensin II in the presence and absence of 1 micromolar AII to radiolabel AII receptor binding sites. The sections were apposed to LKB Ultrofilm for 5-9 days to autoradiographically visualize the distribution of AII receptor binding sites which were defined as film areas in which the exposure exceeded that seen in film areas from the corresponding area of an adjacent section incubated with one micromolar AII. Additional alternate sections were placed in 3% paraformaldehyde in phosphate buffer (pH 7.4) to fix the sections. These sections were then incubated with an antibody to substance P for immunocytochemical staining of substance P in the spinal cord. Sections from a more rostral section of the lumbar spinal cord were also analyzed for AII receptor binding as a control region. At the level of entry of the sectioned root, AII receptor binding in the dorsal horn ipsilateral to the sectioned root was lower than that seen in the contralateral dorsal horn. By comparison, there was no difference in AII receptor binding between the right and left dorsal horn rostral to the segment of the spinal cord with the sectioned root. Immunocytochemical staining of Substance P immunoreactive material suggested that there was a concomitant loss of Substance P in ipsilateral dorsal horn of the segment with the sectioned root. These results suggest that some AII receptors in the dorsal horn of the spinal cord are located on presynaptic terminals of sensory afferent neurons, but that there may be other neuronal elements in the dorsal horn that contain AII receptors.

364.2 ANGIOTENSIN II (AII) RECEPTOR LOCALIZATION IN NEONATAL RAT BRAIN K. E. Moe and R. C. Speth. Dept. Psychology and Coll. Veterinary Medicine, Washington State University, Pullman, WA 99164.

AII receptors are reported to be elevated in neonatal rat brains but it has not been established whether this is due to higher receptor densities in regions known to have AII receptors or a wider receptor distribution in the brain. This study examined the distribution of AII receptors in the brains of neonatal (12 day old) male Sprague-Dawley rats by in vitro receptor autoradiography as described elsewhere in this volume (Dinh et al). AII receptor binding was widespread in the neonatal rat brain. In addition to the areas previously reported to have AII receptors, radioligand binding was intense in the glomerular layer of the olfactory bulb, and the entire medial surface of the olfactory bulb and frontal cortex along the longitudinal anterior cerebral fissure extending caudally to the anterior limit of the corpus callosum, where it was continuous with the induseum griseum dorsally and the organum vasculosum of the lamina terminalis (OVLT) ventrally. AII receptor binding sites continuous with the induseum griseum extended into the anterior cingulate gyrus. Moderate amounts of binding were also present in the external granule cell layer of the frontal cerebral cortex extending along its entire circumference, continuous with high levels of AII receptor binding in the pyriform cortex and olfactory tubercle. AII receptor binding sites continuous with the OVLT extended dorsally along the diagonal band of Broca to the septum and along the median preoptic nucleus to the subfornical organ. Another pathway of AII receptor binding sites extended from the olfactory bulb along the lateral olfactory tract to the nucleus of the lateral olfactory tract and the amygdaloid complex. AII receptor binding sites were present in moderate densities in several areas of the hypothalamus and intensely high densities in the subthalamus and discrete subnuclei of the thalamus over its entire rostro-caudal extent and was continuous with high densities of AII receptor binding sites in the superior colliculus. High binding was also seen in the periaqueductal gray and moderate binding was present in the inferior colliculus in the midbrain. In the pons AII receptor binding was present in the ventral transverse fibers, superior olivary nucleus, and several dorsal nuclei. High densities of AII receptor binding sites were present in the roof nuclei of the cerebellum, while the molecular layer and discrete areas of the granule cell layer of the cerebellar cortex displayed moderate AII receptor binding. In the medulla, AII receptor binding sites were high in the facial nucleus and several of the trigeminal nuclei in addition to previously reported nuclei. These results suggest that AII receptor binding in neonatal rat brain is much more extensive than that seen in adult animals.

364.3 **MICROREGIONAL DISTRIBUTION OF RENIN ACTIVITY WITHIN CATECHOLAMINE-CONTAINING MEDULLARY AND FOREBRAIN REGIONS OF THE DOG BRAIN.** P.A. Bridle*, K.B. Brosnihan, A. Husain*, R.R. Smeby, R. Howard* and C.M. Ferrario. Department of Brain and Vascular Research, Cleveland Clinic, Cleveland, OH 44106.

Although a functional interaction between the renin-angiotensin and catecholaminergic systems has been well documented, the neurochemical basis for an interaction in the central nervous system has not been explored. We have previously demonstrated a heterogeneous distribution of norepinephrine (NE) along the rostrocaudal extent of the catecholamine (CA)-containing nuclear regions in the medulla and hypothalamus of the dog. In this study we have characterized the microregional distribution of renin activity (RA) within the same regions.

The brains and pituitaries of 4 male adult anesthetized dogs were quickly removed and frozen immediately on dry ice. A block of brain stem tissue beginning 6 mm caudal to the obex and terminating at the level of the inferior colliculus was sliced in 2 mm sections. Micropunches (1 to 5 mg) verified anatomically to correspond to the A1 (lateral reticular nucleus), the A2 (dorsal motor nucleus of the vagus, nucleus tractus solitarii), the A5 (ventrolateral medulla at the level of the VII nerve) and A6 (locus coeruleus) were removed and the brain stem tissue remaining was pooled. Forebrain regions were similarly microdissected. Tissues were homogenized in a 0.1M phosphate buffer (pH 7.4) containing 10 mM EDTA, 0.01% NaN_3, 1 mM phenylmethyl-sulfonyl fluoride and N-ethyl maleimide and an aliquot was removed for protein determination. After 3 freeze/thaw cycles and centrifugation, two separate aliquots from the supernatant were incubated for 30 hrs at 37°C and 4°C, respectively, with partially purified species homologous renin substrate. The generated angiotensin I (Ang I) in each sample was measured by RIA and a RA unit (U) was defined as pg Ang I/mg protein/hour.

In the medulla, RA was detectable within all micropunches and averaged 3- to 7-fold higher than that measured in the remaining brain stem tissue (1.9 ± 1.3 U). Within the A2 region the highest level of RA (14.5 ± 3.2 U) was found between 3 and 5 mm rostral to the obex; within the A1 region the peak level of RA (10.3 ± 2.0 U) extended from the obex to 3 mm rostrally. The relative order of RA in the CA-containing regions was A2 > A1 > A5 > A6, the reverse order of average concentrations found for NE in these regions. Within the hypothalamus, RA ranged from 4.2 ± 1.9 U in the lateral basal hypothalamus to 0.2 ± 0.1 U in the anterior hypothalamus (AH). Other forebrain regions had levels of RA similar to the AH. The anterior (697 ± 105 U) and posterior (350 ± 250 U) lobes of the pituitary had substantially higher levels of RA than the hypothalamus. These data demonstrate a differential localization of renin within two CA-containing nuclear regions of the dog's medulla and within restricted regions of the hypothalamus. (Supported in part by NHLBI grant HL-6835).

364.4 RECEPTOR DISTRIBUTION IN THE PET SCAN PLANE OF HUMAN BRAIN: IN VITRO AUTORADIOGRAPHIC STUDIES. B.J. Morton, A.B. Young and J.B. Penney. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

There is a great deal of interest in imaging neurotransmitter receptors in vivo in living humans using positron emission tomographic (PET) scanning. However, there has been little systematic study of which regions of brain could be resolved on receptor PET scans given the low resolution of this technique. We approached this problem using in vitro receptor autoradiography. Whole cryostat sections from a normal human hemisphere were cut in 4 planes parallel to the orbito-meatal line and mounted on 5" x 7" subbed glass slides. The lowest plane went through cerebellar nuclei, amygdala and base of frontal lobe; the second through cerebellar vermis, midbrain, hippocampus, hypothalamus, basal forebrain and temporal and frontal cortices; the third through occipital, temporal and frontal cortices, hippocampus, globus pallidus, caudate, putamen and thalamus; the fourth through primary visual, auditory, somatosensory and motor cortices, thalamus, caudate and putamen. The slides were incubated with tritiated ligands and then exposed to Ultrofilm to produce autoradiograms (ARGs).

We have produced ARGs which display the distribution of total muscarinic receptors with 1 nM [3H]-QNB by the method of Fisher et al. (J. Neurosci. 1:1407, 1981); M1 weighted ARGs (1 nM [3H]QNB plus 300 µM carbachol); M_2 weighted ARGs (1 nM [3H]QNB plus 10 µM pirenzepine); mu opiate (12 nM [3H]naloxone plus 10 nM delta receptor peptide) and delta opiate (12 nM [3H]DADL plus 500 nM morphiceptin) receptor ARGs by the method of Abou-Khalil et al. (Brain Res. 323:21, 1984); benzodiazepine (BDZ; 4 nM [3H]flunitrazepam), glycine (10 nM [3H]strychnine) and GABA (240 nM [3H]muscimol) receptor ARGs by the method of Penney et al. (Eur. J. Pharm. 72:421, 1981); and specific GABA-A (15 nM [3H]GABA plus 100 µM baclofen) and GABA-B (15 [3H] nM GABA plus 40 uM isoguvacine) receptor ARGs by the method of Chu et al. (Neurosci. Abst. 11:725, 1985).

M1 receptors were high in cortex, hippocampus, caudate and putamen; M2 receptors in colliculi, periaqueductal grey, geniculates, basal nucleus and anterior thalamus. Opiate receptors were high in islands of Calleja, medial thalamus, amygdala, insular and association cortices but were low in primary cortices. BDZ receptors were high in layer 4 of cortex (particularly visual cortex) cerebellar molecular layer and moderate in basal ganglia. GABA-A receptors were distributed similarly to BDZ receptors except that they were highest in cerebellar granular layer. Specific glycine receptors were only found in periaqueductal grey. These ARGs can be used to predict how an ideal receptor PET scan might look. Supported by USPHS Grant NS15655.

364.5 Methods for Study of Pre- and Postsynaptic Processes of Dopaminergic System In Vivo Using Positron Emission Tomography (PET), 3-(2'-[^{18}F]Fluoroethyl) spiperone (FESP) and 6-[^{18}F]Fluoro-L-DOPA (FD). M.E. Phelps*, J.R. Barrio, S.-C. Huang, N. Satyamurthy, A. Luxen, R.A. Hawkins, J.C. Mazziotta. UCLA School of Medicine, Los Angeles, CA 90024.

Since short chain 3-N alkylation of spiperone does not significantly alter binding affinity to D_2 sites, we developed a one-step, high-yield, high-specific activity (SA) synthesis for FESP. In-vitro competitive binding with rat striatum confirmed its general binding similarity to spiperone (IC_{50} = 1.5 nM). Tracer kinetic studies were performed in monkeys with intravenous (I.V.) injections of 3-5 mCi of FESP (SA = 500-2000 Ci/mmole; dose = 1.5 to 10 nmole). Serial PET imaging showed ^{18}F activity rapidly accumulating in striatum. Striatum/cerebellum ratios increased linearly over time (2%/min) to a value of 5 at 4 hrs. Plasma clearance of FESP was rapid. HPLC analysis of plasma showed temporal increase of ^{18}F metabolic products. At 3 hrs only 5% of ^{18}F was FESP. In vivo dose response indicated half-saturation of D_2 sites at 10 µg/kg I.V. A tracer kinetic model was formulated from in vivo and in vitro studies in our lab on the mechanisms of spiperone transport, binding, retention and clearance. This model provides estimates of the ratio of receptor density, B_{max} to equilibrium dissociation constant, K_D or the product of B_{max} and association constant, k_a, but not the individual terms. A double injection approach was developed to resolve this ambiguity. First high SA FESP (650-1000 Ci/mmole) was injected I.V. and kinetic PET and blood data were collected for 90 min, followed by a second injection of lower SA FESP (15.7 Ci/mmole) and 90 min data collection. This approach attempts, in a single study, to combine the properties of a dynamic approach (1st injection) providing transport and binding information with those of "equilibrium displacement" (2nd injection) in which a measurable fraction of the receptors is blocked by the unlabeled ligand. Slow ligand dissociation is a necessary condition. This approach yielded B_{max}=11 pmoles/g and k_a=0.86/(min µmole) in good agreement with in vitro values. A high yield synthesis of FD was also developed. FD is a competitive substrate with L-DOPA for neutral amino acid transport and enzymatic conversion to dopamine. Monkeys were injected with 5 mCi of FD for 3 hr serial imaging and blood sampling. ^{18}F activity rapidly accumulated in striatum for 20 min, then slowly decreased. Clearance from cerebellum and cortex was rapid with striatum/cerebellum ratio of 4 by 3 hrs. With physiological and pharmacological interventions, these techniques provide novel approaches for probing dynamic and regulatory biochemical processes of neurotransmitter systems in the intact functioning brain.

364.6 **NONEQUILIBRIUM MEASUREMENT OF RADIOLIGAND-RECEPTOR BINDING IN VIVO USING PET AND 18F-SPIROPERIDOL.** JS Perlmutter*, M Kilbourn*, M Welch*, ME Raichle (SPON: T Videen). Washington Univ Sch of Med, St. Louis, MO 63110

We have previously described a method using positron emission tomography (PET) and 18F-spiroperidol for the measurement of radioligand- receptor binding in vivo. In this investigation, we demonstrate the sensitivity of this technique to alterations in the maximum number of available specific binding sites.

All studies were performed on 2 baboons and repeated studies on each animal were at least 3 weeks apart. Each baboon was anesthetized, paralyzed and then injected IV with unlabeled spiroperidol (.001mg/kg to .040mg/kg) to block a number of specific binding sites. Two hours after pretreatment, regional blood flow and blood volume were measured using PET and 15O-labeled H_2O and CO, respectively. Three hours after pretreatment, 3.5 to 6.5 mCi of no-carrier-added 18F-spiroperidol (containing less than 2 to 4 µg of spiroperidol) were administered IV. Sequential PET scans and measurements of arterial-blood radioactivity due to radioligand and its labeled metabolites continued for at least 2.5 hours. The fraction of radioligand bound in the plasma was measured for each experiment by ultracentrifugal microfiltration.

A three-compartment model describing the behavior of radiotracer and a parameter estimation scheme were used to estimate the combined forward-rate constant [equal to the product of the maximum number of available binding sites (B_{max}) times the association rate constant (k,(1/sec)] and the regional permeability-surface-area product (PS,cc/sec) for the striatum. The results for one of the baboons is listed below.

	pretreatment dose of unlabeled spiroperidol (mg/kg)					
	None	.001	.005	.010	.025	.040
PS	.063	.060	.039	.054	.050	.043
k	.049	.027	.016	.018	.007	.004

These results demonstrate that this nonequilibrium method for estimating radioligand-receptor binding in vivo is sensitive to small alterations in the maximum number of available specific binding sites. The lowest dose of unlabeled spiroperidol used was at least 50 times lower than the amount found to have any detectable clinical effect in people. This technique should be sufficiently sensitive to detect changes in radioligand-receptor binding that are clinically relevant.

364.7 ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF BETA-ADRENERGIC RECEPTORS IN PRE- AND POSTSYNAPTIC PROCESSES OF NEURONS AND IN ASTROGLIA OF THE CORTEX AND NEOSTRIATUM. C. Aoki, T.H. Joh, and V.M. Pickel. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

The β2-adrenergic receptor (BAR) purified from frog erythrocytes has been immunocytochemically localized at postsynaptic densities of the frog hippocampus and rat cerebellum (Strader, PNAS 80:1840). We sought to determine whether other sites on neurons or glia exhibited immunoreactivity for the BAR in areas receiving sparse (neostriatum) or dense laminated (cortex) noradrenergic innervation. Rabbit polyclonal antiserum to the previously purified frog erythrocyte BAR was localized by the peroxidase-antiperoxidase (PAP) method. The cortical and striatal tissues included aldehyde-fixed vibratome sections and freshly prepared synaptosomes.

The most commonly labeled profiles were postsynaptic densities (PSDs) of the perforated and non-perforated type in both sections and synaptosomal preparations from the cortex and striatum. The labeled PSDs constituted 12% of the total recognized junctions in synaptosomal fractions from the cortex. The labeling at postsynaptic sites appeared to be diminished by preincubation with the agonist, isoproterenol (100 uM). In favorable planes of setion, isolated saccules of smooth endoplasmic reticulum and vesicles in dendrites and soma also exhibited peroxidase immunoreactivity. More rarely, myelinated and unmyelinated axons and small (0.1-0.5 um) axon terminals also contained immunocytochemical labeling. The peroxidase reaction product was associated with the plasmalemma and the outer membranes of synaptic vesicles in axon terminals. In addition to labeling in neuronal processes, selective astrocytes also exhibited peroxidase immunoreactivity. The intensity of labeling in astrocytes was considerably greater than in neurons. In astroglia, the peroxidase product was diffusely distributed in the cytoplasm and primarily found along the intracellular surface of plasma membranes. These findings establish that BAR are localized to postsynaptic sites in higher mammals and are detected in areas containing varying amounts of noradrenergic innervation. In addition, the localization within axon terminals and astroglia is consistent with presynaptic and non-neuronal, modulatory functions for BAR. The intracellular localization in both neurons and astrocytes suggests that the antiserum may primarily recognize or be accessible to newly synthesized and/or degraded BAR.

(Supported by grants NSF 8320120 and NS07782-02)

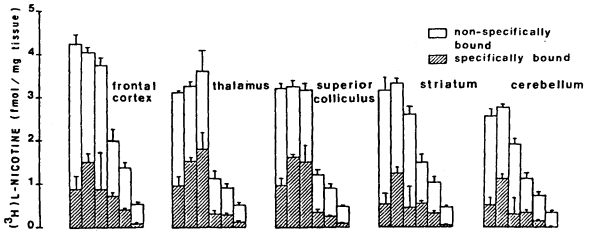
364.8 IN VIVO BINDING OF [^{3}H]L-NICOTINE IN THE MOUSE BRAIN. E. Broussolle*, D. Wong* and E. London (SPON: W. Pickworth). Neuropharmacology Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224.

In vitro studies with [^{3}H]nicotine showed heterogeneous, specific binding in rat brain (Clarke, P. et al., Brain Res. 323:390, 1984; London, E. D. et al., Neurosci. Lett. 53:179, 1985). Labelling was dense in the interpeduncular nucleus, medial habenula, superior colliculus, and thalamic nuclei. The present work aimed to study cerebral nicotinic receptors in vivo.

Adult male ICR mice received [^{3}H]L-nicotine ([^{3}H]N, 5 μCi, i.v., specific activity = 75.7 Ci/mmol), and were killed at various times thereafter. They received saline or unlabeled L-nicotine (5 mg/kg s.c.) 2 min before [^{3}H]N to assess total and nonspecific binding, respectively. Brains were dissected into 13 regions for measurement of radioactivity.

Brain entry of [^{3}H]N was rapid, and radioactivity declined after 7.5 min, as previously reported (Appelgren, L.-E. et al., Acta. Physiol. Scand., 56: 249, 1962). Specific binding, absent at 1 min, appeared at 2.5 min, peaked at 5 min (50% total binding in the thalamus and superior colliculus) and fell nearly to 0 by 30 min. Cytisine was as potent as L-nicotine in displacing [^{3}H]N binding, but mecamylamine was ineffective. Nonspecific binding was heterogeneous. It is possible that nonspecific binding was overestimated because of the limited dose of unlabelled nicotine that could be used due to toxicity, and nicotine's effect to increase cerebral blood flow.

Our findings suggest that [^{3}H]N can serve as a ligand for in vivo studies of cerebral nicotinic receptors. Estimates of nonspecific binding might be improved by using a displacer which lacks nicotinic agonist effects. More accurate and favorable ratios of specific/nonspecific binding may be obtained with quantitative autoradiographic techniques using the in vivo model.



Minutes after [^{3}H]N (left to right: 2.5, 5,7.5, 10, 15, 30 min). Each point shows the mean ± SEM for 4-6 mice.

364.9 TOPOGRAPHICAL ORGANIZATION OF THE CHOLINERGIC INNERVATION, MUSCARINIC RECEPTOR SITES AND cGMP-IMMUNOREACTIVITY IN RAT BRAIN. J.A.D.M. Tonnaer*, B.H.W. Ernste*, H.W.M. Steinbusch and C.L.E. Broekkamp* (SPON: European Neuroscience Association). Dept. CNS Pharmacology, Organon International B.V., 5340 BH Oss, and Dept. Pharmacology, Vrije Universiteit, 1081 BT Amsterdam, The Netherlands.

Employing ^{3}H-hemicholinium (^{3}H-HC), ^{3}H-pirenzepine (^{3}H-PZ) and ^{3}H-quinuclidinyl benzilate (^{3}H-QNB), autoradiographic binding studies were performed to identify and quantitate the localization of high-affinity choline carriers, M1-subtype muscarinic binding sites and a mixed population of M1- and M2-subtypes of muscarinic binding sites, respectively, in rat brain. Immunocytochemical studies with polyclonal antibodies against cGMP were carried out to visualize those sites in the brain where muscarinic receptors are coupled to guanylate cyclase as the effector system.

Labelling of adjacent brain sections with ^{3}H-HC, ^{3}H-PZ and ^{3}H-QNB revealed different topographical patterns of the respective binding sites. ^{3}H-HC binding, which is supposed to reflect cholinergic innervation, was highest in the accumbens nucleus, olfactory tubercle, caudate-putamen, dentate gyrus, amygdala and interpeduncular nucleus. Fimbria-lesioning resulted in a significant reduction of ^{3}H-HC binding in the hippocampus and dentate gyrus. ^{3}H-PZ binding was predominant in the forebrain and marginal in midbrain and brainstem areas. Maximal ^{3}H-PZ binding was found in the accumbens nucleus, in discrete substructures of the hippocampus (e.g. CA1 field of the oriens layer and the subiculum), and in the molecular layer of the dentate gyrus. The binding of ^{3}H-QNB was more evenly distributed over the brain as compared to that of ^{3}H-PZ. ^{3}H-QNB binding clearly exceeded the binding of ^{3}H-PZ in thalamic and hypothalamic areas and in the brain stem.

There was no relation between the density of the cholinergic innervation and the binding capacity of any of the muscarinic sites in the various brain regions. A correlation was found between the topography patterns of the ^{3}H-PZ and ^{3}H-QNB binding sites. However, some brain areas showed preference for one of the two ligands, probably reflecting a distinct localization of M1- and M2-subtypes of muscarinic binding sites.

cGMP-immunoreactive neuronal elements were observed in discrete forebrain areas such as the CA2 field of the hippocampus and the reticular thalamic nucleus. In vitro treatment of hippocampal slices with carbachol resulted in an enhancement of the cGMP staining. A comparison of the topography of muscarinic binding sites and cGMP-immunoreactive areas would suggest that cGMP is a second messenger of M2-type rather than M1-type muscarinic receptors in the rat forebrain.

364.10 AUTORADIOGRAPHIC LOCALIZATION OF ADENOSINE UPTAKE SITES AND ADENOSINE A1-RECEPTORS IN GUINEA PIG BRAIN USING [^{3}H]DIPYRIDAMOLE, [^{3}H]NITROBENZYLTHIOINOSINE AND [^{3}H]CYCLOHEXYLADENOSINE. J. DECKERT*, J.C. BISSERBE* AND P.J. MARANGOS (SPON: D.A. TAYLOR). Unit on Neurochemistry BPB, NIMH and Section on Neurochemistry, LCS, NIAAA, Bethesda, Md. 20892.

Dypridamole and nitrobenzylthioinosine have both been shown to be potent inhibitors of adenosine uptake into guinea pig cerebral cortical slices (Huang M. and Daly, J.W., Life Sci., 14:489, 1974) and binding of [^{3}H] cyclohexyladenosine to guinea pig brain membranes has been shown to demonstrate characteristics of A1-receptors (Bruns, R.F., Daly, J.W., and Snyder, S.H., Proc. Natl. Acad. Sci. USA, 77: 5547, 1980). In this study we show the autoradiographic distribution of the respective binding sites of [^{3}H] dipyridamole, [^{3}H] nitrobenzylthioinosine and [^{3}H] cyclohexyladenosine in guinea pig brain.

Slide-mounted guinea pig brain sections were prepared and binding of the tritiated ligands was performed essentially as described before (Bisserbe, J.C., Patel, J. and Marangos, P.J., J. Neurosci. 5: 544, 1985 and Bisserbe, J.C., Deckert, J. and Marangos, P.J., Neurosci. Lett., in press).

The localization of adenosine A1-receptors as labelled by [^{3}H] cyclohexyladenosine in guinea pig brain resembles their localization as previously described in rat brain with its characteristic distribution in hippocampus (molecular and polymorphic layer) and cerebellum (molecular layer). Similarly the localization of adenosine uptake sites as labelled by [^{3}H] nitrobenzylthioinosine is comparable to that described in rat brain. [^{3}H] nitrobenzylthioinosine labels a high affinity class of binding sites with a Bmax and K_d similar to rat brain (Bmax = 134 ± 19 fmol/mg protein, K_d = 0.59 ± 0.08 nM). On the contrary [^{3}H] Dipyridamole binds to a substantially high number of sites (Bmax = 650 ± 100 fmol/mg protein, K_d = 10 ± 1.5 nM).

In concordance with the Scatchard experiments binding of [^{3}H] dipyridamole to its sites can be inhibited by Nitrobenzylthioinosine in nanomolar concentrations only to about 50% as could be shown in classical binding experiments and in autoradiographic experiments. One of the more striking differences to the previously described distribution of [^{3}H]Nitrobenzylthioinosine binding sites is the finding that there is a high intensity of adenosine uptake sites as labelled by [^{3}H] Dipyridamole in the molecular layer of cerebellum.

This localization in cerebellum is similar to that of adenosine A-1 receptors labelled by [^{3}H] cyclohexyladenosine as one would expect under the assumption of a functional adenosinergic neuromodulatory system.

364.11 **SELECTIVE BINDING OF AN ENDOGENOUS CLONIDINE-LIKE SUBSTANCE TO IMIDAZOLE BINDING SITES AND DISTRIBUTION OF THESE SITES IN MEDULLA OBLONGATA OF THE RAT.** P. Ernsberger, V. Arango, M.P. Meeley, and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., N.Y., N.Y. 10021.

Imidazole binding sites (IMID) labeled by ^{3}H-p-aminoclonidine (^{3}H-PAC) are present in ventrolateral medulla (VLM) (Ernsberger et al., SNA 11:490, 1985). IMID exhibit a high affinity not only for clonidine analogs but also for other imidazoles, and may mediate the vasodepressor action of these compounds in the rostral VLM (Granata and Reis, this meeting). An endogenous clonidine-displacing substance (CDS) inhibits ^{3}H-PAC binding in bovine VLM membranes and acts to lower arterial pressure when microinjected into rostral VLM of the rat (Meeley et al., Life Sci., 38:1119, 1986). We sought to determine whether IMID are localized in the rostral VLM of the rat using quantitative autoradiography, and whether CDS binds selectively to IMID in bovine VLM membranes.

^{3}H-PAC binding sites were labeled in slide-mounted sections of rat medulla (Unnerstall et al., Brain Res. Rev. 7:69, 1985); nonspecific binding was defined by the imidazole phentolamine (10 µM). IMID were identified by displacement of ^{3}H-PAC (2 nM) with imidazole-4-acetic acid (IAA), a compound with 1,000-fold selectivity for IMID over adrenergic sites. Alternate sections were 1) wiped from the slide and counted directly, or 2) exposed to Ultrofilm. Autoradiograms were quantified by image analysis and corrected for autoradiographic efficiency (see Giuliano et al., this meeting). Specific ^{3}H-PAC sites in rat medulla were concentrated in the N. Tr. Solitarii (NTS) (44±4 fmol/mg wet weight), rostral VLM (26±2), and dorsolateral reticular formation (22±2). IAA displaced ^{3}H-PAC binding in medullary sections (direct counting). Dose-dependent displacement was localized autoradiographically in rostral VLM and in NTS.

log [IAA] =	-7	-6.5	-6	-5.5	-4.5
Rat Medulla					
Whole (counting)	78±8	70±11	53±11	38±12	37±17
NTS (imaging)	94±4	97± 9	91±12	89± 5	77± 7
rVLM (imaging)	96±7	84± 3	75± 7	72± 7	73±17
Calf medulla					
VLM membranes	91±3	89± 1	83± 3	73± 3	49±4

Values are % total specific ^{3}H-PAC binding; mean ± SE, n=3-8.

Dose-dependent displacement of ^{3}H-PAC binding by IAA was also observed in bovine VLM membranes. CDS also displaced ^{3}H-PAC binding, interacting with two classes of ^{3}H-PAC sites: 70% had low affinity for CDS, while 30% had 9-fold higher affinity. ^{3}H-PAC sites with a low affinity for CDS were adrenergic, since they were blocked by norepinephrine (10 µM), while norepinephrine did not block high-affinity sites.

We conclude that 1) IAA inhibits ^{3}H-PAC binding and can be used to selectively localize IMID; 2) IMID are present in rat medulla, principally in autonomic nuclei; 3) CDS binds preferentially to non-adrenergic ^{3}H-PAC sites equivalent to IMID in VLM and may act as an endogenous ligand for these sites; and 4) IMID may play a role in central autonomic control.

364.12 DISTRIBUTION OF NEUROTRANSMITTER RECEPTORS IN THE HUMAN VISUAL CORTEX POSTMORTEM. A. Biegon and M. Schenk*. Weizmann Institute of Science, Rehovot, Israel.

The relative importance of various neurotransmitters in higher brain functions may depend, among other factors, on the density of receptors in cortical regions subserving these functions. The distribution of the following receptor types was studied in sagittal sections of the human brain postmosrtem:noradrenergic (alpha and beta) serotonergic (s1 and s2), cholinergic muscarinic, benzodiazepines and GABA. Brains were obtained from drug and neuropathology free subjects with a postmortem delay not exceeding 40 hours. The receptors were labeled by incubation of 40m thick cryostat sections with appropriate tritiated or iodinated ligands (e.g. Biegon et al., Brain Res. 363:91, 1985). Alpha adrenergic receptors were labeled with tritiated prazosin (alpha 1) or paraamino clonidine (alpha 2); with or without excess phentolamine to define non specific binding. Iodinated pindolol was used to label beta adrenergic receptors, with propranolol to block non specific binding. Serotonin-1 receptors were labeled with 4nM 3H-5HT with cold serotonin as displacer, while s2 receptors were labeled with 1251-iodo LSD in the presence of sulpiride (specific dopamine receptor blocker) with excess cold mianserine serving to define non specific binding. Cholinergic muscarinic were labeled with 3H-quinuclidinyl benzilate (QNB) ±atropin, benzodiazepines: 3H-diazepam or midazolam ± excess diazepam, and GABA:3H-muscimol ± GABA. Each and every one of the receptor types examined exhibits a unique cortical distribution and lamination pattern. The cortical lamination of receptor subtypes appears to be complimentary: alpha 1 receptors are dense in external and internal cortical layers while alpha 2 receptors are concentrated in middle cortical layers. S1 binding is highest in cortical layer 1 throughout most of the cortex, and S2 receptors predominate in middle layers. In addition, sharp changes in lamination and density of binding are seen in the primary visual cortex (area 17) with some but not all receptors. The most striking is the S1 receptor, showing extremely high density in the middle layers of area 17, higher than the density observed in layer 1 which otherwise contains the highest concentration of receptors. Since most serotonergic drugs are hallucinogenic, it is possible that hallucinogenic properties of drugs may be correlated with dense and highly laminated binding pattern in the visual cortex.

DEVELOPMENT AND PLASTICITY: PROCESS OUTGROWTH V

365.1 MODULATION OF GROWTH CONE MOVEMENT ON SURFACES COATED WITH FIBRONECTIN AND LAMININ. P. Letourneau, I. Pech*, J. McCarthy*, S. PALM*, and L. Furcht. Depts. of Cell Biology and Neuroanatomy, and Laboratory Medicine and Pathology, Univ. of Minnesota, Minneapolis, MN 55455.

In order to advance across substrata and promote neurite elongation, growth cones must get traction via adhesion to other surfaces. Fibronectin (FN) and laminin (LM) are two glycoproteins of extracellular matrices that may mediate growth cone anchorage in vivo (Rogers et al., Dev. Biol. 98:212, 1983; Dev. Biol. 113:429, 1986). This study probed mechanisms of growth cone adhesion to FN and LM by suing a peptide sequence from FN, heparin and an antibody to a putative cell surface FN and LM receptor (CSAT, provided by A. Horwitz, Univ. of Penn.; Horwitz et al., J. Cell Biol. 101:2134, 1985) as potential inhibitors of growth cone adhesion and movement across FN- and LM-treated substrate. Dorsal root ganglia and spinal cords were removed from 10d and 7d, respectively, chick embryos, cut into explants, and placed into FN- or LM-treated culture dishes. After 24 hr incubation, dense networks of neurites extended from the explants onto the in vitro surfaces. A field of growth cones at the edge of an outgrowth was chosen and recorded by time lapse video microscopy for at least 90 minutes. Then, an aliquot of the FN tetrapeptide (Arg-Gly-Asp-Ser), heparin or the CSAT antibody to the putative FN/LM receptor was added, and videorecording continued for at least 60 minutes. Growth cone velocities were determined from videotapes by tracking growth cone positions at 10 minute intervals. Addition of 10 µg/ml CSAT antibody rapidly stops growth cones from advancing on FN- and LM-treated surfaces, although filopodial motility continues. Similarly, heparin stops growth cones advance in FN and LM. Addition of 2mM FN tetrapeptide stops growth cones on FN, but growth cones continue to advance on an LM-treated substratum in the presence of the FN tetrapeptide. Initial examinations of cell-substratum adhesion by interference reflection microscopy indicate that addition of CSAT interferes with adhesion of growth cones to the substratum. These studies indicate that growth cones adhere to FN and LM by way of surface components that bind to specific domains of the extracellular glycoproteins.

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365.2 ROLE OF EXTRACELLULAR MATRIX AND ENDFOOT "TANGLES" IN AXONAL PATTERNING OF THE AVIAN TELENCEPHALON. M.R. Palmert, M.J. Kujawa, A.I. Caplan, and J. Silver. Depts. of Dev. Genetics and Biol., Case Western Reserve Univ., Cleveland, OH., 44106.

We have examined the role of extracellular matrix (ECM) in patterning axons in the developing chick telencephalon at embryonic days (E) 4,5,6,8, and 10 by investigating the spatiotemporal relationship between axonal growth and ECM using antibodies against: 1) a neural cell adhesion molecule (NCAM, which stains axons and glia); 2) a neural-glial adhesion molecule (NgCAM, which stains only axons); and 3) a component of the ECM, chondroitin 6-sulfate (C6S). TEM was utilized to study the patterning of axons and support cells.

At E4, C6S was localized to the ventral and ventro-medial marginal zone (olfactory and septal regions). The location of axons (determined by NgCAM staining and TEM) resembled the C6S pattern. NCAM was also present in the ventral marginal zone but extended dorsally beyond the axons in both the medial and lateral walls of the telencephalon. In the E5 telencephalon, the C6S was in a ventral band that extended into the intermediate zone; lighter staining appeared more dorsally in both the lateral and medial marginal zones. Axons were localized in the ventral and lateral portions of the telencephalic marginal zone but were excluded from the middle of the medial wall where a dense "tangle" of neuroepithelial endfeet containing invaginated basal lamina was located. NCAM was distributed broadly around the circumference of the hemisphere. At E6, C6S was present in the ventral (piriform), ventro-medial (olfactory and septal), and expanding lateral (striatal) areas but was minimal in the "tangle." Dorsally (in the cortex), 2 distinct bands corresponding to the marginal zone and upper surface of the ventricular zone were evident. The axonal pattern mimicked the localization of the C6S staining except for the absence of axons in the deeper band. Like the C6S staining, axons were absent from the middle of the medial wall, the location of the "tangle." NCAM and axon (NgCAM) patterns were similar except for positive staining of NCAM in the "tangle" where axons were absent. At E8, the ventral C6S band was still intense. C6S staining of the lateral (striatal) and dorsal (cortical) areas was lighter and more diffuse with the 2 dorsal bands less discernable. The "tangle" was no longer present and axons now appeared in the middle of the medial wall. By E10, the C6S staining diminished throughout the telencephalon.

In summary our data demonstrate that the localization of C6S staining closely mimics the pattern of early axons throughout the avian telencephalon. In the medial wall, where early axons do not grow, the "tangle" may serve transiently as a physical and molecular barrier to the passage of axons.

365.3 SUBSTRATES FOR DIRECTED OUTGROWTH FROM DISPLACED GANGLION CELLS INTO THE RODENT SUPERIOR COLLICULUS. M.H. Hankin and R.D.Lund, Dept. of Neurobiology, Anatomy and Cell Science, and Center for Neuroscience, Univ. of Pittsburgh, Sch. of Medicine, Pittsburgh, PA 15261

When embryonic retinae are placed on the midbrain of neonatal rats, axons grow across the surface to innervate subcortical visual centers. We have investigated whether there is evidence of target-directed growth to the tectum, and whether the surface of the brain is an obligatory substrate for growing optic axons as in normal development.

Mouse retinae transplanted to the cerebral aqueduct of neonatal rats matured relatively normally and emitted axons that grew dorsally towards the tectum rather than along the aqueduct surface or the defect caused by introducing the transplant. Fibers leaving the transplant from its dorsal side always projected radially from the graft, whereas fibers exiting ventrally grew around the retina and then radially towards the superficial layers of the superior colliculus. Axons reached the superficial layers of the superior colliculus either by running dorsally along the midline, or by following an arching course along the orientation of radial glial processes.

A series of additional experiments suggested that the outgrowth was target-directed and specific to optic axons. The directed optic outgrowth was probably not due to mechanical factors associated with transplantation first because outgrowth was not seen along the injection tract or aqueduct surface and secondly, because cortical transplants placed in a similar manner showed more diffuse outgrowth. Outgrowth from retinae was oriented toward the tectum even in the presence of a cortical lesion or when enucleation was combined with a cortical lesion. This suggests that the outgrowth was not a generalized response to deafferentation. The earliest dectectable fibers grew towards the tectum.

Thus, it appears that optic axons can use different substrates from normal in their growth to the tectum. It is possible that radial glia, because of their orientation, could fulfill this role, however, they alone are insufficient to support optic axon outgrowth since axons fail to grow out from grafts placed more ventrally in the midbrain tegmentum. We suggest that a neurotropic factor released by the superficial layers of the tectum, in combination with radial glia could provide a substrate for growth.

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365.4 NEURITES GROWING ON AN AXONAL SUBSTRATE ARE SHORTENED BY FABS AGAINST NEURAL CELL SURFACE GLYCOPROTEINS. S.Chang, F.G.Rathjen+, E.B.Grunewald+, and J.A. Raper. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, FRG.

We have developed a new in vitro assay which quantifies the extension of growth cones on axonal substrates. The lengths of chick sympathetic neurites elongating on chick sympathetic axons were measured in the presence or the absence of antibody Fab fragments. Several hundred neurites were measured for each condition and the data analyzed for statistical significance using the Wilcoxon rank test. The Fab fragments we used were directed against the well characterized neural cell adhesion molecule (NCAM), and against two recently purified neural cell surface glycoprotein antigens (E4, F11). The E4 and F11 antigens both have major bands at 135 kDa, and are clearly distinct from NCAM, laminin, or fibronectin.

It was found that anti-NCAM Fabs (1 mg/ml) did not significantly reduce neurite lengths as compared to controls, although NCAM is present on the surfaces of sympathetic axons, and the same anti-NCAM Fabs inhibit retinal cell-cell adhesion.

Anti-E4 Fabs (0.1-1 mg/ml) and anti-F11 Fabs (1 mg/ml) significantly reduce neurite lengths as compared to controls. The average neurite length measured in the presence of anti-E4 Fabs is 60-70 % of the control value; the average neurite length measured in the presence of anti-F11 Fabs is 80% of the control value. Time course experiments show that the reduction in average neurite lengths caused by the Fabs is due to a decrease in the rate of outgrowth. In the presence of anti-E4 Fabs the rate of neurite elongation is about one-third the control value. In the presence of anti-F11 Fabs the rate is about two-thirds the control value. The effect of the two Fabs added totether is the sum of each Fab alone. Outgrowth on laminin substrates is unperturbed in the presence of the Fabs, indicating that their effects are substrate specific, and are not due to a general decrease in cell health or motility.

The E4 and F11 antigens are present in the spinal cord and retina very early in development, after E3-4 through maturity. They are found throughout the white matter of the spinal cord and they stain complementary fiber layers in the E9 retina.

These results suggest that the E4 and F11 antigens play a role in the extension of growth cones on axons.

365.5 CYTOTACTIN, AN EXTRACELLULAR MATRIX PROTEIN OF NEURAL AND NON-NEURAL TISSUES THAT MEDIATES GLIA-NEURON INTERACTION Kathryn L. Crossin*, Stanley Hoffman, Martin Grumet, and Gerald M. Edelman. Laboratory of Developmental and Molecular Biology, The Rockefeller University, New York, New York, 10021

A new extracellular matrix protein, cytotactin, has been identified, isolated, and partially characterized. Cytotactin is involved in glia-neuron adhesion in vitro, but unlike the neuron-glia cell adhesion molecule, it is absent from neurons. It is found at the surface of glia in culture in a cell-associated fibrillar pattern. Cytotactin was isolated from 14-day embryonic chicken brains as structurally-related polypeptides of Mr 220,000, 200,000 and 190,000. The peptides from brain were easily extracted into isotonic buffers without detergent, but appeared to be highly disulfide bonded to each other. In preliminary experiments to investigate cytotactin-mediated binding, beads bearing covalently-coupled cytotactin bound specifically to neurons. Although cytotactin was identified as a molecule that mediates glia-neuron interactions, preliminary immunohistochemical localization of the molecule suggested that it was an extracellular matrix protein with a widespread but nonetheless more restricted distribution than either fibronectin or laminin. Cytotactin appears in the basement membrane of the gastrulating chicken embryo, and in the developing neural tube, notochord and somites in a temporal sequence beginning in the cephalic regions and proceeding caudally. Between 2 and 3 days of development, the molecule is present at high levels in the early neural crest pathways (surrounding the neural tube and somites) but, in contrast to fibronectin and laminin, is not found in the lateral plate mesoderm or ectoderm. At later times, cytotactin is expressed extensively in the central nervous system, in lesser amounts in the peripheral nervous system, and in a number of nonneural sites, most prominently in all smooth muscles and in basement membranes of lung and kidney. Cytotactin appears in adult tissues with distributions that are similar to those seen in embryonic tissues. The survey of the times and sites of cytotactin appearance during embryogenesis raises the possibility that certain extracellular matrix proteins contribute to pattern formation in embryogenesis as a result of their restricted expression in a spatiotemporally-regulated fashion at some sites but not at others. Supported by USPHS Grants AM-04256, HD-16550, HD-09635, NS-21629, and a Senator Jacob Javits Center of Excellence in Neuroscience Grant (NS-22789).

365.6 THE TISSUE SPECIFICITY OF CONTACT MEDIATED AVOIDANCE BETWEEN GROWTH CONES AND AXONS. J.A. Raper and J.P. Kapfhammer+. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, FRG.

Experiments using invertebrate and vertebrate preparations indicate that growth cones are able to distinguish between labels differentially associated with axons. For example, cultured embryonic day 6 (E6) chick retinal neurites prefer not to mix with E8 sympathetic neurites, while E6 retinal neurites mix freely with other retinal neurites, and E8 sympathetic neurites mix freely with other sympathetic neurites. Time lapse studies show that retinal growth cones maintain their normal, flattened, motile morphology when they contact retinal axons. The same is true for sympathetic growth cones contacting sympathetic axons. However, retinal growth cones which contact sympathetic axons, or sympathetic growth cones which contact retinal axons, generally lose their normal motile morphology, and after collapsing, withdraw backwards.

We have cultured a variety of embryonic chick neural tissues together to determine which growth cone-neurite pairs lead to contact mediated retraction. We cultured most of the possible pairings of E6 retina (RET), E4 ventral diencephalon (DEN), E8 sympathetic ganglia (SYM), E8 dorsal root ganglia (DRG), and E8 ciliary ganglia (CIL). We made time lapse films of collisions between growth cones and neurites which could be traced back to their explants of origin. A sufficient number of randomly selected cases were filmed so that the results could be compared quantitatively.

Growth cones meeting neurites of the same type do not retract from each other: e.g. RET growth cones meeting RET neurites, SYM meeting SYM, DRG meeting DRG, and CIL meeting CIL.

Growth cones meeting axons of another type either retract or not depending on the specific pairings involved. The following unlike pairings result in growth cone retraction: RET growth cones meeting SYM neurites, RET meeting DRG, RET meeting CIL, SYM meeting RET, DRG meeting RET, CIL meeting RET, SYM meeting DEN. The following unlike pairings do not result in growth cone retraction: RET growth cones meeting DEN neurites, SYM meeting DRG, DRG meeting SYM, and CIL meeting SYM. Mixed results are obtained with SYM growth cones meeting CIL neurites.

The data thus far collected fit the following rule: peripheral growth cones retract from central neurites, central growth cones retract from peripheral neurites, peripheral growth cones do not retract from peripheral neurites, and central growth cones do not retract from central neurites.

365.7 TARGET SPECIFICATION OF MORPHOLOGICAL CHARACTERISTICS OF IDENTIFIED LEECH NEURONS DURING EMBRYOGENESIS. C.M. Loer, J. Jellies, and W.B. Kristan, Jr. Dept. of Biology, UCSD, La Jolla, CA 92093.

The segmental nervous systems of annelids and arthropods arise from seemingly identical blocks of precursors which are reiterated along the length of the animal. Despite initial similarity, segmentally homologous neurons often differ considerably in morphology, physiology, and consequent function. Such differences could result from variations in the developmental program in different segmental precursors as well as differences in the segmental environment. We have found that the development of some morphological characteristics of an identified leech neuron, the Retzius (Rz) cell, depends upon the presence of its segment-specific target tissue.

A pair of large serotonergic Rz cells is found in each segment of the leech, Hirudo medicinalis. While most Rz cells diffusely innervate body wall of their own segment and each adjacent segment, those in the sex ganglia (5 and 6) densely innervate only the reproductive tissue. In addition to having different peripheral targets, Rz (5,6) do not extend axons into the interganglionic connectives, have smaller cell bodies, and have a less dense arborization within the ganglionic neuropil. Initially, however, Rz (5,6) extend processes into the body wall and connectives. Differences between Rz (5,6) and standard Rz cells arise only after Rz (5,6) processes contact the developing reproductive tissue. We have proposed that some interaction between the reproductive tissue and Rz (5,6) is responsible for the unique development of these neurons. We have tested this hypothesis by ablating the reproductive tissue from embryos and subsequently examining the morphology of Rz (5,6).

Reproductive tissue was removed from early ten day old embryos by a suction micropipet. Control animals included both unoperated siblings as well as embryos in which nearby body wall was removed instead of reproductive tissue. Survival of experimental animals was comparable to that of unoperated controls. After an additional 10 days of development, individual Rz cells were filled with HRP.

Rz (5,6) in animals whose reproductive tissue was removed resembled standard Rz cells. Such Rz cells projected processes into the body wall (10/10) and frequently extended axons into the interganglionic connectives (22/37), something Rz (5,6) in unoperated animals never did. In comparison, processes of Rz (5,6) in operated controls seldom entered the body wall (2/10) or the connectives (1/11). These results suggest that the segment-specific target of Rz (5,6) interacts with these neurons to influence the development of their particular morphology. We are also testing the hypothesis by transplanting reproductive tissue into standard segments to ask whether these Rz cells can be changed.

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365.8 MORPHOLOGICAL CHARACTERISTICS OF THE INTERACTION BETWEEN DEVELOPING IDENTIFIED LEECH NEURONS AND THEIR TARGET ORGANS. K.A. French, S.M. Furgal* and W.B. Kristan, Jr. Dept. of Biology, UCSD, La Jolla, CA 92093.

Paired large, serotonergic neurons, the Retzius (Rz) cells, are found in each of the segmental ganglia of the medicinal leech, Hirudo medicinalis. In all segments, except the two that contain most of the reproductive structures (segments 5 and 6), these neurons diffusely innervate the body wall. In those two segments, the Rz cells heavily innervate the reproductive structures instead: the male reproductive tissue in segment 5 and the female tissue in segment 6. Differences in central morphology accompany this difference in peripheral innervation. Experimental manipulations in embryos have suggested that interaction between the outgrowing processes of Rz cells and their eventual target tissues plays a major role in shaping these morphological differences, so we have begun to characterize the contacts between growing Rz cells and their targets in the periphery as an approach to understanding the mechanism of the interaction.

The primordial reproductive tissue can be recognized in segments 5 and 6 of whole embryos and in histological sections before axonal outgrowth begins. These clusters of undifferentiated cells grow from near the lateral edge of the germinal plate toward the ventral midline at about the time that Rz axons exit the ganglia and grow toward the periphery. The morphology of Rz cells was revealed either by indirect immunological labelling using an antibody to serotonin or by filling individual cells with horseradish peroxidase.

Growth cones of Rz (5,6) in the vicinity of the reproductive primordia were seen to be extended and elaborate, typical of axons that are actively choosing a pathway. In contrast, the growth cones of the Rz cells in other segments of the same embryos appeared thin and simple as they passed through the homologous portion of the germinal plate. Sections through segments 5 and 6 of embryos, examined with light and electron microscopy, showed that labelled Rz axonal profiles approach the outer limiting layer of the reproductive primordia.

These results suggest that growing Rz (5,6) axons actively respond to the presence of the reproductive cells with elaborate growth cones and subsequently redirect their path away from the body wall to follow the reproductive primordia toward the ventral midline. At the time that this choice is being made, Rz processes lie near the outer boundary of the reproductive tissue, where their growth cones may be making contacts that mediate the redirection.

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365.9 AXONAL GUIDANCE PROVIDED BY SINGLE NON-NEURONAL CELLS IN THE LEECH. J. Jellies and W.B. Kristan, Jr. Department of Biology, UCSD, La Jolla, CA, 92093.

Each midbody ganglion in Hirudo medicinalis gives rise to a pair of bilateral roots which quickly branch into four major nerves. An additional nerve between segments 5 and 6, the sex nerve (SN), carries axons of the Retzius (Rz) neurons, as well as those of motor and presumably sensory neurons. Visualization of Rz processes (using an antiserum against serotonin) as well as muscle and muscle precursor cells (using a monoclonal antibody: Zipser and McKay, 1981, Nature, 289:549) in embryos revealed a single bilateral pair of cells in the position of the future SN's. When filled with Lucifer Yellow, these cells showed a characteristic, flat morphology and could be recognized about 12 hours before axons entered the presumptive SN. Cross-sections revealed that axons were later closely associated with each "flat cell."

We examined the role of the flat cell in establishing the SN by ablating it in two ways, mechanical disruption (repeated penetration with a microelectrode) and photoinactivation (illumination after filling the cell with Lucifer Yellow). The flat cell on only one side was ablated, leaving the other as a control for normal SN development. After mechanical disruption about 10% of animals were missing flat cells (n=6) when examined after 3-5 days. Whenever a flat cell was missing, the SN was absent whereas whenever a flat cell was intact, the SN was present.

Photoinactivation (by methods after Miller and Selverston, 1979, Science, 206:702) more specifically eliminated the flat cell. To do this, a single Rz cell in a live embryo was first filled with dye and its processes used as landmarks to find and subsequently fill a flat cell. (Killing Rz cells alone had no effect on SN formation.) Ablation of the dye-filled flat cells by exposure to light always eliminated the appropriate SN (n=5), as determined 3-4 days later by staining axonal bundles with an antiserum directed against tubulin.

These experiments suggest that the flat cell provides a continuous substrate necessary for axons to navigate between segments 5 and 6. We are currently investigating the probable mechanisms underlying this navigation and the ways in which the flat cell first defines the pathway during early embryogenesis.

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365.10 MUTATIONS AFFECTING GROWTH OF LUMBAR NEURONS IN THE NEMATODE CAENORHABDITIS ELEGANS STUDIED IMMUNOCYTOLOGICALLY. Shahid S. Siddiqui & Joseph Culotti*, Dept. Biochem, Molec Biol & Cell Biol and Dept of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201.

A pair of bilaterally symmetric lumbar ganglia are located in the tail of the nematode C. elegans. In adults each lumbar ganglion consists of seven embryonically derived neurons, PHA, PHB, PLM, PVC, PVQ, LUA, and a single neuron PVR in the right ganglion, and five postembryonically derived neurons PHC, PVN, PVW, and PLN, and a neuron PQR in the left ganglion (Sulston, J. & Horvitz, R.H., Dev. Biol., 56:110, 1977; and Sulston, J. et al., Dev. Biol. 100:64, 1983). We previously used anti-HRP antibodies to examine the morphologies of lumbar neurons PHC and PVN in 96 uncoordinated mutants of C. elegans (Soc. Neurosci. Abs., 1985). We recently discovered that a variety of anti-tubulin monoclonal antibodies (provided by T. Arai and by G. Piperno) can be used to stain mechanosensory neurons (Chalfie, M. & Thomson, J.N., J. Cell Biol., 82:278, 1979) including lumbar neuron PLM. In wild type animals, PHC and PVN send axons ventrally to targets in the ventral cord, whereas, PLM sends an axon forward in a subventral position, extending a branch to the ventral cord near the vulva. We have used the antibodies to examine these neurons in uncoordinated mutants representing approx. 90 unc genes. In mutants of 9 genes (unc-13, unc-33, unc-44, unc-51, unc-61, unc-71, unc-73, unc-98, and unc-6) PLM as well as PHC and PVN axons had abnormal morphologies. A variety of PLM errors were observed in these mutants, including premature termination and abnormal positioning or branching of axons. Mutations in unc-53 and unc-76 had similar effects on PLM, but did not affect the growth of PHC or PVN. Thus some unc genes are required for the growth of postembryonic neurons PHC and PVN as well as for embryonic neuron PLM, whereas others are required for PLM but not for PHC and PVN. The differential effects of mutations in unc-53 and unc-76 vs. mutations in the other unc genes on these and other lumbar neurons (see also Soc. Neurosci. Abs. 102.8, 1985 for effects on PHA and PHB) suggest that these two genes may normally act in embryonic but not in postembryonic nerve growth.

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365.11 PUTATIVE GABAERGIC NEURONS AND THE IMMUNOCYTOCHEMICAL IDENTIFICATION OF MUTATIONS AFFECTING AXONAL OUTGROWTH AND GABA EXPRESSION IN C. ELEGANS. S.L. McIntire*, H.R. Horvitz and J.G. White*. Lab. of Molecular Biology, MRC, Hills Road, Cambridge, England, CB2 2QH and Dept. of Biology, MIT, Cambridge, MA 02139.

We have previously described a set of GABA immunoreactive neurons in C. elegans (Soc. Neurosci. Abstr., 1985). The labelled cells include the thirteen VD and six DD motorneurons which are located in the ventral nerve cord as well as a number of identified neurons in the ganglia of the head and tail. The VD and DD motorneurons have processes in both the ventral and dorsal nerve cords that are connected by a circumferential commissure. These motorneurons are thought to act as reciprocal inhibitors, coordinating the alternate contraction of the ventral and dorsal musculature and, hence, the propogation of a sinusoidal wave.

By immunocytochemically screening existing locomotory-defective or uncoordinated ("unc") mutants, we have been able to identify a number of genes affecting VD and DD axonal outgrowth or GABA expression. Mutations in three genes [unc-5(e53), unc-6(e78), and unc-51(e369)] result in a complete failure of the extensions of the VD and DD neurons to reach the dorsal nerve cord. Labelled processes appear in abnormal lateral positions where they frequently interconnect or reenter the ventral nerve cord. The placement of processes in the ventral cord can also appear abnormal. Mutations in another six genes [unc-34(e315), unc-40(e271), unc-76(e911), unc-62(e644), unc-71(e541), and unc-73(e644)] result in less complete defects in commissure formation. In these mutants, the dorsal extensions of DD and VD processes often reach the dorsal cord by atypical paths.

We have identified possible defects in GABA expression caused by mutations in four genes. In unc-25(e156) animals, there appears to be no immunoreactivity in any of the neurons that are normally labelled with antibodies against GABA. In unc-30(e191) animals, there is again no immunoreactivity in the VD and DD neurons; however, there is normal labelling of neurons in the head and tail of the animal. Serial EM reconstructions of unc-30 have shown that the placement of VD and DD processes is abnormal (J.G. White, unpublished observations). In unc-47(e307) animals, the intensity of immunofluorescence appears considerably enhanced in the labelled cells. Mutations in the gene unc-43(e408) result in a diminished intensity of fluorescence in VD neurons.

We are pursuing genetic and molecular analyses of the mutants described here.

365.12 FORMATION OF THE NEUROENDOCRINE TRANSVERSE NERVE DURING EMBRYONIC DEVELOPMENT IN THE MOTH, MANDUCA SEXTA. J. Carr* and P.H. Taghert, (SPON: S. Currie) Dept. of Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110

We are interested in the differentiation of peptidergic neurons during embryonic development. Our studies have focussed on the segmentally repeated transverse nerve (TN) in the moth, Manduca. In the larval stage, this nerve contains 2 motor axons and the axons and terminals of 14 identified neuroendocrine cells. Using 2 different monoclonal antibodies (TN-1 & 2), we have begun to characterize the construction of this nerve during embryogenesis.

The antibodies were derived from hybridomas generated after immunization with adult TN (Taghert et al., 1983, Soc. Neur. 9: 314). TN-1 stains most peripheral axons in Manduca by the time they exit the CNS; one commissural bundle is stained heavily and two lightly. In ovo injections of TN-1 ascites demonstrate that this antigen is present on the surfaces of these axons. TN-1 also stains a variety of mesodermal cells including many that appear to assume the role of glial cells associated with peripheral nerve axons. TN-2 selectively stains 2 neuroepithelial cells that lie at the anterior margin of each segmental ganglion; each sends a single process into the forming TN.

In each segment and before any axons arrive, TN-1 positive mesodermal cells are aligned in a distinctive "Arch" over each segmental ganglion in a form and location that pre-figures the TN itself. This arch inserts into the ectoderm on either side of every ganglion near the anterior margin of each segment. A subset of the TN-1 positive mesodermal cells are identifiable: these lie at specific locations with short cellular processes that mark the directions of future axon pathways or collaterals. In addition, the two TN-2 positive cells have projected into the arch and are expressing their specific antigen before any axons have arrived. Finally and coincidentally, a distinct group of TN-1 positive cells appear at a point between the arch insertion point and the location of the target muscle for the TN motoneurons.

The growth cones of the motoneurons are the first to arrive at the arch (by about 37% of development); the secretory neurons follow at 43% and join in at two distinct locations in the periphery, but do not fasciculate with the motoneurons. One of these locations is the insertion point of the mesodermal arch into the ectoderm. These observations suggest roles for future glial cells in the morphogenesis of this simple nerve and perhaps in the direction of neural differentiation once the neuroendocrine axons have arrived at their final locations.
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365.13 IDENTIFICATION AND DIFFERENTIATION OF TWO PERIPHERAL PEPTIDERGIC NEURONS IN THE EMBRYO OF THE MOTH, MANDUCA. P.H. Taghert and P.F. Copenhaver, Dept. Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO. 63110.

During neuronal development in insects, single neurons undergo a stereotyped program of differentiation. Mature form and synaptic connectivity arise following precise growth cone navigation to target areas; transmitter phenotypes are typically expressed before the completion of morphogenesis and are selected (at least in part) as a result of stereotyped cell lineages. We are interested in the cellular mechanisms that underlie and integrate these various developmental schedules. Here we describe 2 peripherally located, segmentally repeated neurons that lie along nerves in the moth, Manduca. These are large, neuroendocrine neurons that, due to their specific location, are extremely accessible to a variety of descriptive and manipulative approaches during embryonic development.

On either side of each segmental ganglion there are 8-10 neurons located along peripheral nerves. Two of these neurons (L1 and L2) are the only cells that project axons back towards the CNS. L1 is a multipolar neuron with its major axon projecting into the transverse nerve (TN) and 2-4 distal axons projecting towards the heart. L2 is a unipolar neuron that projects into the dorsal (intersegmental) nerve (DN). Intracellular dye fills reveal that both L1 and L2 have extremely stereotyped axonal projections into the TN and DN and into the respective side branches of those nerves. Both cells (but no other peripheral neurons) are positively stained by a monoclonal antibody to the molluscan cardioactive peptides.

Morphologically, L1 and L2 show a pattern of relative error-free growth that is reminiscent of the development of neurons of the insect CNS. Examination of L1 filopodial contacts at the LM level suggests interactions between this cell and specific mesodermal cells that lie at defined locations along the developing TN (cf., Carr and Taghert, this volume). Peptide immunoreactivity appears in L1 and L2 between 55-57% of embryonic development. They are among the first neurons to express this phenotype along with 2 in the brain and 2 in the frontal ganglion. By this point in development, L1 and L2 have projected axons into their respective nerves but have not completed their terminal arbors.

Our results reveal that the normal differentiation of L1 and L2 in many ways mirrors that seen for neurons of the CNS. Studies from this point, in embryo culture, will take advantage of their extreme accessibility to elucidate the mechanisms that underlie this stereotyped differentiation.
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366.1 PRESYNAPTIC INHIBITION RECONSTITUTED *IN VITRO*. H.J. Chiel, D.W. Tank, J.A. Connor, Mol. Biophys. Res. Dept., AT&T Bell Labs, Murray Hill, NJ 07974.

We have constructed a three neuron circuit *in vitro* that, according to several criteria, shows presynaptic inhibition. The circuit was constructed by co-culturing the identified neurons L10, C2, and C4 from *Aplysia californica*. In vivo, these neurons participate in two different circuits showing presynaptic inhibition: (#1) the cholinergic neuron L10 is located in the abdominal ganglion and is presynaptically inhibited by activating L32 neurons, or by application of histamine (Bailey et al., Soc. Neurosci. Abstr. Vol. 9, p. 1025, 1983); (#2) activation of the histaminergic cerebral neuron C2 presynaptically inhibits buccal neuron B18, suppressing B18-induced EPSPs in the cerebral neuron C4 (Chiel et al., Soc. Neurosci. Abstr. Vol. 9, p. 913, 1983). By co-culturing a cholinergic presynaptic cell with C2 and C4, we attempted to reconstruct circuit #2 *in vitro*. L10 was substituted for the *in vivo* presynaptic cell (B18) because B18 is difficult to routinely isolate and culture, both cells are presynaptically inhibited by histamine *in vivo*, and experiments indicated that B18, like L10 is cholinergic: acetylcholine mimicked B18's postsynaptic actions, and the EPSPs B18 induces in C4 are suppressed by the cholinergic blocker hexamethonium (1mM).

Prior to isolation, L10 was identified by its ability to induce large IPSPs in abdominal left upper quadrant cells. The two cerebral neurons, C2 and C4, were identified by morphological criteria. All neurons were isolated from juvenile *Aplysia* (3-5 gms), and appropriate cell combinations were co-cultured using the techniques of Schacher et al. (J. Neurosci. **3**: 2403, 1983). Reconstituted circuits were studied electrophysiologically 2-5 days later.

We found that L10 induced a large facilitating EPSP in C4 (6 of 6 circuits), and an IPSP in C2 (4 of 4 circuits), both of which were enhanced by raising external [Ca^{++}]. Activating C2 hyperpolarized L10 (5 of 5 circuits) and depolarized C4 (8 of 12 circuits). Electrical synapses between C2 and C4 were occasionally observed (4 of 12 circuits). Finally, we found that L10-induced EPSPs in C4 were suppressed 20%-50% by activating C2 (3 of 4 circuits having all 3 cells). This effect persisted when intracellular current injection was used to override C2-induced changes in the soma potentials of C4 (3 experiments) and L10 (1 experiment). Histamine (1mM in artificial seawater, pH 7.6) rapidly perfused onto cells L10 and C4 (cultured without C2) also suppressed the L10-induced EPSPs in C4. These results support the hypothesis that, *in vitro*, histamine released by C2 acts presynaptically to inhibit L10. The similarity of the connections which L10 forms in the *in vitro* hybrid circuit to those of B18 *in vivo* are consistent with the hypothesis that neurons sharing relevant transmitters and receptors *in vivo* can be substituted for each other *in vitro*. This hybrid circuit can be used to analyze the biophysical mechanisms of presynaptic inhibition. (We thank S. Schacher for technical advice on culturing *Aplysia* neurons. H.J.C. was supported by a grant from AFOSR under contract F49620-85-C-0009.)

366.2 SEROTONIN MEDIATES IN VITRO CONDITIONING EFFECTS ON TYPE B PHOTORECEPTORS IN HERMISSENDA. L. Grover and J. Farley. Prog. in Neurosci. and Behav., Princeton Univ., Princeton, NJ 08544.

Light-rotation pairings result in a persistent reduction in phototaxic behavior in Hermissenda. We are interested in the role of serotonin in this conditioning. Previous results show that Hermissenda central ganglia contain serotonin (5-HT) and that neuropil where B photoreceptors and S/E cells terminate contains 5-HT reactive fibers (Auerbach et al., Soc. Neurosci. Abstr., 1985). Previous research has also shown that 5-HT mimics the effects of behavioral conditioning on Type B cells (Farley & Auerbach, Nature, 1986). We therefore asked if 5-HT might mediate cumulative depolarization and increased input resistance (R_M) in Type B cells produced by in vitro pairings of light (110 $\mu W/cm^2$) and statocyst (caudal hair cell) stimulation, which are short-term correlates of conditioning.

Five paired (P) conditioning trials in normal ASW (n=11) produced 6.5±0.6 mV (mean±S.E.M.) of cumulative depolarization, and a mean increase in R_M of 27.7±6.4 MΩ. Five unpaired (UP) control trials (n=7) produced significantly less depolarization (0.4±1.6 mV, $p<.005$) and increase in R_M (9.6±5.1 MΩ, $p<.05$). Bufotenine (100 μM), previously shown to antagonize 5-HT effects on B cells (Wu & Farley, Soc. Neurosci. Abstr., 1985), significantly reduced P - UP differences in cumulative depolarization [P (n=10) = 3.9±1.5 mV, UP (n=8) = 3.0±1.2 mV, n.s.] and R_M increases (P = 14.7±3.9 MΩ, UP = 6.5±11.1 MΩ, n.s.), but had no effect on B cell EPSPs. Depletion of 5-HT by injections of 5,7-DHT (80% depletion of buccal ganglia 5-HT and 35% depletion of cerebropleural ganglia 5-HT were obtained by 3 successive daily injections of 5,7-DHT, as determined by HPLC-ECD) also abolished pairing-specific cumulative depolarization [P (n=6) = 4.4±1.2 mV, UP (n=5) = 5.9±2.8 mV, n.s.] and R_M increases (P = 19.3±4.7 MΩ, UP = 34.6±19.8 MΩ, n.s.). Control animals, injected with ascorbate carrier solution, showed no attenuation of in vitro conditioning effects: cumulative depolarization for P (n=8) = 5.7±1.4 mV, for UP (n=5) = 0.0±2.0 mV ($p<.025$), R_M increases for P = 34.8±7.1 MΩ, UP = 2.2±15.1 MΩ ($p<.025$). Yohimbine (100 μM), an α_2-receptor antagonist, had no effect on pairing-specific cumulative depolarization [P (n=7) = 5.5±0.7 mV, UP (n=7) = -0.2±2.3 mV, $p<.025$], increases in R_M (P = 34.0±11.1 MΩ, UP = -3.3±9.0 MΩ, $p<.025$), or B cell EPSPs. Collectively, these and previous results suggest a direct role for 5-HT modulation of B cell excitability during associative learning. (Supported by NSF BNS 8316707 to JF).

366.3 A SYSTEM FOR THE STUDY OF SYNAPSE SPECIFICITY IN LONG-TERM MEMORY IN APLYSIA. G.A. Clark. H. Hughes Medical Institute; Ctr. for Neurobiol. & Behav., Columbia Univ., College of P&S; and NYS Psychiat. Instit., New York, NY 10032.

Long-term sensitization of the gill- and siphon-withdrawal reflex in Aplysia involves the enhancement of synaptic connections of the LE siphon sensory cells (Bailey and Chen, 1983; Frost et al., 1985), and long-term facilitation produced at these synapses by serotonin, a putative facilitatory transmitter, is blocked by protein synthesis inhibitors (Montarolo et al., 1985; Schacher et al., this volume). The finding that long-term neuronal changes are dependent on the expression of gene products not utilized for short-term memory raises two questions central to the biology of long-term memory. First, must long-term neuronal changes necessarily be cell-wide, or can they occur only at selected synapses of a given cell? Second, can an extracellular neuromodulatory signal induce long-term facilitation when applied only at synaptic regions, or must it be applied directly on the soma itself? To address these questions, I have asked whether serotonin can produce long-term facilitation at synapses of LE siphon sensory cells onto peripheral siphon motoneurons. Because the peripheral synapses are 4 to 5 cm from the soma, it is possible to restrict pharmacological treatments to either central (somatic) or peripheral (synaptic) regions of the sensory cell. Moreover, previous studies have shown that local applications of serotonin can produce short-term facilitation at either central or peripheral synapses independently (Clark and Kandel, 1984). I now report that serotonin can also produce long-term facilitation at peripheral sensory neuron synapses.

The abdominal ganglion and attached siphon nerve with associated peripheral siphon motor neurons were dissected from Aplysia and maintained in organ culture. The synaptic connections from several siphon sensory cells onto peripheral siphon motoneurons were then tested using standard intracellular recording and stimulation techniques. Following testing, experimental preparations (N=8) received 5 exposures of 30 uM serotonin bath-applied onto both the central and peripheral nervous systems; each exposure lasted for 5 min, and was followed by a 15 min wash with normal recording medium. Control preparations (N=9) received 5 applications of recording medium without serotonin, and equivalent washes. When the same synaptic connections were retested 20-24 hr later, the amplitude of synaptic potentials in experimental preparations showed a significant increase relative to controls (61 ± 8% vs. 19 ± 6%, $p<.001$), confirming previous results obtained in dissociated cell culture. I now plan to ask: 1) whether local applications of serotonin restricted to either central (somatic) or peripheral (synaptic) cellular regions can produce long-term synaptic facilitation; 2) whether local applications of protein synthesis inhibitors restricted to either somatic or synaptic regions block long-term facilitation; and 3) whether long-term neuronal changes (if any) produced by local application of serotonin are restricted to the site of serotonin application, as in short-term facilitation, or if instead they are cell-wide.

366.4 A CRITICAL PERIOD OF MACROMOLECULAR SYNTHESIS IS NECESSARY FOR THE EXPRESSION OF LONG-TERM FACILITATION OF SENSORIMOTOR CONNECTIONS IN APLYSIA. S. Schacher, P.G. Montarolo, V.F. Castellucci, E.R. Kandel and P. Goelet. Ctr. for Neurobiol. & Behav. and Howard Hughes Medical Institute, Columbia Univ. College of P&S, and NYS Psychiat. Instit., New York, NY 10032.

A common feature of the retention of long-term memory in mammals is that protein synthesis is primarily required during and just after training (Davis & Squire, 1984). To determine whether this temporal requirement for macromolecular synthesis in long-term memory can be demonstrated at the level of an identified synapse related to learning, we examined the time window of protein or RNA synthesis inhibition on the long-term facilitation of the sensorimotor connections of the gill-withdrawal reflex in Aplysia.

The gill-withdrawal reflex undergoes short- and long-term sensitization, both of which involve the facilitation of the connections between the sensory neurons and their follower cells. The sensorimotor component of this reflex can be reconstituted in vitro and shows both short- and long-term heterosynaptic facilitation (Montarolo et al., 1985). A single application of serotonin (5-HT) induces facilitation that lasts minutes; five repeated applications (over a 1.5 hr period) results in facilitation that lasts 24 hrs. The long-term facilitation but not the short-term is blocked when protein or RNA synthesis is inhibited during the 5-HT applications.

Cultures exposed to 5 applications of 5-HT (1 uM) over a 1.5 hr period showed an increase in EPSP amplitude of 71 ± 31% (N=9) when retested 24 hrs later, compared to a slight decrease in EPSP amplitude of -14 ± 12% in untreated control cultures (N=8). Anisomycin (10 uM) blocks this long-term facilitation (-16 ± 14%, N=7) when added 1 hr before the first 5-HT application and present for 3 hr, until 1/2 hr after the last 5-HT application. Since the inhibition of protein synthesis by anisomycin rapidly recovers to control levels within 4 hrs, we next explored the time window during which new protein synthesis was critical for the expression of long-term facilitation. We shifted the 3 hr exposure to anisomycin putting it either before 5-HT application (by 12 hr) or after the last 5-HT application (0.5 hr or 4 hr). In each case, anisomycin had no statistically significant effect on the 5-HT induced long-term facilitation (65 ± 10%, N=4; 51 ± 19%, N=4; 90 ± 32%, N=4, respectively).

Thus, long-term facilitation of an identified synaptic connection, important for the storage of long-term memory for behavioral sensitization, shows a narrow critical time window for macromolecular synthesis that is comparable to the time window of long-term behavioral memory in intact vertebrates. The restriction of this change to two identified cells and their connection, the ability to induce this change with one transmitter, and the expression of this change during a defined 1 to 3 hr time period should facilitate the search for the macromolecules critical for the expression of the long-term change and allow study of the means whereby the synthesis of these macromolecules is regulated.

366.5 5-HT INDUCES LONG-LASTING EXCITABILITY CHANGES IN CULTURED APLYSIA SENSORY NEURONS. N. Dale*, E.R. Kandel and S. Schacher (SPON: M. Flaster). Howard Hughes Med. Instit., Ctr. for Neurobiol. & Behav., Columbia, P&S, New York, NY 10032.

The gill- and siphon-withdrawal reflex in Aplysia can undergo long-term sensitization after repeated noxious stimuli to the tail. Part of the underlying mechanism involves a long-lasting change in the efficiency of the synapses between the sensory and motor neurons of the reflex (Frost et al., 1985, PNAS 82:8266). Recently, long-term facilitation has been demonstrated with repeated 5-HT applications in sensorimotor synapses reconstituted in culture (Montarolo et al., 1985, Soc. Neurosci. Abstr. 11:795). The locus for this long-term change in synaptic efficiency is not known.

The short-term change at this synapse produced by 5-HT is presynaptic and involves reduction of K^+ current that causes broadening of each action potential and increase in cell excitability. As a first step in determining whether the long-term change in synaptic efficiency is correlated with a similar excitability change, we have investigated the excitability of sensory neurons in culture one day following repeated 5-HT applications. We measured excitability in control and experimental groups of abdominal or pleural sensory neurons by injecting a graded series of depolarizing current pulses (2 sec long) separated by 1 min, and examined the number of spikes evoked as a function of current. The experimental group of sensory neurons was then treated with 4 applications of 5-HT (1 uM) for 5 min at 15 min intervals. Twenty-four hours later the excitability of both groups was reexamined using an identical procedure. The group treated with 5-HT showed an increase in the number of spikes evoked by each injected current pulse. The mean difference (14 spikes) between the maximum number of spikes generated before the 5-HT application and 24 hrs later was highly significant (N=8 cells in 6 dishes, SD=7.15, range +5 to +27; $p < 0.001$). By contrast, the mean difference (3 spikes) in the maximum number of spikes generated in the control group after 24 hrs was not significant (SD=15.6, N=11 cells in 5 dishes, range -16 to +39; $p > 0.5$). In addition, the difference in maximum number of spikes caused by 5-HT treatment was highly significantly different from the mean difference of the control group ($p < 0.001$).

Thus, repeated 5-HT applications that cause long-lasting facilitation of sensorimotor synapses in cuture, can cause a long-lasting increase in the excitability of sensory neurons. If these excitability changes demonstrated in culture also occur in intact animals exposed to long-term sensitization training, they would have two implications: 1) sensory neurons in a sensitized animal might respond more vigorously to tactile stimulation and 2) their serotonin-sensitive outward currents may be reduced, allowing more calcium to enter with each spike, leading to increased transmitter release in a manner analogous to the mechanisms underlying short-term sensitization at the same synapse (Klein et al., 1982, PNAS 79:5713).

366.6 LOSS OF THE REGULATORY SUBUNIT OF THE CYCLIC AMP-DEPENDENT PROTEIN KINASE IN APLYSIA NEURONS AFTER SENSITIZING TREATMENTS. S.M Greenberg, H. Bayley*, V.F. Castellucci, and J.H. Schwartz, Center for Neurobiology and Behavior and Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, New York, NY 10032.

Elevation of cAMP in Aplysia neurons during short-term sensitization activates cAMP-dependent protein kinase, which in turn leads to the closure of a specific K^+ channel and an increase in transmitter release. It was suggested (Science, 218:433, 1982) that cAMP might also be the signal for producing long-term changes in synaptic transmission, perhaps through a long-lasting modification of the cAMP-dependent kinase involved in regulation of the channel.

To investigate the effects of a sustained elevation of cAMP on the kinase, we treated synaptosomes from Aplysia central ganglia with 8-p-chlorophenylthio-cAMP, a permeant analogue. We assayed for regulatory subunits (R) of the kinase by photolabeling with $[^{32}P]N_3cAMP$ and for catalytic subunits (C) by phosphorylation of a heptapeptide substrate. After a 2-hr exposure to the analogue and a 2-hr washout period, the synaptosomes were found to contain about half the total amount of R compared with untreated synaptosomes; the loss was most pronounced for the M_r 47,000 and 105,000 species. In contrast, the treated synaptosomes showed no decrease in C activity. Loss of R most likely results from cAMP-dependent proteolytic turnover rather than down-regulation of R synthesis, since the synaptosomes do not make new protein.

To determine whether a selective loss of R also occurs in sensory neurons as a result of long-term training, we assayed R and C in cells of the abdominal ganglion that mediate the gill-withdrawal reflex. In preliminary experiments, we found that 24 hr after animals were trained for long-term sensitization, these cells contained less R than naive controls, whereas the C activity remained unaffected. Selective loss of the regulatory subunits of the kinase should result in enhanced sensitivity to small amounts of cAMP and thus an enzyme more effective in producing the physiological changes underlying synaptic facilitation. We suspect that this loss is at least in part caused by a cAMP-dependent increase in turnover. To see whether there is also a change in the expression of the kinase genes we have obtained Aplysia R clones with a bovine cDNA for R_I (provided by Stanley Knight) for use as probes.

366.7 DEPLETION OF SEROTONIN IN APLYSIA INTERFERES WITH FACILITATION PRODUCED BY SENSITIZING STIMULI. D.L. Glanzman, S. Mackey* and E.R. Kandel. Howard Hughes Medical Institute, Ctr. for Neurobiol. & Behav., Columbia, P&S, New York, NY 10032.

Pharmacological and anatomical data suggest that 5-HT has an important role in facilitation of synaptic transmission in the abdominal and pleural-pedal ganglia of Aplysia, and in behavioral sensitization of defensive withdrawal responses. Application of 5-HT mimics the effects of tail or nerve stimulation in producing facilitation of sensory-to-motor neuron PSPs, and broadening of spikes and elevation of cyclic AMP in both LE and pleural sensory cells. Furthermore, immunohistochemistry has revealed 5-HT processes surrounding the LE and pleural sensory cells. However, the findings that endogenous transmitters other than 5-HT can also mimic the effects of sensitizing stimuli (Abrams et al., 1984), and that the L29 facilitator cells do not contain 5-HT (Ono & McCamen, 1984; Kistler et al., 1984, 1985), raise the question of 5-HT's relative importance in mediating behavioral sensitization.

We have therefore chosen to lesion serotonergic neurons in Aplysia using a specific 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), and then to examine whether such lesions interfere with the facilitatory effects of sensitizing stimuli on neurons which mediate two types of defensive responses of Aplysia: tail-withdrawal and siphon- and gill-withdrawal. Aplysia were injected with 5,7-DHT (125 mg to 200 mg total dosage). We then compared the effectiveness of sensitizing stimuli in animals receiving either 5,7-DHT or control injections.

We have examined the effects of 5,7-DHT on the facilitation of sensory neuron connections in both abdominal and pedal ganglia. Sensitizing stimuli were, in both cases, a train of electrical shocks to the tail which was left connected to the isolated central nervous system via a single pedal nerve (LP9). For the abdominal ganglion, we measured the monosynaptic EPSPs from the LE cells to their followers. 5,7-DHT substantially reduced the facilitation of these EPSPs which tail shocks could produce. The average facilitation produced in ganglia from animals which had received control injections was 148 $\pm$ 13% (N=6) compared to 2 $\pm$ 9% (N=6) in animals treated with the neurotoxin. This difference was significant ($p < 0.001$, two-tailed t-test). Interestingly, L29 cells appear capable of producing facilitation in 5,7-DHT-treated abdominal ganglia. We have found that 5,7-DHT treatment also reduces the tail-shock induced facilitation of complex EPSPs in pedal motor neurons (115 $\pm$ 26% in controls vs. 15 $\pm$ 9% in treated, $p < 0.02$, two-tailed t-test).

Anatomical examination of the pleural-pedal and abdominal ganglia from Aplysia with the glyoxilic acid histofluorescence indicate that 5,7-DHT significantly reduces central 5-HT in Aplysia. By contrast, dopamine histofluorescence appears normal. We have confirmed these qualitative observations with HPLC. Although we have not completely ruled out a nonspecific effect of the neurotoxin, our results thus far imply that 5-HT plays a prominent role in mediating behavioral sensitization in Aplysia.

366.8 LOCALIZATION OF POTENTIAL SEROTONERGIC FACILITATOR NEURONS BY GLYOXYLIC ACID HISTOFLUORESCENCE AND RETROGRADE FLUORESCENT LABELING IN APLYSIA. R.D. Hawkins. Ctr. for Neurobiol. & Behav., Columbia Univ., College of P&S, and NYS Psychiat. Instit., New York, NY 10032.

Pharmacological data indicate that 5-HT has an important role in facilitation of the LE sensory cells in the abdominal ganglion of Aplysia (Glanzman et al., this volume). However, individual 5-HT facilitator neurons have not yet been identified. To address this issue, I have used a modification of the glyoxylic acid histofluorescence technique combined with retrograde fluorescent labeling to locate potential serotonergic facilitator neurons in the nervous system of Aplysia.

Cryostat sections of ganglia from adult or juvenile Aplysia were bathed in an ice cold glyoxylic acid solution (Barber, 1982) for 5 min, heated at 100° for 5 min, and viewed on a fluorescence microscope. Using this method, serotonin appears yellow-green and catecholamine appears blue. I consistently observed 3 groups of 5-HT neurons in the abdominal ganglion but no 5-HT cells in the region of the L29 facilitator neurons. Furthermore, physiologically identified L29 cells injected with lissamine rhodamine showed no 5-HT fluorescence, while RB cells did. These results agree with the immunocytochemical results of Kistler et al. (1985), and indicate that the L29 cells are not serotonergic. Thus far, stimulation of other neurons in serotonin-containing regions of the abdominal ganglion has not produced facilitation of LE cells.

I have also observed an extensive network of 5-HT processes surrounding most neurons in the ganglion, including the LE cells. When the abdominal ganglion was left connected to the ring ganglia for 7 days in culture medium the fluorescence of 5-HT processes around the LE cells remained normal; by contrast, when the abdominal ganglion was isolated for the same length of time the fluorescence of these processes was markedly reduced. These results suggest that some of the 5-HT processes near the LE cells may come from cell bodies in the ring (pedal, pleural, and cerebral) ganglia.

There are at least 15 5-HT neurons in each cerebral ganglion, and perhaps as many as 100 in each pedal ganglion. To locate that subset of 5-HT neurons in the ring ganglia which send an axon to the abdominal ganglion, I pinned the intact central nervous system of Aplysia in culture medium for 24 hrs with the abdominal ganglion placed in a small well containing either lissamine rhodamine or propidium iodide; I then processed the ring ganglia for 5-HT histofluorescence. Thus far, viewing the sections with rhodamine and 5-HT optics has revealed cells in the pedal ganglia and in the RCB and LCB clusters of the cerebral ganglia which show both fluorescent dye backfilling from the abdominal ganglion and 5-HT histofluorescence. These cells are therefore potential serotonergic facilitator neurons. Mackey et al. (this volume) have begun to test the cerebral neurons physiologically, and have obtained evidence that they produce facilitation of LE cells.

366.9 NEURONS IN 5-HT CONTAINING REGION OF CEREBRAL GANGLIA PRODUCE FACILITATION OF LE CELLS IN APLYSIA. S.L. Mackey*, R.D. Hawkins and E.R. Kandel (SPON: W.N. Frost). Howard Hughes Medical Institute, Ctr. for Neurobiol. & Behav., Columbia University, College of P&S, and NYS Psychiat. Instit., New York, NY 10032.

Pharmacological and anatomical data indicate that 5-HT has an important role in facilitation of the LE sensory cells in the abdominal ganglion of Aplysia (Kistler et al., 1984, 1985; Glanzman, this volume). This has encouraged us to search for specific serotonergic facilitating neurons. Hawkins (this volume) has found 5-HT neurons in the LCB and RCB clusters of the cerebral ganglia that send an axon to the abdominal ganglion. We therefore attempted to determine whether these cells could produce facilitation of LE cells. Candidate cells were selected on the basis of their sending an axon into the pleural abdominal connectives as evidenced by an antidromic action potential. On average two cells in both the LCB and the RCB clusters met this criterion. In the first set of experiments the nervous system was perfused in culture medium containing 100 mM TEA. After obtaining a baseline spike width in an LE cell, the cerebral cell was stimulated for 20-50 seconds (10-100 spikes). In 13 of 25 cells tested, intracellular stimulation of the cerebral cell produced an average of 23% spike broadening in the LE cell. This compared to 32% spike broadening produced by stimulation of the left pleural abdominal connective at 10 Hz for .5 to 5 seconds (N=9).

In the second set of experiments, facilitation of the monosynaptic EPSP from LE to follower cells was tested. The ring and abdominal ganglia were perfused in culture medium and cerebral candidates were identified according to the above criterion. Monosynaptic EPSPs from LE cells to followers were tested once every 5 seconds. In 5 of 9 cells tested, stimulation of a cerebral cell for 20-45 seconds (50-210 spikes) produced an average of 153% facilitation of EPSP amplitude above baseline. This compared to an average of 292% facilitation produced by stimulation of the left pleural abdominal connective at 10 Hz for .5 to 5 seconds (N=2).

We have begun to characterize these cerebral facilitator neurons. In addition to sending an axon in the left pleural abdominal connective, they receive strong excitatory input from the P9 nerve which innervates the tail and from the siphon and branchial nerves. Preliminary results show no synaptic or electrical connections with the L29's or the metacerebral cells. We have also obtained preliminary evidence that the cells we have tested are in fact serotonergic. In one experiment a cerebral cell which produced spike broadening in an LE cell was intracellularly injected with rhodamine and cryosectioned. Staining with glyoxylic acid showed positive fluorescence for 5-HT.

Taken together, the results of these experiments provide preliminary evidence that identified 5-HT neurons in the cerebral LCB and RCB clusters can produce facilitation of the LE cells.

366.10 DISTINCT PROCESSES IN PRESYNAPTIC FACILITATION CONTRIBUTE TO SENSITIZATION AND DISHABITUATION IN APLYSIA: POSSIBLE INVOLVEMENT OF C KINASE IN DISHABITUATION. B. Hochner*, O. Braha*, M. Klein* and E.R. Kandel (SPON: M. Chen). H. Hughes Med. Instit., Ctr. Neurobiol. & Behav., Columbia, P&S, New York, NY 10032.

Presynaptic facilitation of the connections between sensory and motor neurons, that accounts in part for sensitization of defensive withdrawal reflexes, is accompanied by reduction of the "S"-K^+ channel in the sensory neurons. This modulation of ion channels by a cAMP-dependent protein kinase can contribute to facilitation by broadening action potentials and by increasing the excitability of the sensory neurons. We reported earlier (Hochner et al., 1985) that an additional process contributes to facilitation when transmission has been depressed, paralleling the behavioral process of dishabituation. This second component causes no change in the presynaptic outward current or action potential duration.

Serotonin, a facilitating transmitter, also leads to an increase in Ca^{++} transients in the sensory neurons (Boyle et al., 1984). Following up on possible intracellular mechanisms triggered by Ca^{++}, we have obtained preliminary evidence that the second intracellular component of facilitation may utilize a Ca^{2+}-dependent protein kinase, the Ca^{2+} phospholipid-dependent C kinase. Whereas the component of facilitation that is attributable to S channel closure is accompanied by reduction of presynaptic outward current and broadening of the action potential, phorbol ester, an activator of C kinase, can produce facilitation without a change in presynaptic currents. In addition, phorbol ester occludes part of the facilitation induced by serotonin.

The contribution of the second process is magnified when the synapse is in a state of depressed release corresponding to habituation of the reflex. The behavioral significance of the two processes may thus be different. S channel closure contributes a sensitizing component that does not interact directly with the mechanism of habituation. The second facilitating process contributes a dishabituating action, perhaps related to mobilization of transmitter vesicles, that reverses the depression of transmitter release resulting from habituation. Our experiments with phorbol ester suggest that the distinction between the two processes may extend to the molecular level. Thus, whereas S channel closure is a consequence of a single second messenger system (cyclic AMP), the second process may depend on the interaction of two second messenger systems: 1) the cAMP kinase and perhaps 2) the C kinase. More direct evidence for C kinase involvement requires the biochemical demonstration that serotonin translocates and activates the C kinase in these neurons (see Sacktor et al., these Abstracts), that cAMP interacts with the C kinase, and that blockade of the C kinase reduces a component of facilitation. Irrespective of the involvement of the C kinase system in physiological facilitation, our results suggest that activation of this system with phorbol ester can be used as a tool to separate the two facilitating processes from each other, allowing experimental manipulations that can lead to a better understanding of the interaction between them.

366.11 TRANSLOCATION FROM CYTOSOL TO MEMBRANE OF PROTEIN KINASE C AFTER STIMULATION OF APLYSIA NEURONS WITH SEROTONIN. Todd C. Sacktor*, Catherine A. O'Brian*, I. Bernard Weinstein*, and James H. Schwartz, Department of Neurology, Howard Hughes Medical Institute, and Cancer Center, Columbia University College of Physicians & Surgeons, New York, NY 10032.

To examine how parallel second-messenger systems might operate in both short- and long-term memory in the gill-withdrawal reflex, we have developed the capability of assaying the Ca^{2+}/phospholipid-dependent protein kinase (PKC) in the Aplysia nervous system and sensory neurons. DeRiemer et al. (1985, J. Neurosci., 5:2672; Nature, 313:313) have described several endogenous protein substrates of PKC in the cytosol of Aplysia ganglia and suggest that the enzyme induces voltage-sensitive Ca^{2+} currents in bag cell neurons. We use three methods to characterize the kinase in Aplysia nervous tissue: PKC-specific phosphorylation of endogenous protein substrates, phosphorylation of exogenous histone H1, and ^{3}H-phorbol dibutyrate binding. We find prominent endogenous substrates of PKC from cytosol, with M_r x 10^{-3} values of 200, 116, 110, 92, 70, 55, 34, 28, 23, and 14, and from membrane fractions, with values of 80, 29, and 20. Using histone phosphorylation together with phorbol-ester binding assays we are able to examine the distribution of PKC molecules between membrane and cytosol. Under sensitizing conditions (exposure of isolated ganglia to $5x10^{-5}$M serotonin for 5 min), we find that the enzyme moves from the cytosol to the membrane. Studies in other animals have shown that translocation of PKC is associated with activation of the enzyme. We also found that the regulation of the kinase by phorbol ester diminishes, suggesting the appearance of PKC activators in the sample or a change in the enzyme itself.

Studies in Aplysia of both the cAMP-dependent and the Ca^{2+}/calmodulin-dependent protein kinases reveal that after stimulation, these enzymes can become autonomous, persisting in their activity. The changes that we have described for the Aplysia PKC suggest that this kinase also has molecular properties endowing it with the capacity for stimulation-dependent persistence. Given that Hochner et al. (this volume) have found that phorbol esters can contribute to facilitation at sensory neuron synapses, these molecular features of PKC might play a role in prolonging or enhancing short-term sensitization.

366.12 STIMULATION OF C2, AN IDENTIFIED HISTAMINERGIC APLYSIA NEURON, RESULTS IN INCREASED RELEASE OF LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID: POSSIBLE MEDIATORS OF SYNAPTIC MODULATION. D. Piomelli*, S. Feinmark*, E. Shapiro and J.H. Schwartz (SPON: G. Chin), Howard Hughes Medical Inst. and Dept. of Pharmacol., Columbia Univ. Coll. of Phys. & Surg., New York, NY 10032.

Attention has been focussed primarily on cAMP-dependent protein phosphorylation in the modulation of synaptic transmission in Aplysia, but other second-messenger systems are also likely to be active. In mammals, the release of arachidonic acid from membrane phospholipids has been shown to be a receptor-stimulated reaction associated with phosphoinositide hydrolysis, elevation of intracellular Ca^{2+} and activation of guanylate cyclase. The free arachidonic acid is metabolized to prostaglandins, hydroxyacids, and leukotrienes, potent effectors of many cell processes. In the neural components from Aplysia ganglia, we have identified two arachidonic acid metabolites, 12- and 5-hydroxyeicosatetraenoic acids (HETE). A survey of Aplysia tissues shows that while 12-HETE is ubiquitously produced, 5-HETE is 10-100 times more abundant in neural components than in the connective tissue sheath of ganglia and in other non-neural tissues. In addition, isolated identified neurons (R2, L7 and bag cells) and synaptosomes incorporate [^{3}H]arachidonic acid into phospholipids, and convert it to [^{3}H]5-HETE and [^{3}H]12-HETE. [^{3}H]12-HETE and [^{3}H]5-HETE are not destroyed in nervous tissue, but rather are rapidly incorporated into cell glycerophospholipids. In cerebral ganglia labeled by incubation with [^{3}H]arachidonic acid, intracellular stimulation of the histaminergic neuron C2 causes the release of [^{3}H]5-HETE and, to a minor extent, of [^{3}H]12-HETE. Release is blocked by the histamine antagonist, cimetidine, and mimicked by the Ca^{2+} channel agonist BAY K 8644. In addition, application of 50 uM histamine stimulates the production of both [^{3}H]12-HETE and 5-HETE in prelabeled cerebral ganglia, which is also antagonized by cimetidine as well as by omitting Ca^{2+}. Furthermore, the isolated giant cerebral neuron, an identified follower of C2 in the cerebral ganglion, also releases labeled HETE when exposed to histamine. Our results indicate that stimulation of histamine receptors in Aplysia, which can mediate presynaptic inhibition, leads to the release and metabolism of arachidonic acid through both the 12- and the 5-lipoxygenase pathways. The differential activation of the two pathways thus appears to depend on how neurons are stimulated: specificity of input can determine the nature of the predominant metabolite formed.

367.1 DIFFERENT TYPES OF POTASSIUM CHANNELS IN CNS NEURONS OF DROSOPHILA. A. Komatsu, Y.-A. Sun, and C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Analysis of Drosophila mutants with altered membrane currents may provide insights into the molecular mechanisms of ion channels. Dissociated CNS neurons from Drosophila larvae retain functional channels and are suitable for single channel recording. To provide a basis for analysis of mutant channels, we have examined the diversity of single-channel K^+ currents in normal type I and III neurons in culture (Wu et al., J. Neurosci. 3:1888, 1983). Channel activity was recorded from cell-attached patches on the soma with electrodes filled with physiological saline. In some experiments the saline contained TTX, Co^{++}, and Cd^{++} to suppress Na^+ and Ca^{++} channels.

At least five types of voltage-sensitive K^+ channels were identified. The most frequently observed was an outward current resembling the delayed rectifier. It showed a unit conductance of 11 pS with an extrapolated reversal potential of about 20 mV negative to the resting potential. The channel activity increased at more positive membrane potentials and was sustained during prolonged depolarization. The open and closed time did not show a strong voltage dependency. The open time histogram was fit by a single exponential with a mean between 3 to 5 msec. The closed time histogram had at least two components. The time constant of the fast component ranged between 5 to 10 msec. Two additional types of outward currents with distinct kinetic properties were recorded. One occurred in clusters (several seconds of activity interrupted by long silent periods). The other inactivated slowly (activity declining after 10 to 20 secs of depolarization). However, all three types of channels showed similar unit conductances and reversal potentials.

Two inward currents activated by hyperpolarization were also recorded. One was similar to the inward rectifier, showing a unit conductance of 15 pS and a reversal potential near resting. The open time histogram was fit by a single exponential but the closed time histogram required two exponentials to fit. A second type had a smaller conductance (10-20 pS) and a more negative reversal potential (about 40 mV below resting).

Further studies of K^+ channels in mutant neurons are under way. It will be interesting to see if these channel types are also genetically distinct.

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367.2 MODULATION OF K AND Ca CURRENTS BY FMRFamide IN APLYSIA NEURONS IS MIMICKED BY A NON-HYDROLYZABLE GTP ANALOG. V. Brezina* and R. Eckert, Department of Biology, UCLA, Los Angeles, CA 90024.

In certain neurons in the abdominal ganglion of Aplysia californica, the neuropeptide FMRFamide enhances a K current that resembles the serotonin-sensitive 'S' current, $I_{K(s)}$ (Erxleben et al., 1985, Neurosci. Abstr. 11, 710), and simultaneously suppresses the voltage-dependent Ca current, and consequently the Ca-activated K current (Brezina et al. and Erxleben et al., 1985, Biophys. J. 47, 435a). Further experiments (carried out primarily on the left upper quadrant cells L2, L3, L4 and L6, and on cell R2, using two-electrode voltage clamp and puffed application of peptide) reveal that all of these effects of FMRFamide are mimicked by iontophoretic injection into the cell of the non-hydrolyzable GTP analog guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S). Thus, this compound activates a steady outward current at the normal holding potential of -40 mV, and also suppresses the Ca and Ca-dependent K currents elicited by depolarizing voltage steps. The Ca current, isolated in Na-free solution containing 200 mM TEA and 5 mM 4-aminopyridine,is usually suppressed by 50-70% in these cells. These effects of GTP-γ-S, unlike the parallel effects of FMRFamide, are slow to develop (10-20 minutes) and irreversible, and are observed even without prior exposure of the cell to FMRFamide, although, when the peptide is applied before the actions of GTP-γ-S are fully developed, its effects become irreversible and potentiate those of GTP-γ-S. Once the actions of GTP-γ-S have fully developed, FMRFamide does not further suppress Ca current or activate K(s)-like current, although it is still able to elicit the Na-dependent inward current (Ruben et al.,1986,J.Neurosci. 6, 252) that it normally activates in these cells in addition to its other effects.Since GTP-γ-S is known to be an irreversible activator of GTP-binding proteins, these results suggest that suppression of Ca current and enhancement of K(s)-like current (but not activation of the Na-dependent inward current) by FMRFamide in these cells may normally be mediated by such a protein. Supported by NSF BNS 83-16417 and USPHS NRSA GM07185.

367.3 COMPARISON OF ION CHANNELS IN HUMAN MACROPHAGES AND THE U937 CELL LINE. Frances V. McCann, Theodore M. Keller and George R. Stibitz. Dept. of Physiology, Dartmouth Medical School, Hanover, N.H. 03756

The U937 cell line, when activated by treatment with gamma interferon and calcitriol, acquires a high degree of functional similarity to human monocyte derived macrophages (HMDM), including phagocytic ability. The non-activated U937 cell line is an incomplete functional model for HMDM. A comparison of the ionic conductances present in HMDM and the U937 cell line, both activated and non-activated, should help to define the role of these conductances in the effector functions of mononuclear phagocytes.

Patch clamp studies on HMDM and the non-activated U937 cell line reveal several different conductances. In HMDM, a channel was observed that exhibits a slope conductance of 255 pS in the presence of a normal K^+ gradient, 229 pS in a symmetric K^+ gradient and 133 pS in a reversed K^+ gradient. Replacement of Cl^- by acetate did not alter the results. In the inside-outside patch configuration the bath concentration of Ca^{++} was increased from 10^{-8} M to 10^{-7} M (inside surface of the membrane) causing an increase in both the frequency of opening and the percentage of time that the channel was open. Further increasing the bath Ca^{++} concentration to 10^{-6} M resulted in further increases in the frequency of firing and time in the open state. These data confirm the presence of a Ca^{++} activated K^+ channel. We have not observed a conductance of this amplitude or kinetic behavior in the presence of Ca^{++} in over 50 patches from the non-activated U937 cell line.

A second channel with a slope conductance of 30 pS was also observed in HMDM. The frequency of opening and the percentage of time in the open state both increased 10-fold in response to increasing the potential over a range of 100 mV. Replacement of Cl^- by acetate had no effect on this conductance. This channel, insensitive to variation of the bath Ca^{++} concentration between 10^{-8} and 10^{-6} M when in the inside-outside patch configuration, is a voltage gated K^+ channel. A channel was observed in non-activated U937 cells that was nearly identical in: 1) slope conductance; 2) the kinetic response to a potential gradient; and 3) lack of response to changes in internal Ca^{++} concentration.

The presence of a Ca^{++} activated K^+ conductance in HMDM, and its apparent absence in the nonactivated U937 cell line, suggests a possible role for this channel in effector functions which are unique to the mature macrophage. Conversely, the voltage gated K^+ channel present in both cell types seems unlikely to play a significant role in these effector functions. Work is in progress to determine if the Ca^{++} activated K^+ conductance appears in U937 cells which have been activated by treatment with gamma interferon and calcitriol. This work is supported by NIH GM-31423.

367.4 IDENTIFICATION OF INWARD AND OUTWARD RECTIFYING K CONDUCTANCES IN CULTURED HUMAN MACROPHAGES. E.K. Gallin* and L.C. McKinney. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Recent electrophysiological studies of several types of leukocytes have revealed the presence of a variety of voltage gated ion channels. Previously, we described the presence of a large conductance(250 pS in solutions of symmetrical K)Ca-activated K channel in human macrophages (Gallin,Biophys.J.,1984) which is expressed over time when cells are maintained in tissue culture. We here describe the properties of macroscopic ion currents which may be due to the activity of this Ca-activated K channel,and in addition, report the existence of an inwardly rectifying K conductance not previously described in human macrophages. Patch electrodes contained 150mM KCl,10mM NaCl,1.2mM $MgCl_2$,1.1mM EGTA, and varying Ca concentrations (free Ca ranged from 10^{-8} to $3x10^{-6}$M), pH=7.2. Zero current holding potentials (ZCP) ranged from -30 to -65mV and input resistances, measured within 10 mV of the ZCP were 1-4 GΩ. Records obtained in the whole-cell configuration from cells cultured for 2 to 6 weeks revealed outward currents activating at potentials positive to 30 mV. These currents exhibited no time-dependent inactivation, and had an extremely noisy baseline. Addition of charybdotoxin (CTX), a blocker of Ca-activated K channels, to the bath decreased this current by at least 80% (n=3). We verified that CTX inhibits the activity of single Ca-activated K channels by comparing channel activity in excised inside-out patches with and without CTX in the patch electrode. (KCL=150mM; Ca=$3x10^{-6}$M on either side of patch). Control patches (n=7) contained an average of 4-7 Ca-activated K channels per patch which were active in the potential range tested (-50 to 70 mV). Patches (n=5) from the same cells with CTX (25nM) in the recording pipette contained, at most, 1-2 active channels at potentials > 50 mV. Thus, it is likely that the outward current described above results from Ca-activated K^+ channel activity. In 35% of cells, inward currents were observed which activated at potentials negative to -70, showed time-dependent inactivation for steps negative to -120 mV, and were blocked by addition of $BaCl_2$ (2mM) to the bath. Records obtained in the cell-attached patch configuration from the same cells revealed inward single channel current fluctuations which increased in amplitude as the patch was hyperpolarized and did not reverse polarity with depolarizations up to 100 mV. With voltage ramps, channel amplitude was zero was near the ZCP of the cells. Single channel conductance was 28 ± 3pS (n=9). The properties of both the macroscopic and single channel currents are the same as those of the inward rectifying K current previously described in both primary cultures of mouse macrophages and the mouse-derived macrophage-like cell line, J774 (Gallin and Sheehy, J.Physiol.,1985;McKinney and Gallin,Biophys.J.,1985). We thank Dr. C. Miller for the generous gift of CTX.

367.5 POTASSIUM CHANNELS IN T LYMPHOCYTES MEDIATING EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN RATS S.I.V. Judge*, J.Z. Yeh*, M.D. Mannie* and P.Y. Paterson*(SPON: D.H. Harter). Dept. of Pharmacol. and Dept. of Microbiol.-Immunol., Northwestern Univ. Med. Sch., Chicago, IL. 60611.

Primed T lymphocytes, which are selectively reactive to whole myelin basic protein (MBP) (Paterson, Adv. Immunol. 1966) or to synthetic peptides containing the encephalitogenic determinant in MBP (Mannie et al., PNAS 1985) exhibit proliferation-activation and transfer EAE into normal syngeneic Lewis rats following cultures with these antigens. Since voltage-activated K channels have been implicated as necessary for activation and subsequent proliferation of T lymphocytes (Matteson and Deutsch, Nature 1984; DeCoursey et al., Nature 1984; Chandy et al., J. Exp. Med. 1984), we examined in the role of K channels in T cell activation and subsequent EAE induction. Using the gigohm-seal whole-cell variation of the patch clamp, we studied voltage-activated K channels in T cells of MBP or encephalitogenic continuous cell lines. These cells were studied in the resting state, during activation with blastogenesis and enlargement, and at rest following proliferation.

Potassium conductances for all resting T cells began to be activated between -20 and -30 mV and for blast T cells between -30 and -40 mV. Resting T cells attained maximum K conductances on the order of 2 nS. The maximum K conductance was increased 5-fold in MBP activated T cells and 10-fold in the MBP peptide activated T cells. During the 4 day period of activation in cultures, K conductances increased over time and directly correlated with the extent of T cell activation, as evidenced by the size and relative number of blast cells in the cultures. Both resting and blast cells exhibited potassium inactivation, which appeared to be dependent on the external K concentration. In 160 mM K, the time constant for inactivation was slower and the steady-state inactivation was less complete than in normal K (4.6 mM). The K currents were susceptible to tetraethylammoniun block. In addition, the Ca channel blocker D600 (methoxyverapamil) caused voltage dependent block of K channels and accelerated the decay phase of the K current. In contrast, another Ca channel blocker, nifedipine, had very little effect.

In summary, our results show that K channel function in rat T cells is modulated during the time course of stimulation with encephalitogenic antigens. The K currents represent the currents flowing through delayed K channels, and not Ca-dependent K channels. It remains to be tested whether blocking of K channels prevents activation of T cells and inhibits their capacity to transfer EAE.

367.6 POTASSIUM CURRENTS IN NEUROSECRETORY CELL BODIES. C. Onetti*, R. Valdiosera*, E. Garcia* and U. Garcia* (SPON: M. Pacheco). Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Apdo. Postal 199, 28000, Colima, México and Dept. of Physiology, CINVESTAV, Apdo. Postal 14-740, 07000 México, D.F.

Outward currents in response to membrane depolarizations were recorded at room temperature (22°C) from axotomized neuron somata of the crayfish (Procambarus clarkii) X-organ, using the whole cell clamp technique (Hamill et al, Pflug. Arch. 391: 85, 1981). Cells disection and experimental procedures were as previously reported (Garcia et al, Soc., Neurosci, Abstr., Vol.11, Part 1, p. 520, 1985). Composition of the pipette solution was (in mM): KCl 210, K_2EGTA 10 and HEPES-KOH 10; and the bathing solution: TRIS-HCl 210, $CaCl_2$ 10, KCl 5.4 and $CdCl_2$ 2 (pH = 7.4).

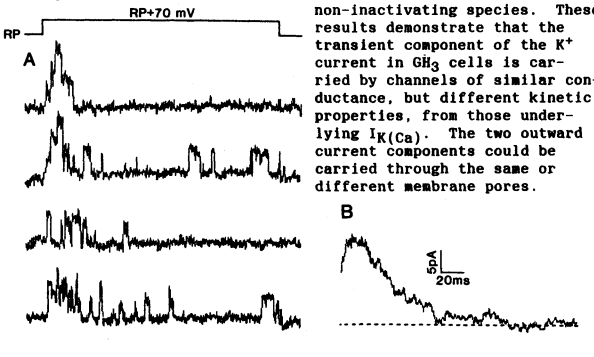
Two components of outward currents were distinguished on the basis of their voltage dependence, time course and different sensitivity to K channel blockers. One of them, activated at membrane potentials between -40 and -30 mV, showing a sigmoidal time course. It did not inactivate during 400 msec and was blocked (90%) by 20 mM TEA in the bath. Presumably, this current is a K current through the delayed rectifier. The other component was a transient current, only seen when the test pulse was preceded by a hiperpolarizing pulse. It was not affected by 20 mM TEA in the bath and was blocked by 2 mM 4AP. This current has the characteristics described for I_A. With respect to its voltage dependence and kinetics we have consistently observed two groups of neurons. In one group (n = 23), the A-current had a threshold of -57 ± 4 mV (mean ± S.D.), the slope of the I-V relationship was 1.8 ± 0.4 mS/cm^2 and the steady state inactivation midpoint was -94 ± 10 mV. In the other group (n = 22), the I_A activated at -42 ± 7 mV, the slope of the I-V curve was 1 ± 0.2 mS/cm^2 and the steady state inactivation midpoint was -72 ± 5 mV.

We do not know whether this finding is due to the existence of two populations of neurons or is caused by two different metabolic states of these cells.

367.7 **PATCH CLAMP RECORDING OF TRANSIENT POTASSIUM CHANNELS IN CLONAL ANTERIOR PITUITARY CELLS.** **Michael A. Rogawski and Jeffery L. Barker. Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.**

Voltage clamp recordings from GH_3 clonal anterior pituitary cells have revealed the existence of two kinetically distinct outward current species. Virtually all cells exhibit a sustained Ca^{2+}-dependent K^+ current [$I_{K(Ca)}$] that is sensitive to inorganic Ca^{2+} channel blockers and tetraethylammonium. We have previously reported that a 55-90 pS Ca^{2+}- and voltage-sensitive K^+ channel underlies this current. In addition, some GH_3 cells possess an inactivating component to the outward current, referred to as $I_{K(V)}$. In this communication, we report patch clamp recordings of the channels underlying $I_{K(V)}$.

Single channel recordings were carried out in the cell attached configuration from GH_3 cells (kindly provided by B. Dufy). The bathing medium and patch electrode contained 5.5 mM K^+, 10 mM Ca^{2+} and 1 mM Mg^{2+}. Step depolarization of the patch (200-300 msec) invariably activated $I_{K(Ca)}$ channels whose open probability and mean open-time increased strongly as the patch was depolarized in the range 10-50 mV from resting potential (RP). These channels opened randomly so that in ensemble averages the mean current was time invariant during the step. However, occasionally, with large depolarizations (50-90 mV from rest) a kinetically distinct population of channels was observed (Fig. A). Ensemble averages of these channels during repetitive voltage steps demonstrated that their probability of being in the open state was maximum 5-20 msecs following the onset of the depolarization, and the probability decayed rapidly within 30-90 msec (Fig. B). The conductance and mean open time of these channels is comparable to those of the non-inactivating species. These results demonstrate that the transient component of the K^+ current in GH_3 cells is carried by channels of similar conductance, but different kinetic properties, from those underlying $I_{K(Ca)}$. The two outward current components could be carried through the same or different membrane pores.



367.8 MEMBRANE LIPIDS AND SINGLE CHANNEL PROPERTIES OF A VOLTAGE-SENSITIVE K^+ CHANNEL ON NG108-15 CELLS. R. McGee. Zoology Dept., Univ. of Nottingham, Nottingham NG7 2RD, England.

Currently, little is known regarding the extent to which single channel properties of voltage-sensitive ion channels are determined by the lipid environment of the cell membrane. To examine this question, NG108-15 neuroblastoma x glioma hybrid cells were grown in the presence of arachidonic acid (20:4) which caused substantial enrichment of the membrane phospholipids with 20:4 at the expense of oleic acid (18:1) (Cell. Molec. Neurobiol. 5: 333-352, 1985). Using the cell-attached patch clamp technique a voltage-sensitive channel with apparent selectivity for K^+ (observed in about 10% of the patches) was studied. Recordings were made with electrodes (4-10mΩ) which contained the same saline which bathed the cells (150 NaCl, 5.4 KCl, 1.0 $CaCl_2$, 0.8 $MgCl_2$, 10 HEPES, pH 7.4). Channel openings were observed as outward currents using a List EPC-7 amplifier and recorded on video tape. Recordings containing only a single channel were later filtered at 3khz, digitized at 50μs intervals and analyzed using a PDP 11/27 computer. Channel openings were elicited by stepping the pipette holding potential from +60mV (to remove inactivation) to values ranging from -30 to -120mV. The first channel opening usually occurred within 1-10ms. Subsequent channel kinetics were complicated; closed times ranging from < 0.25ms to > 10 sec were observed and the total time before inactivation ranged from < 50ms to > 20 sec. The primary conductance of the channel was 18pS, but subconductance states were clearly visible. Enrichment with 20:4 did not appear to alter channel conductance. Preliminary comparison of the latency to first channel opening for 1 patch each from control and 20:4-enriched cells indicated times of 3.52 + 1.97ms (SD n = 29) and 4.81 + 3.16 (n = 27) ms, respectively. These initial results suggest that 20:4 enrichment may cause a longer and more variable latency time, but additional recordings will have to be analyzed before this conclusion can be confirmed. A detailed analysis of the channel kinetics for the voltage-sensitive K^+ channel from control and 20:4-enriched cells also will be presented.

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367.9 MEMBRANE PROPERTIES OF GUINEA-PIG CULTURED INTRINSIC CARDIAC NEURONS. D.J. Adams* and L.A. Fieber* (SPON: K. Magleby). Dept. of Pharmacology, Univ. of Miami Sch. Med., Miami, FL 33101.

Parasympathetic ganglion cells prepared from dissociated atrial myocardium of newborn guinea-pigs and grown in tissue culture for 8-15 days (Hassall, C.J. & Burnstock, G., Neurosci. Lett., 52:111, 1984) were studied using whole-cell and single channel recording techniques. Cultures of intrinsic cardiac neurons occurred either in isolation or in clusters of 20-30 nerve cell bodies that were 25-35μm in diameter and were positively identified with a fluorescein-conjugated antiserum to tetanus toxin. Whole-cell patch clamp recording with electrodes filled with 140mM KCl and 0.5mM K_2 EGTA was used to investigate passive and active membrane properties. Cardiac neurons had resting potentials in the range of -45 to -65mV and input resistances of >500 Mohm. Although cultured cardiac neurons were normally quiescent, action potentials (>80mV amplitude) could be evoked with brief depolarizing current pulses. Single channel recordings from cell-attached membrane patches were made using either 5mM or 140mM KCl-filled pipettes containing 1-10μM acetylcholine (ACh). ACh-induced ionic currents were observed in >50% of cultured neurons, consistent with the distribution and density of the muscarinic ligand, [^{3}H]-propylbenzylcholine mustard, binding sites found on these nerve-cell bodies and processes (Buckley, N.J. & Hassall, C.J., unpublished observations). The current-voltage relationship exhibited marked inward-going rectification and the reversal (zero-current) potential for the ACh-activated channel was similar to the K^+ equilibrium potential. In response to depolarizing voltage steps, at least two types of outward single channel currents were also observed in most neuronal membrane patches. Reversal potential measurements suggest that potassium is the major charge carrier for both currents which would act to repolarize the membrane potential and may contribute to maintaining neuronal excitability. (Supported by AHA grant-in-aid, Florida Affiliate).

367.10 c-AMP DEPENDENT PROTEIN KINASE OPENS SEVERAL K^+ CHANNELS FROM MAMMALIAN BRAIN. R.Reeves*, J.Farley, & B.Rudy,(SPON: W.E. Sullivan) Prog. in Neurosci. & Behavior, Princeton Univ., Princeton, NJ 08544 & Dept. of Physiol. & Biophys., NYU Medical Ctr., NY,NY 10016.

It has been suggested that phosphorylational control of ionic channels might underly persistent changes in neuronal excitability in the brain, and that the target for such modulation might be channels themselves. To test the plausibility of this notion, we have assessed the effects of the purified, catalytically-activated subunit of c-AMP-dependent protein kinase upon K^+ channels incorporated into artificial membranes formed on the tips of patch clamp electrodes. The source of channels was a synaptosomal membrane preparation obtained from whole rat brain. Three distinct K^+ selective ion channels, distinguished in previous studies (Farley & Rudy, Soc. Neurosci. Abstr. 1985; Biophys. J. 1986) on the basis of their unitary conductance, voltage-dependence, sensitivity to calcium, and pharmacology were studied. A fourth channel, not previously described, was also characterized. A-kinase in a final bath concentration of $6X10^{-8}$ M resulted in consistent, reliable, and persistent increases (>50% of baseline levels) in the open time of several of these channels. The large (264 ± 64 pS; mean ± SEM) TEA-sensitive, calciumactivated K^+ channel (Type I; BK) was opened in 9 of 9 separate experiments. An intermediate-sized (Type II; 123 ± 11 pS) calcium-activated, TEA-insensitive K^+ channel was opened in 9 of 11 experiments; while in 1 experiment , open-time increases < 50% were observed. A smaller (Type IV; 71 ± 5 pS) K^+ channel, whose voltage- and calcium-sensitivity have not yet been determined, was opened in 7 of 8 experiments. No consistent effect upon a 50 pS K^+ (Type III) channel, which appears to be calcium- and voltage-insensitive, were observed. In 5/13 experiments, the channel was opened, in 4/13 the channel closed, in 4/13 experiments no effect was observed. In 6 separate experiments in which several of the above channels were present in the membrane, heat-inactivated kinase was without effect. An interesting finding was that while no exogenous ATP was required for the A-kinase effects, A-kinase effects could be prevented if ATPase (.002 - .17 units: .02 - 2.3 μM) were added prior to kinase addition. Measurements of ATP indicated free concentration levels < 10 μM, an order of magnitude less than ATP's K_m value for A kinase. Thus, it is unlikely that free ATP serves as the phosphate source in these experiments. It is possible that membrane-bound/compartmentalized ATP is the source. Finally, it should be noted that the opening of channel Types II and IV by A-kinase stands in contrast to the closure of these channels by the calcium/phospholipid enzyme, protein kinase C (Lipkin et al., this volume). Supported by NSF BNS-8316707 to J.Farley

367.11 PROTEIN KINASE C EFFECTS ON SINGLE K^+ CHANNELS FROM MAMMALIAN BRAIN. S.Lipkin*, J.Farley, & B.Rudy (SPON:K.R.Jennings) Prog. in Neurosci. & Behavior, Princeton University, Princeton, NJ 08544 & Dept. of Physiol. & Biophysics, NYU Medical Ctr., NY,NY 10016.

Phosphorylational control of ionic conductances has been suggested to be a widespread mechanism for long-term changes in neuronal excitability. We have examined the effects of the calcium/phospholipid sensitive enzyme (protein kinase C; PKC) upon several varieties of K^+ channels from mammalian brain, inserted into artificial membranes formed on the tips of patch clamp electrodes. These channels have been previously characterized on the basis of their unitary conductances, calcium- and voltage-sensitivity, and pharmacology (Farley & Rudy, Soc. Neurosci. Abstr. 1985; Biophys. J. 1986).

Final bath concentrations of 1-2 units of PKC (1 unit transferred 400 pmols of phosphate), resulted in decreases (of 50% or more) in the open-time of a calcium dependent, TEA-insensitive, intermediate-sized K^+ channel (100 ±3 pS; Type II channel) in 13 of 13 cases. In contrast to the closure of the Type II channel, PKC addition resulted in increases (> 50%) in the open time of another smaller K^+ channel (52 ± 1.5 pS; Type III) in 11 of 12 experiments. This channel appears to be both calcium- and voltage-independent. PKC was without effect on the large (BK) calcium-dependent potassium channel in several experiments. Heat-inactivated kinase was without effect in 5 of 5 experiments. Similarly, bath concentrations of 10 μ M imipramine, a reported PKC inhibitor, prevented the effects of PKC in 3 of 3 experiments.

As in companion studies on the effects of c-AMP PK (Reeves et al., this volume), we found that addition of exogenous ATP was not required for PKC's effects. However, addition of ATPase (.04 -.08 activity units) prevented PKCs effects. Measurements of free ATP (in the absence of ATPase) indicated the bath concentrations to be no higher than 10 μM, well below the K_m value for C kinase. These results suggest that a membrane-bound/compartmentalized source of ATP served as the phosphate source. It is also of interest to note that ATPase itself had detectable effects upon several of the channels.

It is interesting that PKC effects upon the Type II (closure) and Type III (opening) channels were the opposite of c-AMP PK. These results suggest that ion channels can serve as a common substrate for various protein kinases, and that these kinases may act to dually regulate the functional state of the channels in vivo.

Supported by NSF grant BNS 8316707 to J. Farley.

367.12 **CALCIUM CURRENT IN ACUTELY ISOLATED ADULT RAT SUPERIOR CERVICAL GANGLION NEURONS.** Stephen R. Ikeda, Geoffery G. Schofield, and Forrest F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The somata of neurons in the superior cervical ganglia were isolated from adult (100-300 g) rats using proteolytic enzymes (Ikeda et al., *J. Neurophysiol.* **55**: 527-539, 1986). Current-clamp recordings using the whole-cell patch-clamp technique in normal physiological solution revealed resting membrane potentials around -44 mV. Large overshooting action potentials could be elicited by depolarizing pulses for up to 5 hours following isolation of the cells.

The Ca^{2+} current was recorded using the whole-cell patch-clamp technique. Isolation of the Ca^{2+} current was accomplished using ion substitution and pharmacological agents. The external solution contained (mM): TEA-Cl 140, HEPES-CsOH 10, $MgCl_2$ 0.8, $CaCl_2$ 10.0, glucose 10, and TTX 0.003. The internal solutions contained (mM): N-methylglucamine-HCl 120, HEPES 10, EGTA 11, $CaCl_2$ 1.0, and $MgCl_2$ 2.0. In addition, the internal solution contained MgATP 4 mM, diTRIS-phosphocreatine 20 mM, and creatine kinase 50 U/ml to maintain Ca^{2+} currents (Forscher P. and Oxford G.S., *J. Gen. Physiol.* **85**:743-63, 1985). In these solutions, depolarizing pulses from a holding potential of -80 mV generated a sustained inward current at potentials more positive than -30 mV. Maximal inward current amplitude was attained near +10 mV; positive to this potential the inward current decreased and approached a zero current asymptote. A low threshhold transient Ca^{2+} current was not observed even with more negative holding potentials (-100 mV). Removal of Ca^{2+} from the external solution reversibly abolished the inward current. Inclusion of 500 μM Cd^{2+} in the external solution reversibly reduced the inward current to less than 5% of control. Substitution of external Ca^{2+} with Ba^{2+} increased the maximal inward current and shifted the peak of the I-V curve to near 0 mV.

The use of the whole-cell patch-clamp technique in these isolated neurons allows better current isolation and spatial clamp control than conventional intracellular electrodes in intact ganglia preparations. In addition, the preparation should be suitable for the study of the actue and chronic effects of pharmacological agents as well as the effects of internal messengers and neurotransmitters on Ca^{2+} currents in an adult mammalian neuron.

367.13 **THREE POTASSIUM CURRENTS IN ACUTELY ISOLATED ADULT RAT SUPERIOR CERVICAL GANGLION CELLS.** Geoffrey G. Schofield, Stephen R. Ikeda and Forrest F. Weight. Laboratory of Preclinical Studies. National Institute on Alcohol Abuse and Alcoholism, Rockville, Md. 20852.

Single superior cervical ganglion (SCG) somata were acutely isolated by a modified enzymatic dispersion technique (Ikeda *et al.*, J Neurophysiol **55**: 527 1986) from male rats (100-300g). The neurons were voltage-clamped using the whole-cell patch-clamp technique.

In solutions designed to isolate K^+ currents, depolarizations from a holding potential of -80 mV elicited two distinct outward current components. An initial component activated at potentials more positive than -60 mV, rose rapidly, and then decayed. A second sustained current component was observed at potentials more positive than -20 mV.

The initial transient current was inactivated by holding the membrane potential at -50 mV, whereas the second current component was reduced only slightly at this holding potential. The transient current decayed with a time constant of approximately 20 msec. When the holding potential was -50 mV, the transient current could be fully reactivated by a conditioning prepulse to -100 mV. The time constant of reactivation at -100 mV was approximately 36 msec. This current was not blocked by 0.5 mM Cd^{2+}-containing or Ca^{2+}-free external solutions, but could be markedly reduced by 2 mM 4-aminopyridine. The transient current had the characteristics of I_A which has been observed in intact rat SCG (Wanke *et al.* J Physiol **358**: 91 1985).

The sustained outward current could be further separated into two outward current components. Depolarizations from -50 mV elicited a current that activated at potentials more positive than -20 mV, and was blocked by superfusion with a 0.5 mM Cd^{2+}-containing or Ca^{2+}-free external solution. Digital subtraction revealed that the Ca^{2+}-sensitive current activated with an exponential time constant of 20 to 40 msec over the potential range 0 to +10 mV. The current that remained in the Ca^{2+}-free external solution also activated at potentials more positive than -20 mV and was reduced by superfusion with 10 mM TEA-Cl.

We conclude that under the conditions of these experiments there appear to be at least 3 outward currents in acutely isolated neuronal somata from adult rat SCG: (i) a transient K^+ current with the characteristics similar to I_A; (ii) a sustained Ca^{2+}-sensitive K^+ current similar to $I_{K(Ca)}$; and (iii) a sustained Ca^{2+}-insensitive, TEA-sensitive K^+ current that appears to be similar to the delayed rectifier or I_K.

367.14 Elevated K^+ Shortens Action Potential Duration by Inducing Transient K^+ Current in Cultured Chick DRG Neurons. J. Yang, C.E. Zorumski, and G.D. Fischbach, Depts. of Anatomy & Neurobiology and Psychiatry, Washington University, School of Medicine, St. Louis, MO.

Dissociated embryonic chick DRG neurons maintained in culture exhibit a mixed NA^+/Ca^{++} action potential (AP). The characteristic "shoulder" on the repolarizing phase is due to the relatively prolonged inward Ca^{++} current. DRG neurons grown in elevated K^+ medium (20mM vs 4.8mM) lack the plateau phase of the AP (Dev. Biol. 78:173, '80). This alteration in repolarization may be due to a decrease in an inward depolarizing current or, alternatively, an increase in an outward repolarizing current. To distinguish between these mechanisms, we investigated membrane currents of voltage clamped DRG neurons with whole cell patch clamp techniques.

Experiments were performed on DRG neurons grown in 20mM K^+ for periods of time ranging from 6 hrs-4 days. Upon transfer to the recording solution containing 4.8mM K^+, the neurons recovered their usual RMP (-50mV) within 10 minutes. Voltage clamp analysis with recording electrodes containing CsCl/TEA and TTX in the bath to block K^+ and Na^+ currents revealed prominent Ca^{++} currents in neurons from both K^+ treated and control cultures. Outward currents were recorded using KCl containing electrodes with Cd^{++} and TTX in the bath. Step depolarization from -50mV holding potential resulted in a delayed K^+ current in both control and K^+ treated cells. However, when the holding potential was increased to -80mV, depolarizing steps revealed a rapid, transient outward current blockable by 4-aminopyridine in K^+ treated but not in the control neurons. We have not yet quantitatively characterized this transient outward current but it is likely that it can account for the rapid AP repolarization in K^+ treated neurons. In current clamp, puffer application of micromolar concentration of 4-aminopyridine to K^+ treated cells resulted in broadening of AP with the characteristic plateau indistinguishable from AP elicited in control cells.

Since the effect of K^+ was observed in neurons cultured for a relatively short time (~6 hours), we were able to investigate the action of a protein synthesis inhibitor. Puromycin (10μg/ml) failed to block the K^+ induced shorteneing of AP suggesting no involvement of protein synthesis. The mechanism of induction of this new current by the elevated K^+ culture condition and a possible role of second messenger are under investigation.

367.15 POTASSIUM SINGLE CHANNELS IN CULTURED MOUSE SPINAL CORD NEURONS: A MACROSCOPIC ANALYSIS. J.E. Kimura*, B. McIver* and C.L.-H Huang* (SPON: E. Hendley) Dept. of Physiol. and Biophys., Univ. of Vermont, Coll. of Med., Burlington, VT 05405.

Averages of 100 to 200 traces of single channel current records from outside-out patches revealed a "macroscopic" current trace similar to the potassium current recorded from squid giant axon in sodium free tetrodotoxin containing solutions. A slope conductance of 8.7 pSiemens was obtained for the linear region of the current-voltage (I-V) relationship of the open channels positive to - 40 mV. This relationship saturates at positive potentials. Short open times are observed at these potentials even at 10 C and open channel currents are difficult to determine. Consequently, the non-linearity in the I-V relationship may in part be due to limitations in bandwidth. Inactivation of the average record at 0 mV was not observed even after 100 msec. Strictly for descriptive purposes, the best fit of the Hodgkin-Huxley potassium gating scheme requires the power (X) to be 3 at -20 mV and 5 at 30 mV. The mid-point of the activation curve, assuming X=4 is approximately -40 mV and that of the "open probability curve" is 5 mV. Tau-n is 16 msec at -40 mV and falls to 3 msec at 50 mV at 10 C. At a similar temperature, tau-n varies from 7 to 1 msec over the same voltages in squid giant axon. The following expressions describe the relationship between the forward and back rate constants with potential.

$$\alpha_n = \frac{0.9\gamma}{e^{\gamma}-1} \qquad \beta_n = \frac{0.04\delta}{e^{\delta}-1}$$

$$\gamma = \frac{0.1 - V_m}{0.03} \qquad \delta = \frac{0.4 + V_m}{0.3}$$

α_n changes slowly with voltage, while β_n is steeply dependent on voltage, rising from 0.02 $msec^{-1}$ at -40 mV to 0.3 $msec^{-1}$ at 40 mV.

During a voltage clamp step channels open and remain open until repolarization to the holding potential when channels would close. Flickering was observed at potentials positive to 0 mV and the rate increased with potential. Mean closing times during flickering decreased with potential. The time to first event histograms could be characterized by a rising phase followed by a falling phase, which was well defined by a single exponential distribution. The time constant of this distribution became shorter with voltage.

This work was supported by NSF grant BNS 82-17484 and grants from the Whitaker and Epilepsy Foundations to J.E.K.

367.16 MEMBRANE CURRENTS IN RAT CORTICAL NEURONS IN CELL CULTURE. M. Dichter, C. Zona*, M. Frosch, G. Pironne*, G.F. Ayala. Chairs of Neurophysiology and Human Physiology, Department of Experimental Medicine, University of Rome, Tor Vergata, Rome, Italy.

Rat cortical neurons maintained in dissociated cell culture (Dichter, Brain Res. 149:279, 1978) were recorded in the whole cell patch clamp mode. Neurons were studied from 3 days to 4 weeks in culture. Under physiological conditions, depolarizing commands from holding potentials more negative than -40 to -50 mV produced a large, rapid, transient inward current which had a threshold at approximately -30 to -40 mV and a peak current as high as 6000 pA. This was followed by at least two outward currents; a persistent current and a transient current which was activated at membrane potentials more negative than -60 mV and which lasted only approximately 10 msec. All three of these currents were present from at least 3 days in vitro. Under conditions where outward K and inward Ca currents were blocked (Cs and TEA in the electrode, 4-AP (2 mM) and Cd (0.2 mM) in the bath), depolarizations subthreshold for the large transient Na current, produced a slowly inactivating inward current. This current had a low threshold, an onset within msec of the depolarization and was TTX sensitive. Thus, as had been shown for neocortical neurons in slices, these cells possess a slowly inactivating Na current which is activated close to the RMP (Connors et al, J. Neurophysiol. 48:1302, 1982; Stafstrom et al, J. Neurophysiol. 53:153, 1985). The cortical neurons also had two kinds of Ca currents when studied under appropriate conditions (see Zona et al, this meeting).

367.17 EFFECTS OF REPETITIVE ACTIVATION AND CHANGES IN IONIC MILIEU ON CA1 PYRAMIDAL CELL AHPS. V. Tancredi C. Franck,* M. Dichter. Chairs of Neurophysiology and Human Physiology, Department of Experimental Medicine, University of Rome, Tor Vergata, Rome, Italy.

The large, longlasting AHP seen in hippocampal pyramidal neurons has been postulated to be responsible for the hyperpolarizing potential which occurs after epileptiform depolarizing shifts (post-DS HP). It has been proposed that an increase in extracellular K causes the post-DS HP to decrease and ultimately reverse during the transition from interictal discharges to seizures. Alternatively, the post-DS HP could decline during repetitive activation because of intrinsic factors. We have tested these hypotheses by recording AHPs from hippocampal CA1 neurons in the in vitro slice preparation. Under baseline conditions (3.25 mM K, 2 mM Ca physiological saline, 33-35 C), neuronal RMP was -76 mV and maximal AHPs evoked by 500 msec depolarizing pulses of 0.8 to 2 nA were 10 mV large and lasted approximately 1.2 seconds. They were associated with a 25-45% decrease in membrane resistance (after accounting for anomalous rectification.) When evoked at increasing membrane potentials, the AHPs either became isoelectric without reversing or reversed at approximately -99 mV. When extracellular K was raised to 10 mM, RMP declined from -76 mV to -66 mV, but AHPs remained hyperpolarizing (mean amplitude 6.0 mV). Reversal potential for the AHP in 10 mM K was -89 mV.

When AHPs were repetitively evoked in control media at ISIs of 5 seconds for 1-2 minutes, no decrement was seen. When ISI was decreased to 3 and 1 second, membrane potential became clamped at hyperpolarizing values and no decrement was seen after 2 minutes. Repetitive activation in high K or in high K (10 mM) combined with low Ca (0.5 mM) failed to cause a decrement in the AHP.

Thus, if AHPs are responsible for the post-DS HP, some mechanism other than extracellular K accumulation and Ca decrement, or repetitive activation must be postulated to account for their decline during the transition to seizures.

367.18 CA CURRENTS IN RAT CORTICAL NEURONS IN CELL CULTURE. C. Zona*, M. Dichter, G. Pironne*, G.F. Ayala. Chairs of Neurophysiology and Human Physiology, Department of Experimental Medicine, University of Rome, Tor Vergata, Rome, Italy.

Rat cortical neurons maintained in dissociated cell culture were recorded in the whole cell patch clamp mode. A number of intrinsic voltage sensitive membrane currents were studied in these cells, including the rapid transient Na, a slowly inactivating Na, and at least two K currents (see Dichter et al, this meeting). When outward K currents were suppressed by intracellular Cs and TEA and extracellular 4-AP and Na currents by TTX, depolarizing step commands to between -40 and 0 from holding potentials between -40 and -100 mV revealed an additional inward current which may be divisible into two separate components. The first occurred with lower threshold depolarizations (threshold approximately -40 mV), activated within msec of the depolarizing command, was smaller (between 20 and 500 pA) and had a very slow inactivation at depolarized membrane potentials. The second had a higher threshold, was larger in amplitude (up to 2000 pA) and had a faster decay (over several hundred msec.) The latter is likely to be the current responsible for the Ca dependent action potentials seen in these cells under comparable conditions in current clamp recordings. Both currents were present in 5 uM TTX or when Ba was substituted for Ca, and both were reduced or abolished by 0.2 mM Cd application, indicating that they were most likely Ca currents.

Ca currents tended to deteriorate over several minutes of recording in the WC mode, as has been reported in other systems. Addition of 2 mM ATP and 1 mM Mg to the internal electrolyte slowed this inactivation. Holding the membrane at -60 mV slowed the "wash out" even further, and under these conditions we have recorded stable currents for more than 30 minutes.

367.19 ISOPROTERONOL REDUCES A LONG-LASTING AHP IN NEURONS FROM CAT SENSORIMOTOR CORTEX IN VITRO. R.C. Foehring, P.C. Schwindt and W.E. Crill, Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle WA 98195

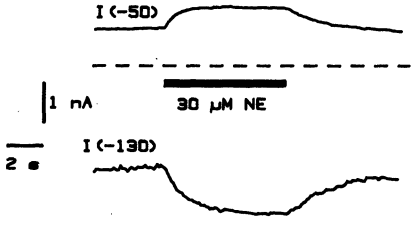
Layer V neurons from cat sensorimotor cortex were studied in the neocortical slice by impaling cells with microelectrodes filled with 3M KCl or K-acetate. Repetitive firing behavior and membrane electrical properties were examined before and after application of a microdrop containing 10^{-2}M Isoproteronol (Iso), a B-noradrenergic agonist, to the surface of the slice. The microdrop was used to avoid oxidation of Iso in the superfusate. The actual concentration of Iso in the slice was likely << 10^{-2}M. Repetitive firing in response to 1s current injections was studied in 9 cells. In all cases Iso increased firing rate at a given current level, the slope of the f-I relation usually increased, and early spike-frequency adaptation was reduced. Most striking was a reduction in the duration of an afterhyperpolarization (AHP) which decayed in > 2s following trains of spikes. Both the AHP and its reduction by Iso were most clearly seen following summation of slow AHPs by repetition of 1s current pulses (5 to 7 nA) every 2s. In 14/15 cells the summated AHP decreased in duration by 6.6-67% (mean 36.4%) following Iso. Similar results were obtained following single 1s trains of spikes. In 17/19 cells application of Iso resulted in a small depolarization (1-8mV, mean 3.4mV), which also occurred in the presence of TTX and when using K-acetate electrodes. Synaptic noise usually increased with Iso. Input conductance decreased slightly in 8/10 cells. Effects on single action potentials were variable: The fast AHP following a single spike decreased in amplitude in 6/8 cells and decreased in duration in 4/8 cases. In two cells tested, the response increased with increased concentration of Iso in the drop (10^{-6}, 10^{-4}, 10^{-2}M). The Iso effects were not seen in the presence of B-blocking agents (propranolol, timolol). No response was seen in 2 cells tested with phenylepherine, an a-noradrenergic agonist. Preliminary voltage clamp analysis indicates that Iso results in decreased K^+ currents. Supported by NIH grants NS16972, NS22410 and NS07097.

367.20 NOREPINEPHRINE-INDUCED BACKGROUND CURRENT IN BULLFROG SENSORY NEURONS. L. J. Regan* and B. P. Bean. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

We used whole-cell recording to study currents in bullfrog sensory neurons. Cell bodies were isolated from dorsal root ganglia by treatment with collagenase (1 mg/ml) in Ca^{++}-free saline that contained albumin (1 mg/ml). Cells were voltage-clamped with patch pipettes containing (in mM) 120 K glutamate, 10 K_2EGTA, 5 $MgCl_2$, 5.5 ATP, 1.1 GTP, 10 HEPES, pH 7.4. The external solution was 2 or 10 $CaCl_2$, 150 NaCl, 4 KCl, 2 $MgCl_2$, 10 HEPES, pH 7.4, 22°C.

The average resting potential of the cells was -75 mV. The cells seemed to retain many of their channels and transmitter receptors after enzymatic dispersion; they had large voltage-activated Na^+, Ca^{++}, and K^+ currents, and the Ca^{++} currents were sensitive to modulation by norepinephrine (NE), GABA, ATP, leu-enkephalin, and acetylcholine.

In addition to decreasing the Ca^{++} current, NE produced an increase in the resting conductance of the cell (usually without changing the resting potential much). To test for the presence of the NE-induced conductance increase, cells were held at -50 mV and stepped to -130 mV for 50 msec at 100 msec intervals. In 17 out of 46 cells tested, 30 uM NE induced an outward current at -50 mV and an inward current at -130 mV. Although complete solution exchanges were accomplished within 200 msec, the onset and recovery of the NE-induced current took seconds. The NE-induced current had a roughly linear I-V relationship, and reversed at -74 ± 3 mV (n=8). When the K^+ concentration of the external solution was raised from 4 to 16 mM, the reversal potential shifted to -50 ± 3 mV (n=5), suggesting that the current is largely (but not purely) a K^+ current. In three out of 46 cells, NE decreased rather than increased the background conductance.



368.1 SPATIAL DISTRIBUTION AND KINETIC DIFFERENCES QF Ca^{++} CURRENTS ACROSS THE SOMA MEMBRANE IN MOLLUSCAN NEURONS. S. Thompson and J. Coombs*. Hopkins Marine Station,Pacific Grove,CA 93950

Neurons isolated from the central ganglia of the nudibranch Doriopsilla were voltage clamped with two microelectrodes. Membrane currents were recorded simultaneously from the whole cell and from a restricted region of the soma by a moveable loose-patch current recording electrode. The area of membrane within the patch electrode was measured electrically and corresponded to about 0.01% of the cell surface area. Ca currents were recorded in a 60 mM Ca, zero Na saline containing TEA and 4-AP. The spatial distribution of Ca channels was determined by recording patch currents in response to standard voltage clamp pulses as the patch electrode was moved to various sites from the cap of the cell, antipodal to the axon, to the region of the axon hillock. The amplitude of the Ca current and, therefore, the density of Ca channels increased along this gradient. Also, the kinetics of the Ca current near the soma cap were very different than the kinetics of Ca currents recorded near the equator or near the axon. Ca currents near the cap do not inactivate during prolonged depolarization or during conditioning depolarizing pulses while currents near the axon and the whole cell current show inactivation. These findings indicate that during depolarization the Ca ion load delivered near the top of the cell where the nucleus sits is different than the load delivered near the axon spike initiation zone.

368.2

WITHDRAWN

368.3 INACTIVATION MECHANISMS OF CALCIUM CHANNELS IN HORIZONTAL CELLS ISOLATED FROM THE CATFISH RETINA. B. N. Christensen, Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Cone horizontal cells enzymatically isolated from catfish retina have several types of voltage sensitive membrane channels including sodium, potassium and calcium. The sodium and calcium channels are activated during cell depolarization and are responsible for the action potential seen in the isolated horizontal cell. The sodium channels inactivate rapidly, but the calcium channels appear to turn off only slowly. The long life time of the calcium channel and the absence of an outward potassium current is believed to play a role in maintaining the long lasting plateau region during the action potential. The membrane potential during the plateau may remain positive indefinitely, returning to the negative resting membrane potential only following extrinisic current injection. How the inward calcium current is turned off is not clear and the possible mechanisms are the subject of this paper.

All experiments are done under voltage clamp using the single patch electrode in the whole cell configuration. The three possibilities under investigation include voltage dependent inactivation, a calcium activated potassium current, and calcium dependent inactivation. It seems unlikely that there is a calcium activated potassium conductance since neither 30 mM TEA nor the bee toxin apamin alter the slow decline in the inward current. Substitution of 25 mM barium for calcium also results in a slow decrease in the inward current suggesting that the decrease may be due to accumulation of unbuffered intracellular barium. Consistent with this hypothesis, the inward barium current can be depressed in two ways. In two pulse experiments, a test pulse produces the smallest inward current when the conditioning pulse activates maximally the calcium channel. As the conditioning pulse approaches E_{Ca} reducing the inward barium transport, the inward current becomes larger during the test pulse. When both conditioning and test pulse activate maximally the calcium channel, the inward current during the test pulse is smallest when the interval between pulses is zero and becomes larger as the interval increases indicating that barium entry during the first pulse depresses entry during the second. These results suggest that the slow turn-off of the calcium current in isolated horizontal cells is due, at least, in part to a calcium dependent inactivation.

This research has been supported in spirit by grant DHHS EY-01897.

368.4 **INDUCTION OF A CALCIUM CHANNEL IN ASTROCYTES BY cAMP.** L.L.Y.Chun*, B.A.Barres, and D.P.Corey (SPON: X.O.Breakefield) Program in Neuroscience, Harvard Medical School, Howard Hughes Medical Institute and Department of Neurology, Massachusetts General Hospital, Boston, Ma.

Astrocytes in culture are thought to have a voltage-sensitve calcium conductance, because they can fire a slow action potential when the bath composition is modified to increase and to isolate calcium current (MacVicar, Science 1984). In order to observe this conductance and to further analyze its properties, we used the whole-cell patch clamp technique to record from purified cultures of cerebral cortical astrocytes. The current was not observed under normal culture conditions, but could be induced by agents that activate a cAMP second messenger system.

Cultures were prepared from postnatal-day-1 rat cerebral cortex using the technique of McCarthy and deVellis and were further purified by the procedure of Noble and Raff. They were immunohistochemically verified to be greater than 98% pure type 1 astrocytes. Solutions for electrophysiological recording were designed to reveal even a small calcium current. The bath contained (in mM) 10 Ca, 120 TMA methanesulfonate, 20 Cl, 5 HEPES; the pipette solution was 120 TEA methanesulfonate, 1 Mg, 2 Cl, 10 EGTA buffered to 10^{-9} M Ca, 5 HEPES; both pH 7.4. Under these conditions, voltage-sensitive calcium current could not be detected.

In MacVicar's experiments, electrophysiologic recording was facilitated by preincubating the astrocytes for 2 to 8 hours in dibutyryl cyclic AMP. This induced the normally flat astrocytes to round up. Accordingly, we repeated our experiments after first incubating the cells in substances known to increase cAMP levels in astrocytes: forskolin (10 uM) or 8-br-cAMP (1 mM) for 0.5 to 2 hours. This treatment induced morphologic transformation of the cells. Under these conditions, all cells displayed a voltage-sensitive calcium current ranging from 50 to 200 pA in peak inward current. The conductance activated with depolarizations to -30 mV or more, had only a slow voltage-independent inactivation, and was blocked by cobalt. This conductance appears identical to the "L" or long-lasting calcium channel described by Nowycky *et al.* (Nature, 1985) in DRG neurons. It appears as early as 15 minutes after forskolin exposure and remains as long as 2 hours after forskolin wash-out. Earlier and later times are being investigated.

Thus far, the induction of calcium current appears to be specific to type 1 astrocytes. Type 2 astrocytes, which normally express the "T" or transient calcium channel (Barres *et al.*, Soc. Neurosci. Abst. 1985), cannot be induced to express the "L" channel with forskolin. Similarly, a calcium current cannot be induced in cultured meningeal (pial) cells, although they too round up in response to forskolin.

The induction of an otherwise unexpressed calcium current by cAMP raises two questions. First, what is the mechanism of induction? Second, can physiological substances known to increase cAMP levels in type 1 astrocytes (norepinephrine, VIP, somatostatin) also induce this channel?

368.5 KINETICS OF THE CALCIUM-DEPENDENT ACTION POTENTIAL OF PAPILLARY MUSCLE FROM NORMAL AND CARDIOMYOPATHIC HAMSTERS. K. L. Rossner, and F. E. DiBenedetto*. Dept. of Physiology, New York College of Osteopathic Medicine, Old Westbury, N.Y. 11568

Ca2+ - dependent action potentials (Ca-APs) of papillary muscle from the left ventricle of 10-14 day old normal (BIO F_1B) and cardiomyopathic (BIO 14.6) hamsters were studied in order to evaluate the condition of the slow inward (calcium) current in myopathic tissue in an early stage of the disease. In addition, the effect of isoproterenol (ISO) on Ca-APs was examined. After decapitating a hamster, the papillary muscle was dissected and secured in a 1 c.c. capacity chamber, superfused with aerated (95% O_2:5% CO_2) Tyrode solution at 35°C, pH 7.3, and allowed to equilibrate for 30 minutes. Membrane potentials were recorded with flexibly mounted glass microelectrodes filled with 3M KCl (10-20 MΩ). Fast Na+ channels were inactivated by depolarization with 25 mM KCl Tyrode solution (equimolar subtraction of NaCl), which produced resting potentials between -45 and -50 mV. With 0.2 mM $BaCl_2$ added to the solution, Ca-APs with all-or-none properties were evoked with suprathreshold stimulation applied via a monopolar platinum electrode. Stimulus strength and duration were adjusted to maintain a near-constant latency between the stimulus artifact and the peak of the differentiated record of Ca-AP upstroke velocity (Vmax). In 0 and 0.01 μM ISO, Vmax for myopathics equalled (mean ± SEM) 4.2 ± 0.4 V/s, and 5.3 ± 0.3 V/s (n = 8), respectively. These values were significantly lower for normals, equalling 6.7 ± 0.5 V/s and 7.3 ± 0.3 V/s (n = 8), respectively. No significant interstrain difference in Vmax was seen in 0.1 and 1.0 μM ISO, although both strains exhibited significant dose-dependent increases in Vmax. ISO increased total amplitudes of Ca-APs and durations measured at 50% repolarization, but no significant differences emerged between strains for these parameters. Prior to ISO, the time constant to recovery from inactivation (τ_{rec}, measured from Vmax records) was significantly lower in papillary muscle of myopathic hamsters (128 ± 12 ms) as compared to normals (76 ± 5 ms). ISO (0.01 μM) decreased τ_{rec} from pre-ISO values to an observable but not significant degree in normals (71 ± 7 ms). In myopathics, however, there was a significant 25% reduction in τ_{rec} (95 ± 12 ms) from pre-ISO values. In myopathics 1.0 μM ISO produced further reductions in τ_{rec} such that the statistically significant interstrain difference in this parameter disappeared. In summary, before exposure to ISO, Vmax was reduced and τ_{rec} prolonged to abnormal extents in papillary muscles of myopathic hamsters. ISO had the effect of altering these kinetic properties of the slow inward current to bring them to within range of values found in papillary muscle of normal hamsters. Funding: HL36064

368.6 VARIATION OF CALCIUM CHANNEL PROPERTIES IN NEURONS FROM DIFFERENT BRAIN REGIONS. Alan R. Kay* and Robert K.S. Wong (SPON: J. Madden), Dept. Physiol. & Biophys., Univ. of Texas Med. Branch, Galveston, TX 77550.

Variations have been found in the firing patterns of neurons from distinct regions of the brain, suggesting differences in the distribution of ionic channels. The inability to adequately voltage clamp differentiated neurons has limited the exploration of the basis of these differences. Distinct types of calcium currents have been described in neurons (Carbone & Lux, Nature 310: 501, 1984; Nowycky et al., Nature 316: 440, 1985). We report here on the distribution of calcium currents in different regions of the mature guinea pig brain.

We have recently described a procedure for isolating neurons from the adult CNS (Kay & Wong, J. Neurosci. Methods, in press, 1986). These cells lose most of their distal dendritic tree, reducing space clamp problems, however, enough of the proximal dendrites remain to allow identification of the cell type.

Currents were measured using the whole-cell patch clamp technique. The intracellular medium proved to be important in holding these cells in good condition after penetration: Tris-phosphate proved most effective in this regard. Mg-ATP (2 mM, Byerly & Yazejian, J. Physiol. 370: 631, 1986) retarded the wash out of calcium currents, while intracellular leupeptin (100 μM) reduced the incidence of cell-rounding. K currents were blocked by intracellular Tris and substitution of Cs for K in the extracellular medium. Na currents were blocked by TTX or by the substitution of choline for Na. Under these conditions no active currents were elicited in the range -90 to 30 mV, after the addition of 0.2 mM $CdCl_2$.

In pyramidal cells from the CA1 region of the hippocampus a single class of channels predominated, with a threshold of activation of -40 mV and a slow phase of inactivation (30% decline over 250 ms). In contrast cells from the habenula possessed a current that activated at about -60 mV and inactivated rapidly in a voltage dependent fashion, together with a persistent calcium current. Furthermore, the persistent component inactivated more slowly than its counterpart in the hippocampus.

(Supported by NS14546).

368.7 TWO TYPES OF CALCIUM CONDUCTANCES IN CULTURED MAMMALIAN HIPPOCAMPAL NEURONS. Y. Yaari, B. Hamon* and H.D. Lux*. Department of Neurophysiology, Max-Planck-Institute for Psychiatry, Am Klopferspitz 18A, 8033 Planegg, FRG.

The entry of calcium through voltage-gated membrane conductances, g(Ca)s, is important for neuronal integration. Studies of mammalian brain slice preparations have indicated the coexistance of more than one type of g(Ca)s in some CNS neurons. We have employed patch-clamp techniques on cultured rat hippocampal neurons (dissociated from 18-19-day old embryos) to investigate whether this is also a feature of mammalian cortical neurons. Whole-cell calcium currents were pharmacologically isolated from other voltage-dependent membrane currents and further identified by their sensitivity to blockade by cadmium and manganese.

We found that two distinct types of g(Ca)s develop during neuronal growth and differentiation in culture. First to appear (3-5 h after plating) is a low (-50 to -40 mV) voltage-activated (LVA), fully-inactivating somatic g(Ca). It is metabolically stable and conducts calcium better than barium. The second, which appears in conjunction with neurite outgrowth (1-2 days in culture) and eventually predominates in the dendrites, is a high (-20 mV) voltage-activated (HVA), slowly-inactivating g(Ca). It exhibits a rapid run-down and is more permeable to barium than to calcium.

In their voltage-dependence, activation and inactivation kinetics and selectivity, the LVA and HVA g(Ca)s of differentiated hippocampal neurons resemble the two somatic g(Ca)s found in rat sensory neurons. This suggests the ubiquity of LVA and HVA g(Ca)s in the mammalian nervous system. In the function of a hippocampal neuron the two g(Ca) types most probably play different roles. The apparent wider dendritic distribution of the HVA g(Ca) suggests it modulates synaptic input to the neuron, e.g., boosts excitatory postsynaptic potentials. Its high activation threshold would favor transmission of larger postsynaptic events, thus increasing the signal-to-noise ratio of neuronal responsiveness. The LVA g(Ca), being in proximity to the axon trigger zone, may be involved in the electrogenesis of rhythmic neuronal firing.

368.8 **TWO Ca^{2+}-DEPENDENT K^+ CHANNELS, I_{Kc}, I_{Ac}, AND OCCULT Ca^{2+} CHANNELS IN DROSOPHILA.** A. Wei and L. Salkoff. Dept. Anat. & Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

The developmental appearance of currents in an excitable membrane can be studied by two-electrode voltage-clamp measurements of indirect flight muscles of Drosophila pupae. We report here the early presence of two current systems, a slow activating calcium dependent potassium current (I_{Kc}) and an inactive "occult" calcium current (I_{Ca}), prior to the appearance of other currents. "Occult" calcium currents can be unmasked by intracellular injection of adenosine triphosphate (ATP) or ethyleneglycol tetraacetic acid (EGTA).

We have nov demonstrated the presence of at least seven independent current carrying systems in Drosophila muscle. It is interesting to note that this membrane has a Ca^{2+}-sensitive counterpart for each of the two voltage-activated K^+ channels, i.e. I_{Kc} is similar to I_{Kv} and I_{Ac} is similar to I_{Av}. I_{Kc} develops two days prior to the fast transient Ca^{2+}-dependent K^+ current, I_{Ac}, which develops in early adulthood. I_{Kc} is blocked by TEA and has kinetic properties similar to I_{Kv}, yet is separable from I_{Kv} by recording from younger fibers (55 hrs) before I_{Kv} appears. I_{Kc} is also independent of I_{Av}, being observable in Shaker mutants which lack I_{Av}. I_{Kc} can be evoked in Ca^{2+} free saline by intracellular injection of Ca^{2+} prior to voltage step pulses. Activation of I_{Kc} is thus dependent on both intracellular Ca^{2+} and membrane voltage. Recordings from very early fibers (50 hrs), reveal the presence of I_{Kc} before the appearance of I_{Ac}.

I_{Ca} is normally not present in fibers till its abrupt appearance just prior to adult eclosion (96 hrs). However, large inward currents carried by either Ca^{2+} or Ba^{2+} are observed in younger fibers injected with either ATP or EGTA. These inward currents have voltage-dependent and kinetic properties indistinguishable from later appearing I_{Ca}. This current is unmasked in fibers as early as 50 hrs, before the earliest appearance of I_{Av}. The membrane of early fibers thus appear to possess inactive Ca^{2+} channels which can be made to function by manipulations which may act to lower intracellular Ca^{2+} concentrations. However, direct effects of ATP on Ca^{2+} channels cannot presently be ruled out.

Supported by NIH Postdoctoral Grant T32 NS07057-08 (A.W.), NSF Grant BNS 8311024 and the Esther and Joseph Klingenstein Fund (L.S.).

368.9 THE SPIKE OF A SLOW CRAB AXON HAS SODIUM AND CALCIUM COMPONENTS. P.J. Stephens and P.J. Church*. Villanova University, Department of Biology, Villanova, PA 19085

In the Californian shore crab (Pachygrapsus crassipes) the limb bender muscle is innervated by two excitatory axons, a fast (FBE) and a slow (SBE). Glass microelectrodes were used to impale single axons in the exposed limb nerve in the merus of autotomized walking limbs. Graded stimulus shocks were applied to the limb nerve and identification of the impaled axon as that of the SBE was based upon 1) the simultaneous appearance of an axon spike and an epsp in bender muscle fibers known to be innervated by only the SBE; 2) the spike was followed by a pronounced depolarizing afterpotential 3) passing depolarizing current through the microelectrode produced SBE spikes and concomitant epsp's in bender muscle fibers. In the present study we impaled the SBE axon with 2 microelectrodes, one to record and the other to pass current to produce spikes or correct changes in membrane potential produced by bathing preparations in modified saline solutions.

In normal crab saline, a train of spikes could be provoked by passing depolarizing current through a microelectrode in the SBE axon. Increasing the intensity of the stimulus caused a decline in the amplitude of the spikes and a splitting of each spike into two components. The components were recorded at different frequencies, indicating that the channels have different opening-closing kinetics.

Bathing preparations in crab saline made up with 10mM cadmium (to block calcium channels) resulted in a decline in spike amplitude. In these preparations, only a single component was observed when the SBE axon was depolarized with high intensity stimulation through the microelectrode. In other preparations, replacing calcium with barium did not change spike amplitude.

Replacing sodium ions in the saline with choline, or bath application of TTX, decreased the size of the spike but did not abolish it. Depolarizing current passed through the microelectrode evoked only single spikes, irrespective of the intensity or the duration of the stimulus shock.

We conclude that the SBE axon spike has two components, one dependent upon sodium the other on calcium.

This work was supported by a grant from the Whitehall Foundation.

368.10 VOLTAGE-GATED MEMBRANE CONDUCTANCES IN MATURE SOLITARY NEURONS ISOLATED FROM SALAMANDER SPINAL CORD. A. Hernandez-Cruz* and P.R. MacLeish , Lab. of Neurobiology, The Rockefeller University New York, N.Y. 10021.

Previous voltage-clamp experiments on mature vertebrate motoneurons have provided evidence for the presence of a fast Na current, several outward currents and a sustained inward current, presumably carried by Ca ions (Barrett E.J. et.al. J. Physiol 304:231, 1980 ; Schwindt P.C. & Crill W.E., J. Neurophysiol. 43:1700, 1980). However, these conductances have been difficult to isolate because of the problems derived from diffusional restriction and the presence of ionic regulatory mechanisms in the intact spinal cord tissue, as well as the complicated geometry of the cells themselves. The following experiments were aimed to circumvent these problems by studying individual solitary spinal cord cells in culture, where control of both the external ionic environment and the internal milieu can be achieved.

Neurons from the lumbar region of the spinal cord of tiger salamander (Ambystoma Tigrinum) were enzymatically dissociated and plated at low density on glass coverslips coated with Sal-1, a monoclonal antibody that increases adhesion and neurite extension of salamander neurons (MacLeish et.al. PNAS 80:7014, 1983). During the first two weeks, neurons extended their truncated processes forming phase-dark neurites which ended in growth cones. Cells could be kept for periods exceeding 10 weeks in salamander growth medium. In general neurons retained their excitability throughout as determined by the generation of fast action potentials observed within one hour after plating and for periods extending up to 60 days in culture. Whole-cell voltage-clamp recordings were obtained from distinct large cells, morphologically similar to the ventral horn neurons in the intact salamander spinal cord (Van Gehuchten, A. Arch.Biol ,Liege 15:599, 1897). In freshly dissociated cells, (V holding -65 mV) several components of outward current were isolated: a sodium-dependent component IK(Na), a calcium-dependent component IK(Ca), and two voltage-and time-dependent components with different kinetics and pharmacological sensitivities. The outward currents were greately reduced by adding the K-channel blockers TEA (10 mM) and 4-AP (3 mM) to the bathing solution, and almost completely suppressed when the impermeant cation N-Methyl Glucamine replaced K^+ in the electrode internal solution. Two components of inward current were also observed: a fast, transient, Na^+-dependent component (INa) and a slowly-activated, sustained component (ICa). All these currents were similar to those reported to exist in adult cat and frog motoneurons supporting the assumption that the salamander neurones were mature at the time of dissociation. ICa was studied in isolation in solutions containing TEA, 4AP and 0 Na. Its peak amplitude varied with the external Ca^{++} concentration and it was abolished by addition of Co^{++} (3 mM) or Mn^{++} (1 mM). The rise time of ICa was voltage-dependent and little inactivation was observed during pulses up to 500 ms in duration. At depolarizing levels beyond +10 mV the ICa kinetics were obscured by the coactivation of a small remaining voltage and time-dependent outward current. However, analysis of the tail currents revealed that a large fraction of ICa does not inactivate even at large depolarizing levels.

Certain membrane properties seemed to change with time in culture: The ratio ICa/INa (peak amplitude) increased as the cells formed neurites and growth cones, supporting a proposed role of Ca^{++} entry in the growth process (Anglister L. et.al. Dev. Biol. 94:351 , 1982)). On the other hand, ICa was not enhanced by equimolar substitution of Ba^{++} for Ca^{++} in freshly plated cells, whereas it was clearly augmented in cultures older than 2 weeks, suggesting either a change in selectivity for Ca channels or the expression of a new type of Ca channel. These experiments show that mature spinal cord neurons can be grown in culture and their membrane conductances isolated and characterized. This model also seems appropriate for the study of the role of membrane conductances in the control of neuronal growth in mature CNS neurons.

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368.11 ION CHANNELS IN CHICK AND HUMAN FIBROBLASTS. L.L. Stockbridge and A.S. French. Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7.

It has been demonstrated that fibroblasts have an active response to mechanical and electrical stimuli thought to be mediated by calcium-activated potassium channels (1,2). In an effort to find a mechanosensitive channel similar to that reported in chick skeletal muscle (3), we have attempted to characterize potassium channels in fibroblasts.

Tissue cultures of chick sclerotome fibroblasts (72 hr embryo) and human skin fibroblasts (Detroit 551) were studied with the patch clamp technique using cell-attached and inside-out patches. Patch pipettes contained (mM): KCl 140, $MgCl_2$ 2, EGTA 11 and HEPES 10 (pH 7.4). The bath solution contained (mM): NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1 and HEPES 10. To determine ion selectivity, Na+ and K+ concentrations in exchange solutions were varied while keeping the total monovalent cation concentration constant. To determine mechanosensitivity, 0-5 PSI suction was applied to the patch pipette with a 1cc syringe and monitored with a pressure transducer.

Single channel recordings revealed at least 6 channels common to both cell types which were separable by current-voltage relationships, reversal potentials and kinetic properties:

Channel	Conductance	Minimum Kinetic States
A (n=6)	245±28 pS	2 open, 2 closed
B (n=12)	190±60 pS	1 open, 2 closed
C (n=5)	101±27 pS	1 open, 2 closed
D (n=4)	79±13 pS	2 open, 2 closed
E (n=7)	39±15 pS	2 open, 2 closed
F (n=4)	19±14 pS	2 open, 2 closed

A, C and D are predominantly selective to K+ ions, but will also conduct Na+ ions. The permeability ratio in these channels is at least 3:1 K+/Na+. B appears to be a calcium-activated potassium channel with little voltage dependence. However, it is not mechanosensitive.

None of these channels evinced mechanosensitivity to suction. However, a mechanosensitive channel has appeared in one chick fibroblast patch. It has not been fully characterized, but appears to be permeable to both monovalent cations.

1. Nelson, P.G. et al. J. Gen. Physiol. 60:58-71, 1972.
2. Hosoi, S. and Slayman, C.L. J. Physiol. 367:267-290, 1985.
3. Guharay, F. and Sachs, F. J. Physiol. 352:685-701, 1984.

Supported by Canadian Medical Research Council and Alberta Heritage Foundation for Medical Research.

368.12 **CALCIUM AND POTASSIUM CONDUCTANCES EXIST IN PHEOCHROMOCYTOMA CELLS OF THE RAT.** **R.Y.K. Pun and M.M. Behbehani. Dept. of Physiol. and Biophys., Univ. of Cincinnati, Cincinnati, OH 45267-0576.**

A clonal cell line (PC12) from the rat adrenal pheochromocytoma has been studied extensively as a model for cellular differentiation and excitation-secretion coupling. Several lines of evidence indicated the presence of voltage-sensitive calcium channels in these cells. Release of catecholamines is dependent on Ca, ^{45}Ca is taken up upon depolarization of cells, and both phenomena are blocked by Ca channel antagonists. In this communication, we report the presence of Ca and K conductances in these cells as measured by electrophysiological methods.

PC12 cells were provided by Dr. G. Guroff (NICHD, NIH), and grown in Dubbecco's Modified Eagle Medium supplemented with 10% horse serum and 10% fetal calf serum. The cells were not exposed to Nerve Growth Factor. Bath solution was a HEPES buffered medium (140 mM Na, 4 mM K) with 3 mM Ca and 1 mM Mg (pH 7.4, 325 mOs). Whole-cell recordings were made with patch-electrodes filled with the following solution (mM): KCl or KF (140), $MgSO_4$ (2), EGTA (1.1), HEPES (10), glucose (10), pH 7.10, 300 mOs. Results obtained with a mixture of KCl (125) and KF (15) were similar. Drug solutions were made up in bathing medium and applied onto cells with pressure through a blunt-tipped pipette of tip diameter 5-10 µm, positioned near the cell. Studies were initiated only if the seal resistance between the pipette and the membrane was greater than 1 GΩ, and the cell membrane potential was -40 mV or more negative. Under these conditions, average membrane potential of the cells was 51.9 ± 0.4 mV (mean ± S.D., n = 17). The membrane time constant as measured from the voltage deflection to 0.1 nA hyperpolarizing current of 50-150 msec duration was 22.6 ± 0.7 msec (mean ± S.D., n = 11). Membrane resistance was 692 ± 28 MΩ (mean ± S.D., n = 13); this value probably reflects the small size of the cells. In all studies, at resting membrane potential, a depolarizing pulse produced a regenerative slow depolarization of about 10-20 mV followed by delayed rectification. The voltage deflection evoked by a depolarizing current pulse was always smaller than the voltage deflection produced by a hyperpolarizing pulse even when the depolarizing pulse was of greater intensity. This phenomenon is highly suggestive of the presence of a delayed rectifier. In several cells, hyperpolarizing pulses evoked an anomalous (inward) rectification. A slow TTX insensitive spike followed by a afterhyperpolarization (AHP) could be elicited by a short current pulse (< 10 msec) or when the cell was hyperpolarized (negative than -90 mV) and then stimulated. This slow spike was blocked by Cd (2 mM) but not by Co (3 mM). TEA (25 mM) and Ba (10 mM) were found to prolong the duration of the slow spike. These observations indicate that the slow spike is mediated by influx of Ca, probably through voltage-sensitive Ca channels. The blockade of the AHP by Ba further suggests the presence of a Ca-mediated potassium conductance in these cells.

368.13 WHOLE CELL RECORDINGS FROM CULTURED NEURONS OF NEONATE RAT SUPRAOPTIC NUCLEUS. P. Cobbett and W.T. Mason* AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

The neurons of the supraoptic (SON) and paraventricular (PVN) nuclei which project to the neurohypophysis have characteristic firing patterns which enable efficient release of hormone (oxytocin or vasopressin) from their terminals. Some of the mechanisms underlying these firing patterns have been elucidated using in vitro preparations. To enable a more detailed study of these events, we have made whole cell voltage and current clamp recordings from neurons in cultures of neonate hypothalamus.

Cells in tissue blocks containing the SON and a small surrounding area, from 3-4 day old rats, were dispersed mechanically and enzymatically. Cell suspensions were plated out, and cultures were maintained in Dulbecco's modified Eagle's medium supplemented with 25% new born calf serum.

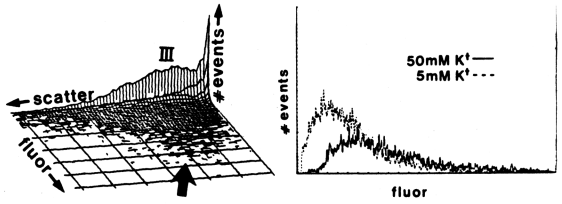
Recordings were made 10-21 days later from the soma of neurons over 10μm in diameter with less than four major processes. (Neurons of similar appearance in the same cultures were immunoreactive to a neurophysin antiserum.) Using low resistance electrodes (~10MΩ) the recorded cell was dialysed with electrode solution. In this way the ionic content of the internal and external solutions was controlled. In current clamp mode, with K as the major internal cation and with Na but not Ca externally, neurons had resting potentials in the range -53 to -70mV (n=10; -62.5±6.1mV, mean ±SD), input resistance of $4.8\pm2.5 \times 10^{10}\Omega$, and fired spontaneous or current evoked action potentials at frequencies up to 20Hz without significant inactivation. The action potential amplitude was 66.5±3.4mV and its duration was 4.2±0.6ms. Action potentials were in some instances superimposed on spontaneous depolarising voltage fluctuations. In two neurons phasic bursts of action potentials, characteristic of vasopressin cells, were recorded. Under voltage clamp, voltage activated K and TTX sensitive Na currents were recorded. Isolation of the Na current (n=4), using Na as the sole external cation and Cs as the internal cation, showed that its properties reflected those of the action potential. Its threshold was -55mV and it was less than 50% inactivated at frequencies up to 20Hz. The peak current (2.6±0.7nA) was recorded at about -40mV and the reversal potential was calculated to be about 60mV.

These properties indicate that viable cultures of the neonate SON region can be obtained and that this preparation appears to be suitable for the further study of the properties of SON neuroendocrine cells.

P.C. is a Beit Memorial Fellow.

368.14 FLOW CYTOMETRIC ANALYSIS OF MEMBRANE EXCITABILITY IN EMBRYONIC RAT MOTONEURONS. A.E. Schaffner, R.N. Mandler*, E.A. Novotny*, G.D. Lange and J.L. Barker, LNP, NINCDS, NIH, Bethesda, MD 20892.

We have combined retrograde fluorescent labeling techniques with voltage-sensitive oxonol dye staining to examine, with flow cytometric methods, the development of excitable membrane properties in embryonic motoneurons (MNs). As previously described (Schaffner et al.), the limbs of E14 rats were injected with FITC-conjugated lectin, then maintained at 35°C overnight, following which cell suspensions from the spinal cord (SC) were prepared for oxonol staining and flow cytometric analysis. The dual-parameter histogram of SC cells from injected embryos (see below) shows that a detectable portion of the live cell population (peak III) exhibits fluorescence accumulated in MNs (arrow). Five-minute incubation of the SC cell suspension with oxonol, a dye that stains inversely with membrane potential, was used to probe the distribution of MN membrane potentials under resting conditions and in the presence of elevated [K+] or batrachotoxin (BTX), which activates [Na+] channels. Histograms of oxonol signals associated with MNs show a relatively broad and complex distribution. Incubation in high [K+] or BTX shifted the distribution of MN fluorescence intensities to higher values, the response to BTX exhibiting tetrodotoxin sensitivity. The results indicate that at E14/15 functional [K+] and [Na+] channels are already expressed at the cell body level of many MNs.

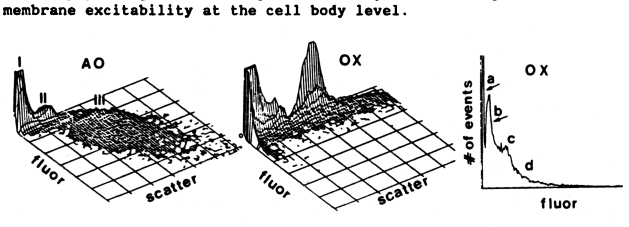


Legend: A, Three-dimensional histogram of the fluorescence signal in labeled MNs. B, Single-parameter histogram of oxonol fluorescence signals of labeled MNs at rest and in elevated [K+].

Schaffner et al. (1983) Soc. Neurosci. Abstracts 9:7.

368.15 FLOW CYTOMETRIC ANALYSIS OF MEMBRANE POTENTIAL IN POPULATIONS OF EMBRYONIC RAT SPINAL CORD CELLS. E.A. Novotny*, R.N. Mandler*, A.E.Schaffner, G.D. Lange and J.L. Barker, (SPON: C. Colton) LNP, NINCDS, NIH, Bethesda, MD 20892

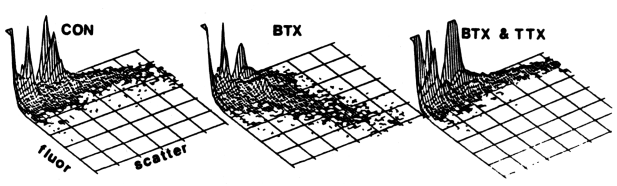
Initially, the development of membrane properties in excitable cells has been studied in monolayer culture using electrical recordings from individual cells. We have recently developed a complementary optical method for monitoring membrane potential in cell suspensions of embryonic spinal cord (SC) and dorsal root ganglion cells (DRG). Cells were suspended in physiological saline, then stained with an anionic indicator (oxonol) dye that partitions inversely with membrane potential. Three-dimensional histograms (below) of cell suspensions stained with either vital (acridine orange, AO), non-vital (ethidium bromide, EB) or a voltage-sensitive indicator dye (oxonol, OX) show that under resting conditions particles (peak I), dead or leaky cells (peak II), and live cells (peak III) all exhibit complex but similar levels of OX fluorescence. Thus, OX stains independently of light-scatter. When only live cells are considered in the subsequent analysis, it is clear that the fluorescence signals associated with live SC and DRG cells are variably distributed into 4 domains (a,b,c,d). When cell suspensions are incubated in depolarizing media containing either elevated [K+] or batrachotoxin, which activates Na+ channels, only the oxonol fluorescence intensities of the live cells increases. Thus, the flow cytometric strategy can be used to complement electrophysiological techniques to study the development of membrane excitability at the cell body level.



Legend: Three-dimensional histograms of AO and OX fluorescence distributions in SC cells as a function of forward-angle light scatter under resting conditions; dual-parameter histogram of OX fluorescence on AO+ (live) cells.

368.16 FLOW CYTOMETRIC DEMONSTRATION OF FUNCTIONAL Na^+ CHANNELS IN THE DEVELOPING EMBRYONIC RAT NERVOUS SYSTEM. R.N.Mandler*, A.E.Schaffner, E.A.Novotny*, G.D.Lange and J.L.Barker (SPON: R.J.Lederman). Lab of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

We have used voltage-sensitive oxonol dyes in conjunction with flow cytometry to probe the developmental expression of functional Na^+ channels in cell populations of embryonic spinal cord (SC) and dorsal root ganglia (DRG). Cells were dissociated from E12-E16 rat SCs and DRGs, recovered for two hours, then incubated in physiological medium with and without 0.1-1 uM batrachotoxin (BTX), an activator of Na^+ channels, and with and without 1uM tetrodotoxin (TTX), a blocker of Na^+ channel activation. At E12 there was little, if any change in the oxonol fluorescence signal distribution associated with live SC and DRG cells in the presence of 1uM BTX. At E15 there was a considerable and complex increase in the signals distributed in the SC (but not DRG) "live-cell" population alone, as shown below. There appeared to be a direct relationship between light-scatter and degree of shift in oxonol signal distribution. The entire shift was blocked by co-incubation in TTX. Thus, functional TTX-sensitive Na^+ channels develop between E12 and E16 in rat SC cells at the level of the cell body. It should now be possible to study relatively expeditiously the development and the function of Na^+ channels in different areas of the nervous system.



Legend: Three-dimensional histograms of oxonol fluorescence distribution in SC as a function of forward-angle light scatter under control conditions and in the presence of 1uM BTX with and without 1uM TTX.

368.17 NA, K AND CA CURRENTS IN IDENTIFIED LEECH NEURONS IN CULTURE. R.R. Stewart, W.B. Adams and J.G. Nicholls. Biocenter, University of Basel, 4056 Basel, Switzerland.

Individual leech neurons, maintained in culture, have been voltage clamped to determine the ionic currents and conductance changes underlying action potential generation. Retzius (R), Anterior Pagoda (AP), Pressure (P), and Nociceptive (N) cells in culture have characteristic electrical properties by which they can be recognized unambigously. For example, P cell action potentials overshoot to near +40 mV and then repolarize to below the resting potential for about 50 ms, while R cell action potentials overshoot by only 5 to 10 mV and have a longer undershoot (100 ms). What are the ionic currents giving rise to these differences in action potential shape?

Voltage clamped cells are held at -50 mV and presented with depolarizing and hyperpolarizing steps. A depolarizing step to 0 mV, leads to an inward current, then an outward current. From earlier studies it was known that the action potentials in these cell depends on Na rather than Ca. As expected the initial inward current is influenced predictably by changes in external Na. The outward current is carried by K; addition of 25-30 mM TEA, reversibly eliminates nearly all of the current. In R, P, N and AP cells the Na current peaks in 1-3ms and inactivates in 5-10 ms. In constrast, activation and inactivation kinetics of the K currents vary widely. The time to peak K current in N and P cells is more than 70ms; in R cells between 8 and 12 ms; and in AP cells between 2 and 4 ms. The K currents in all four cell types show inactivation. In R cells the principal K current is activated by Ca entry; it is blocked by 1mM Cd and reduced by 3Mn. In P cells the principal outward current appears to be a delayed rectifying K current.

Ca currents have been recorded in 0 Na, 25 mM TEA and 20 mM Ca, Ba, or Sr. With Ca, Ba or Sr the currents have a time to peak of 10-15 ms. These currents, presumably through Ca channels, are blocked by Cd and partially blocked by 3mM Mn.

Thus the differences in action potentials in these leech neurons represent, primarily, differences in the kinetics of their K currents.

Supported by Swiss National Research Foundation Grant No. 3.525-1.83 to J.G.N. and by NIH Grant No. 1 F32 NS07920-01 to R.R.S.

368.18 SINGLE CHANNEL ANALYSIS OF K+ SELECTIVE CHANNELS DURING THE DEVELOPMENT OF PURKINJE NEURONS IN CULTURE. A.J. Yool, V.E. Dionne*+ and D.L. Gruol. Divn. of Preclinical Neuroscience, Scripps Clinic Research Fndn., La Jolla CA; and +Dept. of Medicine, Univ. Calif. San Diego, La Jolla CA 92037.

The physiological development of Purkinje neurons (PNs) in vivo and in culture is characterized by predictable changes in the patterns of spontaneous activity, implicating a developmental sequence in the expression of ionic conductances during differentiation. Several types of ionic conductances contribute to the complex activity patterns of mature PNs, including at least 3 types of K+ selective channels, distinguished by single channel conductance, voltage dependence and sensitivity to TEA (Gruol, Neurosci. Abst. v10). In this study, the cerebellar cultures prepared from rat embryos at one day before birth provided access to identifiable PNs throughout their development, which has been shown to be comparable in morphology and physiology to the postnatal development of PNs in the rat (Gruol and Franklin, Neurosci. Abst. v11). Extracellular recordings from single PNs showed that spontaneous activity is sensitive to K+ channel blockers (TEA, 4-AP) as early as 6 days in vitro (DIV), and that the effects of these agents change with the development of more complex activity patterns. Single channel recordings in the cell-attached and outside-out configurations were used to identify the types of K+ selective channels present in the soma and dendrites at different stages of development. In immature PNs at 6 DIV, the two types of K+ selective channels seen most frequently showed relatively small conductances of approximately 20 and 40 pS. By 14 DIV, most PNs had initiated dendritic growth, and displayed at least four types of K+ selective channels (classified by average conductance values of 20, 40, 70 and 100 pS) in both the somal and dendritic regions. K+ selective channels representative of these four conductance levels persisted through 24 DIV and beyond, a stage at which PNs were morphologically and functionally mature. These data suggest that changes in K+ channel types or relative abundance may be involved in the changes in spontaneous activity patterns, and in the differential sensitivity to K+ channel blockers observed during the development of the PN. Further studies will analyze the pharmacological and voltage sensitivities of the K+ channels present at earlier developmental stages. (Supported by NINCDS grant NS21777 to DLG)

368.19 INTRACELLULAR ANALYSIS OF SPONTANEOUS AND EVOKED ACTIVITY IN THE SOMATIC AND DENDRITIC REGIONS OF PURKINJE NEURONS IN CULTURE. D.L. Gruol, Div. Preclin. Neurosci. and Endocrin., Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Purkinje neurons (PN) differentiated in culture express complex electrophysiological properties as reflected in the patterns of spontaneous activity and type of spike events generated (Gruol, Brain Res. 1983). In the present study, intracellular voltage recordings and ion channel blockers were used to investigate mechanisms contributing to these properties. Cultures were prepared from 20 day rat embryos and maintained for several weeks before use. In intracellular recordings from the somatic region, PNs exhibited spontaneous activity that could be divided into distinct patterns based on the regularity of the activity and the predominant spike event. Spike events were categorized as simple spikes, doublet spikes or complex spikes. Regular patterns of simple spikes were the most common firing mode. Polarization of the membrane by intracellular current injection revealed that: 1) firing pattern and type of spike generated were highly membrane potential dependent, 2) PNs could exhibit more than one type of firing pattern and spike event and 3) transitions from a regular simple spike, to regular doublet spike, to regular complex spike pattern occurred in some PNs as the membrane potential was hyperpolarized. In intracellular recordings from the dendritic region, the regular patterns of activity characteristic of the somal recordings were uncommon. Instead, dendritic recordings were characterized by extensive synaptic activity. All three types of spike events were associated with synaptic potentials. As in the somatic region, firing pattern and the type of spike event were dependent on membrane potential. In both somatic and dendritic recordings, all spontaneous activity was blocked by TTX and Mg++. In dendritic recordings, depolarizing current pulses evoked action potentials that were sensitive to TTX or Mg++, suggesting that action potentials mediated by more than one ionic mechanisms are generated in this cellular region. In somatic recordings, action potentials evoked by depolarizing current pulses were blocked by TTX but not Mg++. However, anodal break Ca++ spikes were evident in TTX containing media, suggesting that a low threshold Ca++ conductance contributes to the electrophysiological properties of the somatic region. These data indicate that membrane properties of PNs are complex in both the depolarizing and hyperpolarizing membrane potential ranges, that PNs possess the capability for several different firing patterns, that membrane potential is a major factor in defining the firing pattern, that Na++ and Ca++ conductances in both the somatic and dendritic region contribute to the firing patterns and that high threshold Ca++ conductances are present in the dendritic but not the somatic region. (Supported by NINCDS Grant NS21777)

368.20 A LOW THRESHOLD SPIKE MEDIATES mPRF FIRING PATTERN RESPONSES R.W. Greene, H.L. Haas, and R.W. McCarley. Lab. Neuroscience, Dept. of Psychiatry, Harvard Med. Sch./Brockton VAMC, Brockton, MA 02401

Since the first recordings of neurons during the behavioral state alterations of the sleep-wake cycle a central neurophysiological problem has been to determine the mechanisms controlling the different firing patterns and responsiveness in different behavioral states. Alterations in firing rate may result not only from changes in synaptic input but also from intrinsic membrane properties of the neuron. However, while it is known that baseline membrane potential (MP) of medial pontine reticular formation (mPRF) neurons depolarizes 7-10mV on passage from waking (W) and slow wave sleep to desynchronized sleep (D), the consequences of this behavioral-state specific change for firing patterns and responsiveness and the underlying mechanisms have not previously been investigated, probably because of the lack of a suitable experimental preparation. This is in spite of electrophysiological and pharmacological evidence for the importance of mPRF in mediating essential events of both W and D. We here report that experiments using a novel in vitro preparation, the mPRF brain stem slice of young rats, indicate that one population of mPRF neurons alters firing pattern responses with baseline MP change as a result of the presence of a low threshold spike (LTS).

As determined by response to intracellularly applied current, 9/17 mPRF neurons displayed an inactivating LTS and 8 were non-LTS neurons. The LTS inactivated at MPs more positive than -65 mV and thus LTS neurons have the capability of generating both tonic and bursting modes of AP firing patterns. Non-LTS neurons showed only a tonic firing mode qualitatively unaffected by baseline MP. Ca dependency of the LTS was demonstrated by its elimination in the presence of antagonists to a Ca flux across the membrane (0 mm Ca and 10 mM Mg; or 500 uM Cd). Other currents influencing firing pattern in both LTS and non-LTS neurons were shown by application of perfusion fluid containing Mg (10mM), Ca (0mM) or Cd (200mM) or TEA (5mM). This resulted in an increase in excitability as measured by the number of APs generated by an excitatory current pulse starting from the same MP. The resting input resistance was unaffected. There was a reduction of the AHP, an increase in AP duration, and a reduction of AP amplitude. The latter two became more prominent with successive APs which suggests that the increase in AP duration resulted from I_K inactivation revealed in the absence of Ca^{2+} -dependent K^+ current. The reduction of AHP could also have lead to Na^+ current inactivation and the observed reduction of AP amplitude. Finally, an early outward transient rectification was observed to be antagonized by 4-AP. Thus, repetitive firing in mPRF neurons appears to be influenced by not only a low threshold Ca-current (for LTS neurons) but also a Ca-dependant and 2 voltage-dependant potassium currents.

369.1 4-AMINOPYRIDINE DEPRESSES KCL TASTE RESPONSES RECORDED FROM RAT CHORDA TYMPANI NERVE. M. Kim* and C. M. Mistretta (SPON: B. E. Bradley). Schools of Nursing and Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

Investigators have reported that neural taste responses to NaCl are mediated at least in part by amiloride sensitive Na-channels located in taste receptor membranes. To learn whether K-channels have a role in transduction of taste responses to KCl, we used a potassium channel blocker, 4-aminopyridine (4-AP).

Electrophysiological recordings were made from the whole chorda tympani nerve in 35 adult rats and taste responses were recorded before and after a 5 min application of 5mM 4-AP to the tongue. Taste stimuli included KCl, NH4Cl, and NaCl (0.025 to 0.75M); KBr, KH2PO4, CsCl, and RbCl (0.025 to 0.10M); and sucrose (0.1 and 0.5M). Summated response magnitudes were measured at 10 sec after stimulus application. Response magnitudes after 4-AP were expressed as percentages of the original responses, before application of the channel blocker.

Taste response magnitudes to 0.025, 0.05 and 0.1M KCl were reduced to 0, 20 and 60% of original responses, respectively. Responses to KBr and KH2PO4 were affected to a similar extent. Responses to 0.025 - 0.10M CsCl and RbCl were reduced to 10 - 80% of original magnitude. All responses recovered completely by 60 min after 4-AP; recovery was more than 80% complete by 30 min. Responses to higher concentrations of KCl and to all concentrations of NaCl and sucrose were not affected by the blocker. Responses to 0.025 and 0.05M NH4Cl were reduced to 60 and 80% of original magnitude; responses to 0.1M NH4Cl and higher concentrations were not altered.

When the application time of 4-AP on the tongue was shortened to 1 min, response magnitudes to 0.025, 0.05 and 0.1M KCl were reduced to 35, 65 and 85% of original. Recovery was complete in 30 min. Responses to higher concentrations of KCl were not affected, nor were responses to any concentrations of NH4Cl or NaCl.

These results demonstrate a selective effect of 4-AP on taste responses to low concentrations of potassium salts and two other alkali metals, CsCl and RbCl. Responses to very low KCl concentrations are essentially abolished by the channel blocker. This suggests that K-channels have a role in mediating the neural response to potassium in taste receptor membranes. Preliminary data from our laboratory indicate that sensitivity of the KCl taste response to 4-AP increases during development. Thus, K-channels might be added to taste membranes postnatally; this could relate to the increasing responsiveness of chorda tympani nerve fibers to KCl in postnatal rats.

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369.2 AN ANALYSIS OF THE SUPPRESSED NaCl TASTE RESPONSE FOLLOWING AMILORIDE B.K. Formaker*, D.L. Hill and P.S. Lasiter. Dept. of Psychology, Univ. of Toledo, Toledo OH 43606.

Previous investigations have shown that the sodium transport blocker, amiloride, suppresses NaCl responses recorded from the chorda tympani nerve. It has been suggested that the response to NaCl is mediated by amiloride-sensitive and amiloride-insensitive sodium receptor components. However, behavioral investigations from our laboratory indicate that the residual response of NaCl following amiloride may be due to the chloride ion, rather than an amiloride-insensitive sodium channel.

To examine if the chloride ion is responsible for the residual response to NaCl following amiloride, we recorded multiunit responses from the chorda tympani to a chemical concentration series (0.05M, 0.1M, 0.25M, & 0.5M) of NaCl, NaBr, sodium acetate (NaAc), sodium bicarbonate ($NaHCO_3$), choline chloride (ChCl), NH_4Cl and ammonium acetate (NH_4Ac), before and after lingual application of 500 µM amiloride hydrochloride. Responses to NaCl and NaBr were suppressed 65%-90% following amiloride treatment. In contrast, responses to NaAc and $NaHCO_3$ were suppressed 90%-100%. The responses to 0.25M NaCl and 0.25M ChCl were approximately equal following exposure to amiloride. Lineweaver-Burk analyses were conducted to determine the nature of competetive interactions following amiloride treatment. These analyses showed that a) amiloride's effect was competetive only for sodium-containing chemicals and b) the residual response intercepts of halogen-containing chemicals and non-halogen-containing chemicals were distinct; intercepts for ChCl, NaBr and NaCl were statistically equivalent and these intercepts were reliably different from non-halogen containing chemicals (NaAc, $NaHCO_3$). These results indicate that the residual response to NaCl observed following amiloride treatment may be due to a specific halogen-sensitive mechanism rather than to an amiloride-insensitive sodium component on the receptor membrane. The near complete suppression of NaAc or $NaHCO_3$ cannot be predicted from a two-channel model of sodium transduction.
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369.3 THE UNIT ORGANIZATION OF TASTE BUDS: DYE-COUPLING BETWEEN MUDPUPPY TASTE CELLS J. Yang* and S. Roper. Dept. of Anatomy, Colorado State University, Ft. Collins, CO 80523 and Rocky Mt. Taste & Smell Center, Denver, CO 80262.

The functional unit of vertebrate taste buds is not well-established: individual cells may be the primary units, but it is also possible that a stimulus may activate a subset of taste cells which are electrically coupled and respond as a unit. We have investigated the existence of such electrically-coupled units by injecting taste cells with lucifer yellow (LY): LY-coupling has been shown to indicate the presence of electrical junctions between cells. Additionally, we injected lingual epithelial cells to test for dye-coupling between epithelial and taste cells.

The lingual epithelium from mudpuppies (*Necturus maculosus*) was removed and placed in amphibian Ringer solution. Taste cells were impaled with glass microelectrodes which contained LY. Hyperpolarizing current was passed through the microelectrode for 1-3 minutes to inject LY into cells. Taste cells generate impulses (Roper, 1983); thus, we confirmed stable impalements in taste buds by the presence of action potentials before, during, and after injecting LY.

In **taste buds**, from a sample of 82 impalements we detected LY-coupling in 14 cases: dye-coupled pairs of cells were observed in 11 cases, and trios of cells in 3 cases. Larger subsets of coupled cells (>3) were not observed. Since the average number of cells in the mudpuppy taste bud is 100, this means that approximately 38% of the cells are dye-coupled, and in groups of 2 or 3 cells. It must be recognized, however, that weak electrical junctions may not be revealed by LY-coupling, and thus these figures represent a conservative estimate of the extent of electrical coupling between taste cells. West & Bernard (1978) also reported electrical coupling in *Necturus* taste cells, but with their methodology they were unable to examine the number of cells to which any one cell was coupled.

In **epithelial cells**, we examined dye-coupling in the superficial (mucosal) and basal (germinative) layers. Extensive LY-coupling was found in the basal layer (15/15 cases), and in most instances the injected cell was coupled to 3-5 adjacent cells. In the superficial epithelium, dye-coupling was rare (1/35 cases). No dye-coupling was observed between epithelial cells and taste cells (i.e. at the taste pore region).

We conclude that extensive electrical coupling in groups of 2-3 cells occurs in the mudpuppy taste bud. Coupling may occur selectively between identical types of taste cells (dark, light, etc.) but this remains to be determined.

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369.4 ULTRASTRUCTURE OF MOUSE FOLIATE TASTE BUDS. S.M. Royer and J.C. Kinnamon. Dept. of MCD Biology, University of Colorado, Boulder, CO 80309.

We have used high voltage electron microscopy and computer-assisted three-dimensional reconstruction techniques to characterize the ultrastructure of murine foliate taste buds and to explore synaptic and other interactions between taste cells and nerve fibers. Our observations have demonstrated that murine foliate taste buds are generally similar in ultrastructure to those of the mouse circumvallate papilla. Like the latter, cells of the foliate papilla may be grouped into dark, light and intermediate types, all of which make afferent synapses onto nerve fibers. Reconstructions from serial sections have revealed that these synapses may be quite complex in morphology. The active zone often has an annular or branching structure and may extend over an area a few micrometers in diameter. In the taste cell, both subsurface cisternae and mitochondria are frequently found in close association with the presynaptic region, and these may actually lie within interruptions of the active zone. Dense-cored vesicles also occasionally lie among the clear synaptic vesicles and sometimes appear immediately adjacent to the presynaptic membrane specialization.

Subsurface cisternae and mitochondria are also intimately associated with the taste cell membrane at non-synaptic regions of close apposition between taste cells and nerve fibers. Such mitochondria are generally very different from other mitochondria in the taste cell in that they have expanded, tubular or vesicular cristae. Reconstructions of these mitochondria indicate that they are unusually large, and their outer membranes may lie parallel to the inner leaflet of the taste cell membrane for a span of a few micrometers where the taste cell membrane is adjacent to that of the nerve fiber. These atypical mitochondria resemble mitochondria found in certain tumor cells and other cells with a high level of respiratory activity. Their presence at sites of taste cell-nerve fiber contact may indicate a specialized, energy-requiring metabolic activity in these regions. We have also observed such atypical mitochondria in taste cells of murine vallate and fungiform papillae.

The nerve fibers within the taste bud often contain aggregations of both clear and dense-cored vesicles. We have not yet seen any evidence of efferent synapses from nerve fibers onto taste cells or any other possible sites of release for the vesicles. Consequently, the significance of such vesicles within the nerve fibers remains obscure.

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369.5 HUMAN FUNGIFORM TASTE BUD DENSITY: AGE AND GENDER. Inglis J. Miller, Jr. (Dept. Anatomy, Bowman Gray Sch. of Medicine, Wake Forest University, Winston-Salem, NC 27103)

The objective of this study is to determine control densities of human taste buds in order to establish reference points for understanding taste function in normal subjects and patients with taste dysfunction. Studies are underway on tongues from 57 human cadavers ranging in age from premature infants to adults 95 yrs old. Photography is used to document morphological features on the tongue. Areas of the tongue measuring about 1 cm sq are sectioned serially with 20um frozen sections, mounted on slides,stained with H&E,and examined by light microscopy. Taste bud density (per sq cm surface), number of gustatory papillae (with taste buds) and number of taste buds / papilla have been counted for two regions of the anterior portion of the tongue: 1. tip and 2. midregion about 2-3 cm caudal from the tip on the lateral margin. Quantitative data are present for adult males N =10 ranging in age from 22 to 80 yrs, and preliminary observations are incomplete on 8 adult females aged 26-90 yrs at death. The mean age for males is 51.5 yrs,and the mean age for females is 57.6 yrs.Taste bud density on the tip averages 116.5+132(SD, N=18)tb/cm sq and 23.3+27 tb/cm sq (midregion) for all subjects. The density on the tip is nearly identical for males (116.3+154, N=10) and females (116.8 + 23.5, N=8). Midregion densities are 24.5+23.5 (10 males) and 21.0+17.1 (8 females). There are differences between individuals of both sexes in taste bud density of two log. units (range 3.6- 514 tb/cm sq, for males; and 3.0-329 tb/cm sq for females) on the tip. The distribution of tip taste bud densities across subjects is bimodal with modes near 10 tb/cm sq and at about 150 tb/cm sq. By dividing the population into "high" and "low" density halves, one can compare the proportion of subjects by age and sex in each half. Low density subjects range from 3.0 to 74.5 tb/cm sq with a mean of 23.1+23 (N=9) on the tip and 1.8-22.9 tb/cmsq with a mean of 7.6+8.4 on the midregion. The high density subjects range from 113.6-514 tb/cm sq on the tip with a mean of 209+130tb/cm sq (N=9) on the tip. A range of 13.9-85.9 tb/cm sq with a mean of 39.0+24.3 (N=9) is found on the midregion in the high density group. Neither the distribution of ages (high mean: 56.0+22, low mean: 55.1+22 yrs) nor the distribution of sexes (high:3 females/6males;(low: 4 males/ 5 females) is different for the high and low subjects. Thus, these differences in human taste bud densities seem not attributable to the age or sex of the subjects.

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369.6 AUTONOMIC CONTROL OF SECRETORY GRANULE DEPLETION IN VON EBNER'S GLANDS IN THE RAT. Suat Gurkan* and Robert M. Bradley. Dept. Oral Biology, Sch. of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

Von Ebner's lingual salivary glands drain into the clefts of the circumvallate and foliate papillae. Because the majority of the lingual taste buds are found in the epithelium lining the clefts of these papillae, the glands supply the fluid environment of most of the tongue taste buds. Since little is known about the neural control of von Ebner's glands, we have employed morphometric analyses to characterize the extent of parasympathetic and β-adrenergic control of the gland acini.

Rats were starved overnight to cause accumulation of the secretory granules in gland acini. Rats in five groups of 10 each were injected intraperitoneally with the β-adrenergic agonist isoproterenol, dissolved in saline, at 7.5, 15, 20, 30, and 60 mg/kg. Another five groups of rats were injected with 100, 200, 300, 400 and 500 μg/kg of the parasympathetic agonist carbachol. Control rats were injected with saline. Two hours later the rats were sacrificed by cervical dislocation and the von Ebner's glands were rapidly removed, fixed, embedded in plastic and sectioned at 1 μM. Five randomly selected acini from each rat were photographed at 100x and the areas of each acinar cell and its granules were measured using computer planimetry.

Isoproterenol produced a depletion in secretory granules that was dependent on dose. At doses of 7.5, 15, and 20 mg/kg there was no reduction in granule content of the cells, compared to controls. The higher doses of 30 and 60 mg/kg produced a highly significant reduction in granules ($p < 0.0001$). Carbachol produced a significant reduction in granules at all doses ($p < 0.0001$). However, with both drugs even the higher doses did not cause complete depletion of granules. In only a few acini did the depletion approach 90%, the mean value being about 50%.

These results indicate that granule depletion in the acini of von Ebner's glands is under β-adrenergic and parasympathetic control. It is possible, therefore, that depending on the relative activation of β-adrenergic and parasympathetic nerve supplies, the composition of the saliva bathing the taste buds could be quite different.

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369.7 CHEMORECEPTORS OF PARAMECIUM. J.L. Van Houten, R.R. Preston*, S. Schulz*, J.M. Sasner*, and R. Smith*. Department of Zoology, University of Vermont, Burlington, VT 05405.

Paramecia are chemoreceptor cells that, like primary neurons of taste and olfaction, respond to external stimuli by transducing external chemical information into electrical information that is useful to the organism. Folic acid and cAMP are two stimuli that are detected by paramecia and elicit a hyperpolarization. We previously reported that there is no dependence of this change in membrane potential (ΔE_m) on external K or Na and no obvious voltage dependence. The ΔE_m is not purely a surface charge phenomenon, however. Agents such as the polycation ruthenium red do not perturb the stimulus-induced hyperpolarization, nor does a surface charge mutant (Satow and Kung,J. Membr. Biol. 59 179, 1981) show an abnormal ΔE_m in response to stimuli. This and other evidence indicate that surface receptors must bind the stimuli and this binding is transduced into E_m. Paramecia have specific, saturable ^{3}H-folate binding sites that are correlated with chemoreception (J. Comp. Physiol. 155 113, 1984). Similarly, there is low affinity, ^{3}H-cAMP binding to the cell body membrane, which is specific and saturable with a K_d (300 μM), close to the concentration for the half-maximal, stimulus-induced hyperpolarization and attraction response. The number of protein binding sites for folate have been narrowed to 3 by folate affinity chromatography and further to 2 by ^{125}I labeling of surface exposed proteins. An antifolate antibody has been produced that is being used to further narrow the number of candidates for the folate chemoreceptor to those that can be crosslinked to folate. Crosslinking of whole cells to an N-hydroxyl succimide derivative of folate ("activated" folate) specifically inhibits attraction to folate: cells treated with "activated" folate show no attraction to folate (I_{che}= 0.54+/- 0.06 n = 12), but normal attraction to acetate (I_{che}= 0.61+/- 0.06 n = 6). Whereas, control cells treated with folate are attracted to both folate and acetate (I_{che}= 0.72+/- 0.06 n = 12; I_{che}= 0.67+/- 0.06 n = 6). The folate receptor should be among the surface proteins crosslinked. Therefore, crosslinking provides us with a way to circumvent problems of using conventional biochemistry on low affinity binding proteins. Azido derivatives of cAMP can be photolyzed onto whole cells and they variably inhibit attraction to cAMP. Only one cAMP specific binding protein (48,000 Mr) among the cells' membrane proteins elutes from cAMP affinity columns and only 2 proteins are ^{32}P-labeled in preliminary experiments with ^{32}P-azido-cAMP. The larger protein (~250,000 Mr) is an "immobilization antigen" that coats the cell membrane and the other is a 48,000 Mr protein and is not precipitated with antibody against the immobilization antigen. It is possible that this cAMP binding protein is the cAMP chemoreceptor.

369.8 CODING OF ODORANT QUALITY BY THE OLFACTORY SYSTEM OF THE LOBSTER: BEHAVIORAL AND NEURAL ANALYSIS OF DISCRIMINATION OF QUALITY OF SINGLE CHEMICALS AND CHEMICAL MIXTURES. M.-N. Girardot, J.B. Fine* and C.D. Derby. Dept. of Biology, Georgia State University, Atlanta, GA 30303.

Toward understanding neural mechanisms responsible for behavioral discrimination of odorant quality, we are using the olfactory system of the spiny lobster (Panulirus argus). To analyze behavioral discrimination, we have developed a learning paradigm involving aversive conditioning, which uses a visual/mechanical stimulus as the unconditioned aversive stimulus. Behavioral responses to two concentrations of attractive chemicals (taurine, glutamate, glycine, AMP, betaine) or chemical mixtures (artificial mixtures of crab, shrimp, oyster, mullet) were compared before, during, and after pairing the aversive stimulus with one of the chemicals or mixtures. Conditioning for about 7 days normally resulted in a clear passive or active avoidance of the conditioned chemical, with no or little avoidance of the nonconditioned chemicals. This demonstrates the ability of these animals to discriminate among these chemicals. The degree of perceived similarity and dissimilarity among these chemicals is presently being analyzed.

The analysis of neural mechanisms involved in such behavioral discriminations is based on responses of primary chemoreceptor neurons in the antennules and of interneurons in the brain to these same chemicals and mixtures. The ability of these neurons to differentiate among odorants using a population code (i.e, across-neuron pattern code) was tested by using correlation analysis to compare the similarities in responses of all neurons at a given level to the same chemicals and mixtures used in the behavioral study. Preliminary analysis shows that the across-neuron patterns for some single compounds that were shown to be behaviorally discriminable (e.g., taurine and glutamate) are dissimilar from each other, and this dissimilarity is independent of stimulus concentration. We are continuing with this analysis as well as analyzing the contribution of narrowly-tuned neurons to the discrimination.

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369.9 PURINERGIC AND TAURINERGIC CHEMORECEPTION IN THE SPINY LOBSTER: PHYSIOLOGY AND BIOCHEMISTRY. H. G. Trapido-Rosenthal*, R. A. Gleeson*, W. E. S. Carr*, S. M. Lambert*, and M. L. Milstead* (Spon: S. K. Fisher). C. V. Whitney Laboratory and Department of Zoology, University of Florida.

The olfactory organ of the spiny lobster, Panulirus argus, consists of a group of aesthetasc sensilla that are present on the antennule of this marine invertebrate. These sensilla are densely packed with dendrites of primary chemosensory neurons. Electrophysiological data indicate that many of the neurons are differentially excited by purine nucleotides (AMP, ADP, and ATP), and by taurine (Tau), when these compounds are present in seawater bathing the sensilla. Responses of the neurons sensitive to purine nucleotides are mediated by several classes of purinergic receptors with different ligand specificities and affinities. Adenosine (Ado) is only slightly stimulatory to these neurons. There are also at least two classes of taurinergic neurons that differ in the degree to which their electrophysiological responsiveness to Tau can be modulated by the presence of other amino acids.

Biochemical studies have shown that the aesthetasc sensilla contain ectonulceotidases that dephosphorylate the stimulatory purine nucleotides, generating the much less active nucleoside Ado; Ado is then specifically taken up into an intracellular compartment. The ectonucleotidases within sensilla have characteristics similar to those of ectonucleotidases on membrane surfaces of mammalian cells. The sensillar uptake system for Ado differs somewhat from that in mammals in that it is relatively insensitive to inhibition by dipyridamole and nitrobenzylthioinosine, potent inhibitors of Ado uptake in mammals. Whereas excitatory nucleotides in the sensilla are inactivated by dephosphorylation prior to uptake, the excitatory Tau molecule is internalized directly by a specific uptake system with characteristics similar to that of the Tau uptake system in mammals. Guanidinoethanesulfonate is a potent inhibitor of Tau uptake in both systems.

Our studies thus indicate that the olfactory sensilla of the lobster have both receptors and inactivation mechanisms for purine nucleotides and for taurine that are very similar to those found in internal tissues of mammals where these substances have neuroactive effects.

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369.10 CHEMICAL ALTERATION OF THE SENSE OF SMELL IN AVIAN SPECIES. SELECTIVE HYPOSMIA IN STARLINGS (Sturnus vulgaris) FROM TWO-STEP MODIFICATION OF THE OLFACTORY EPITHELIUM. Thomas Hellman Morton,[1] L. Clark,*[2] and J.R. Mason*[2] [1]Department of Chemistry, University of California, Riverside, CA 92521 and [2]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

Behavioral assay of the sense of smell in birds has been developed based on classical cardiac conditioning. Selective olfactory deficits result from direct chemical treatment of the olfactory epithelium. Heart beat rate was measured in starlings by subcutaneous implantation of three 0.007 mm stainless steel wires, one on the right wing and one on each leg. The bird was then wrapped in a strip of dry chamois and its bill placed in the odorant delivery port of a flow-dilution olfactometer. A conditioning session consisted of sequential presentations of three odorants in randomized order, with a 5-minute period between each trial. Two of the odorants (S+) were promptly followed by a 0.5 sec, 10 V electric shock (UCS) passed through the foot electrodes, while the third (S-) was not paired with UCS. Heart beat rate was monitored for three 15 sec intervals, (1) immediately prior to odorant, (2) during odorant presentation, and (3) immediately after odorant. When UCS was delivered between intervals (2) and (3), heart beat rate was significantly greater during (3) than during (1). After a few trials heart beat rate increased significantly during interval (2) when S+ odorant was presented. Significant discrimination between S+ and S- was acquired in a single session with 10 trials for each odorant.

After conditioning a subject was anesthetized with IP injection of Equithesin (2 mL/kg), and two-step lavage (0.1 mL aqueous acetoacetic ester followed by 0.1 mL aqueous $NaBH_3CN$) was administered to the olfactory sac, followed by irrigation with 1 mL of normal saline. A typical subject recovered from anesthesia quickly enough that a postlavage test session could be initiated within 1 hr after lavage. Mean heart beat rates for a typical female subject with cyclohexanone [$(CH_2)_5CO$] and ethyl butyrate [PrCOOEt] as S+ odorants and n-butanol [n-BuOH] as S- are tabulated below for 60 trials:

	PRELAVAGE INTERVALS			POSTLAVAGE INTERVALS		
	(1)	(2)	(3)	(1)	(2)	(3)
$(CH_2)_5CO$	7.8 Hz	9.0 Hz	8.8 Hz	7.3 Hz	7.4 Hz	8.3 Hz
PrCOOEt	7.7 Hz	9.1 Hz	8.5 Hz	7.4 Hz	9.0 Hz	8.8 Hz
n-BuOH (S-)	8.0 Hz	7.7 Hz	7.7 Hz	7.4 hz	7.4 Hz	7.4 Hz

Two step modification impaired the subject's ability to detect cyclohexanone. Responding to the other S+, ethyl butyrate, was not affected: for the postlavage session the difference in heart beat rate between intervals (1) and (2) was significant ($p < 0.000005$). For cyclohexanone the difference between (1) and (2) was not ($p > 0.5$).

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369.11 ON A SECOND MESSENGER IN OLFACTORY TRANSDUCTION. I. Vodyanoy* and V. Vodyanoy*.(SPON. M. Cahalan). Dept. of Physiol. and Biophys., Univ. of Calif. Irvine, Irvine, CA 92717.

We report a model system to study the initial events in chemoreception by the mammalian olfactory epithelium: membrane fragments from rat olfactory epithelial homogenates were incorporated into planar, essentially solvent-free bimolecular lipid membranes (BLM's). This artificial membrane exhibits a chemosensitive response, measured in terms of electrical current flow (at the constant voltage applied across the membrane) to a nanomolar concentration of odorant (diethyl sulfide). The effect of diethyl sulfide is dose-dependent with threshold of about 10 pM.

The response of the reconstituted system to odorant is determined by a potassium channel (Vodyanoy and Murphy, Science, 220, 717, 1983). Chemosensitivity is manifest as a change in the mean open time of single channel events in response to the odorant.

These chemosensitive channels may operate either using an intrinsic sensor and odorant as a primary transmitter, or using the remote sensor and an internal second messenger.

We studied the involvement of adenosine triphosphate (ATP), guanosine triphosphate (GTP) and adenosine 3',5'-monophosphate (cyclic AMP) in the chemical transduction according to the scheme (Lancet, Ann.Rev.Neurosci,1986; Shepherd, Nature, 316, 214, 1985): odorant-receptor complex -> G-protein (GTP) -> adenylate cyclase (ATP) -> cyclic AMP -> (protein kinase) -> channel protein. The membrane electrical properties were studied in chamber BLMC-2 (Forward Technology Research Laboratory,Inc, N.J.).

We found that the conductivity of BLM treated with olfactory epithelium homogenates increases in response to the odorant if the buffer solution used for homogenate preparation or the membrane bathing solutions contain ATP and GTP. There was no such conductance increase if ATP and GTP are not added or when ATP alone was added after the odorant addition. Not until the GTP was delivered to the system did the conductance increase.

Cyclic AMP initiates the conductance increase which is much like the conductance response to the odorant. The amplitude of cyclic AMP effect is dose-dependent with a threshold of about 1 uM.

These observations are consistent with an indirect action of odorant involving a remote sensor and the internal second messenger in the initial step of olfactory transduction.

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369.12 DIFFERENTIAL INTERACTION OF LECTINS WITH CHEMOSENSORY RECEPTORS. D.L. Kalinoski, R.C. Bruch* and J.G. Brand*. Monell Chemical Senses Center, Philadelphia, PA 19104.

L-Amino acids are effective stimuli that elicit both gustatory and olfactory electrophysiological responses in the channel catfish (Ictalurus punctatus). Furthermore, L-alanine (L-ala) and L-arginine (L-arg) bind with equal affinity (K_d 10^{-7} - 10^{-6} M) to receptors in both taste membrane preparations and in isolated olfactory cilia preparations. Since lectins are useful reagents for identifying and characterizing cell-surface glycoproteins, three peroxidase-conjugated lectins were used to identify the glycoprotein components of the chemosensory membranes. The lectins used were concanavalin A (conA), wheat germ agglutinin (WGA), and peanut agglutinin (PNA). In both gustatory and olfactory membranes, about half of the protein components visualized by silver staining of polyacrylamide gels were labeled by conA and WGA. By contrast, only one predominant component was labeled by PNA in the olfactory cilia, whereas about half of the proteins of taste membranes were labeled by this lectin. The relatively large number of PNA-labeled components in taste membranes contrasted sharply with the labeling patterns of olfactory cilia and nonchemosensory membranes from catfish, such as liver and brain, which contained few PNA-sensitive components. When the same (nonconjugated) lectins were preincubated with isolated olfactory cilia, 70% of L-ala and L-arg receptor binding was inhibited by conA and WGA, whereas PNA inhibited L-ala but not L-arg binding. Inhibition of binding to these receptors was both time- and dose-dependent. By contrast, using the same conditions, no inhibition of either L-ala or L-arg receptor binding in taste membranes was observed with any of the lectins. The differential labeling of the chemosensory membranes and the differential inhibition of receptor binding by lectins suggest that, despite ligand similarity, the chemosensory receptors in these membranes are not identical molecular species. These results also suggest that lectins may be useful reagents for identification and further characterization of the structure and function of chemosensory receptors at the molecular level.

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369.13 POSSIBLE EXPRESSION OF ODORANT RECEPTOR PROTEINS IN XENOPUS OOCYTES INJECTED WITH RAT OLFACTORY EPITHELIAL mRNA. Nathan Dascal*2, Judith Heldman*1, Eli Gershon*2 and Doron Lancet1. 1Dept. of Membrane Research, the Weizmann Institute of Science, Rehovot, and 2 Dept. of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Israel.

Olfactory reception has recently been shown to be mediated by odorant-induced activation of adenylate cyclase in the sensory neuronal membrane (Pace et al., Nature 316:255 (1985)). Reconstitution studies with this transduction enzyme could help to identify the yet unknown olfactory receptor proteins. Xenopus oocytes constitute an ideal system in which olfactory receptors may be functionally expressed. These oocytes were shown to respond to adenosine-stimulated intracellular elevation of cAMP by hyperpolarizing potassium currents (Lotan et al., Mol. Pharmacol. 28:170 (1985)). Thus, it is expected that injection of olfactory epithelial mRNA would lead to the synthesis and membrane insertion of odorant receptor proteins, that could couple to the endogenous adenylate cyclase of the oocyte. Odorant stimulation could then be monitored electrophysiologically in single cells.

Olfactory epithelia, freshly dissected from 14 days old Sprague-Dawley rats, were processed for mRNA preparation by LiCl-urea, Phenol-chloroform extraction, followed by oligo-dT cellulose affinity chromatography. mRNA was characterized by agarose gel electrophoresis and by cell-free translation in the reticulocyte lysate system. Fully grown, immature follicular Xenopus laevis oocytes were injected with 50-60 nanoliters of 1-2 ug/ml poly (A)+ mRNA. After incubation at 23°C for 36-48h, responses to bath-applied ligands were recorded under voltage clamp in frog Ringer's solution. It was found that the odorants n-amyl acetate and citral, as well as their equimolar mixture with cineole and l-carvone (0.5-1mM), gave rise to small (10-20nA) depolarizing currents in control oocytes, injected with water or with mRNA from rat brain. In contrast, some oocytes injected with olfactory epithelial mRNA and stimulated with odorants displayed small (10-30nA) hypepolarizing currents, as expected for receptor-mediated cAMP elevation. Following bath application of a suboptimal concentration ($5X10^{-7}$M) of forskolin, a ligand that activates adenylate cyclase directly, enlarged odorant-induced hyperpolarizing currents (50-150nA) could sometimes be monitored, superimposed on a forskolin-induced prolonged hyperpolarization. Oocytes that did not respond to odorants were often equally non-reactive to adenosine, possibly due to an impairment of the transduction cascade. The results suggest functional expression of odorant receptor message, and could form a basis for mRNA enrichment, en-route to molecular cloning.

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369.14 BUTYRYLCHOLINESTERASE HISTOCHEMISTRY MARKS THE PERIPHERAL DISTRIBUTION OF OLFACTORY AND VOMERONASAL NERVES IN HAMSTER NASAL MUCOSA. T.A. Schoenfeld, A.N. Clancy and F. Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Bojsen-Møller (1975) demonstrated that trigeminal afferent and postganglionic autonomic efferent nerves to the nasal septal mucosa of the rat stain positively for acetylcholinesterase (AChE). He also showed staining of vomeronasal nerves and of nerves from the septal organ but little or no staining of olfactory nerves. We have discovered that the presence or absence of staining of the olfactory, septal organ and vomeronasal nerves in hamster septal mucosa is dependent on the use of a butyrylcholinesterase (BuChE) inhibitor (ethopropazine) in incubations for AChE. Including ethopropazine prevents staining of olfactory, septal organ and vomeronasal nerves, whereas eliminating ethopropazine results in intense staining of these nerves and provides a marker for their peripheral distribution in the septal mucosa. Incubation with a substrate having low affinity for AChE but high affinity for BuChE (with or without AChE inhibitor) also results in the staining of olfactory, septal organ and vomeronasal nerves, confirming that these nerves have BuChE, not AChE. Incubations for BuChE also stain trigeminal and perivascular nerves but not nasal glands. Thus, olfactory, septal organ and vomeronasal nerves stain positively only for BuChE, parasympathetically-innervated nasal glands only for AChE, and trigeminal and perivascular nerves for both classes of cholinesterase. By incubating together frozen sections of septal mucosa and olfactory bulb, we have also found that BuChE is confined almost exclusively to the peripheral (nasal) portion of olfactory and vomeronasal nerves. The central (cranial) portion of these nerves is almost completely devoid of staining under conditions that result in intense BuChE staining of peripheral olfactory and vomeronasal nerve profiles and of central fiber tracts (e.g., anterior commissure, corpus callosum). Thus, BuChE is not associated with the terminal portion of olfactory and vomeronasal nerves and therefore is not a generalized marker (like olfactory marker protein) for the full course of olfactory and vomeronasal axons. BuChE is associated with these axons where they pass through regions of moderate to high concentrations of AChE that correspond to glandular and vascular smooth muscle targets of parasympathetic acetylcholine (ACh), whereas it is absent where there is little or no AChE en passant. BuChE is known to hydrolyze ACh, but because of a low affinity between enzyme and substrate, degradation occurs only at relatively high ACh concentrations. Perhaps BuChE serves to protect olfactory and vomeronasal nerve peripheral axons from the effects of high titers of ACh released into the mucosa during prolonged parasympathetic outflow.

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369.15 ATLAS OF THE HAMSTER NASAL CAVITY AND THE DISTRIBUTIONS OF NEURAL AND NON-NEURAL TISSUES OF THE PERIPHERAL OLFACTORY SYSTEM. A.N. Clancy, T.A. Schoenfeld and F. Macrides. Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545.

Previous research using the fluorescent stilbene isothiocyothionate retrograde tracer SITS revealed a topographically organized pattern of projections of the peripheral olfactory receptor neurons into the main olfactory bulb. In the course of this research, a stereotaxic atlas of the male Syrian hamster nasal cavity was generated. The atlas, which was constructed in the coronal plane of section, extends from the nares to the caudal-most extreme of the nasal cavity and is based on material collected from ten animals. For purposes of comparison, representative photographs of material cut in the sagittal and horizontal planes of section were prepared. The atlas depicts and provides relevant stereotaxic coordinates of the nasal septum, endoturbinates and ectoturbinates, vomeronasal organ, septal organ, septal window and various ducts, including the Hardarian duct and nasopalatine duct. The distributions of olfactory and vomeronasal sensory epithelia, respiratory epithelium, and other types of tissue within the nasal cavity were fully documented by means of light microscopic charting of hematoxylin-azure-eosin stained material and by immunohistochemical staining for olfactory marker protein, a protein which is specifically associated with olfactory and vomeronasal receptor neurons. Additional studies of the peripheral olfactory system by cholinesterase histochemistry are presented in a companion poster. Computer-assisted morphometric analyses were used to determine the average surface areas invested with these various tissue types and the average volumes of the air spaces within the nasal cavity. The atlas and morphometric results may be of value in studies of olfactory chemoreception by providing an objective framework to study air flow to sensory surfaces within the nasal cavity. Air turnover and clearance rates may also be modeled as a function of respiratory rates and sniffing cycles.

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369.16 HISTOLOGICAL ORGANIZATION OF THE VOMERONASAL ORGAN OF HABU, TRIMERESURUS FLAVOVIRIDIS. S. Takami* & K. Hirosawa. Dept. of Fine Morphology, Inst. of Medical Science, Univ. of Tokyo, Tokyo 108, Japan.

The vomeronasal organ (VNO), which is involved in the perception of species-specific odors, is known to be well developed in many reptilian species, especially in snakes.

Nine mature Habu captured in the Amami Island were fixed by perfusion from the left carotid artery. The VNO were embedded either in celloidin or Epon-Araldite. Celloidin sections were used for three-dimentional reconstruction of the VNO with a COSMOZONE (Nikon, Japan). Epon-Araldite embedded specimens were processed for light and electron microscopy.

Habu VNO was grossly divided into a dome-shaped sensory epithelial part (SE) and a hemispherical mushroom body, the latter being non-sensory. A narrow lumen, leading to the oral cavity via the vomeronasal duct, was made between the mushroom body and the SE. The luminal region of the SE consisted solely of sensory dendrites and supporting cells (supporting cell layer), while a large number of sensory cells piled up in a columnar fashion beneath the supporting cell layer. Well developed vascular plexuses were found around the neck and bottom of the columns. The height of the columns reached its maximum in the middle of the dorsal part (over 300μm), but gradually decreased toward the periphery. Each column contained the basal, undifferentiated, and bipolar cells. Occasional mitotic figures were encountered among the basal cells. The bipolar cells, characterized with well developed sER and prominent lipofuscin granules, were arrayed in a string-like arrangement: cells situated in the neck region of columns contained a larger amount of the organelles. Dendrites of bipolar cells, running through the cytoplasmic meshwork of supporting cells, terminated as small swellings from which long microvilli protruded to the lumen. Junctional complexes were made between supporting cells and also between dendrites and supporting cells. Axons of bipolar cells formed the nerve bundles in the submucosa.

Present findings confirm that the VNO of Habu has the columnar organization similar to that reported in other snakes, suggesting that this organization is generally present in snakes.

369.17 INTENSITY CODING VIA ACROSS-FIBER PATTERNS IN CHEMORECEPTORS. B.R. Johnson, C.L. Merrill*, R. Voigt*, P.F. Borroni*, and J. Atema. Boston University Marine Program, Marine Biological Lab., Woods Hole, MA 02543.

The antennules and legs of the lobster, Homarus americanus function in olfaction and taste. Lobster prey contain mM concentrations of stimuli, for which seawater backgrounds are µM to nM. Thus chemoreceptors may provide intensity information over a six-log concentration range. We examined the stimulus-response (SR) curves for three narrowly tuned populations of receptors; olfactory hydroxy-L-proline (Hyp) and taurine (Tau) cells; and gustatory ammonia (NH_4) cells. We determined whether patterns of afferent activity in these receptor populations could contain information about stimulus intensity.

Single cell SR curves were based on extracellular recordings from small nerve bundles. Tau cells were tested at four 1-log concentration steps. Threshold was below $3x10^{-9}M$ and saturation level was $3x10^{-7}M$ with a response rate of 48 spikes/5s. Hyp cells were tested at six 1-log concentration steps: threshold was below $3x10^{-8}M$ and saturation was at $3x10^{-5}M$ with a response rate of 20 spikes/5s. Most Hyp and Tau cells responded at all test concentrations. The shape of the individual SR curves varied greatly within these two cell populations. NH_4 cells were tested over a 4 log concentration range in which they did not saturate (maximum response rate = 115 spikes/10s). Individual SR curves of NH_4 cells were more homogeneous than those of Hyp and Tau cells.

Pearson's correlation coefficient (α =0.01) demonstrated differences (based only on the total number of spikes elicited in each cell) between responses evoked across each receptor population at different stimulus concentrations. Across Tau cells, each test concentration elicited a significantly different pattern of neural activity. Hyp cells distinguished high ($3x10^{-3}$, $3x10^{-4}M$), medium ($3x10^{-5}$, $3x10^{-6}M$), low ($3x10^{-7}M$), and very low ($3x10^{-8}M$) concentrations. NH_4 cells distinguished differences only between the lowest and highest concentrations.

This analysis of neural activity in lobster chemoreceptors reveals that a population code for stimulus intensity indeed exists. Such a code may be needed for neurons which have low maximum firing rates and thus low capacity for information transfer over large stimulus ranges. An across-fiber pattern code in Tau and Hyp cells may have its basis in variability of shapes of individual SR curves. Neural patterns of stimulus intensity may combine with patterns of stimulus identity to code overall quality of naturally occurring stimulus mixtures.
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CHEMICAL SENSORY SYSTEMS: CNS

370.1 NORADRENERGIC MODULATION OF RESPONSES EVOKED IN THE MAIN OLFACTORY BULB BY OLFACTORY NERVE STIMULATION. C. R. ALMLI, H. PEREZ*, and A. HERNANDEZ*. Program in Neural Science, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110, and Institute of Nutrition and Food Technology, University of Chile, Santiago 11, Chile.

The locus coeruleus (LC) noradrenergic system has been implicated in the modulation of neuronal activity of several brain regions. Since reduction of brain noradrenaline levels alters odor-guided behaviors, and most of the noradrenergic input to the main olfactory bulb (MOB) can be attributed to the centrafugal projections from the LC, the present study determined the effects of electrical stimulation of the LC on the modulation of MOB field potentials evoked by orthodromic olfactory nerve (ON) or antidromic lateral olfactory tract (LOT) stimulation.

Adult Wistar rats were anesthetized with alpha-chloralose and D-tubocurarine, and a bipolar recording electrode was positioned on the surface to 0.8 mm below the MOB surface. Stimulating electrodes were positioned within the ON, LOT, and LC. A micropipette, positioned near the recording electrode in the MOB, was used to mechanically inject alpha-(prazosin) and beta-(propanolol) adrenergic blockers.

Field-evoked potentials from the MOB in response to ON or LOT stimulation were measured with and without conditioning stimulation of the LC noradrenergic system. The LC conditioning stimulus suppressed or inhibited the late components of the MOB potential evoked by orthodromic ON stimulation. This inhibitory effect was suppressed or abolished by the microinjection of the alpha-adrenergic blocker prazosin into the MOB. There was little, or no, effect of LC conditioning stimulation on the MOB response evoked by LOT stimulation.

The present results indicate that noradrenergic fibers projecting from the LC exert modulatory influences on neuronal networks underlying orthodromic evoked responses in the MOB. It is suggested that the suppressive influence of LC stimulation on MOB activity is mediated by a disruption of the closed-loop sustained activity between granule cells and mitral/tufted cells.
(Supported by Grants B-2018-8522 and BRSG 5389).

370.2 OLFACTORY NERVE AXON ORGANIZATION: BRAIDED OR NEIGHBORLY? G.D. Adamek and M.M. Daston*. Dept. of Anatomy and Cell Biology, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267

The unmyelinated axons of vertebrate olfactory nerves project as bundles of several hundred tightly packed in Schwann cell invaginations. The axons are 0.2 μ in diameter and there is little extracellular space. This organization is unique among sensory nerves and suggests that if neighboring axons are simultaneously active, increases in extracellular potassium will lead to depolarization and sodium conductance inactivation. Measurements indicate that the increase in extracellular potassium concentration is long lasting and that in these receptor neurons adaptation may be primarily in the action potential generation processes rather than in the receptor mechanism. There is some experimental support for the hypothesis that receptors sensitive to particular chemical stimuli are spatially segregated. If axons of the neurons in these patches travel to the olfactory bulb as neighbors, they might mutually inhibit spike propagation in each other. As stimulus concentrations increase, transmission would become progressively less efficient. Signals evoked by substances which cause general disorganization of the receptor membrane would be highly attenuated. Electron and fluorescence light microscopy were used to examine the configuration of olfactory nerve axons. Wheat germ agglutinin conjugated to horse radish peroxidase (WGA-HRP), other electron-dense tracers, and fluorescent dyes were injected into the receptor epithelium and in the axon terminal layer of the olfactory bulb of anesthetized frogs to study the following issues: (a) Do the axons of cells lying near each other in the receptor epithelium lie within the same Schwann cell fascicle? (b) For what distance do neighboring axons remain proximate within the olfactory nerve? (c) Do axons within a single Schwann fascicle remain together when they pass into the neighboring Schwann cell? (d) Do axons which terminate close to each other in the olfactory bulb project as neighbors in the olfactory nerve? Dyes and WGA-HRP were pressure-injected using a 5-μ pipette pulled to a tip diameter of 5 μ. For receptor epithelium injections, the pipette tip was positioned in the cell soma region about 50 μ below the tight junctional layer formed by the supporting cell apices and the receptor dendrites. For bulb injections, the micropipette tip was positioned in the glomerular layer. The amount of marker injected was small enough to fill only a few cells in the immediate vicinity of the injection site. After one hour, the olfactory nerve was fixed by immersion in paraformaldehyde and glutaraldehyde, then dissected out and prepared for electron or light microscopy. Individual labeled axons were traced through serial electron micrographs or within fluorescence micrographs of longitudinal sections. Preliminary results indicate that axons which are neighbors in one cross section remain close to each other within the same fascicle over extended lengths of the nerve.

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370.3 HORSERADISH PEROXIDASE (HRP) LABELING OF OLFACTORY NERVE TERMINALS IN PIGEON OLFACTORY BULB. L. C. Miller* and J. C. Walker* (SPON: J. Robinson).R. J. Reynolds Tobacco Co., Winston-Salem, NC 27102.

Structural and functional study of normal and regenerating olfactory systems could provide insights into the mechanisms of sensory coding in this modality. At least three requirements must be met in order for such an approach to be applied to an animal model: 1) reliable methods for the testing, in isolation, of olfactory and nonolfactory (trigeminal) odor sensitivity; 2) neuroanatomical structure that allows complete transection or resection of olfactory and trigeminal afferent pathways from the nasal cavity; and 3) morphological procedures to determine reliably the degree and pattern of innervation of the olfactory bulb in normal and nerve-transected animals. Since the White Carneaux pigeon now meets conditions 1 and 2, the purpose of this work was to develop the necessary tools for labeling olfactory afferents in this animal. A procedure was developed to use the anterograde transport of horseradish peroxidase (HRP) to define the terminal field of olfactory nerve axons in the olfactory bulb. Under Chloropent^{R} anesthesia, the left olfactory nerve was transected and the proximal stump was placed, for 1 hour, in a Teflon "well" containing HRP (Sigma VI, 75% solution). Following perfusion-fixation 48 to 72 hr later, both olfactory bulbs were sectioned parasagitally at 40 um, and the tissue was processed with tetramethyl benzidine (Mesulam, 1978). Under light microscopy a relatively uniform band of reaction product, somewhat denser on the ventral surface of the bulb, was visualized consistently in only the olfactory nerve and glomerular layers of the left, but not the right, olfactory bulb. These results indicate that the same general method employed to visualize the connection of the olfactory receptor neurons with the olfactory bulb in hamsters (Meredith et al., 1980) can be adapted for studying the avian olfactory system. This method can be used to describe the time course of reinnervation of the pigeon olfactory bulb following nerve transection and to determine the degree and pattern of reinnervation necessary for functional recovery, as measured by performance on specific odor psychophysical tasks.

370.4 HRP study: Projections to olfactory bulb. R. Guevara-Aguilar and O.A. Donatti-Albarrán. Depto. de Fisiología, Facultad de Medicina, UNAM, México 04510, D. F.

We have reported the projections from vagus nerve to olfactory bulb (OB) and the results show that the pathway travel through the nucleus of the tractus solitarius (NTS) (See this Meeting). The aim of this paper was to demonstrate with retrograde transport of HRP direct projections to OB from NTS. Experiments were conducted on twelve Wistar rats anesthetized with cloral hydrate (400 mg/kg). Horseradish peroxidase (type VI, 25%) was applied unilaterally in the OB and in the olfactory tubercle (OT). The HRP was delivered by microiontophoretic ejection by positive DC current (2.0-2.5 µA, 10 min.) from glass micropipettes with tips of 25-40 µM. After 24-48 hrs of survival time the animals were fully anesthetized and their brains perfused. The histological sections 50 µM were processed following blue reaction of Mesulam. No labelled neurons were identified in the NTS when the HRP was applied in the OB, but a lot of labelled neurons were observed only homolaterally in the NTS when the HRP was applied in the OT. In our previous published papers we have postulated OT as a final common pathway from influences of reticular formation and hypothalamus, and this HRP study support the idea of OT acts as relay station of influences from visceral organs to OB.

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370.5 CELL INTERACTIONS IN THE OLFACTORY BULB OF THE AWAKE RAT. F.H. Eeckman* and W.J. Freeman. Dept. of Physiol.-Anatomy, U.C. Berkeley, Berkeley, CA 94720.

We performed simultaneous recordings of multi-unit pulses and EEG at various locations in the olfactory bulb of awake rats. No stimulation was used. Both the EEG and the spikes were recorded with chronically implanted 100 micron stainless steel electrodes with sharpened tips. The EEG electrode was positioned in the granule cell layer, while the unit electrode was at the level of the mitral cells (interelectrode distance was .5 mm). The EEG was digitized at 1 ms intervals and bandpass filtered at 10-300 Hz. The pulse train was analyzed with a threshold device, set to emit a standard pulse, 5V 1 ms for every signal above twice the noise level. Individual traces often showed 3 to 4 distinct action potential waveforms. Data were stored on tape and processed off-line on a Perkin Elmer 3220 supermini. We used the 2 dimensional conditional pulse probability table to find correlations between units and EEG wave amplitudes.

Earlier we reported results in anesthetized animals (neuroscience poster, 1985). Here we found units correlated with the dominant frequency of the EEG at 30-60 Hz. In the awake animal however the dominant peak occurred at 70-80 Hz. The unit spikes were found to correlate well with this higher frequency. The percentage of modulation was much higher in the awake animal (between 60-90% versus 10-30% under anesthesia). The pulses correspond to mitral cell firings. The EEG is generated almost exclusively by the granule cells. All other cells in the bulb generate closed fields.

The data are explained in terms of Freeman's model for burst generation in the Olfactory Bulb (Freeman 1975, 1979). Our experiments showing a fixed phase relationship with quarter cycle time lead for pulses to waves provide further evidence for this model. They also show clear differences between the awake and anesthetized groups both in frequency and percentage modulation.

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370.6 INVESTIGATIONS ON THE INTERACTIONS OF NEURONS IN THE OLFACTORY BULB AND THE ANTERIOR OLFACTORY NUCLEUS BY MEASURING THE CONCENTRATIONS OF CHOLECYSTOKININ-mRNA D.K. Meyer* and C.G. Bönnemann* (SPON: W. Lichtensteiger). Dept. of Pharmacol., Freiburg Univ. Med. Fac., D-7800 Freiburg, FRG.

It is of basic importance for the understanding of the function of the olfactory bulb (OB) to know how intrinsic neurons interact with centrifugal afferents. Recently, cholecystokinin-like immunoreactivity (CCK-IR) has been described to be present in rat OB. It has been found in efferent cells (i.e. tufted cells) as well as in some centrifugal afferents, which seem to come from the anterior olfactory nucleus (AON) (Seroogy et al., J. comp. Neurol. 239:373, 1985). This finding offers the possibility to study the neuronal interactions in the OB with biochemical methods. For this purpose, we have combined surgical as well as chemical lesions with the measurement of CCK-IR and of mRNA specific for cholecystokinin-peptides (CCK-mRNA) in rat OB and AON. A filter hybridisation method was used to quantitate the CCK-mRNA which had been extracted by use of a lauryl-sulfate/proteinase K procedure.

Seven days after surgical deafferentiation of the right OB, concentrations of CCK-IR in OB and AON of the lesioned side were significantly decreased, i.e. by approximately 50%. There were no changes in the structures of the contralateral intact side. Surprisingly, concentrations in CCK-mRNA were lowered by approximately 50% in the OB's of both sides.

Five days after injection of ibotenic acid, a neurotoxic agent, into the right OB, the concentrations of CCK-IR in the OB's and AON's of both sides were significantly enhanced . After this injection, the concentrations of CCK-mRNA were elevated in the AON's of both sides.

The data confirm that in the OB intrinsic neurons and centrifugal afferents are closely functionally connected. The comparative measurement of peptide contents and concentrations of mRNA seems to be a good tool to study these neuronal interactions.

370.7 GUSTATORY AND OLFACTORY ABLATION EFFECTS ON CHEMOSENSORY LEARNING IN GOLDFISH. R.J. Arcement and D.J. Ingle. Teachers College, Columbia University, New York, NY 10027 and Eye Research Institute, Boston, MA 02114.

Chemoreception in fish is problematical because gustatory and olfactory stimuli are both perceived in a liquid medium making a distinction between the two senses elusive. Contrary to other studies, this investigation showed that transection of the olfactory tract (OT) does not interfere with food (beef and brine shrimp) extract discrimination. Furthermore, to the authors' knowledge, no study has been carried out involving ablations of the taste centers, facial lobe (FL) and vagal lobes (VL), combined with chemosensory learning. Obstacles to such studies are the location of the medulla and the large mass of brain tissue taken up by the VL.

Training and surgical procedures were developed to address these problems. The training protocol employed habituation and reward using food extracts. Histological analysis showed that surgery was effective in completely ablating the FL in one large group of fish and leaving remnants in only two in another large group. VL ablations were carried out such that as much of the lobes as possible was removed without damaging the vagal nerve. Surgery was effective in that histology showed the bulk of the VL in all subjects to be removed with some showing complete ablations while others showing small lobe remnants.

Training and surgical protocols were used on groups of goldfish to assess their chemoreceptive ability in food extract discrimination after a series of ablations involving the OT, forebrain (F), FL and VL. Removal of the OT or F had no effect on the performance of some members of a group which were distinguishing well. In order to appraise the roles of the taste centers the following ablation groups were prepared: FL and OT; FL; and sham. Testing showed no difference among these groups. A final group was assembled which had their FL and VL removed. Since these fish were still able to discriminate, their OT were then ablated. In the first test performance was poor with only three fish showing some discrimination. In the second test about one-half showed evidence of discrimination yet in the third test only four performed well. Histological analysis showed a correlation between the size of the VL remnants and continued food extract discrimination.

In summary it was found that chemoreception of food extracts can occur without the medullary taste centers or without the olfactory pathway. Furthermore it was shown that chemoreception can occur when both the OT and FL are ablated. Pilot studies with three fish also indicated that it can occur when the OT and VL are ablated but larger numbers are needed for confirmation.

370.8 EFFECTS OF CHEMORECEPTOR STIMULATION ON GLUCOSE UTILIZATION BY THE RABBIT CAROTID BODY IN VITRO. A. Obeso,* C. Gonzalez, B. Dinger and S. Fidone. Dept. Physiology, School of Medicine, Univ. Valladolid (SPAIN) & Dept. Physiology, School of Medicine, Univ. Utah, Salt Lake City, Utah (USA)

The mechanisms of sensory transduction in carotid body chemoreceptors remains largely unknown. A link between intermediary metabolism and chemosensation in this organ has for many years been implied by various metabolic hypotheses of chemoreception, but few studies have related the energetic metabolism of the carotid body to its chemoresponsiveness. In the present study, we have measured the rate of glucose consumption by measuring the accumulation of 2-deoxy-^{3}H-glucose-6-phosphate from tracer amounts of labelled 2-deoxy-^{3}H-glucose (^{3}H-2DG), in an effort to demonstrate a unique sensitivity of carotid body elements to natural chemosensory stimuli.

Glucose utilization in vitro was measured in rabbit (New Zealand white) carotid body and in a non-chemosensitive neural tissue, the nodose ganglion. The tissues were preincubated for 25 min (37°C) in 4 ml of Tyrode's solution equilibrated with 100% O_2 (pH=7.4) and containing 3 mM glucose. Tissues were transferred to mini-vials containing 750 µls of Tyrode's media with 3 mM glucose and 2 µM ^{3}H-2DG. Incubation media were equilibrated either with 100% O_2 or 20% O_2 in N_2. Samples were washed at 0-4°C for 2 min to remove extracellular ^{3}H-2DG, and processed either for determination of the phosphorylated sugar analogue (Gonzalez and Garcia-Sancho, Anal. Biochem. 114: 285, 1981), or for autoradiography of water diffusible substances in carotid body tissue (Fidone et al., Chemoreception in the carotid body, Springer-Verlag, 1977, pp. 106-113).

The rate of ^{3}H-2DG phosphorylation in carotid body (64 nmols/g/min) and nodose ganglion (42 nmols/g/min) was linear for more than 30 min during incubation in 100% O_2-equilibrated (control) media. Carotid bodies incubated for 5 or 10 min in media equilibrated with 20% O_2 in N_2 exhibited increases in ^{3}H-2DG phosphorylation of 34% ($p<0.001$) and 45% ($p<0.02$), respectively; nodose ganglia similarly treated yielded values (+11% and -3%, respectively) which were not significantly different from those obtained with control media. LM autoradiography of carotid bodies incubated in ^{3}H-2DG revealed a preponderance of silver grains localized over lobules of type I cells.

These data suggest that the mechanisms for low O_2 chemotransduction in the mammalian carotid body are linked to the intermediary metabolism of the type I cells in this sensory receptor.

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370.9 INTERACTIONS BETWEEN THE NERVUS TERMINALIS AND THE OLFACTORY SYSTEM J. White and M. Meredith. Dept. of Biological Science, Florida State University, Talahassee, FL 32304

The nervus terminalis (n.t.) is a ganglionated cranial nerve of unknown function connecting the forebrain with the nasal cavity. Its peripheral connections and ganglion cell anatomy suggest that the nerve may be chemosensitive and/or affect the physiology of the nasal mucosa via nerve endings in the olfactory epithelium or onto nasal glands and blood vessels. Ganglion cells contain high levels of LHRH and accumulating circumstantial evidence suggests that the nerve may be concerned with behavioral and physiological reproductive responses to chemical signals - but it is not clear whether it is chemosensitive itself or operates via interconnections with the olfactory system. We are examining this system in the bonnethead shark where the n.t. is separate from the olfactory system and independent stimulation and recording are possible. Chemical stimulation of the olfactory mucosa by ourselves and others has produced no obvious electrophysiological response in the nerve but it is possible that the response is conveyed by some of the small unmyelinated fibers seen in the nerve with EM - and would have been missed with the electrodes used. In order to evaluate possible interactions with the olfactory system, mediated either by the peripheral or central terminations of the n.t., we have recorded from single units in the shark olfactory bulb while stimulating the n.t. electrically. Electrical stimulation at intensities that produce a clear compound action potential in the nerve and with the nerve connected either centrally or peripherally, produced no change in activity of 18 units recorded at various positions in the lateral lobe of the olfactory bulb. Electrical stimulation, both before and during olfactory stimulation, was repeated multiple times and the activity of bulbar cells averaged to allow detection of small changes in firing rate. No significant changes in spontaneous activity, nor in response to chemical stimulation were seen, suggesting that any interactions between the systems directed from the n.t. towards the lateral olfactory bulb are minimal. The possibility that there are interactions in the opposite direction, from the olfactory system towards the n.t. is currently being explored. Experiments where the central connections of the n.t. were cut, cooled or stimulated show that there are efferent fibers originating in the brain that can influence the activity of n.t. ganglion cells. The influence of electrical stimulation of the olfactory system on the activity of these fibers and of the ganglion cells is now being tested.

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370.10 OPTICAL RECORDING OF THE PIRIFORM CORTEX ELECTRICAL ACTIVITY INDUCED BY LATERAL OLFACTORY TRACT STIMULATIONS IN THE RAT. M. Cattarelli and L.B. Cohen. Dept. of Physiology, Yale Univ. School of Medicine, New Haven, CT 06510-8026, USA and Physiologie Neuro-Sensorielle, Universite Claude Bernard 69622 Villeurbanne cedex, France.

As shown by Orbach et al. (1985), optical recording methods can be used for monitoring the electrical activity induced in mammalian visual and somatosensory cortices by sensory stimulations. We have used this method for monitoring the piriform electrical activity induced by stimulations of the lateral olfactory tract (LOT) in the rat and for studying its spatial distribution on that cortex. Surgical procedures, i.e. tracheotomy and exposure of the latero-basal brain surface, were performed on Wistar SPF male rats anaesthetized with equithesine. The anaesthesia was continued during the experiment. In addition the animals were curarized and artifically ventilated. After having removed the dura, the piriform cortex was stained with a voltage-sensitive dye (RH414 or RH795, kindly supplied by R. Hildesheim and Dr. A. Grinvald). Cortical fluorescence was measured with a 124 element photodiode array using epi-illumination while various spatially localized electrical stimulations were delivered through a five wire electrode located on the LOT. Usually eight or sixteen trials were averaged. Optical signals with a duration of half-height of from 15 to 100 msec were recorded in response to LOT stimulations. Their time course and their spatial distribution varied somewhat according to the location of the LOT stimulating electrodes. However, a large overlapping was always noted in the distribution of the signals. These results suggest that in addition to a large spreading out of the LOT inputs to the piriform cortex there is also some spatially organized projection of this tract to the piriform cortex.

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370.11 A COMPUTER SIMULATION OF PIRIFORM CORTEX. M. Wilson*, J. Bower[1], J. Chover* & L. Haberly (SPON: J. Hind), Departments of Anatomy, Electrical Engineering, and Mathematics, University of Wisconsin, Madison, WI 53706. [1]Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Using parameters obtained from anatomical and physiological experiments (Haberly, Chem. Senses 10:219, 1985), a computer model of piriform (olfactory) cortex has been developed that simulates physiological responses obtained using a variety of recording methods. The model consists of a linear array of pyramidal cells interconnected by a highly divergent/convergent system of associational fibers. Excitatory input is delivered to the cells along a single afferent pathway with characteristics similar to the lateral olfactory tract projection to piriform cortex from the olfactory bulb. Two forms of inhibition are incorporated in the model: a feedforward, long latency, long duration hyperpolarizing potential which simulates the K+ mediated potential observed in piriform cortex (see Tseng & Haberly, this volume), and a feedback, short latency current shunting type inhibition simulating the well characterized Cl- mediated process. The simulation incorporates the different propagation velocities of the separate fiber pathways, synaptic delays, refractory periods, time constant estimates for inhibitory and excitatory events, and spatial variations in the distribution of different excitatory and inhibitory influences. This model has been shown to simulate a variety of known intracellular and extracellular responses. In response to continuous odor-like stimulation, the model generates a series of oscillatory extracellular bursts comprised of a dominant 40 Hz sinusoid. Single shock stimulation gives characteristic EPSP/IPSP intracellular responses and extracellular field potentials at appropriate latencies. In the presence of "noise", weak single shock stimulation produces damped oscillatory extracellular potentials. Both spontaneous and induced interictal/ictal epileptiform events can be generated after blocking inhibitory processes, or strengthening specific associational pathways. The model suggests that the transition from interictal to ictal events can be controlled by the long latency, long duration hyperpolarizing potential. Having successfully used this model to generate physiological responses characteristic of piriform cortex, the next objective will be to explore the significance of the specific structural and physiological features of this cortex for the processing of complex spatial and temporal olfactory information and their implications for general associative memory processes. Supported by NSF grant NBS-8311118 and NIH grant NS19865 to L.B.H.

370.12 ULTRASTRUCTURE OF PRIMARY AFFERENT FIBER TERMINALS WITHIN THE GUSTATORY LOBES OF THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. T.E. Finger and M. Womble*. Rocky Mountain Taste & Smell Center and Dept. Cellular & Structural Biology, Univ. Colorado Medical Sch., Denver, CO 80262

The taste buds on the barbels of ictalurid catfish are innervated by branches of the facial nerve. Some of these fibers are mechanosensitive only while others are both mechanically and chemically sensitive (Caprio, 1978). The barbel nerves terminate centrally in the facial (gustatory) lobes, which are paired specializations of the medullary special visceral sensory column. The present report represents preliminary findings of a study of the ultrastructure of the chemosensory and mechanosensory primary afferent fibers.

Horseradish peroxidase (HRP) was applied to the barbel nerves in their course through the orbit. Following a survival time of 3-5 days, the animals were perfused with a mixed aldehyde fixative, and the brains prepared for ultrastructural histochemistry with DAB or Hanker-Yates substrate serving as the chromogen.

Myelinated labeled fibers, ranging in diameter from 0.5 to 1.5 μm, enter the base of the facial lobe and turn outward toward the surface. At varying depths, individual fibers lose their myelin sheath and ramify within a limited extent of the lobe, covering a volume of about 250 μm^3. Labeled fibers often contact one another within the facial lobe; in some cases membrane specializations appear at the point of contact. Whether these represent functional contacts or merely adherent complexes could not be determined in the labeled material.

Most labeled synapses within the facial lobe involve small (approx. 1 μm across) presynaptic elements forming asymmetric contacts onto very small processes (less than 0.5 μm) which contain numerous pleiomorphic vescicles. Many of these postsynaptic elements are tentatively identified as dendritic spines. Occasionaly, a labeled terminal was observed to contact a dendritic shaft or soma. The synaptic vescicles in the presynaptic terminals are spherical, the majority being approximately 30 nm in diameter. In many cases, the labeled presynaptic terminal was observed to form a terminal complex synapsing simultaneously on several small, vesicle-filled postsynaptic processes. These synaptic complexes resembled glomeruli but were not enclosed in a glial envelope. In a few cases, unlabeled, vesicle-filled elements were presynaptic to labeled fibers. This may provide a means for central modulation of gustatory afferent activity.

In addition to labeled small terminals, some labeled fibers give rise to large, bulbous formations, 2-3 μm in diameter. These bulbous structures were both pre- and post-synaptic to diverse elements in the surrounding neuropil.

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370.13 ORAL INNERVATION IN THE RABBIT: AFFERENT AND EFFERENT PROJECTIONS OF THE GLOSSOPHARYNGEAL NERVE. M. L. Pensak*, T. Hanamori* and D. V. Smith. Dept. Otolaryngol. Maxillofac. Surg., Univ. Cincinnati Med. Ctr., Cincinnati, OH 45267.

The glossopharyngeal (IXth) nerve innervates taste buds in the circumvallate and foliate papillae on the posterior portion of the mammalian tongue. In addition to its' chemosensory function, the IXth nerve also carries mechanosensitive afferent fibers and parasympathetic efferent fibers. In rats and hamsters the IXth nerve projects into the nucleus of the solitary tract (nst) caudal to the projection of the VIIth nerve, but overlapping with it to some extent. In the first of a series of studies to delineate the brainstem organization of the innervation of the oral cavity in rabbits, we applied crystallized HRP (Sigma Type VI) to the proximal portion of the lingual-tonsillar branch of the IXth nerve. The animals were allowed to survive for 3 days and the brains were then processed for HRP reaction using the TMB method. Afferent fibers from the IXth nerve entered the brainstem between 0.6 and 1.0 mm rostral to the caudal extent of the dorsal cochlear nucleus (dcn) at the level of the facial nucleus. The major area of afferent termination within the nucleus of the solitary tract (nst) was from 0.2 mm rostral to the caudal border of the dcn to 2.6 mm caudal to this landmark. In the more rostral extent of this afferent termination within the nst, afferent label was seen primarily medial and ventral to the solitary tract (ST). As the projection proceeded caudally, the afferent label was seen ventral and lateral to the ST, within the interstitial nucleus. From this area of major termination, sparse afferent label was seen to extend within the ST into the cervical spinal cord as far as 5.6 mm caudal to the dcn. In addition to this primary projection within the nst, afferent label was evident in the spinal tract (STV) and nucleus (sVi and sVc) of the trigeminal nerve from 2.0 to 4.6 mm caudal to the dcn. Cell bodies giving rise to efferent fibers in the IXth nerve were labelled retrogradely within the inferior salivatory nucleus (isn) from 1.8 mm rostral back to the level of the caudal border of the dcn. Efferent fibers from these cells projected in a dorsomedial direction from the isn to form a sharp genu under the floor of the IVth venticle ventromedial to the medial vestibular nucleus. This projection was evident from 0.6 to 1.6 mm rostral to the caudal extent of the dcn, from the rostral extent of the nst to the caudal border of the genu of the facial nerve. Cells in the isn are known to control secretions of the parotid and von Ebner's glands in the rat.

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370.14 RESPONSE CHARACTERISTICS OF LAMB NUCLEUS OF THE SOLITARY TRACT NEURONS TO TACTILE, CHEMICAL AND THERMAL STIMULATION OF THE ORAL CAVITY AND EPIGLOTTIS. R.D. Sweazey and R.M. Bradley. Dept. Oral Biology, Sch. of Dentistry, Univ. Michigan, Ann Arbor, MI 48109.

Using anatomical techniques we have shown that the primary afferents innervating the lamb caudal oral cavity and upper airway have overlapping terminations in the solitary tract nucleus (NST). We have now recorded neural responses from these regions of the NST to stimulation of the soft palate, caudal tongue and epiglottis with tactile, temperature and chemical stimuli.

Recordings have been made from 46 neurons in or immediately ventral to the lamb NST. Twenty-four percent received sensory information exclusively from the caudal tongue, 7% from the soft palate and 52% had receptive fields restricted to the epiglottis. The remaining neurons received converging sensory inputs from more than one receptor area. Most of the convergence observed in the NST occurred between the caudal tongue and soft palate. Of the 22 neurons with receptive fields located in the caudal oral cavity 6 received converging tactile information from the tongue and soft palate. In all cases the receptive field on the tongue was directly opposed to the receptive field located on the soft palate. Only 2 neurons have been identified as receiving converging inputs from the epiglottis and caudal oral cavity. As was the case for neurons receiving converging inputs from the tongue and palate, the convergence between epiglottis and caudal oral cavity occurred in neurons responsive to tactile stimuli. No convergence in chemosensory information from the epiglottis and caudal oral cavity has been observed; the location of cells responsive to chemical stimulation of the epiglottis is anatomically separate from those responsive to chemical stimulation of the caudal oral cavity.

Generally, NST neurons responded to more than one stimulus modality. Thirty-three percent of the neurons responded to one, 48% responded to two and 19% responded to all three stimulus modalities. Tactile stimuli were the most effective for both the oral cavity and epiglottis. Over 90% of the isolated neurons responded to a tactile stimulus, and most of these responses were rapidly adapting. Chemical stimuli evoked responses in 67% of the isolated neurons. The most effective chemical stimulus for a particular neuron was dependent upon the location of the neuron's receptive field. NH_4Cl was the most effective chemical stimulus for neurons with receptive fields in the caudal oral cavity; KCl if the receptive field was located on the epiglottis. Thermal stimuli were the least effective for both areas. Of the 14 neurons responsive to thermal stimuli, 11 responded to cooling, 3 to warming.

The presence of multimodal neurons suggests that the region of the NST being investigated is important in the integration of afferent information about complex stimuli in the caudal oral cavity and upper airway. Furthermore, the information provided by these neurons may be important in the initiation and control of upper airway reflexes such as swallowing and coughing.

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370.15 GUSTATORY AND OLFACTORY CHANGES ASSOCIATED WITH DEPRESSION. S.H. Ackerman*, S. Mattis*, J.A. Sechzer, M. Russ*, L. Burton*, and J.B. Henriques*. Department of Psychiatry, Cornell University Medical College and the Eating Disorders Institute, New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605

Depression is commonly associated with a decrease in appetite. In studying this change in appetite we tested the hypothesis that perceptual changes specific to taste and smell occur in depression. Twelve subjects (6 depressed patients and 6 normal subjects) were studied twice, at 6 week intervals. Depressed patients were tested first after admission to the hospital and then retested after treatment.

Subjects tasted 6 supra-threshold concentrations of sucrose (sweet), NaCl (salty), citric acid (sour) and quinine (bitter), and smelled 6 concentrations of pyridine (rancid) and phenylethyl-methylethyl carbinol (PEMEC) (floral). Each group of tastes and smells was interspersed randomly with 6 intensities of a 1000 Hz tone (49-100 Db). Subjects were asked to estimate stimulus intensity by magnitude matching, a ratio scaling procedure in which intensity differences among tones are compared with intensity differences among specific tastes (e.g., salty) or smells. To assess the hedonic value of the stimuli, subjects were asked to use a 9 point scale to rate the pleasantness or unpleasantness of the taste of different sucrose solutions, the smell of pyridine and PEMEC solutions and the sounds of different tones (38-115 Db).

Because sensory testing might be confounded by the effects of depression on cognition or motivation we used a signal detection paradigm, employing verbal and olfactory cues, to quantify subjects' response biases (β) and sensitivity (d').

The major significant finding was that patients underestimated increments in stimulus intensity for sour and bitter tastes and for the highest NaCl concentration. They did not differ from controls on estimates of sucrose concentration, odor intensity or decibels of tone. Results of signal detection and hedonic ratings suggest that the underestimation of taste intensity is not an artifact of response bias, sensitivity or perceived pleasantness/unpleasantness.

These results suggest a sensory specific disturbance in the perception of supra-threshold intensities of bitter, sour and salty tastes, associated with depression. A sensory specific disturbance during depression has not, to our knowledge, been described previously.

DEVELOPMENTAL DISORDERS

371.1 DIAGNOSTIC LEVELS OF ULTRASOUND MAY DISRUPT MYELINATION. D.E. Palmer*, M.H. Ellisman, M.P. Andre* (SPON: J.M. LeBeau). Lab. Neurocytology, Depts. Neurosci. and Radiol., Sch. of Med., Univ. of Calif. San Diego, La Jolla, CA 92093.

Although diagnostic ultrasound imaging is generally considered innocuous, mounting evidence from epidemiological and animal studies suggests the potential for numerous adverse effects. The bioeffects noted by previous investigators indicate the possibility of subtle neurologic deficits in exposed children. In order to test for possible bioeffects on myelination we exposed unanesthetized rat pups to ultrasound doses consistent with those typically seen by a human fetus. We used 3-5 day old rat pups since the development of myelination in the neonatal rat is similar to that of a human fetus of about 4-5 months in utero. Animals were exposed to ultrasound from a 3.5 MHz transducer (from an ADR 2130 real-time linear array scanner), mounted in a waterproof housing. The animals were 8.5 cm away from the transducer. The spatial-peak temporal-average (SPTA) intensity of our applied field was 0.14 mW/cm^2, the spatial-average temporal-average (SATA) intensity was 0.05 mW/cm^2. Single litters were divided into experimental groups that were bound and exposed for 30 minutes, and control groups, that were either bound and placed in the tank, but not exposed for 30 minutes, or taken straight from the cage. Experimental animals were sacrificed and fixed either immediately post-exposure or after a recovery period of 24 hours. Sham exposure rats were sacrificed and fixed immediately. Other controls were sacrificed and fixed directly from the cage on the following day. Rats were sacrificed by decapitation. In normal development, sequential wrappings of the myelinating Schwann cells terminate at forming nodes of Ranvier by attaching to the axolemma in a series of septate-like junctional complexes referred to as the glial-axonal-junctions (GAJ). Tight junctions develop between each of these terminal loops of Schwann cell cytoplasm, sealing off the space between layers of myelin and the GAJ. In experimental animals, this junctional arrangement is perturbed. Enlargement of the periaxonal space is visible with separation of adjacent paranodal loops and disruption of focal contacts linking the paranodal axolemma with not yet compacted Schwann cell processes. Evidence that this type of ultrastructural effect of ultrasound exposure may lead to complete paranodal disruption was also noted, as in several nodes the GAJ's were broken and paranodal demyelination was evident. Nodes exhibiting this microedematous morphology were apparent in roots from all exposed animals, even after a 24 hour recovery period. The disruption, and subsequent consequences for nervous system development may explain some of the adverse effects indicated in epidemiological studies of children exposed to fetal ultrasound observation.

371.2 VOLTAGE-ACTIVATED SODIUM CONDUCTANCE IN NORMAL AND TRISOMY 16 FETAL MICE NEURONS. Carlos B. Orozco*, Sandra A. Smith†, Carole H. Latker†, Charles J. Epstein† and Stanley I. Rapoport. (SPONSOR: H. Levitan). Laboratory of Neurosciences. National Institute on Aging. Bethesda MD 20892, and Depts. of Pediatrics and Biochemistry and Biophysics. University of California. San Francisco, CA 94143.

Trisomy 16 in the mouse is a model for trisomy 21 in humans (Down syndrome). We have described differences in the electrical membrane properties between fetal trisomy 16 and control dorsal root ganglia (DRG) neurons (Orozco, et al, submitted). Trisomic cells have a higher resting potential and faster rate of depolarization of the action potential, which can be due to an enhanced sodium and/or calcium membrane conductance. To test the first hypothesis we use the patch clamp technique to characterize the sodium current during activity.

Trisomic and euploid (control) mouse embryos were obtained as previously described (Orozco, et al, Soc. Neurosci. Abstr. 11:398). DRG neurons maintained in primary tissue culture were used to obtain current and voltage clamp recordings (L/M EPC-7, List Electronics, Germany). Na^+ current was recorded in the "Whole Cell" configuration. Single and double pulse protocols were used to measure the steady state activation and inactivation. Three different external concentrations of Na were used (150, 54, and 30 mM). The internal concentration of sodium was always 30 mM Na. The sodium equilibrium potential calculated in these conditions was +40, +16 and 0 mV respectively. Isotonicity of the external solution was compensated with sucrose. NaF (28 mM) intracellularly and $CdCl_2$ (1-2 mM) extracellularly were added to block calcium currents. Intracellular K^+ was replaced by TRIS; 2 mM TEA was included in the internal solution to suppress the potassium currents.

Sodium current in control and trisomic neurons was similar to that described in other excitable cells. However, half of the activation was attained at -60/-50 mV and 50% of the inactivation at -85 mV in both neurons. The current was blocked by tetrodotoxin (TTX). The specific membrane conductance for Na^+ varied directly with its external concentration and for control cells was 0.3 mS/cm^2 in 30 mM Na, 0.6 mS/cm^2 in 54 mM Na and 1.8 mS/cm^2 in 150 mM Na. Results obtained at the two lower concentrations of sodium indicate that trisomic neurons possess higher sodium conductance than do control cells (0.63 mS/cm^2 (n=3) in 30 mM Na and 0.95 mS/cm^2 (n=6) in 54 mM Na). The voltage dependence observed for activation and inactivation makes unlikely that TTX-sensitive Na conductance contributes significantly to rate of depolarization of the action potential of these fetal neurons. However, the results indicate that differences in Na conductance between normal and trisomic neurons already exist at this stage of development. Experiments are now being conducted to confirm this observation and to elucidate the possible mechanisms involved.

371.3 ELECTRICAL MEMBRANE PROPERTIES OF CULTURED FETAL DORSAL ROOT GANGLIA NEURONS FROM NORMAL HUMAN AND DOWN'S SYNDROME. K.Nieminen and S.I.Rapoport (SPON:W.Alberts). NIH, NIA, Lab. of Neuroscience Bethesda, Md 20892.

Down's syndrome (DS) is expressed as an abnormality in the electrical membrane properties (Emp) of cultured human dorsal root ganglia (DRG) neurons from fetal tissue (Scott B.S. et al; Develop. Brain Res. 2, pp. 257-270, 1982). In present study Emp of cultured fetal DRG neurons have been compared between normal and DS (confirmed trisomy 21) with whole cell patch clamp technique. DRG neurons were obtained from abortuses 15 to 19 weeks old. Cells were dissociated with trypsin, and cultured for 5 to 20 days. Electrical recordings were done at 24 C in normal physiological Ringer (Nieminen K. et al.; Biophys. J., 49, 2, p. 170a, 1986). Characteristics of action potentials (AP) were recorded in the current clamp mode. A slow, voltage dependent, Ca-independent K-component was recorded in the voltage clamp mode and by blocking the inward currents with 2uM tetrodotoxin and 2 mM CdCl2. A fast, Ca-dependent K-component was calculated by subtracting the slow outward component from whole currents. Results are summarized as follows :

	unit	control (n=39)		DS (n=29)		p
		mean	sd.	mean	sd.	
Age	days	10.1	4.3	9.1	4.9	ns
Cell diameter	um	23.7	4.7	24.2	5.5	ns
Resting membr.potential	mV	-52.9	3.3	-54.8	3.1	ns
Input resistance	Mohm	184.2	17.2	163.0	18.3	ns
Depolar.spike amplitude	mV	76.9	7.6	85.7	13.2	ns
Afterhyp.spike ampl.	mV	9.0	2.1	13.0	1.4	.001
Max dV/dt of AP	V/s	75.6	19.9	140.3	50.4	.005
Min dV/dt of AP	V/s	-19.0	7.0	-38.8	10.8	.001
Duration (50 % spike)	ms	4.2	0.9	2.2	1.0	.001
Fast K-conductance/area	mS/cm2	1.8	0.7	2.8	0.8	.005
Slow K-conductance/area	mS/cm2	2.69	0.51	1.95	0.67	.005

The slow, voltage dependent K-conductance is increased. The action potentials of DS have accelerated rising and falling phases associated with shorter duration of depolarisation spike. Our records disagree with Scott also in the change of the after hyperpolarisation; it is significantly increased in DS. The initial results indicate that these changes are due to the increased fast K-current in DS's DRG neurons. These findings are from the peripheral neuron, but their excistence in the central nervous system might explain the mental retardation found in DS.

371.4 NEURULATION: A BIOCHEMICAL ANALYSIS OF MICRO-DISSECTED NEUROEPITHELIUM IN NORMAL AND ABNORMAL NEURULATION. T.M.Johnson*,R.G.Higbee*,W.Goossens*,D.G.McLone* and P.A.Knepper*(Spon:R.Berry).Division of Neurosurgery,Children's Memorial Hospital,Northwestern Univ.Med.Ctr.,Chicago, IL 60614.

The cytoskeleton, cell surface glycoconjugates and the extracellular matrix play vital roles in the process of neurulation. Current studies seek the cellular mechanisms which characterize the events of neural tube closure in normal and abnormal neurulation. A genetic model of abnormal neural tube closure, the splotch mouse mutant (Sp/Sp), was studied on gestation days 10,11, and 12 by scanning electron microscopy and correlated with biochemical analysis of micro-dissected neural folds and/or neural tubes.

The Sp/Sp embryos exhibited myeloschisis of the sacral region of varying degrees, primarily as a large defect extending to the caudal cell mass, and approximately one-half of these embryos also had cranioschisis. In order to determine biochemical changes in the neuroepithelium, open and/or closed neural tissue of gestation day 12 Sp/Sp, Sp/+ and C-57 embryos were placed in 2% agar without fixation or in several types of fixatives -- 100% ethanol for 24 hr at 4°C, 4% paraformldehyde for 12 hr, or 2% glutaraldhyde for 2 hr -- to stabilize the tissues for micro-dissection of the neuroepithelium. The dissection technique, as verified by light microscopy, yielded dissected tissues which were >90% neuroepithelium. The neural tissue was solubilized in Laemmli sample buffer for one-dimensional (1D) or lysis buffer for two-dimensional (2D) polyacrylamide gel electrophoresis. The gels were stained with silver (Bio-Rad) or Coomassie blue. Ethanol prepared neural tissue gave the best results on 1D or 2D polyacrylamide gel electrophoresis. The 2D profile of the neuroepithelium of Sp/Sp versus control embryos showed differences in the apparent pI and molecular weights of several proteins. Additional studies are in progress to further characterize these differences by Western blot.

These results indicate that biochemical studies on micro-dissected neuroepithelium are feasible, so that comparisons in the time of appearance, type and amount of proteins can be made on the basis of stage of neurulation or of abnormal neurulation, i.e., myeloschisis or cranioschisis.

371.5 EFFECTS OF ISOTRETINOIN ON NEURAL CREST CELL BEHAVIOUR IN VITRO. L.C. Smith-Thomas, M. Bronner-Fraser and I.Lott*. Developmental Biology Center and Division of Pediatric Neurology, University of California, Irvine 92717

Isotretinoin (13-cis retinoic acid, Accutane) is a Vitamin A derivative that has been used successfully as an anti-acne medication. Recently, this drug has been found to cause severe birth defects in humans whose mothers were exposed to the drug in the early stages of pregnancy. Malformations include cleft palate, mandibular underdevelopment as well as central nervous system and ear defects. Most of the affected structures receive contributions from the cranial neural crest. It therefore seems likely that the teratogenic effects of isotretinoin could be due to disturbances in cranial neural crest migration.

In order to investigate the mechanism of isotretinoin-induced birth defects we have developed an in vitro animal model system. Cranial neural crest explant cultures were grown on fibronectin-coated culture dishes in medium containing 20 ug/ml of isotretinoin. Differences between control and experimental cultures were observed by 1 day after exposure to isotretinoin. Control cultures displayed a confluent morphology with well-flattened cells. This morphology persisted for the duration of the experiment. In contrast many rounded cells were present in isotretinoin treated cultures. Two days after drug addition centrally located cells were poorly attached to the substratum and gaps were often observed between aggregates of cells. 3 to 5 days after addition of isotretinoin, large gaps were observed between aggregates or sheets of cells that were tenuously attached to the substratum. At these times many of the cells in the centrally located aggregates became pigmented. The changes in cell morphology observed in treated cultures suggest that isotretinoin decreases the adhesion of cranial neural crest cells to their substratum.

In order to determine the cell type specificity of isotretinoin we have examined the effects of the drug on other cell types such as neural tube and cardiac fibroblasts. In contrast to neural crest cells both these cell types were relatively unaffected by exposure to isotretinoin. Neural tube explants comprised cells that remained flattened and closely associated with each other in the presence of isotretinoin. Both control and isotretinoin treated cardiac fibroblast cultures comprised confluent outgrowths of cigar shaped cells. We have also examined the effects of trans-retinol on the behaviour of neural crest cells and compared them to those of isotretinoin. The effects of these two retinoids were similar under our experimental conditions.

These results suggest that isotretinoin affects interactions between neural crest cells and their substratum. Therefore, isotretinoin induced birth defects may result from defective neural crest cell-extracellular matrix interactions and consequent abnormal neural crest cell migration during early embryonic development. (Supported by USPHS HD-15527 and March of Dimes Grant 1-896)

371.6 A LACK OF TRANSFERRIN IMMUNOREACTIVITY IN THE CNS OF MYELIN DEFICIENT RATS. J.R. Connor[1,2] K.D. Barron[3] R.E. Fine[4] and C.K.Csiza[5]. [1]Dept. of Physiology, George Washington Univ. and [2]V.A. Medical Center, Washington D.C. 20422, [3] Dept. of Neurology, Albany Med. Coll., NY, [4] Dept. of Biochemistry, Boston Univ., Sch. of Med. Boston MA, and [5] New York State Department of Health, Albany NY.

Transferrin is a 80,000MW glycoprotein whose major function is associated with iron transport and mobilization. Recently, we have shown that transferrin (Tf) is a specific marker for oligodendrocytes in the rat nervous system (Connor & Fine, Brain Res. 368:319, 1986) and is developmentally associated with myelination. Furthermore, Tf enters the brain from the blood through receptor mediated endocytosis.This study examines Tf levels in the CNS of a strain of Wistar rats which have a genetic mutation characterized by myelin deficiency (Csiza and de Lahunta Am. J. Pathol. 95:215-224, 1979). The md rats have ataxia and tremors and are consequently easily distinguished from their normal littermates which serve as controls. In the youngest animals (10 days) the diagnosis of md is made histologically by staining for myelin with toluidine blue on 1 micron sections. No myelin is present in the cervical spinal cord in the md rat. The animals were anesthetized and then perfused with either 4% paraformaldehyde or a formalin-ethanol-acetic acid (FEA) mixture. The tissue was cut on a vibratome (50 um) or a cryostat (8 um) and reacted for immunohistochemistry according to either the PAP method of Sternberger or the ABC Vectastain procedure. In both cases 3,3' diaminobenzidine was the chromogen. A more intense reaction was achieved by using the FEA as a fixative compared to the paraformaldehyde. The antibodies used in this study were anti-Tf (1:1000), anti-Galactocerebroside (1:500, gift of Dr. L. Rome, UCLA) and glial filament acidic protein antiserum (1:1000, gift of Dr. L. Eng, Palo Alto, CA). Beginning at 10 days after birth, both Tf and galactocerebroside (GalC) immunoreactivity were present in oligodendrocytes in the white matter of the spinal cord in the nonaffected animals, but no Tf or GalC staining was present in the md rats. Astrogliosis was observed in the md rat in both the gray and white matter of the spinal cord as early as 10 postnatal days and the intensity of astrogliosis continued until by 30 postnatal days the white matter of the spinal cord and corpus callosum was almost entirely filled with reaction product. Neither Tf nor GalC immunoreactivity was observed in the CNS of the md rats at any age group (10, 20, 30, 40, 50 daysof age). Preliminary results indicate that liver synthesis of Tf in the md rat is normal.The results of this study demonstrate that Tf is not present in the CNS of animals which are myelin deficient. (Supported by NS 22671 and the Veterans Administration).

371.7 ABERRANT GLYCONJUGATE BIOSYNTHESIS DURING DIFFERENTIATION OF DYSTROPHIC AVIAN PECTORAL MUSCLE CELLS IN CULTURE. E.L. Hogan and K.C. Leskawa*. Department of Neurology, The Medical University of South Carolina, Charleston, SC 29464-2232

We have previously reported that the labeling of triosylceramide and the Forssman hapten are transiently increased during chick muscle cell differentiation at the time of myoblast membrane fusion (Hogan, Chien and Leskawa, Soc. Neurosci., 1983). To further explore these observations, we have studied the biosynthesis of four major classes of complex carbohydrates (glycopeptides, glycosaminoglycans (GAGs), neutral glycosphingolipids (GSLs) and gangliosides) during differentiation of normal and dystrophic muscle cells. Primary cultures from chick pectoral muscles were harvested at various times as myoblasts differentiated to multinucleated myotubes, after incubation with 3H-galactose. Lipids were extracted, saponified, isolated by reverse-phase chromatography and separated on columns of DEAE-Sephadex and silicic acid. The protein residue was digested with papain and the GAGs separated from glycopeptides with cetylpyridinium chloride. Recoveries were greater than 92%, determined by adding 3H-lactosylceramide, 3H-GM1 ganglioside or 3H-asialosylfetuin to unlabeled samples.

Glycopeptide (GP) and GAG biosynthesis increased steadily during normal myoblast differentiation. With dystrophic muscle cells, however, GP and GAG labeling was significantly elevated in the single cell and myotube phases (several times greater than normal values), but decreased to near-normal values during cell contact and membrane fusion.

With normal muscle cultures, biosynthesis of total GSLs increased significantly during the periods of cell contact and initial membrane fusion. Both ganglioside and neutral GSL labeling increased and then decreased so that myotube values were similar to myoblasts. Ganglioside labeling was higher than neutral GSL labeling at all phases, but the reverse was true for dystrophic cells and ganglioside labeling did not vary during myogenesis. During initial membrane fusion, neutral GSL biosynthesis by dystrophic cells increased dramatically, to levels 9 times greater than normal values. Unlike normal cultures, labeling of dystrophic neutral GSLs did not decrease after formation of myotubes but remained greatly elevated.

This increased glycosylation of macromolecules by dystrophic muscle cells was despite decreased intracellular pools of monosaccharide (60% of normal cultures). No differences were seen in intracelluar pools of 3H-leucine or 3H-thymidine.

These results support our hypothesis of a role for membrane GSLs, including gangliosides, in muscle differentiation and suggest a defect in the control of glycosylation in muscular dystrophy.

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371.8 DEVELOPMENTAL EXPRESSION OF THE HYPERTROPHIED LOCUS COERULEUS TERMINAL ARBOR IN THE MUTANT MOUSE TOTTERING. Eric Phillips* and Pat Levitt. (Spon: H. Pearson) Dept. of Anatomy, Medical College of Penn., Philadelphia, PA 19129.

Tottering (tg/tg) is an autosomal recessive neurological mutation in mice that results in a late postnatal (3-4 weeks) expression of spontaneous focal motor and spike-wave absence seizures. The locus coeruleus noradrenergic neurons hyperinnervate their target regions, and have been shown previously to be responsible in part for the expressed epilepsy. In this study, the developmental time course of expression of the hyperinnervation was investigated immunocytochemically to define how the onset of the abnormal terminal arbor relates to the delayed postnatal expression of the epilepsy. TG/tg and wild type (+/+) mice, birth through postnatal day (P) 35 were prepared for tyrosine hydroxylase immunocytochemistry. Terminal arbors were analyzed in the hippocampus and cerebellum at 7 different ages quantitatively by determining the fraction area occupied by TH-immunoreactive fibers. At birth, few fibers were present in either terminal region and no difference in the axonal arbors were detected. By P5, however, the number of stained axons increased dramatically in both normal and mutant mice. At this stage, clear distinctions between groups were evident in the number of immunoreactive axons, approximately 30% greater in the tg/tg mouse. By P14, arbors both in the hippocampus and cerebellar cortex of tg/tg mice were double of those stained in +/+ mice. This ratio remained constant, with the extent of innervation reaching adult levels both in normal and mutant mice between 2-3 weeks postnatally, well before the onset of the epilepsy. The data indicate that the tg gene defect results in a developmentally anomolous growth of LC noradrenergic axons and this event is not linked temporally to the onset of the pathophysiology. This suggests that other factors, possibly postsynaptic receptor maturation, must occur in addition to the hypertrophic axon growth to reach the spontaneous epileptic state in the adolescent mouse.

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371.9 WATER MAZE LEARNING DEFICITS IN THE TWITCHER HETEROZYGOTE Charles E. Olmstead, UCLA Mental Retardation Research Group, Lanterman Developmental Center, Pomona, CA 91769

Individuals heterozygous for the recessively transmitted Globoid cell leukodystrophy (GCL) have been reported to perform poorly on subtests of intelligence scales. Notably Christomanou et. al. (Human Genetics (1981)58) have reported impaired spatial cognition in obligate heterozygotes for GCL disease. We have recently (Soc. Neurosci. Abst.(1985)11) developed evidence that the murine mutant "twitcher" (C57Bl/6J-twi) is, in addition to being an enzymically correct model for the galactosylceramidase deficiency (Suzuki and Suzuki,Am. J. Pathol.(1984)111), also an excellent tool for studying heterozygote risk for subtle neurological disease.

We have used several variations on the Morris water maze (Learn Motiv. (1981)12) to investigate the spatial abilities of normal (+/+) and heterozygous (twi/+) female mice. Genotype was determined by microassay of galactosylceramidase in 0.5cm segments of clipped tail. Animals of both genotypes readily learned the standard task as evidenced by decreased latencies in reaching the submerged platform and the shorter paths taken. Initial learning reached stable latencies and errors by the 8th 5-trial day. Changing the position of the platform in the field or the relative salience of distal and proximal clues had significantly more effect on the heterozyotes than the normal. Not only did they show increased errors and latencies in reaching the platform, but became increasingly more variable and unstable following successive reversals of platform position. When platform color and/or the degree of opacity of the water was changed the heterozygotes were seen to perform best when the proximal cues were most salient (i.e. Clear water and colored platform), but trial to trial variability was still higher than in the normal littermates. Finally a variation of the Whishaw "learning-set" paradigm (Physiol. Behav.(1985)35) required the animals to learn a new spatial location of the platform every 2 trials for 5 positions per day. Heterozygotes had significantly more errors and longer latencies than the normals across the first five days. After that the two groups were not distinguishable on the basis of error and lantency. However, there were differences. The normal animals adopted a position preference for 1st trial responding that readily changed once the new location was determined. This was shown by decreases in previous and preferred sector errors on the 2nd trial. Heterozygotes perseverated on the position preference on both the 1st and 2nd trials. They did, however, usually show shorter 2nd trial latencies. These data are further evidence supporting the utility of the "twitcher" model for heterozygote risk and demonstrate that the Whishaw method is sensitive to the different stratagies adopted by animals when solving the the Morris water maze.

371.10 METHYLMERCURY-INDUCED MOVEMENT AND POSTURAL DISORDERS IN THE DEVELOPING RAT: REGIONAL ANALYSIS OF AROMATIC AMINE SYSTEMS. J.R. O'Kusky, B.E. Boyes* and E.G. McGeer. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The toxicity of methylmercury (MeHg) in the human nervous system during prenatal and early postnatal development has been associated with neurological disorders resembling cerebral palsy. Clinical signs in affected infants and children include psychomotor retardation, persistence of primitive reflexes, spasticity, ataxia, choreoathetoid movements, myoclonus and generalized tonic convulsions. Similar signs of neurological impairment have been reported in the developing rat following the postnatal administration of MeHg (O'Kusky, J.R. & E.G. McGeer, Develop. Brain Res., 21: 299, 1985). The present study was conducted to determine changes in tissue concentrations of the catecholamines, serotonin and their metabolites in the spinal cord, neostriatum and cerebral cortex of MeHg-treated animals.

Sprague-Dawley rat pups received subcutaneous injections of methylmercuric chloride in physiological saline (5 mg Hg/kg) at 24-hour intervals beginning on postnatal day 5 and continuing until the onset of neurological impairment (day 21-25). Normal and weight-matched controls were injected with equivalent volumes of saline. The concentrations of norepinephrine (NE), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by electrochemical detection after separation by HPLC.

Levels of NE in MeHg-treated animals were significantly increased in the spinal cord (51-53%) and neostriatum (142-161%), but in the cerebral cortex they did not differ significantly from normal or weight-matched controls. Tissue levels of 5-HT were significantly increased in the spinal cord (24-50%), but not in the neostriatum or cerebral cortex. However, the ratio of 5-HIAA/5-HT in the neostriatum was significantly increased (41-49%), suggesting increased turnover of 5-HT in this region in MeHg-treated animals. Tissue levels of DA did not change significantly in the neostriatum and they were below the limit of detection in the spinal cord and cerebral cortex. However, the ratio of (DOPAC + HVA)/DA decreased significantly in the neostriatum (68-70%), suggesting decreased turnover of DA in the MeHg-treated animals. Ongoing morphological studies indicate that these results may be secondary to a lesion of GABAergic interneurons throughout the cerebral cortex and a disinhibition of cortical efferents to the brainstem.

(Supported by the United Cerebral Palsy Research and Educational Foundation and the British Columbia Health Care Research Foundation).

371.11 METHYLMERCURY POISONING OF THE DEVELOPING NERVOUS SYSTEM: EFFECTS ON SOMATOSTATIN IMMUNOREACTIVITY IN CEREBRAL CORTEX AND STRIATUM. J.M. Radke*, J.R. O'Kusky and S.R. Vincent (SPON: J. Weinberg). Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The toxicity of methylmercury (MeHg) in the central nervous system of the developing rat has been shown to result in a relatively selective degeneration of GABAergic neurons in the cerebral cortex and striatum. In view of the reported co-localization of GAD- and neuropeptide-immunoreactivities (SRIF, NPY) in some nonpyramidal neurons of the cerebral cortex (Hendry et al., Proc. Natl. Acad. Sci., 81: 6526, 1984), it appears likely that such coexistence defines subpopulations of GABAergic neurons. The present study was conducted to document changes in tissue levels of somatostatin (SRIF) in MeHg-treated animals to determine the extent to which SRIF-containing neurons are involved in these neurotoxic lesions.

Sprague-Dawley rat pups received subcutaneous injections of methylmercuric chloride (5 mg Hg/kg) at 24-hour intervals beginning on postnatal day 5 and continuing until one of three stages of MeHg toxicity, defined as follows: Stage I (day 18) when MeHg-treated rats continue to gain weight although less rapidly than normal controls, Stage II (day 20-22) when a given rat exhibited a loss of body weight, and Stage III (day 22-25) at the onset of neurological impairment. Normal and weight-matched controls were injected with equivalent volumes of saline. Tissue levels of SRIF were measured in the cerebral cortex (occipital region) and striatum by radioimmunoassay.

In the cerebral cortex levels of SRIF in MeHg-treated animals did not differ significantly from normal or weight-matched controls at any stage of the experiment. In the striatum there was a significant decrease in SRIF at Stage II (49-54%) and Stage III (55-57%). Immunohistochemistry of SRIF-positive neurons in MeHg-treated animals at Stage III revealed a marked cell loss along the dorsolateral margin of the striatum. Surviving neurons were atrophic, exhibiting a reduction in cell size and in complexity of dendritic arborization. In the cerebral cortex of MeHg-treated animals the morphology and distribution of SRIF-positive neurons appeared normal.

These results demonstrate reduced tissue levels of SRIF and a degeneration of SRIF-positive neurons in the striatum following exposure to MeHg. In the cerebral cortex the subpopulation of GABAergic neurons, which are presumed to be co-localized with SRIF, appear to be spared.

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371.12 MATERNAL NEUROBEHAVIORAL TOXICITY OF AN ACUTE ETHANOL DOSE DURING PREGNANCY IS GREATER IN MICE SHOWING TERATOGENIC EFFECTS. R. Haddad, R. M. Dumas* and G. S. Fisch*. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

A single large dose of ethanol (5.8 g/kg) was given to C57Bl/6J mice by gavage on gestation day (GD) 9 (vaginal plug = GD 1). Following treatment, food consumption was measured daily until sacrifice. An individually matched set of pair fed mice (nutritional control) were gavaged with an equivalent dose of an isocaloric dextrose solution on GD 9, given access to comparably limited amounts of food for the following 24 h, and then fed ad lib. Untreated control mice formed a third group. All mice were sacrificed on GD 19. The ethanol treated mice had significantly more litters containing malformed fetuses than both the pair fed (p less than .02) and the untreated control mice (p less than .001). The control groups did not differ significantly from one another. However, no malformations were detected in 8 of the 31 litters in the ethanol treated group. Possibly this subgroup had more food in their stomachs at the time of treatment. This would have reduced the rate of absorption of the ethanol and resulted in a lower peak blood ethanol concentration.

In an attempt to reduce the variability among litters, a further group of 18 pregnant mice were food and water deprived for 4 h prior to treatment on GD 9. (In this group we also restricted all matings to a one h period to enhance the uniformity of gestational age at the time of treatment).

All the mice were comatose after treatment but differed noticeably in their "sleeping time". When sacrificed on GD 19, 12 litters were found to contain malformed fetuses, but no anomalies were observed in 6 of the 18 litters. Comparison of the "sleeping times" of the six dams with litters that appeared normal with "sleeping times" of the 12 dams that had litters in which malformations were evident, showed a significantly longer "sleeping time" (Mann-Whitney test, p less than .01) for the affected group.

The longer "sleeping time" of the mice that were later found to have malformed fetuses suggests that both may have resulted from a greater transport of ethanol to the brain and to the fetuses. Whether this was a consequence of a higher blood ethanol concentration in the more affected mice or of a difference in diffusion rates from the blood into other tissues caused by differences in blood composition at the time of treatment (e.g., lipid content) remains to be investigated.

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371.13 PRENATAL CAFFEINE CAUSES LONG LASTING BEHAVIOURAL AND NEUROCHEMICAL CHANGES IN THE OFFSPRING. B. Frieder and V.E. Grimm*. Center for Neurosciences and Behavioural Research, The Weizmann Institute of Science, Rehovot, ISRAEL 76100

The effects of prenatal caffeine on later development, behaviour and brain neurochemistry were studied. Different doses of caffeine (25, 50 and 75 mg/kg) were give to rat dams during the last week of pregnancy. Prenatal caffeine resulted in a number of behavioural and neurochemical changes in the offspring which were long lasting and dose related. The low dose of prenatal caffeine caused hyperactivity (measured in an open field). The high dose of caffeine caused learning disabilities in complex visual or auditory discrimination learning paradigms but not in simple motor learning or in a spacial orientation task. Both male and female offspring showed some behavioural effects of caffeine exposure. The neurochemical studies carried out on adult animals revealed an increase in protein concentration in the cortex and hippocampus of the animals treated with the highest dose of caffeine and an increase in choline uptake in the hippocampus of animals treated with the lower doses.

371.14 HYPERACTIVITY DUE TO NEONATAL VIRUS INFECTION IN MICE: SITES OF INFECTION IN THE BRAIN. L.S. Crnic and L.I. Pizer* Dept. of Pediatrics, Psychiatry and Microbiology, Univ. of Colo. Sch. of Med., Denver, CO 80262.

We report here three experiments in which newborn mice were injected subcutaneously with a host range mutant herpes simplex type 1 virus. This mutant had been selected to grow on primate but not mouse cells in vitro and so produced a mild infection. Control mice were injected with a killed virus preparation. In the first study, mice were given 10^6 plaque forming units (PFU) of virus on the day of birth. Whole litters were sacrificed at 5, 10, 15, and 20 days of age for determination of brain virus titers. The percent of mice with detectable virus in their brains was lowest at 5 days of age, peaked at 10 days of age (which was also the time of peak mortality) and declined thereafter. Virus titers followed the same pattern. Microscopic examination of the brains of animals in a subsequent experiment showed that the decline in percentage of infected brains at the later ages was due to resolution of the infection. Mice sacrificed at 20 days were tested in an open field and their activity was higher than that of control mice ($p < .05$).

The effect of age of infection was determined by giving mice 5×10^6 PFU on days 1, 2, 3, 4 or 5 of life. Hyperactivity resulted from infection on days 1-4. Mortality due to the virus was lowered by injection after day 1. Activity of mice infected on days 1-4 was significantly different from controls at $p < .01$.

In a third study, mice were shown to be hyperactive when housed for 24 hours in a Digiscan apparatus as well as in the open field, showing that the hyperactivity is not just a reaction to the novel environment of the open field. Brains taken from 33 mice at 8, 10, 15, 20 and 60 days of age were stained for HSV 1 or with Nissl stain. The virus travels up the spinal cord from the peripheral injection site. It infects both the vermal cortex and lateral hemispheres of the cerebellum. It is found in the deep cerebellar nuclei and vestibular nuclei and in the cerebellar peduncles. It reaches the superior and inferior colliculi, lateral nuclei of the thalamus, and in some cases the striatum. The infection destroys cells in these areas and slows the migration of granule cells in the cerebellum. While the areas infected are not the principal areas controlling activity, they are areas known to have modulatory effects upon activity.

371.15 ULTRADIAN PERIODICITIES IN CARDIAC AND RESPIRATORY RATE AND VARIABILITY IN SIDS VICTIMS. R.M. Harper, V.L. Schechtman, K.A. Kluge*, A.J. Wilson*, and D.P. Southall*. Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024; Dept. of Medical Physics, University of Sheffield, Sheffield, U.K.; and Cardiothoracic Institute, Brompton Hospital, London, U.K.

Siblings of victims of the sudden infant death syndrome (SIDS) manifest disordered patterns of periodic sleep state organization (Harper et al., Science, 213,1030, 1981). These disturbances in state patterns should be reflected in the cyclic organization of physiological variables constituting sleep states, including periodicity in heart and respiratory rate and variability. We examined, using spectral techniques, the cyclic organization of low-frequency ultradian variation of cardiac and respiratory parameters in control infants and in infants who subsequently died of SIDS.

Twenty-four hour recordings of ECG and respiratory movements were obtained from 7000 infants. Of these, 20 recordings from infants aged 2-65 days who succumbed to SIDS and 55 recordings of age-matched infants who survived were submitted to analysis. Twelve-hour nighttime (7pm-7am) portions of minute-by-minute values of cardiac and respiratory median rate and variation (interquartile range) were submitted to low-pass digital filtering to remove high-frequency artifacts, subtraction of the mean, and tapering. The data were then subjected to spectral decomposition using the fast Fourier transform. Summed log spectral values representing activity at .12-.35 cycles/hr ("feeding" rhythm) and .7-1.8 cycles/hr ("sleep" rhythm) were submitted to ANOVA. In addition, the frequency of the highest peak below .59 cycles/hr was compared between groups.

Periodic components were observed at low frequencies (approximately 3-hr rhythm), and at periods approximating the sleep-waking rhythm (.5-1.5 cycles/hr). No significant differences were found between SIDS victims and controls on the spectral power measures or on assessment of the peak low-band frequency, even after covarying for age.

We conclude that, if any disturbance in periodic organization at these bands exists in infants who later succumb to SIDS, that disturbance may not be reflected as a simple difference in power. These findings do not exclude the possibility that momentary phase disturbances may mask the overall spectral results, or that very-high-frequency (>2 cycles/hr) spectral estimates may differentiate the groups.

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371.16 LONG-TERM ALTERATIONS IN DOPAMINE RECEPTOR SENSITIVITY IN SOCIALLY SEPARATED RHESUS MONKEYS. M.H. Lewis, A. Beauchamp*, R.B. Mailman and J.P. Gluck*. Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27514 and Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Social separation can have important neurobiological consequences for the developing organism. Long-lasting effects on behavior have been observed following prolonged social separation of certain species of non-human primates during early development. While little is known about the neurobiological sequelae of such differential early social experience, perturbations of central dopamine pathways appear to be involved. The present study tested the hypothesis that early social separation results in long-term alterations in dopamine receptor sensitivity. Rhesus macaques (15 to 20 years old), which had been socially isolated during all or most of their first year of life, were challenged with the direct acting dopamine agonist, apomorphine. Alterations in the frequency of occurrence of spontaneous eye-blinking, stereotyped behavior (SB) and self-injurious behavior (SIB) were measured in socially separated monkeys and age-matched, socially-reared control animals using a modified time-sampling technique.

Following accumulation of baseline data, socially separated and control animals were administered either vehicle (0.1% ascorbic acid), or apomorphine (0.1 or 0.3 mg/kg I.M.). Socially separated monkeys exhibited slightly higher blink rates during baseline than observed in control animals, and substantially greater, dose dependent increases in blink rate following apomorphine administration. Dose-dependent increases in SIB were also observed in socially separated monkeys, whereas little or no SIB was observed in control monkeys in any of the test conditions.

Baseline levels of SB were found to be significantly higher in socially separated monkeys. Socially reared controls demonstrated a dose dependent increase in SB following apomorphine administration. Similarly, socially separated monkeys exhibited a significant increase in SB following 0.3 mg/kg apomorphine. However, these animals exhibited below baseline levels of SB following 0.1 mg/kg apomorphine. The two drug doses also resulted in markedly different behavioral topographies, with whole body stereotypies (rocking, swaying, pacing, etc.) being observed at the higher dose and largely oral stereotypies (chewing, lip smacking, etc.) at the lower dose. These data provide evidence for dopamine receptor supersensitivity and support the idea that early prolonged social separation results in long-term alterations in dopamine activity.

371.17 VISUAL PROSTHESES: RELEVANCE OF STIMULI WITH RESPECT TO SPATIAL-MOTOR BEHAVIOR IN INFANT MACAQUES. B.J. Sonnier, E.R. Strelow*, D.H. Warren*, and A.H. Riesen. Dept. of Psychology, Univ. of California, Riverside, CA 92521

We are examining infant stumptail macaques (M. Arctoides)reared blind from birth to three months with electronic sonar sensory substitution devices to assess correlations between development of spatial-motor behavior and development of auditory, motor, and visual areas of the cortex. Behavioral and cortical development after prolonged early use of a sensory prosthesis could be of considerable importance to their eventual use by humans, and brain-behavior relationships are difficult, if not impossible, to assess in human infants.

Groups of animals are reared as follows: 1) blind infants with the Trisensor Aid (TSA) in continuous use, blind control groups with 2) silent, dummy versions of the TSA or 3) sound-making, but non-functional versions of the TSA, and 4) sighted, colony-reared animals. Cage observations and tests of spatial-motor behavior are made throughout the three-month period, then animals are sacrificed and cortical development is examined, using Golgi-Cox and computer (Bioquant II) techniques. We now have preliminary behavioral results from group 3 to compare with those reported for group 1 at Neuroscience last year, and neuroanatomical analyses are in progress.

Relevance of sound stimuli of the TSA with respect to spatial-motor behavior appears to be extremely important. The sound-making, but non-functional aspects of the device worn by group 3 animals certainly does not promote development of normal spatial-motor behaviors, and may even interfere, although group 2 animals will have to be examined to determine this with any certainty. Most of the infant's time in group 3 is spent clinging to the surrogate, and not much exploration of the home cage occurs. Self-clutching, rarely observed in group 1 and 4 infants, is evident. Finding the surrogate when moved to a different location, a fairly easy task for the group 1 animals, is extremely difficult in group 3.

The general lack of locomotor activity in the group 3 infant indicates that some reduction in cortical motor areas will occur with respect to neuronal development, but the constant input of sound stimulus may prevent this in primary auditory cortex (Area 41). This did not occur in group 1 in either primary auditory or motor areas in comparison to group 4 animals, in fact, some increases in cell size were evident in primary auditory areas in group 1.

Neuronal changes do occur in conjunction with TSA use, but the behavior of group 1 animals compared to those in group 3 indicate that the relevance of the stimulation with respect to spatial-motor behavior is of utmost importance to the sensory prosthesis user.

371.18 SEROTONIN "AGONISTS" IN AN ANIMAL MODEL OF ATTENTION DEFICIT DISORDER (ADD). J.T.Concannon, Dept. of Physiology/Pharmacology, Southeastern College of Osteopathic Medicine, North Miami Beach, FL 33162.

Although the dopamine (DA)-depleted developing rat animal model of ADD has been well-investigated, considerable controversy still exists concerning the ability of psychostimulants and other drugs to decrease overactivity and increase learning ability in this model. Interestingly, a recent report (Heffner and Seiden, Brain Res.,1982,244:81) has shown that the hyperactivity-reducing effect of d-amphetamine was blocked by a serotonin (5-HT) receptor blocker, but not by a putative DA receptor blocker. Hence, 5-HT may in part mediate amphetamine's effects on locomotor activity in this model, although the role of purported 5-HT agonists in reversing attentional/learning deficits characteristic of ADD has yet to be examined.

In the attempt to replicate and expand these findings, I utilized intracisternal injections of 6-hydroxydopamine (6-OHDA) or its vehicle in 5-day-old rat pups after desmethylimipramine pretreatment and examined locomotor activity, passive-avoidance and T-maze learning during development. Time-sampled behavioral activity was measured at 15,20,25,and 30 days of age, using a repeated measure design, after an intraperitoneal injection of either saline, d-amphetamine (0.5 mg/Kg), or the purported 5-HT agonists fenfluramine (3.0,6.0 or 9.0 mg/Kg), quipazine (1.0,3.0,or 6.0 mg/Kg), or amitryptiline (1.0 or 3.0 mg/Kg). Animals in these same groups then received shock-motivated passive-avoidance (at 31 days of age) and T-maze learning (33 days of age).

In line with my previous research, 6-OHDA-induced hyperactivity occurred in saline-injected animals at 20 days of age, but was not detected thereafter (i.e., at 25 or 30 days of age). Only d-amphetamine affected (i.e., increased) activity in vehicle-treated rats at 20 days of age, while both d-amphetamine and fenfluramine increased activity in the 6-OHDA-treated, hyperactive rats. Furthermore, neither d-amphetamine nor any dose of fenfluramine, quipazine, or amitryptiline returned 6-OHDA-induced hyperactivity to the control level, suggesting neither a calming nor a "paradoxical" calming effect of any of these agents. The only 6-OHDA-induced learning deficit was seen in the T-maze for a subset of animals trained at 23 days of age, and it was not reversed by amphetamine (0.5 mg/Kg) or fenfluramine (9.0 mg/Kg). The results suggest that the putative 5-HT agonists are clinically useless in the 6-OHDA, DA-depleted animal model of ADD, since they do not decrease hyperactivity or increase learning/attention, replicating in principle a recent study at NIH using fenfluramine in ADD children (Rapoport et al, Psychopharmacol. Bull., 1985, 21: 232.).

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371.19 LATERALIZATION AND VARIABILITY OF CORTICAL ACTIVITY: EVENT-RELATED POTENTIAL INDICANTS OF READING ABILITY IN BOYS. M. R. Harter, Dept. Psych., Univ. N. C., Greensboro, N.C. 27412

Event-related potential (ERP) indicants of Reading Disability (RD) and Attention Deficit Disorder (ADD) were investigated. Fifty-two 8-12 year old boys constituted four groups: 25 children without RD (divided into two groups--17 without and 8 with ADD); and 27 children with RD (divided into two groups--11 without and 16 with ADD). There were three tasks ("games") designed to assess simple sensory discrimination (black vs white) and two levels of letter discrimination (2 letters vs 2 patterns and 12 letters vs 12 patterns). ERPs recorded over the left and right occipital, central and frontal regions were measured for 1000 msec following each type of stimulus. Various controls insured that group differences in ERPs could not be attributed to age, intelligence, performance on the tasks, or eye or body movement.

The amplitude of a central-frontal positive component peaking at about 240 msec (P240) differentiated the boys with vs without RD. Boys with RD had smaller amplitude P240 over the left central hemisphere. This component was approximately the same amplitude over both hemispheres for boys with RD but was greater over the left than right hemisphere for normal readers. The reduced left central P240 in boys with RD was similar for all tasks, indicating it was not specific to reading per se. It was interpreted as reflecting a left central hemisphere neural deficit in how incoming sensory information is related to memory.

The within subject variability (standard devidation) of the individual samples, which were averaged for each ERP, also was measured for 1000 msec following each type of stimulus. This variability was smaller for children with RD as compared to without RD. In contrast, to the ERP amplitude differences, this group difference 1) was not restricted to any one ERP component but was evident for the entire ERP waveform (although it was greatest at about 330 msec after stimulation), and 2) was not restricted to anyone electrode location, e.g. left central. The reduced ERP variability in RD children eliminates increased variability as a possible explanation of the reduced ERP amplitude in these children. The increased variability in children without RD may reflect a decrease time locking of cortical activity with incoming sensory information. This could be due to the effects of endogenous cortical processes not directly under sensory control, those associated with memory and the interpretation of sensory information.

371.20 PET-SCAN STUDIES DURING READING IN DYSLEXIC AND NON-DYSLEXIC ADULTS. K. Gross-Glenn*, R. Duara, F. Yoshii*, W.W. Barker*, J.Y. Chang*, A. Apicella*, T. Boothe* and H.A. Lubs (SPON: L. Potter). Univ. of Miami School of Medicine, Miami, FL 33101 and Mt. Sinai Medical Ctr., Miami Beach, FL 33141.

We have used positron emission tomography (PET) as a means of studying regional cerebral glucose metabolism (rCMRglc) during continuous oral word-reading in groups of age-matched adults with and without a childhood history of developmental reading disabilities. Earlier behavioral studies of reading have suggested that adults with childhood histories of familial developmental dyslexia use different reading strategies than do adults without such history (Gross-Glenn, et al., 1985).

Three groups of subjects were studied, namely 4 dyslexic subjects and 6 age-matched non-dyslexic subjects who were studied while reading words and 5 age-matched non-dyslexic subjects who were studied while viewing colored pictures (non-verbal visual task). Global CMRglc values in mg/100g/min in these three groups were 7.17 $\pm$ 2.4, 6.69 $\pm$ 2.2 and 8.87 $\pm$ 0.9, respectively.

When normalized values of rCMRglc were compared among the three groups using ANOVA, significant effects were found only in the right and left insular cortices. For the non-dyslexic readers, dyslexic readers and subjects viewing pictures respectively, the values were 1.17, 1.06 and 1.03 in the right insula and 1.16, 1.06 and 1.05 in the left insula. The metabolism in insular cortex was bilaterally increased in non-dyslexic readers compared to the other two groups ($p < 0.05$). These results suggest that the insular cortex is important in normal reading and may be functionally abnormal in dyslexics.

Preliminary data on 6 normal readers and 4 dyslexics suggest that word-reading strategy may be reflected in a more asymmetric activation pattern of rCMRglc among dyslexics than normal readers. Laterality indices [100 X (right - left) / (right + left) /2] were calculated for regions of rCMRglc that were observed to be most activated by the reading task in normals. The absolute (i.e., unsigned) laterality index during reading was significantly greater for dyslexic than non-dyslexic subjects in the region of the anterior superior temporal cortex ($t = 4.36$, 8 df, $p = .002$). Whereas normals showed nearly equal bilateral activation of this region, dyslexics showed more asymmetry, some with greater left, others greater right superior temporal activation. The observed temporal lobe metabolic asymmetries during reading correspond with post-mortem studies that show greater than normal anatomical asymmetries in area tPt for dyslexic brains (Geschwind and Galaburda, 1985).

VISUAL CORTEX V

372.1 PROJECTION OF THALAMIC NUCLEUS CENTRALIS TO PRIMARY VISUAL CORTEX IN THE CAT. S. LeVay and E.T. Cunningham, Jr. Salk Institute, P.O. Box 85800, San Diego, CA 92138.

The nucleus centralis (NC), comprised of centralis medialis (CM), paracentralis (PC) and centralis lateralis (CL), is a major component of the thalamic intralaminar complex. It projects widely to striatum and neocortex, and is thought to be involved in the control of arousal. In the cat, the existence of a projection to area 17 has been denied in several studies. We re-examined this question with retrograde and anterograde tracers, at light- and electron-microscopic (EM) levels.

Large injections of HRP into area 17 (with spread to 18) led to heavy retrograde labeling throughout NC, with almost half of its neurons being labeled. Following an injection of (^{3}H)proline into CM we observed anterograde labeling of layers 5 and 6 in areas 17 and 18. The labeling was fairly sparse in both areas. Injections of WGA-HRP that covered CM, PC and CL led to similar anterograde labeling of cortical layers 5 and 6, and also to a weak additional band in the outermost part of layer 1. The pathway from NC to visual cortex was circuitous: fibers first traveled forward in the anterior limb of the internal capsule, then backward in the cingulum bundle. There was no retrograde labeling of cells in area 17 or 18, indicating that the projection is non-reciprocal.

WGA-HRP-labeled terminals in area 17 were examined in the EM. In all three layers of termination (1, 5 and 6) they contained 50 nm clear vesicles and formed type 1 (asymmetric) synapses on dendritic spines. These spines generally contained an elaborate spine apparatus and, based on their laminar locations, probably belonged to pyramidal cells.

We conclude that NC does project to area 17. The laminar organization suggests that NC primarily influences corticofugal rather than corticocortical systems. The type 1 synaptic morphology is consistent with evidence that NC neurons use an excitatory amino acid transmitter (Ottersen et al., Neurosci. Lett. 42: 19). The results offer a possible connection between the sleep/wake modulation of tonic activity in NC (Glenn and Steriade, J. Neurosci. 2: 1387) and the enhanced visual responsiveness of neurons in the deep layers of area 17 during wakefulness (Livingstone and Hubel, Nature 291: 554).

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372.2 A COMPARISON OF SUBCORTICAL AFFERENTS TO VISUAL CORTICAL AREAS V4, TEO AND IT IN THE MACAQUE. S.B. Fenstemaker*, A.M. Sauceda*, T.D. Albright, and C.G. Gross (SPON: J.A. Movshon). Department of Psychology, Princeton University, Princeton, NJ 08544

We have previously reported that macaque visual cortical areas V4, TEO and IT can be distinguished on the bases of cortical connections, visuotopic organization, receptive field size, and stimulus preferences (Fenstemaker et al., ARVO 25:213, 1984; Neurosci. Abs. 11:1012, 1985). Here we describe the subcortical afferents to these areas as revealed by paired injections of distinguishable retrograde fluorescent tracers. Injections were placed in dorsal and ventral parts of V4, TEO or IT in each of 11 hemispheres. The portion of the visual field represented at the injection site in V4 was determined by concurrent electrophysiologcial recording. Following all three groups of injections, subcortical labeling was restricted to the pulvinar, the claustrum, and the amygdala.

In the pulvinar, V4 and TEO injections labeled cells in the inferior and lateral divisions, while IT injections labeled cells in only the most medial and caudal parts of the lateral and medial pulvinar. Comparison of the locations of labeled cells with a standard map of the pulvinar suggested a topographic projection to V4, a crudely organized projection to TEO, and a diffuse projection to IT.

The ventral claustrum contained labeled cells following all injections. V4 injections produced patches of label which suggested the presence of a coarsely visuotopic projection from the caudal 5mm of the claustrum, but no topographic order more rostrally. Injections into TEO and IT labeled cells throughout the same extent of the claustrum, but the pattern of labeling seen caudally suggested little organization in the projections to TEO, and none to IT.

In the amygdala, all injections produced labeled cells in the lateral, basolateral and basal accessory nuclei. IT injections also labeled a few cells in the cortical nucleus and produced the heaviest amygdaloid labeling overall. No amygdalo-cortical projection was topographically organized.

In summary, V4, TEO and IT receive projections from the same subcortical structures, but the fine patterns of projections from these structures vary with the cortical target. Projections from the pulvinar and claustrum to V4 showed evidence of topographic order while those to IT did not. In accord with our previous results showing that TEO is intermediate between V4 and IT in degree of retinotopic organization, the present results indicate that subcortical projections to TEO have an intermediate degree of topographic organization.

372.3 ANISOTROPIC REPRESENTATION OF THE VISUAL FIELD IN V1 AND V2 IN THE CEBUS. A.P.B. Sousa*, M.G.P. Rosa* and R. Gattass* (SPON: R. Desimone). Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, 21941, Brasil.

The visual topographies of V1 and V2 were studied in 10 New World monkeys, Cebus apella, anesthetised and paralysed, in repeated recording sessions (Gattass et al. Soc. Neurosci. Abstr., 10: 474, 1984). These results were shown to be similar to those described for the Macaca (Gattass et al. J.Comp. Neurol., 201: 519, 1981). In both areas the representation of the lower visual field is larger than that of the upper field. Both in V1 and V2 receptive-field size increases with increasing eccentricity. However, both the slope and the "y" intercept for the function relating receptive-field size with eccentricity in V2 are larger than those obtained for V1.

For the calculation of the magnification factors the cortical distances were measured directly on 3-D models (7.5x) reconstructed from either coronal or parasagittal sections. The analysis of the magnification factor of these areas in different portions and along different dimensions of the visual map revealed that the representation of the visual field in both areas is anisotropic. No difference was found in the magnification of corresponding regions of the upper and lower visual field in both areas. However, the magnification along the isopolar dimension was different from that along the isoeccentric dimension in the cortical map. In the peripheral representation of V1 the magnification along the isopolar dimension is larger than that along the isoeccentric one. In V2 this difference is present throughout the area and is even larger than that observed in V1. The cortical point image size (PIS), estimated by multiplying receptive-field size by cortical magnification factor at each eccentricity, decreases with increasing eccentricity, in both areas; however, for a given eccentricity PIS in V2 is larger than in V1. In peripheral V1 and in V2 PIS calculated along the isopolar dimension is larger than that calculated along the isoeccentric one.

The anisotropy observed in V1 may suggest the presence of ocular dominance columns in the Cebus, similar to those described in the Macaca. In V2 this anisotropy may be related to the orientation of the cytochrome oxidase (CO) bands found in this area (umpublished observations). The PIS in the isopolar dimension is compatible with the dimension of a full cycle of CO-bands.

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372.4 VISUOTOPOGRAPHY OF THE OPOSSUM'S STRIATE CORTEX: CORRELATION WITH THE DISTRIBUTION OF GANGLION CELLS AND THE EFFECT OF COMMISSUROTOMY. E. Volchan*, R.F. Bernardes*, L. Gleiser*, L.G. Gawryszewski*, C.E. Rocha-Miranda* (SPON: L.R.G. Britto). Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, RJ, Brazil, 21941.

In primates the projection of the fovea establishes a correspondence between the retina and the visual field. Since the opossum (Didelphis aurita), like other nonprimate mammals, does not have a fovea we have sought to determine reference axes which might prove to be of methodological and functional significance. We chose the axes of binocular coincidence (ABC), which were based on the disparieties between multunit response fields recorded in striate cortex. The ABC forms an angle of 29.1 degrees (mean value) with the projection of the optic disk at the horizontal plane. "Vertical reference meridians" orthogonal to the ABC were defined. Based on these references we studied the visuotopography along the horizontal meridian for each eye in the striate cortex. We found a representation for center/border of the response fields for the opposite and the same eye, respectively, of 8/20 degrees and 6/14 degrees into the ipsilateral field, near the limit of striate with peristriate. An equivalent ipsilateral field representation was found in animals that had the anterior commissure cut, which in this marsupial disconnects the hemispheres, previously to the recording session. This result suggests that the ipsilateral hemifield can be represented for both eyes via the ipsilateral optic tract. This hypothesis is supported by recent work on the decussation pattern of optic axons in the opossum.

The opossum's "area centralis" cannot be described as a point but rather as a plateau of highest ganglion cell density. The ABC is aligned with the temporal portion of this area ("point P") near the site where 50% of the cells have crossed axons and 50%, uncrossed. The visuotopic organization having P as a boundary between nasal and temporal retina implies in a balanced ratio between the contra- and ipsilateral retinal projection. It also implies that point P itself contributes with less channels than neighboring points to central visual representation. Some analogies with the scotopic system in primates can be drawn.

It may be concluded that the reference axes determined by the response field disparities are associated with other properties of organization of the retino-geniculate-striate system. They may be as important for the visual system of nocturnal animals as the visual axes associated with the foveas are for the diurnal species.

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372.5 INTRINSIC CONNECTIONS IN CAT AREA 7 AS REVEALED BY RETROGRADE AND ANTEROGRADE AXON TRACING TECHNIQUES AND INTRACELLULAR INJECTION OF HRP. E.C. Callahan* and L.B. Haberly (SPON: L. Tong), Neurosci. Training Program and Department of Anatomy, University of Wisconsin, Madison, WI 53706.

Area 7 on the crown of the suprasylvian gyrus of the cat is a large region of polysensory or "association" cortex. Our previous electrophysiological and anatomical experiments (Soc. Neurosci. Abstr., 1984, 10:475) have demonstrated that area 7 possesses a widespread, intrinsic, horizontal (parallel to cortical lamination) fiber system. In order to more fully describe the organization of this horizontal fiber system, we have used both extracellular and intracellular axon tracing methods.

Injections of WGA-HRP and tritiated amino acids into area 7 revealed that the horizontal connections have a complex organization, with retrogradely labeled cell bodies and anterogradely transported label concentrated in patches. The patches are irregular in shape and do not form any obvious pattern. While the transported label is concentrated in patches, large numbers of retrogradely labeled cells and significant quantities of anterogradely transported label are found in the spaces between patches. The long associational connections originate from pyramidal cells in layers 2-6 and project to all cortical layers. The axons run predominantly in the rostral and caudal directions and are especially concentrated in layers 5 and 6.

Area 7 pyramidal cells in layers 2, 3, 5 and 6, filled by intracellular injection of HRP, have a profusion of fine local axon collaterals with many boutons and en passant swellings that have the appearance of synaptic terminals. Axons of pyramidal cells have been observed to give rise to large collaterals in layers 5 and 6 that appear to be the source of the long associational connections.

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372.6 VISUAL AND AUDITORY ASSOCIATION AREAS OF CAT POSTERIOR ECTOSYLVIAN GYRUS. E.M. Bowman* and C.R. Olson (SPON: B.A. Campbell). Dept. of Psychology, Princeton, NJ 08544.

The cortex of the feline posterior ectosylvian gyrus (PEG) is thought to serve high-order auditory or visual functions, but has not been thoroughly studied by modern methods. To characterize the afferent connections of the PEG as a whole and to distinguish subregions within it, we have carried out a retrograde tracer study. Seventeen deposits of distinguishable fluorescent tracers were deposited in or around the PEG of nine cats.

Our essential finding is that the cortex of the PEG contains two parallel, longitudinally oriented strips. These strips were defined by a statistical procedure, based on cell counts in twelve thalamic nuclei, whereby injection sites were grouped into clusters on the basis their connectional similarity. The anterior strip, referred to here as the auditory belt, is affiliated with the auditory system, but is clearly distinct in its connections from adjacent tonotopic auditory areas. The posterior strip, referred to here as the visual belt, is affiliated with the visual system and closely resembles in its connections the adjacent visual areas in the lateral bank of the suprasylvian sulcus.

Auditory Belt. Strong afferents derive from known or suspected auditory association areas, notably AII, and light afferents originate in all tonotopic auditory areas. Thalamic afferents originate from cells scattered irregularly through several nuclei including the lateral division of the posterior nucleus, outlying divisions (dorsal superficial, ventrolateral and medial) of the medial geniculate complex and an acetylcholinesterase-dense moiety of the lateromedial-suprageniculate (LM-Sg) complex. Ventral sites within the auditory belt receive input from the laterodorsal tip of the lateral nucleus of the amygdala.

Visual Belt. Cortical projections originate in visual areas, including 19, 21a, 20 and PS, and in association areas closely related to the visual system, notably area 7, the cingulate gyrus, the frontal eye fields and the granular insula. The insular projection is particularly strong. Thalamic cells projecting to the visual belt are concentrated in the acetylcholinesterase-pale moiety of the LM-Sg complex. Ventral sites within the visual belt receive input from the mediodorsal tip of the lateral nucleus of the amygdala.

In conclusion, our findings indicate that the posterior ectosylvian gyrus consists of two dorsoventrally elongated belts, one affiliated with the auditory system and the other with the visual system.

372.7 CORTICAL CONNECTIONS OF VISUAL CORTICAL AREAS 17 AND 18 IN GREY SQUIRRELS. K. L. Johanson,* L. A. Krubitzer, and J. H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

Cortical connections of area 17 (V-I) and area 18 (V-II) were investigated in grey squirrels with restricted injections of WGA-HRP, ^{3}H-proline, or fluorescent dye. Surface-view patterns of connections were revealed by artificially flattening cortex and cutting sections parallel to the brain surface. In sections stained for myelin from these same brains, it was possible to consistently identify areas 17, 18, somatosensory areas, auditory areas, and several fields of unknown significance lateral to area 18. Within area 18, bands of dense and light myelination were found oriented perpendicular to the 17/18 border.

Single injections confined to area 17 resulted in ipsilateral label in several distinct band-like foci in area 18, in distinct foci in one or both of two adjoining densely myelinated fields along the outer margin of area 18, and in limbic cortex medial to area 17. Dense contralateral projections were found along the 17/18 border. Injections extending from area 17 into area 18, and injections almost confined to area 18 and not extending into cortex lateral to area 18 produced a more extensive pattern of label. Ipsilaterally, transported label was found over a restricted and retinotopically appropriate portion of area 17. Other ipsilateral label was widespread in distinct foci over perhaps one-third of area 18, in the two densely myelinated fields lateral to area 18, limbic cortex, and a region of cortex caudolateral to area 18. Contralaterally, label was widespread in area 18 and in cortex lateral to area 18. We conclude that area 18 projects back to area 17, provides a widespread but uneven distribution of intrinsic connections within area 18, projects to one or more fields lateral to area 18, and has callosal connections with area 18 and one or more fields lateral to area 18. Since area 18 represents the contralateral visual hemifield, and callosal inputs over much of area 18 must reflect neurons with receptive fields in the ipsilateral hemifield, these callosal inputs apparently are not reflected in the excitatory receptive fields of area 18 neurons.

These studies help define subdivisions of visual cortex in squirrels and suggest that these rodents have at least five or six cortical visual areas.

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372.8 VISUAL AREAS IN THE POSTERIOR SUPERIOR TEMPORAL SULCUS IN THE CEBUS. R. Gattass*, M. Fiorani Jr.*, M.G.P. Rosa* and A.P.B. Sousa* (SPON: C.G. Gross). Instituto de Biofísica Carlos Chagas Filho, Rio de Janeiro, RJ, 21941, Brasil.

The visual topography of the areas in the posterior superior temporal sulcus (STS) was studied with multi-unit recordings in eight Cebus apella, anesthetized and paralysed.

In the posterior bank of STS there is an oval-shaped area (70 mm^2), topographically organized named MT. In each hemisphere, MT contains a representation of the contralateral visual hemifield up to 50 degrees. The representation of the vertical meridian forms its lateral border and that of the horizontal meridian runs horizontally across the posterior bank of STS. The upper field is located ventral and the lower field dorsal. The foveal representation is lateral in the posterior bank of STS. MT is coextensive with two myeloarchitectonically distinct zones: one homogeneous and heavily myelinated, located in the posterior bank of STS and another more pale, with visible inner and outer bands of Baillarger in the junction of the posterior bank with the anterior bank. In all animals the representation of the visual field in MT was continuous across these two zones. In one animal the foveal representation of MT was not restricted to the homogeneous zone, but it was coextensive with a region in which the two bands of Baillarger are proeminent. In MT receptive-field size increases linearly with increasing eccentricity while cortical magnification follows a power function with a slope similar to that of V1. No difference was found in the magnification of corresponding regions of the upper and lower visual field in MT. Point-image size decreases with increasing eccentricity with values similar to those obtained in corresponding portions of V1.

Lateral to MT, there is a thin strip of cortex (1-2 mm wide) which contains a representation of the lower visual quadrant up to 50 degrees eccentricity, with the vertical meridian represented anteriorly and the horizontal meridian posteriorly. On myeloarchitectonic grounds this area is difficult to be distinguished from MT in the region of the representation of the central 10 degrees. However, it can be differentiated from a more laterally located area which is less densely myelinated.

Anterior to MT there is a heavily myelinated zone with large directional selective receptive fields and no clear visual topography, which may correspond to MST of the macaque.

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372.9 RETINOTOPIC MAPPING AND RESPONSE CHARACTERISTICS OF NEURONS IN THE WULST OF THE GREAT HORNED OWL. B. J. Frost, B. Morgan*, Department of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

It has now been fairly clearly established that the "Visual Wulst" of the telencephalon of birds has numerous anatomical and physiological similarities to area 17 of mammals. Within the avian class the Wulst is particularly well developed in owls where it occupies a very large area extending over the dorsal-frontal telencephalon. Previous reports on Barn Owls (Tyto alba) in particular have shown that Wulst cells, like mammalian striate cortical cells, respond selectively to stimulus orientation, direction of movement and binocular disparity (Pettigrew and Konishi, 1976). Here we report similar findings for the Great Horned Owl (Bubo virginianus) and describe a very large magnification of the retinotopic representation of the fovea and the immediately adjacent binocular zone.

Three Great Horned owls were anesthetized with Ketamine/Rompun, positioned in a stereotaxic instrument, and visual landmarks such as the pecten oculi, ora terminalis and fovea of both eyes carefully plotted on a tangent screen. Extracellular recordings were taken from a large number of penetrations at various positions across the Wulst using computer-generated visual stimuli. In one series of penetrations encompassing approximately 57% of the Wulst surface, all receptive fields were clustered within a 76.9 square degree area around the fovea. This suggests a cortical magnification factor of between 83:1 to 100:1. Retinotopic organization was found to be similar to the Barn Owls with superior and inferior regions of visual field represented caudally and rostrally respectively, and midline and peripheral regions represented laterally and medially respectively. All cells were very selective for orientation of dark or light lines, and had orientation tuning curves < 30°, and were all directionally specific. Thirty-eight percent of units were monocularly driven with 32% responding only to stimulation through the contralateral eye and 6% through the ipsilateral eye. Of the 62% binocular cells, 46% belonged to the "binocular obligate" class that could only be stimulated with binocular input.

These results confirm Pettigrew's (1979) idea that the visual Wulst in owls has undergone extensive development for the processing of binocular information.

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372.10 EFFECTS OF LYSERGIC ACID DIETHYLAMIDE AND MESCALINE ON FLASH EVOKED POTENTIALS IN THE INTACT, AWAKE RAT. J.T. Eells and D.M. Wilkison. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

Lysergic acid diethylamide (LSD) and mescaline are potent psychoactive agents capable of eliciting profound alterations in visual perception. As part of our ongoing study of the neurophysiological mechanisms underlying drug-induced alterations in sensory perception and neuronal integration, we investigated the effect of LSD and mescaline on the flash evoked potential (FEP) in rats.

Hooded Long Evans rats were anesthetized with pentobarbital and indwelling epidural screw electrodes were stereotaxically implanted over the right and left visual cortices at points 7 mm posterior to bregma and 4 mm lateral to the midline. Ground and reference electrodes were placed 2 mm anterior and 2 mm lateral to bregma. Following a one-week recovery period, recording sessions were initiated. FEPS were recorded in response to 10 µsec flashes at a rate of 1 flash per second. Rats received cumulative doses of LSD (25-200 µg/kg) or mescaline (5-20 mg/kg), intraperitoneally. Full input/output curves at 3 luminescence intensities were established prior to drug administration and 30 minutes after the administration of each dose of LSD or mescaline.

The averaged FEP consisted of a primary (P20-N30) component and a (P50-N70-P100) component followed by a slower negative wave (N150). Mescaline (20 mg/kg) produced a marked attenuation (60-80%) of the primary (P20-N30) component of the FEP at the low and moderate flash intensities, but not at the high flash intensity. These data suggest that mescaline increases the threshold and the slope of the visual response to flash in the rat. In contrast, LSD had no significant effect on the FEP at any dose or flash intensity employed in this study.

The magnitude and the specificity of the effects of mescaline on the P20-N30 primary response suggest that the drug produces deficits in conduction through the retino-geniculato-cortical system. This study was supported by NIDA ROI DA 03785.

372.11 EFFECT OF GABA, BICUCULLINE, AND L-GLUTAMATE ON THE CAT AND RABBIT CALLOSAL PROJECTION AS DETERMINED BY CORTICAL PATTERN-REVERSAL EVOKED CORTICAL POTENIALS (PREPs)

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In the cat and rabbit, borders of areas 17 and 18a in both cortical hemispheres are reciprocally linked through the corpus callosum which is suggested to have an important role in processing visual information. These experiments were designed to examine the role of excitatory and inhibitory neurotransmitters in these visual cortical regions using the PREP as an indicator of visual function. GABA, bicuculline and L-Glutamate were injected at the 17-18a border area in one hemisphere of cat and rabbit. PREPs were recorded at the corresponding region in the contra- or ipsilateral hemisphere.

When GABA was applied, the waveform and amplitude of contralateral PREP changed in both species, the amplitude increased 48% after 6 min., while N1 latency increased 8%. The amplitude of the ipsilateral PREP decreased 57% for cat after 9 min. and 75% for rabbit after 8 min., and the latencies did not change. When bicuculline was applied, the amplitude of the contralateral PREP decreased 76% after 17 min. If the bicuculline was washed out by artificial cerebrospinal fluid, the amplitude recovered to 90% after 3 min. However, amplitude of the ipsilateral PREP increased 194%. When L-Glutamate was applied, the amplitude of the contralateral PREP decreased 59% after 2 min, then recovered gradualLY to 91% after 9 min. The latency increased slightly to 104%. However, if L-Glutamate was applied at the corresponding region in the ipsilateral hemisphere, the amplitude of the PREP increased sharply at first then gradually increased to 155% of the control. Latencies did not change. When increasing concentrations of KCl (0.3M, 1 M, 3 M) was applied at the corresponding region of the contralateral hemisphere the amplitude of the PREP decreased 12% 26% and 40%, respectively. Latency did not change. If 3M KCl was applied at the corresponding region in the ipsilateral hemisphere the amplitude of the PREP decreased 84% in 1 min. Therefore 3M KCl completely depressed nerve conduction. When a reversible cooling was applied at the region of 17-18a border in the opposite hemisphere, the amplitude of PREP decreased 50% after 7 min. After cooling was removed the PREP amplitude recovered to 87% after 17 min.

Since GABA caused remarkable excitation of PREP in contralateral visual cortex and Bicuculline and L-Glutamate caused remarkable inhibition, the collosal projection of visual cortex may have a complex circuit with both excitatory and inhibitory synapses.

372.12 RECEPTIVE FIELD PROPERTIES OF CORTICO-CORTICAL PROJECTION NEURONS IN VISUAL AREA I OF THE AWAKE RABBIT.

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Although a prominent projection from V-I to V-II is seen in all mammals, the visual response properties of this cortico-cortical efferent system have not been characterized in any species. In the present work the receptive field properties of cortico-cortical projection neurons were studied in awake rabbits. All initial surgery was performed under deep pentobarbital anesthesia. Cortico-cortical neurons were identified by antidromic activation following electrical stimulation of V-II. Receptive fields were classified according to the scheme of Murphy and Berman (J. Comp. Neurol., 188:401, 1979). Eye position was monitored $\pm$ 0.2°.

Most microelectrode penetrations were made into the binocular portion of V-I, in cortex representing < 15° from the vertical meridian. Thirty-four ipsilateral projection neurons were studied in this binocular zone whereas five neurons were studied in monocular cortex. The majority of these cortico-cortical projection neurons had simple receptive fields (17 S-I, 8 S-II). The remaining visually driven neurons consisted of one complex cell, one concentric cell and one directionally selective (but not orientation selective) cell. Eleven neurons could not be reliably driven by visual stimulation. The majority of the simple cells (67%) were directionally selective and 41% were end-stopped. Most cortico-cortical projection neurons had relatively long antidromic latencies (median = 4.8 msec, range = 1.15 - 14.6 msec), corresponding to a median conduction velocity of approximately 1 m/sec.

372.13 COMPARATIVE MODIFICATIONS OF CORTICAL CELL RESPONSES FOLLOWING INACTIVATION OF THE CONTRALATERAL CORTEX AND VISUAL AREA I.

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In the past several years we have studied influences of the corticofugal fibers. The corpus callosum is one of the major pathways which contains efferent coritcal fibers linking both hemispheres. In addition, the multiple representations of visual fields on the cortex is well established. Various histological investigations have revealed reciprocal connectivity which constitutes a neuronal substrate for functional relationships between both hemispheres as well as between visual areas. But, the physiological relationships between these areas are far from being elucidated. The aim of the present investigation is two fold, 1) to study the influence of visual area I on visual area II and 2) to analyse the relationships between both hemispheres. Rats were anesthetized with urethan and paralysed. Single units were recorded from the cortex while a retinotopically corresponding neuronal ensemble of V1 and contralateral homotopic site were temporarily inactivated. The local chemical inactivation of V1 was performed with a pressure injection of 50 to 100 ml of xylocaine. The influence of the contralateral cortex was studied by cryoblockade of the homotopic site. In both types of experiments, each unit was tested for its responses to moving light slits and stationary rectangles positioned at various sites in the receptive field (RF) in order to determine its spatial profile. So far data suggest that direction-selective cells are particularly sensitive to area V1 inactivation. Indeed, the responses declined remarkably leading to a virtual extinction. Comparatively, the response of the same cell to stationary on-off stimuli restricted to various areas of the receptive field showed less of a decrease. In contrast to the first type of experiments, the majority of highly directional - selective cells exibited a facilitation to blockade of the contralateral cortex (5/6), while in the same units, the on-off responses remained unaffected. Cells with a weak directional-selectivity showed a decrease in their firing with cortical blockade (23/30). This decline occurred for both stationary and moving stimuli. The spatial profile of the RF was also observed to expand with cryoblockade. Results permit us to conclude that many cells of visual areas (V2 and V1/V2 border) receive a fairly large input from complementary cortical areas.

Supp. NSERC and FCAR

372.14 BEHAVIORAL ACTIVATION OF VISUAL NEURONS IN MONKEY STRIATE CORTEX.

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Since neurons in prestriate cortex exhibit activation when a monkey attends to stimuli in the cells' receptive fields (Fischer and Boch, Brain Res., 345:111-123, 1985) it was important to know if such an effect were also present in striate cortex.

Rhesus monkeys were trained to fixate a small fixation point by using a dimming paradigm. They then learned to respond to the dimming of a stimulus 1° to 4° in the periphery. The stimulus appeared while the fixation point was present, but dimmed only after the fixation point disappeared. The monkeys were rewarded for detecting the dimming regardless of whether or not they made a saccade to the target, and they tended to intermix trials with saccades to the target with trials in which they continued to gaze at where the fixation point had been.

145 neurons were recorded in striate cortex of 4 hemispheres while the monkeys were performing this task. Most neurons gave a phasic on-response to the stimulus. 55 % of these phasic neurons showed a subsequent reactivation when the fixation point went out and the monkey had to respond to the peripheral stimulus, whether or not it made a saccade to it. The reactivation latency was much longer than the on-response latency, (mean 210 ms, measured from the fixation point offset), but shorter than the saccadic reaction times (250-500 msec). It was not due merely to the disappearance of the fixation point since it was absent if the stimulus were placed outside of the receptive field. Its occurence cannot be explained by a simple center-surround interaction since behaviorally irrelevant bright stimuli placed between the receptive field and the fixation point had no effect.

This activity could reflect the process of interruption of the previous fixation or, as far as this is not the same process, the shift of visual attention. Preliminary results indicate that even in the posterior parietal cortex and in the prefrontal cortex in the area of the principal sulcus a comparable reactivation can be displayed. It remains unclear whether this activity in different areas has a common source.

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372.15 SMALL LESIONS IN SUPRASYLVIAN CORTEX PRODUCE CONTRALATERAL VISUAL NEGLECT AND A LOWERED INCIDENCE OF DEEP LAMINAE VISUAL CELLS IN THE SUPERIOR COLLICULUS. Stephen C. Hardy and Barry E. Stein. Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, Virginia 23298.

Previously, Sprague (Sprague,J.M.,Science,153:1544-1547,1966.) demonstrated that posterior neodecortication in cats produced contralateral visual neglect. This effect was due to the loss of corticotectal facilitatory influences on the ipsilateral superior colliculus (SC) and its inhibition by the opposite SC. Thus, removal of the opposite SC restored visually-guided behavior in the previously blind hemifield. Because (a) SC-mediated visually-guided behavior is thought to reflect the function of deep laminae cells and (b) these cells are influenced primarily by posterior suprasylvian cortex (PSSC-Ogasawara, K., McHaffie, J.G. and Stein, B.E.J. of Neurophysiology,52:1226-1245,1984.), it seemed likely that damage to PSSC was primarily responsible for these lesion-induced visual deficits. To test this possibility we examined the visually-guided behavior of cats (n=20) before and after restricted lesions in PSSC and after a subsequent lesion of the contralateral SC in 12 of these animals. In addition, the incidence and response properties of ipsilateral SC cells were examined before and after the PSSC lesions in 8 of these cats.

Animals were trained to fixate on a baited stimulus (food) 60 cm away at 0°. A second stimulus (a white ball on a thin rod) was randomly introduced 8-16 times at each 15° visual angle from 0° to 105° nasal and temporal to the fixation point. When released, the animal could approach the fixation point or the ball to receive food. Preoperative animals approached the ball as it was introduced on nearly 100% of the trials. Several variations of the method were used to examine which methods were most applicable.

Small lesions in PSSC induced profound contralateral visual neglect in all animals when examined as described above. These deficits were much less obvious, or not apparent, at all if the animal was not required to move toward the stimulus or if food was not present at the fixation point. Visually-guided behavior was restored in the contralateral hemifield following removal of the opposite SC. These same PSSC lesions reduced the incidence of visual cells in deep laminae of the ipsilateral SC and fewer of the remaining cells were directionally-selective. Yet, the incidence and response properties of superficial laminae SC cells remained unchanged. These data indicate that only a small corticotectal region within the ipsilateral PSSC need be damaged to compromise the integrity of deep laminae SC cells and to produce visually-guided deficits in the contralateral hemifield.

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372.16 THALAMIC CONNECTIONS OF THE SUPERIOR TEMPORAL REGION IN RHESUS MONKEY. D.N. Pandya, D.L. Rosene and A.M. Galaburda. Department of Anatomy and Neurology, Boston University School of Medcine, Boston, MA 02118, Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA 01730 and Harvard Neurological Unit, Beth Israel Hospital, Boston, MA 02215.

On the basis of architectural and connectional analysis, the superior temporal region (STR) is divided in three distinct, parallel lines, designated the "root", "core" and "belt" lines. Within each of these three lines, there are four separate rostro-caudally arranged subdivisions. The second auditory area (AII) is located within the root line whereas the primary auditory area (AI) is in the core line. The auditory association cortices in superior temporal gyrus (STG) occupy the belt line (Galaburda and Pandya, J. Comp. Neurol., 221: 169-184, 1983).

Thalamic connections of different subdivisions of each of these lines were studied using anterograde (3H aminoacids) and retrograde (WGA-HRP and fluorescent dyes) tracers. The results show that the area AII in the root line mainly projects to and receives afferents from the suprageniculate nucles (SG) and the magnocellular portion of the medial geniculate nucleus (MGNmc). There are only minor connections with the parvocellular MGN (MGNpc) and the pulvinar nucleus. In contrast the primary auditory area (core line) has extensive connections with the MGNpc and only minor connections with SG and pulvinar nuclei. The thalamic connections of the auditory association areas (belt line) are predominately with the pulvinar, intralaminar and mediodorsal nuclei. Additionally, a differential rostro-caudal pattern is imposed upon this basic arrangement of thalamic connectivity. Thus the rostral subdivision of the core line in the supratemporal plane (STP) mainly sends projections to and receives from the caudal MGNpc whereas caudal subdivision of STP (AI) is connected with the rostral MGNpc. Likewise the rostral subdivision of the belt line (STG) mainly receives afferents from and projects to the caudal and medial part of the medial pulvinar whereas the caudal subdivisions of the belt line is connected with the rostral part of the medial pulvinar and has additional connections with the lateral pulvinar, intralaminar and mediodorsal nuclei. The mid portion of STG, in contrast, has connections with the oral pulvinar, the intralaminar nuclei and MGN.

Thus it seems that the different lines and subdivisions of STR have a distinct pattern of thalamic connections that is differentiated in accord with their cortical connectivity.

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372.17 THALAMOCORTICAL CONNECTIONS OF THE SUPERIOR TEMPORAL SULCUS IN THE RHESUS MONKEY. E.H. Yeterian and D.N. Pandya. Department of Psychology, Colby College, Waterville, ME 04901, Department of Anatomy, Boston University School of Medicine, Boston, MA 02118, and E.N. Rogers Memorial Veterans Hospital, Bedford, MA 01730.

On the basis of architecture and cortical connections, the cortex of the superior temporal sulcus (STS) has been shown to contain both unimodal and multimodal areas (Jones and Powell, 1970; Seltzer and Pandya, 1978). Moreover, behavioral and physiological investigations have demonstrated that the cortex of the STS is involved in a variety of complex functions (Petrides and Iversen, 1978; Bruce et al., 1981; Perrett et al., 1984; Baylis et al., 1985). In order to delineate the thalamic input to the cortex of the STS, as well as to compare these thalamic connections with those of parasensory association areas, we placed WGA-HRP injections into the upper bank and the rostral and middle regions of the lower bank of the STS. Some of these injections involved the adjacent gyral cortex. The brains were processed according to the method of Mesulam (1978). The results indicate that the rostral sector of the upper bank of the STS receives input only from the most medial part of the medial pulvinar nucleus (PM). The middle portion of the upper bank, which contains the multimodal area TPO, receives connections from central as well as medial regions of PM. It also receives input from the oral pulvinar nucleus (PO), the nucleus limitans (Li), the dorsal part of the medial geniculate body, and the dorsomedial nucleus (MD). Caudally, the cortex of the dorsal bank has the same basic pattern of input as the middle region, except that there is stronger input from the suprageniculate nucleus (SG). The extreme caudal portion of the dorsal bank, where it adjoins the cortex of the inferior parietal lobule, has more diverse afferents: from PM, PO, the lateral pulvinar nucleus (PL), SG, Li, the lateral posterior nucleus, the ventral posterolateral nucleus, the central lateral nucleus, the central lateral superior nucleus, and the paracentral nucleus. In contrast to the dorsal bank, the cortex of the ventral bank of the STS receives less extensive thalamic input. The rostral part of the ventral bank receives input from ventromedial PM. The thalamic afferents of the middle portion of the ventral bank are also from ventral PM, and from PL and the inferior pulvinar nucleus as well. Thus, the multimodal portion of the STS--area TPO--and the most caudal portion of the upper bank, which are known to receive complex cortical input, have relatively extensive thalamic afferents. In contrast, other areas of the STS receive thalamic input from nuclei related to modality-specific regions of the cortex.

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372.18 FRONTAL LOBE CONNECTIONS OF THE SUPERIOR TEMPORAL SULCUS REGION IN THE RHESUS MONKEY. Benjamin Seltzer and Deepak N. Pandya. V.A. Hospital, Bedford, MA 01730

Although interconnections between the frontal lobe and cortex of the superior temporal sulcus (STS) have previously been described, the precise sources and destinations of these projections have not been established. In this study, 22 discrete injections of isotope or WGA-HRP were made in cytoarchitectonic divisions of the STS region (Seltzer and Pandya, 1978), and the resulting pattern of labelled terminals or cells in the frontal lobe analyzed.

The results show that the rostral tip of the STS (area Pro) projects only to the orbital (areas Pro, 12, 13, and 14) and medial (area 25) surfaces of the frontal lobe. The rostral upper bank of the STS (area TPOr) projects not only to the orbital (areas Pro, 11, 12, and 13) and medial (areas 24 and 25) surfaces, but to the lateral frontal lobe as well. Here it projects to the dorsal portion of area 10 in the frontal pole, area 9, and ventral area 46. The mid-upper bank of the STS (area TPOm) projects only to the lateral surface (areas 9, 10, 12, and 46) with an additional projection to dorsal area 8. The projection to area 10 from this region is extensive as compared to that from rostral area TPO. The caudal upper bank of the sulcus (area TPOc) projects to the caudalmost area 46, dorsal area 8, as well as dorsal area 6. Some of these efferent projections from the upper bank of the STS are reciprocal, notably those to the frontal pole (area 10).

The rostral lower bank of the STS (areas TEa and TEm) projects to the orbital (areas 11, 12, and 14) and lateral (ventral area 46) surfaces of the frontal lobe. The mid-portion of the lower bank (areas TEa and TEm) projects only to area 46. Some of these projections are reciprocal.

Thus sectors of the STS have frontal lobe connections similar to those of the surrounding temporal pole, superior temporal gyrus, and inferotemporal area. The mid-portion of area TPO, a sensory convergence zone (Jones and Powell, 1970; Seltzer and Pandya, 1978), however, has distinct connections with the rostralmost frontal lobe (area 10), and the caudal portion of area TPO also projects to area 6.

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372.19 TYPE II MT NEURONS SHOW PATTERN-MOTION DIRECTION SELECTIVITY. T.D. Albright, H.R. Rodman* and C.G. Gross Dept. of Psychology, Princeton University, Princeton, NJ 08544.

We previously reported that neurons in macaque extrastriate visual area MT can be divided into two classes on the basis of the relationship between their preference for direction of motion and that for orientation (Albright, T.D., J. Neurophysiol., 52:1106, 1984). Type I neurons (60%) have an orientation preference that is roughly at right angles to the preferred direction of motion and are like those seen at earlier stages in the visual system. Type II neurons (30%), by contrast, have an orientation preference that is roughly parallel to direction preference and had not been previously reported.

Some MT neurons are selective for the direction of motion of two-dimensional patterns rather than the oriented components within those patterns (Gizzi et al., ARVO Abs., 1984). We have shown that models of the neural mechanism that might underlie pattern-motion selectivity have Type II properties (Albright, op. cit.). In the present study we sought to determine whether, in fact, Type II MT neurons are pattern-motion selective.

Macaques were anesthetized with N_2O and immobilized. We studied in detail 33 single neurons. Each neuron was tested for pattern-motion selectivity as described by Gizzi et al., that is, direction selectivity for single oriented gratings was compared to that for patterns formed by the intersection of pairs of gratings. In accordance with their observations, we found that pattern-motion selective neurons made up 30% of the sample and component-motion selective neurons 33%. The remaining 36% did not fall into either category. Each neuron was also classified as Type I or II as described above. Consistent with our own previous results, Type II neurons made up 24% of the sample and Type I neurons another 45%. The remaining 31% could not be classified as either Type I or II. All but one of the Type II neurons were found to be pattern-motion selective. In contrast, two-thirds of the Type I neurons were classified as component-motion selective. Finally, of those neurons not classifiable as either Type I or II, most were also not classifiable as either component- or pattern-motion selective.

In summary, we previously observed some MT neurons to have the unusual property of maximal sensitivity to an orientation parallel to the preferred direction of motion. Models of neural circuitry exhibiting pattern-motion selectivity have Type II properties among their characteristics. We have now demonstrated, in accordance with predictions from these models, that Type II neurons are pattern-motion selective. These results support a specific functional role for Type II neurons and suggest that the circuitry we have proposed underlies the selectivity of MT neurons for motion of two-dimensional patterns.

372.20 RESPONSES OF NEURONS IN VISUAL AREA MT AFTER REMOVAL OF THE SUPERIOR COLLICULUS. H.R. Rodman*, C.G. Gross and T.D. Albright (SPON: C. Olson). Dept. of Psychology, Princeton University, Princeton NJ 08544.

Many neurons in macaque visual area MT retain responsiveness, direction selectivity and binocularity following removal or inactivation of striate cortex. Preliminary studies suggested that the residual activity might depend on inputs from the superior colliculus relayed by the pulvinar (Rodman et al., Neurosci. Abs., 11:1246, 1985). We have now examined the response properties of MT neurons after combined lesions of striate cortex and the superior colliculus and after lesions of the colliculus alone.

Superior colliculus lesions were made using radio-frequency current in two monkeys which had previously been studied following bilateral removal of striate cortex. The animals were anesthetized with N_2O and immobilized during recording. 161 single- and multi-units were tested for responsiveness using spots and slits of light and dark edges. Within the portion of the visual field representation included in both lesions, virtually no responsiveness was found. In an animal with partial striate removal, units with receptive fields in the portion of the representation covered only by the colliculus lesion showed apparently normal responsiveness and direction selectivity.

In two additional monkeys, MT responses were studied following unilateral lesions of the superior colliculus alone. In most respects, the responses of MT neurons deprived of their colliculus input resembled those of neurons in normal MT. Over 50 single units were tested extensively for direction and orientation selectivity. As in normal MT, nearly all neurons were strongly responsive to both a moving spot and a moving slit of light, and mean direction tuning bandwidth did not differ significantly from that obtained in normal MT; mean index of directionality was likewise similar to that obtained in normal MT. Over three-quarters of the cells tested also responded to a stationary flashed slit, and mean orientation tuning bandwidth did not differ from that obtained in the normal animal. The proportion of neurons exhibiting sustained responses to the stationary slit was slightly greater than that of cells exhibiting transient ones, in contrast to the predominance of transient responses to stationary stimulus presentation in normal MT. Finally, nearly all neurons were about equally well activated from both eyes, again resembling MT cells in the normal animal.

In conclusion, it appears that inputs from the superior colliculus are sufficient but not necessary for visual responsiveness, direction selectivity and binocularity in this region of extrastriate cortex.

372.21 RESPONSE SELECTIVITIES OF CELLS IN THE MACAQUE'S MEDIAL SUPERIOR TEMPORAL (MST) AREA: UNDERLYING MECHANISMS. K. Tanaka, H. Saito and Y. Fukada*. NHK Sci. and Tech. Res. Labs., Setagaya-ku, Tokyo.

We have studied response properties of cells in the DSR region of the macaque MST area, using monkeys (M. fuscata) anesthetized with N_2O/O_2 and immobilized with gallamine triethiodide. There have been found three types of directionally selective cells with large receptive fields; D-cells responding to a straight movement of patterns in an equidistant plane from the monkey, S-cells responding to a size change, and Rf-cells responding to a rotation in an equidistant plane (Saito et al., J. Neurosci. 6: 145-157). Since MST receives strong fiber projection from the MT area, we hypothesized that the receptive fields of D-, S- and Rf-cells were constructed by integrating regional direction signals provided by MT cells. In the present study, we further studied response properties of S- and Rf-cells to test this hypothesis.

About a half of S-cells responded to a size change of a ring as well as to that of a wide dotted pattern, while the remaining half responded to a dot pattern much stronger than to a ring. Their response magnitude to a wide-field movement was not changed by exchanging the dot pattern for concentric rings, indicating that the shape of pattern is not important for their responses. We named the former cells Non-selective type, and the latter cells Field type. Rf-cells were also halved into Non-selective and Field types (a cross, dot pattern and windmill were used in this case).

Since a size change and rotation of a wide textured field contain several factors other than the arrangement of regional direction signals, i.e., the difference in speed between central and peripheral parts of the stimulus (for both movements), the size change of individual components of the texture (for size change), and the acceleration of movements toward the center (for rotation), these non-direction factors might be used to make up selectivities of Field type cells. We prepared artificial motion patterns consisting of four quadrants, in each of which dots moved straight in parallel with the same speed. The directions of movements in four quadrants were arranged so that the combination simulated either radial movements of a real size change or circular movements of a real rotation. Although the motion patterns did not contain the non-direction factors, Field type S- and Rf-cells responded to them as strongly as to a real size change or rotation of a dot pattern. We conclude that appropriate arrangement of directions of regional movements (Condition 1) + simultaneous stimulation of a wide visual field (Condition 2) are enough for their activation.

Non-selective type S- and Rf-cells also responded well to the artificial motion patterns, indicating that Condition 1 is enough for their activation. The difference between the two types may exist in the characteristics of spatial summation of inputs.

372.22 OBJECT-CENTERED ENCODING OF FACES BY NEURONS IN THE CORTEX IN THE SUPERIOR TEMPORAL SULCUS OF THE MONKEY M.E.Hasselmo*, E.T.Rolls, and G.C.Baylis, Dept. of Experimental Psychology, University of Oxford, Oxford OX1 3UD, England

A population of neurons in the cortex in the anterior part of the superior temporal sulcus has been described, the responses of which may convey information about the identity of faces (Baylis, Rolls and Leonard, 1985, Brain Res. 342: 91-102). The responses of these neurons show considerable invariance over changes in the size and contrast of face stimuli (Rolls and Baylis, 1986, Exp. Brain Res., in press). This we take as evidence that the encoding is not in retinal coordinates. Alternatives are that it is in viewer-centered or object-centered coordinates.

Some neurons with responses selective for faces only respond if the face is moving (Perrett et. al., 1983, Perception 12: A35). It is noted that encoding of gestures is likely to be important in the social behavior of primates. We took advantage of the fact that these neurons respond to moving faces to investigate whether the encoding of faces by these neurons is in viewer-centered or object-centered coordinates. For 6 neurons it has been shown that the neuron responds to particular movements which can only be described in object-centered coordinates. For example, one neuron responded vigorously to a head undergoing ventral flexion, irrespective of whether the head was viewed full face, to either profile, or even from the back of the head. These different views could only be specified as equivalent in object-centered coordinates. Further, for half of the neurons that were tested in this way, the movement specificity was maintained across inversion, responding for example to ventral flexion of the head irrespective of whether the head was upright or inverted. In this procedure, retinally encoded or viewer-centered movement vectors are reversed, but the object-centered description remains the same. It was of interest that 3 of these neurons generalized across different heads performing the same movements.

Further evidence supporting the hypothesis that some of the neurons in this region use object-centered descriptions is that their selectivity between the faces of different individuals is maintained across anisomorphic transforms of the stimulus. However, in most cases (7/9 neurons) although the identity of the face was reflected in the neuronal response, viewing angle also influenced the response. It is possible that these latter neurons represent an intermediate stage in the computation of object-centered descriptions.

Also consistent with object-centered encoding is the finding of neurons which respond to images of faces of a given absolute size, irrespective of the retinal image size. These neurons thus show size constancy.

373.1 PATTERN OF INTERHEMISPHERIC CONNECTIONS IN NEOCORTEX OF MICE WITH CONGENITAL DEFICIENCIES OF THE CALLOSAL COMMISSURE.
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In neonatal mammals, callosal cells are distributed diffusely throughout the sensory neocortex, while in the adult they are restricted to specific regions that form distinctive patterns in the plane tangential to the cortical surface. The developmental mechanisms that specify these patterns are unknown, but studies of the interhemispheric pathway in congenitally anophthalmic mice reveal that input from the eyes normally serves only to fine-tune the callosal pattern in visual cortical areas. Thus, central portions of the visual pathway appear to be innately endowed with the ability to specify at least a coarse version of the normal visual callosal pattern.

Normally the callosal commissure extends over much of the rostrocaudal extent of the interhemispheric fissure and the fibers within it are arranged topographically. Visual fibers are confined to the caudal portion of the corpus callosum, while fibers from more rostral regions of the cortex pass through successively more rostral portions of the corpus callosum. This orderly arrangement of fibers is also present in neonatal animals, suggesting that the rostrocaudal position and extent of the callosal commissure could play a role in helping growing axons find their appropriate targets in the opposite hemisphere.

We tested this idea by studying the distribution of callosal cells in BALB/c mice, a strain in which around 30% of individuals exhibit a congenital deficiency in the rostrocaudal extent of the corpus callosum. Callosal cells were retrogradely labeled by making single or multiple intracortical injections of horseradish peroxidase into the contralateral hemisphere. Our results confirm an earlier report that the number of callosal cells is reduced in BALB/c mice with deficient callosal commissures. In addition, we find that no labeled cells are present in the neocortex of acallosal BALB/c mice. However, in BALB/c mice with deficient callosal commissures, the overall distribution of callosal cells appears normal, even in those cases in which the callosum is displaced anteriorly and reduced to about 20% of its normal size. For example, in visual cortex callosal cells are concentrated at the border between cortical areas 17 and 18a, at the lateral border of area 18a, and in area 18b. The laminar distribution of callosal cells also appears normal in these mice. Thus, gross abnormalities in the extent and placement of the corpus callosum do not prevent normal specification of the visual callosal pattern during development.

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373.2 MIRROR TOPOGRAPHIC CONNECTIONS OF VISUAL CORTEX IN BILATERALLY ENUCLEATED RATS.
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In normal rats both the cells of origin and terminal distribution of the callosal pathway connecting the two visual cortices are restricted to the border of areas 17 and 18a. By contrast callosally projecting neurons are distributed throughout area 17 in neonatal rats. The progression from diffuse to more restricted distribution can be limited by surgical manipulations along the optic pathway,(Cusick, C.G., and Lund, R.D., J. Comp. Neurol. 212: 385-398, 1982). For example, following bilateral enucleation at birth, scattered cells are found throughout the normally acallosal portion of area 17 and terminals also invade a large part of area 17. Is this enlarged projection the result of callosal connections between mirror topographic regions of area 17 (i.e. the lateral part of area 17 projects to the lateral portion of contralateral area 17 and the medial part of area 17 projects to the contralateral medial portion of area 17)?

Rats were bilaterally enucleated at birth. One month later iontophoretic injections of horseradish peroxidase (HRP) were made into either medial or lateral parts of area 17. Normal litter mates were used for comparison.

In enucleated rats with injections restricted to the lateral portion of area 17 HRP labelled cells and terminals were located contralaterally in a mirror topographic position in the lateral region of area 17, but were also present medially within area 17. Following injections in the medial part of area 17 labelled cells and terminals were found within the contralateral cortex in medial and lateral portions of area 17. In normal animals similarly restricted lateral injection results in mirror topographic labelling only.

These results support the hypothesis that prior to the final organization of the visual callosal pathway, neurons located within area 17 on one side are connected with mirror and non-mirror topographic portions of the contralateral area 17.

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373.3 ACTIVITY DEPENDENT MECHANISMS IN THE DEVELOPMENT OF VISUAL CALLOSAL PROJECTIONS IN THE RABBIT.
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In the rabbit, as in many other mammals, visual callosal projections are exuberant at the time of birth and callosal cells are found throughout the mediolateral extent of area 17. This exuberant projection pattern normally retracts during the first postnatal month to become restricted to the 17/18 border. However, this retraction process is dependent on normal afferent input. Total absence of retinal ganglion cell activity resulting from monocular enucleation (ME) at birth results in an exuberant callosal projection in the adult similar to that seen in the neonate. However, total lack of visual experience resulting from dark rearing results in a callosal projection which is much more sparse than that observed normally and which extends into the mediolateral extent of area 17 further than in the normal adult but not as extensively as in the neonate or the ME rabbit. In the present study, we investigated the effects of blocking all activity of retinal ganglion cells by injecting tetrodotoxin (TTX) in citrate buffer into the eye every 3 days from the 7th day postnatal. Pupillary reflexes were tested daily to ensure adequacy of the injection. Citrate buffer alone was injected into control animals. When animals were aged 1-2 months, HRP was injected into the visual cortex ipsilateral to the injected eye. Animals were perfused 24 hours later, the cortex processed for the HRP reaction, and the optic nerve, retina and thalamus processed to assay damage resulting from the injections. Both controls and experimental animals evidence significant but equivalent damage resulting from the repeated injections. However, in controls the callosal projection was normal with a band of callosal cells visualized at the 17/18 border. In TTX animals, the callosal projection was very sparse and extended partially into the medial extent of area 17. Thus, the effects of the TTX injections cannot be attributed to retinal damage, and closely resemble those of dark rearing.

Critical periods for the effects of TTX have not yet been established. However, the critical period for the effects of ME on exuberant callosal projections in the rabbit is over by day 7 i.e. the callosal projection of an adult rabbit enucleated on day 7 are normal. In contrast, our TTX effects resulted from injections beginning at day 7, just before maturation of contacts between retinal ganglion cells and cells of the inner nuclear layer of the retina. The results suggest that critical periods for, and the effects of, ME differ from the effects of modification or abolition of activity (by TTX or dark rearing) in an intact system.

373.4 ABNORMAL CALLOSAL CONNECTIONS IN SIAMESE CATS ARE INDEPENDENT OF STRABISMUS.
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Siamese cats have abnormal visual callosal pathways connecting the two striate cortices (Shatz, '77), and many of them also have pronounced convergent strabismus. It has been thought that the abnormal callosal connectivity in Siamese cats is secondary to abnormal striate cortical topography, but strabismus can result in abnormal callosal connections in normally pigmented cats (Lund & Mitchell '79; Innocenti & Frost, '80; Berman & Payne, '83), raising the possibility that strabismus, rather than abnormal cortical topography, is the responsible factor. To examine this, we made quantitative comparisons of the striate callosal cell zone (CCZ) and callosal terminal zone (CTZ) between normally pigmented and Siamese neonatal kittens and between normal cats, normally pigmented cats with convergent strabismus, and Siamese cats.

Both callosal zones of Siamese kittens were indistinguishable from those of normally pigmented kittens at the time of eye opening. This indicates that any abnormalities in the callosal connectivity of Siamese cats emerge at a time when experience could affect their maturation.

In normal adult cats, considerable variability exists both in the extent (mediolateral width) and number of supragranular cells that constitute the CCZ. In extent, the CCZ's of normally pigmented strabismic cats and of Siamese cats were similar, occupying the upper limit of the normal range. However, the CCZ of Siamese cats was quantitatively different from that of normally pigmented animals, irrespective of overt strabismus: in Siamese cats many fewer callosal cells were present (~25% of normal).

In normal adult cats, the CTZ was restricted to the extreme lateral border of area 17. The CTZ of normally pigmented strabismic cats and of Siamese cats was more widespread. However, in Siamese cats its extent may be ~5 times that of normal, matching the CCZ, while in normally pigmented strabismic cats the expanded CTZ was never this wide.

Finally, both callosal zones were indistinguishable between Siamese cats exhibiting obvious, manifest convergent strabismus and those with no obvious eye misalignment.

Taken together, these results indicate that the abnormalities in the callosal zones of Siamese cats appear late enough in development for experience to be a factor, but that the presence of strabismus cannot account for those abnormalities.

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373.5 EFFECTS OF DARK REARING ON THE MATURATION OF FELINE VISUAL CALLOSAL CONNECTIONS. D. O. Frost and C. Dean.* Section of Neuroanatomy, Yale Medical School, New Haven, CT 06510.

In the visual cortex of normal, adult cats, neurons projecting through the corpus callosum (callosal neurons; CN's) are located within a limited region (callosal zone; CZ) spanning the area 17/18 boundary. At birth the CZ is much wider and extends throughout areas 17 and 18. The CZ achieves its adult form by a gradual narrowing during the first 3 postnatal months (Innocenti & Caminiti, Exp. Br. Res., 38:381, 1980). Visual experience during this period affects the mature number and distribution of callosal neurons: rearing kittens with strabismus, monocular enucleation or monocular eyelid suture stabilizes some callosal connections that would normally have been eliminated and thus results in an abnormally wide CZ (Innocent & Frost, Nature, 280:231, 1979). In contrast, rearing kittens with binocular eyelid suture (BD) or binocular enucleation (BE) enhances the normally occuring reduction in the number of callosal neurons; surprisingly, this effect is greater for BD than for BE, and although CN's are less widespread than normal in BD cats, they are more widespread than normal in BE cats (Innocenti & Frost, Exp. Br. Res., 39:365, 1980).

Here, we study the effects of dark rearing (DR) on callosal development. Six cats were born and raised in total darkness for 96-208 days. Two days before sacrifice, each cat received multiple, unilateral injections of horseradish peroxidase (HRP) in its visual cortex (the posterolateral and lateral gyri were completely filled with HRP), and serial sections of the brains were processed with tetramethyl benzidine to reveal retrogradely labeled CN's in the contralateral hemisphere. The number and distribution of CN's were determined using computer-aided reconstructions. Two normal adult cats were similarly processed.

At the 17/18 border of DR cats, the number of CN's is about 70% that of normal cats (compared to about 53% of normal values in BD cats; [Innocenti, et al., J. Neurosci., 5:255, 1985]). The CZ appears slightly narrower than normal. Thus, no visual stimulation of any sort is necessary to initiate the normally-occuring partial elimination of immature callosal connections. However, normal visual experience is necessary for the stabilization of a normal complement of CN's. DR, like BD and BE, exaggerates the normal reduction in callosal neuron number. Since DR and BE cats both have more CN's than BD cats, it is possible that the absence of visually-elicited retinal ganglion cell activity contributes to the greater stabilization of immature callosal connections in BE as compared to BD cats.

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373.6 FATE OF VISUAL CORTICAL NEURONS AFTER HETEROCHRONIC TRANSPLANTATION. S.K. McConnell. Salk Inst., La Jolla, CA 92138, and Dept. of Neurobiology, Harvard Med. School, Boston, MA 02115.

Neurons in the same layer of the visual cortex tend to have similar morphologies, projection patterns and physiological properties; moreover, cells in a given layer tend to be generated at similar times during development. This raises the possibility that the ultimate fate of a cortical neuron might be determined early in development, perhaps prior to migration. To assess the relative contributions of cell birthday and local environment to this determination, cells normally destined to become deep-layer neurons were taken from the cerebral ventricular zone of embryonic ferrets and transplanted into the brains of newborn hosts, in which supragranular neurons were being generated. Thus the transplanted neurons were challenged to alter their fate: would they recognize their correct deep-layer positions or migrate to the superficial layers along with host neurons?

For these heterochronic transplants, neurons normally destined to populate layers 5 and 6 were labeled by intrauterine injection of [^{3}H] thymidine on E32. The occipital cerebral mantles were then placed in culture medium containing a second label (Fast Blue), dissociated into a single-cell suspension, and finally injected back into the ventricular zone of newborn host ferrets. The dissociation and transplantation procedures were identical to those used previously for isochronic (newborn-to-newborn) transplants (McConnell, Science 229: 1268, 1985).

As in isochronic transplants studied after short survivals, there was a population of dye-labeled neurons in the heterochronic transplants that migrated to, then differentiated in the host cortical plate. When the [^{3}H] thymidine marker was used to examine the final positions of transplanted cells after 1-2 months survival, however, two striking differences between isochronic and heterochronic transplants were revealed. First, about 75% of labeled cells in heterochronic transplants had failed to migrate and were found clustered near the lateral ventricle (less than 10% of isochronic cells were found here). These were either layer 5/6 neurons unable to migrate in an older brain, or stem cells whose normal program of mitotic divisions was arrested upon dissociation. A second difference between the two types of transplants was in the distribution of labeled neurons found within the visual cortex. In isochronics, 99% of labeled cells in cortex were found in layer 2/3, their normal laminar position. In the heterochronic transplants, 60% were found in layer 2/3, and the remaining 40% were distributed among the deeper layers (primarily in layers 5 and 6). So it appears that after transplantation, a minority of E32 neurons were able to recognize their normal laminar position in the deep layers and there differentiate. For most, however, environmental cues appeared to be more important: these transplanted neurons migrated to the same destination as their host counterparts. It will be interesting to see whether heterochronically transplanted cells nevertheless elaborate axonal projections typical of deep-layer neurons. (Supported by NSF BNS 85-10425 to S. LeVay.)

373.7 EFFECTS OF DESIPRAMINE ON NOREPINEPHRINE CONTENT AND PLASTICITY OF KITTEN VISUAL CORTEX. E. E. Allen, P. Q. Trombley*, L. J. Blakemore*, and B. Gordon Institute of Neuroscience and Dept. Psych., Univ. Oregon, Eugene 97403.

The ability of 6-hydroxydopamine (6-OHDA) to decrease visual cortical plasticity may depend on its uptake into norepinephrine (NE) nerve terminals, the destruction of those terminals, and NE depletion. To test this hypothesis we attempted to prevent 6-OHDA uptake by pretreatment with desipramine (DMI) which blocks NE uptake sites.

Four kittens about 5 weeks of age received 1.2 mg/day of intraventricular 6-OHDA for 4 days. Each of these kittens was pretreated with intraperitoneal DMI one half hour before 6-OHDA injection. Two of these 4 kittens received 5.0 mg/kg/day DMI during the 4 days of 6-OHDA treatment. The remaining 2 kittens received 7.5 mg/kg/day DMI over 8 days (2 prior to, 4 during, and 2 after 6-OHDA administration). NE content, measured by high performance liquid chromatography with electrochemical detection, was depleted by about 90% relative to untreated controls in all 4 cats. At the doses of 6-OHDA used here, DMI did not prevent NE depletion. We chose a total dose of 4.8 mg of 6-OHDA because smaller doses have little effect on plasticity (Kasamatsu and Pettigrev, J. Comp. Neurol., 1979; Trombley et al. J. Neurosci., 1986). Doses of DMI larger than 7.5 mg/kg/day are not tolerated by kittens.

In another experiment, we found that DMI alone decreased NE content. DMI at 5.0 mg/kg/day for 14 days decreased cortical NE content by about 80%. This depletion, in contrast to that produced by 6-OHDA, was transient; NE content increased several days after cessation of DMI treatment. DMI at 5.0 mg/kg/day for 6 days depleted NE by about 45%. Because DMI induced NE depletion was less than that achieved by 6-OHDA in combination with DMI, we do not think the depletion following the combination of 6-OHDA and DMI resulted solely from DMI; part was due to 6-OHDA itself.

Because the 6-OHDA induced decrease in visual cortical plasticity has been attributed to NE depletion, we examined the effect of DMI alone on the response to monocular deprivation (MD). Six kittens received DMI for 14-21 days and were monocularly deprived for the last 7 of these. The NE content of 4 of these animals was depleted by more than 55%, while 2 others showed no NE depletion. Irrespective of level of NE depletion, recording from area 17 indicated that these animals retained neuronal plasticity; the nondeprived eye dominated cells in the visual cortex as strongly as in MD cats receiving no drug treatment.

We conclude that DMI, in doses tolerated by kittens, fails to protect them against the NE depleting effects of 6-OHDA. Furthermore, DMI alone partially depletes NE from visual cortex, but this depletion does not reduce visual cortical plasticity.

373.8 TIMING OF 6-OHDA ADMINISTRATION: EFFECTS ON PLASTICITY OF KITTEN VISUAL CORTEX. B. Gordon, E.E. Allen, L. J. Blakemore*, and P.Q. Trombley* Dept. Psych. and Inst. Neurosci. Univ. Oregon, Eugene, OR 97403.

Kasamatsu and colleagues (J. Comp. Neurol. 1976, 1979) reported that 6-hydroxydopamine (6-OHDA) decreased the effects of monocular deprivation (MD) in the kitten visual cortex. They hypothesized that this decrease in plasticity resulted from depletion of norepinephrine (NE). We previously reported a qualitative, but not quantitative, replication of their experimental finding (Trombley et al., J. Neurosci. 1986). We disagreed with their interpretation because we found that the dose of 6-OHDA required to decrease plasticity was much greater than the dose required to deplete NE. We now report that although NE depletion lasts at least 2 weeks following the completion of 6-OHDA treatment, 6-OHDA's effect on plasticity has a much shorter duration.

We compared 3 groups of kittens receiving both intraventricular injections of 6-OHDA and MD for one week. MD began at about 6 weeks of age. (1) These kittens received 0.2 mg of 6-OHDA during the 4 days prior to MD and 4.8 mg 6-OHDA during the 7 days of MD (N=5). (2) These kittens received 4.8 mg 6-OHDA during the 6 days prior to MD with the last injection on the day prior to eye suture (N=4). (3) These kittens received 4.8 mg of 6-OHDA over 6 days with the last injection one week prior to eye suture (N=5). A fourth, control group received intraventricular vehicle solution (N=6).

Immediately following one week of MD, we recorded from area 17 of the visual cortex contralateral to the deprived eye. For each kitten we made an ocular dominance histogram and calculated the proportion of cells driven by the deprived eye, weighted binocularity, and weighted shift (Kasamatsu et al. J. Neurophysiol., 1981). Each of these 3 measures indicated that plasticity decreased (MD had less effect) as 6-OHDA treatment and monocular suture approached concurrence. An analysis of variance followed by post hoc tests showed that kittens receiving 6-OHDA throughout the suture period were significantly less plastic ($p<.01$) than control kittens or kittens receiving 6-OHDA treatment that terminated a week prior to suture. The plasticity of the other group was intermediate.

Immediately after the recording session, samples of visual cortex were removed for analysis of NE content using high performance liquid chromatography with electrochemical detection. All 3 groups receiving 6-OHDA were equally depleted (85-90%) relative to controls.

We conclude that 6-OHDA administration during MD increases its effect on plasticity. Therefore, 6-OHDA must affect plasticity by a mechanism other than NE depletion.

373.9 NORADRENERGIC AND CHOLINERGIC INTERACTION IN OCULAR DOMINANCE PLASTICITY. K. Imamura and T. Kasamatsu. The Smith-Kettlewell Eye Research Foundation, 2232 Webster St., San Francisco, CA 94115

Direct infusion of kitten visual cortex with a catecholamine (CA)-related neurotoxin, 6-hydroxydopamine (6-OHDA) suppresses the shift in ocular dominance following monocular lid suture (ocular dominance plasticity). Subsequent infusion with noradrenaline (NA) of the kitten visual cortex, pretreated with 6-OHDA, restored ocular dominance plasticity to the aplastic cortex (Kasamatsu et al., J. Comp. Neurol., 185, 1979). We further showed that the plasticity can be suppressed in a concentration-dependent manner by directly infusing kitten visual cortex with a β adrenoreceptor antagonist, propranolol (Shirokawa and Kasamatsu, Neuroscience, 1986, in press). However, significant depletion of NA in kitten visual cortex, by various means other than the cortical 6-OHDA infusion, does not necessarily decrease the plasticity (e.g., Daw et al., J. Neuroscience, 5, 1985). Bear and Singer recently reported that the plasticity disappeared from kitten visual cortex only when both NA and thalamic cholinergic inputs had been reduced substantially, implying that the two systems may functionally compensate for one another when one of the two is damaged (Nature, 320, 1986).

In the present study, we wanted to examine further the involvement of the cholinergic system in ocular dominance plasticity. First, the two hemispheres of kitten visual cortex (5-7 weeks old) were locally infused with 4 mM 6-OHDA (in an osmotic minipump) for a week. Upon termination of the 6-OHDA infusion, one hemisphere was infused with a muscarinic cholinergic agonist, bethanechol chloride (1-5 mM), through the same implanted cannula, for the second week in conjunction with monocular lid suture. The other hemisphere received the infusion of the vehicle solution. We obtained the shift in ocular dominance (binocularity, 0.2-0.33) in the bethanechol chloride-infused hemisphere, while binocularity remained relatively high (0.57-0.67) as expected in the control side. The difference was statistically significant. We concluded that bethanechol chloride restored the plasticity to the cortex which was aplastic due to the preceding 6-OHDA infusion. Next, we carried out similar studies using 10 mM propranolol in place of 6-OHDA. In this design, the cortical infusion of 1-5 mM bethanechol chloride did not restore the plasticity; binocularity stayed high (>0.5) and less than 18% of visually active cells responded exclusively to stimulation of the nondeprived eye.

In summary, the present study confirmed the involvement of the cholinergic system in the regulation of ocular dominance plasticity. Furthermore, it suggested that the presence of β adrenoreceptor is needed for cholinergic mechanisms to be involved in the regulation of the plasticity. (Supported by USPHS grant EYO-5549 and The Smith-Kettlewell Eye Research Foundation.)

373.10 DEVELOPMENT OF GABA NEURONS AND SYNAPSES IN MONKEY VISUAL CORTEX. A. Hendrickson. Departments of Biological Structure and Ophthalmology, University of Washington, Seattle, WA 98195.

Immunocytochemical staining has been used to identify the developmental sequence of neurons using gamma aminobutyric acid (GABA) as a neurotransmitter in Macaca monkey visual cortex. Primary visual cortex has been examined at fetal days 130, 140, and 160, and postnatally at 1,7,14,28,42,56, and 168 days. Light microscopic immunocytochemistry was done using primary antisera to GABA or to glutamic acid decarboxylase (GAD) which synthesizes GABA. Staining was revealed by peroxidase/antiperoxidase reacted with diaminobenzidene and hydrogen peroxide. Corresponding sections were stained for cytochrome oxidase (CO).

GAD: At F130 there is very little staining in most layers of visual cortex, but some cell bodies and processes can be identified in layer 1. Neuropile staining slowly increases in all layers until birth, and rapidly thereafter. By 14 days after birth, the amount of GAD has increased markedly in both cell bodies and neuropile in all layers. Perisomatic terminals are apparent on many neurons, some of which also stain for GAD. At 42 days, the visual cortex is similar to adult in GAD distribution with heavily stained neuropile in layers 1A,3,4A,4C and 6. However, the staining is significantly more intense in the young cortex. GAD positive cell bodies are much more obvious than in the adult, and the neuropile staining suggests that a larger number of GAD positive synaptic terminals are present. GAD staining intensity slowly returns to the adult value by the end of the first year.

GABA: At F140 GABA positive cell bodies are found only in layer 1, but by birth they are particularly prominent in all layers. The neuropile is lightly stained, corresponding more closely to GAD distribution at these ages. The number of GABA neurons remains high throughout the early postnatal period.

CO: Before and just after birth, CO staining in present in the neuropile, but differs from the adult in that 4A does not stain and 4C is divided into three bands of equal width. The inner and outermost bands are heavily stained while the middle band is very light. Enface sections show the layer 3 dots to be present, but very light. 4A staining appears at 14 days. Lamination in 4C is reduced at 42 days, and 4C is uniformly dense by 56 days.

This study indicates that GABA positive neurons are present in the visual cortex well before birth, but synapses in the neuropile of most layers develop in the first 6-9 weeks after birth. The distribution of GABA, GAD and CO are quite different during development, but all reach adult patterns by 6-9 weeks after birth. The number of GABA synapses, however, is reduced after this time to reach adult levels late in the first year. Supported by NIH grants EY-01208 and -04536 and by the Dolly Green Scholar award from RPB.

373.11 GENESIS OF GABA IMMUNOREACTIVE NEURONS IN THE FERRET VISUAL CORTEX. Jean D. Peduzzi. School of Optometry, The Medical Center, University of Alabama at Birmingham, Birmingham, Alabama 35294.

Immunohistochemical and ^{3}H-thymidine autoradiographic techniques were combined to study quantitatively the neurogenesis of gamma-aminobutyric acid immunoreactive neurons (GABAergic neurons) in the primary visual cortex of the ferret. While GABAergic neurons follow the same general inside-out pattern of neurogenesis characteristic of the majority of neurons in the ferret visual cortex (Peduzzi & Hickey, Soc. Neurosci. Abstr. 11: 806, 1985), the radial distribution of neurons born on a given day appears qualitatively different for GABAergic neurons. In general, GABAergic neurons born on a given day are more broadly distributed ("scatter") across the radial axis of the cortex than are non-GABAergic neurons born at the same time. More quantitative analyses have now confirmed our earlier qualitative observations.

In this study, each ferret received a single injection of ^{3}H-thymidine on one of the following days: embryonic day 22 (E22), E26, E30, E34, E38, postnatal day 2 (P2), P6, or P10. Each animal was sacrificed at 3 months of age or older. Frozen sections (15 μm) were incubated in antisera (Immunonuclear) to GABA and processed using the PAP technique. The resulting sections were then processed using standard autoradiographic procedures.

While the final locations of GABAergic and non-GABAergic neurons generated on the same day overlap extensively along the radial axis of the visual cortex, the radial "scatter" of neurons born on a given day is, to a very large degree, magnified by the GABAergic neurons, especially during the second half of cortical neurogenesis. The distribution of GABAergic neurons generated very early in development do not contribute as much to such "scatter" since few of the interstitial neurons (ie., those found in the white matter below layer VI) are GABAergic. In general, however, GABAergic neurons do play a major role in magnifying the "scatter" seen in the radial distribution of neurons with common birthdates.

In addition to varying widely in their final radial position in the cortex, GABAergic neurons generated on the same day also exhibit a wide range of soma shapes and sizes, as well as a variety of dendritic orientations.

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373.12 THE DISTRIBUTIONS OF MUSCARINIC AND NICOTINIC ACETYLCHOLINERGIC BINDING SITES IN THE DEVELOPING CAT VISUAL CORTEX. G. Prusky and M. Cynader, Departments of Psychology and Physiology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

There are at least two types of acetylcholinergic (ACh) receptor binding sites: muscarinic (M) and nicotinic (N). Activation of M receptors results in effects that are slow in onset and long in duration. N receptor activation results in rapid transient responses. We have characterized the ontogenesis of M and N binding sites in cat visual cortex, using [^{3}H]QNB and [^{3}H]-DL-nicotine as selective ligands with in vitro autoradiographic techniques (Young and Kuhar, 1979; Herkenham and Pert, 1982).

Our results confirm previous reports (Shaw et al., 1984), showing that M ACh receptors are specifically localised in layer IV of primary visual cortex early in post-natal life, and this distribution alters during the physiologically defined critical period. These receptors reverse their lamination so that by 8 weeks of age the adult pattern is reached with layer IV containing the fewest receptors. N receptors can be visualized in layer IV of primary visual cortex at the onset of the critical period. There is no change in the laminar distribution of the N binding sites as the kitten ages, but the number increases and by adulthood they occupy layer IV of all extrastriate cortical areas with the highest concentration in primary visual cortex.

The patterns of development for M and N receptors are complimentary. As the M receptors decrease in layer IV during the critical period, the N sites begin to occupy that layer. The anatomical specificity of both the M and N receptors for the target layer of the incoming afferent fibers from the lateral geniculate nucleus (LGN), suggests that these receptors may regulate LGN input at different times. The slow-acting M ACh receptors may regulate the LGN input early when the system is "plastic", but as the system moves through the critical period and becomes "hard-wired", it utilizes the efficient N ACh receptors. Observations of primary auditory cortex indicate that a similar pattern of ACh receptor development exists in this system, therefore this phenomenon may be a general characteristic of all developing primary sensory cortices in the cat.

373.13 Maturation of the Somatostatin System in Rat Visual Cortex. Michael R. Harris*, Lois K. Laemle and Susan C. Feldman. Department of Anatomy, UMDNJ-New Jersey Medical School, Newark, N.J. 07103.

The development of the rat visual cortex (VC) was studied by analyzing change in the number and distribution of neurons containing immunoreactive somatostatin (ISRIF). Brains, at least two, from rats aged E20, day 0 (day of birth) and postnatal days 2,4,6,8,10, 15, and 21 were fixed in Bouin's solution, embedded in paraffin and sectioned in the coronal plane. ISRIF was localized by light-microscopic immunocytochemistry using two antisera to the peptide; all staining was judged specific. For each animal, the distribution of ISRIF neurons and fibers were mapped in representative sections throughout the rostral-caudal extent of VC using a camera lucida. For each section, the depth of the cortex from pial surface to white matter was measured, the cortex was then subdivided into thirds and the position of each ISRIF-containing cell was indicated.

From E20 to day 21, there was a four-fold increase in depth of VC from 4½-8 mm to 14-20½ mm. With maturation, there was a change both in the number of ISRIF neurons and in their distribution. From E20 to day 4, two types of ISRIF-containing cells were evident: round cells which stained intensely and cells with one or two thick processes which were usually oriented radially with respect to the pial surface. After day 4, all of the ISRIF cells resembled neurons. The number of ISRIF "neuron-like" cells was very high on days E20 and 0; at these ages 20-50% of the cells were present in the upper third of VC and all of the round cells were present in the bottom third near the white matter. On day 2, very few ISRIF neurons were seen; depending on the A-P level these cells were present mainly in the middle third. The majority of the round cells, i.e. two-thirds, were present in the bottom third of the cortex. From days 4-21 there was a progressive increase in the number of ISRIF neurons and a redistribution from that seen on day 4 when less than 10% of the ISRIF neurons were present in the upper third to that seen by day 21 when 25% of the ISRIF neurons were present in the first third, 30-40% in the middle third and the remainder in the bottom third. Although there was a decrease in the number of ISRIF neurons from late gestation to day 2, the density of ISRIF fibers in the neuropil was greatest at early postnatal ages.

Our results suggest that there are two populations of ISRIF-containing neurons in VC: one late prenatal-early postnatal and a second, post-natal population, which appears about day 4. Whether these represent new cells or the synthesis of ISRIF by pre-existing cells is under investigation. The presence of substantial numbers of ISRIF neurons early in cortical maturation, especially the prominent plexus of ISRIF fibers and processes, suggests a role for these neurons in the organization of VC.

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373.14 FROM BASIC NETWORK PRINCIPLES TO NEURAL ARCHITECTURE: EMERGENCE OF ORIENTATION CELLS AND COLUMNS. R. Linsker * (SPON: S. M. Goldin). IBM Thomas J. Watson Research Center, Yorktown Heights, NY 10598.

The functional architecture of mammalian visual cortex has been elucidated in impressive detail by experimental work of the past 20-25 years. The origin of many of the salient features of this architecture -- e.g., the formation of orientation-specific cells and their columnar organization -- has, however, remained unexplained. How much of this architectural complexity depends upon detailed developmental rules that are not yet known, and how much can be derived from network-development considerations that are quite simple and possibly quite general, yet biologically plausible?

To approach this question, we simulate the progressive maturation of a system composed of a few layers of cells. Each cell receives input via initially random connections from a neighborhood of cells of the previous layer. The system develops according to a connection-modification rule (e.g., of Hebb type). Despite the limited amount of 'genetic' information needed to specify this system, we find that the range of developmental options for the cells of each layer is constrained, and that the mature cell types that emerge have biologically useful information-processing functions.

We explicitly demonstrate the emergence of spatial-opponent cells, orientation-selective cells (whose receptive field types match those of the 'simple' cells of Hubel and Wiesel), and orientation-cell columns. The emergence and organization of more complex cell types, when one extends this system to more layers, are also discussed.

These architectural features emerge even though no orientation preference is specified to the system at any stage, and none of the basic developmental rules is specific to visual processing. They emerge even in the absence of visual experience.

We discuss the correspondence between these results and experimental findings. We emphasize the biologically-important geometric and functional character of the mature cell types and organization that emerge, show how and why these types develop, and how the emergence of new feature-analyzing cell types occurs as one proceeds from each layer to the next, even though the underlying development rules are unchanged. We shall also comment on how the analysis of what we call "modular self-adaptive networks" -- of which this system is an example -- allows one to understand the emergent properties of complex highly-parallel networks in terms of far more simply-described circuits.

373.15 **MODELS FOR THE FORMATION OF OCULAR DOMINANCE COLUMNS SOLVED BY LINEAR STABILITY ANALYSIS.** K.D. Miller, J.B. Keller and M.P. Stryker (SPON: D.M. Waitzman). Depts. of Physics, Neurosciences, and Mathematics, Stanford University, Stanford, CA 94305, and Dept. of Physiology, UCSF, San Francisco, CA 94143.

The development of ocular dominance columns (or patches), in mammalian visual cortex and in fish and amphibian optic tectum, provides an important example of apparent activity-dependent synaptic remodeling in the central nervous system. Here a large-scale structure develops as a result of interactions among small-scale elements: the "decisions" to strengthen or weaken a synaptic connection must be made locally at the individual cell or synapse. We have examined what kinds of activity-dependent interactions, on the small scale, could give rise to the large-scale patterns observed.

We have developed a mathematical model that incorporates, as special cases, several mechanisms of cellular interaction that have been suggested in the literature. In addition, we incorporate simple forms of two other factors often invoked to explain column formation: retinotopy, and constraints on the total synaptic strength a single cell can support. We have examined the stability, under small perturbations, of the state in which both eyes equally and uniformly innervate cortex or tectum. In this way, we have developed an expression to predict the approximate width of periodicity that should emerge in terms of the afferent arbor widths, distances over which same-eye afferents have correlated activity patterns, and distances over which intra-cortical interactions occur.

Two arrangements of intra-cortical interaction will support column formation under this model: if the cortical interactions include sufficient inhibition, then column width is largely determined by the peak of the fourier transform of the function describing the interaction, though column width can be modulated by arbor width and correlation length. If the cortical interactions are purely excitatory and of a sufficiently long range, column formation can be caused by constraints on the total synaptic strength that an afferent can support. In this case, column width should be determined primarily by the arbor width.

These distinctions provide, at least in principle, a means to experimentally distinguish between different models. The distances associated with a proposed mechanism may be measured to determine whether they are consistent with the column width observed. In addition, parameters may be measured across species or experimental preparations to see whether a principal parameter responsible for column width can be identified.

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373.16 THE MATURATION OF THE EFFERENT PROJECTION FROM THE CORTEX TO THE VENTRAL NUCLEUS OF THE LATERAL GENICULATE BODY IN SYRIAN HAMSTERS. J. Hahm*, J.J. Ramirez*, S. Jhaveri*, and G.E. Schneider. (SPON: L.I. Benowitz). Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

Wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) when used in a 2% solution of dimethylsulfoxide, provides a sensitive label for tracing anterograde (and retrograde) neuronal projections in neonatal and adult animals. To study the development of the efferent projections from the visual cortex to the ventral nucleus of the lateral geniculate body (LGv), we injected the posterior cortex of hamsters (aged P2-P10, P14, P21 and adults) with WGA-HRP, allowed them to survive for 30 hrs. and processed their brains as described in the accompanying abstract (Ramirez, Hahm, Jhaveri and Schneider).

On P2 and P3, the dorsal nucleus of the lateral geniculate body and the lateral posterior nucleus are very heavily labelled. HRP-filled axons can be followed along the internal capsule and into the external medullary lamina, just medial to the LGv. However, the LGv itself is devoid of reaction product. A day later, on P4, a band of HRP reaction product, primarily axonal labelling, can be traced dorsoventrally through the middle of the nucleus. By P6, scattered grains of reaction product, present over the entire mediolateral extent of the nucleus, indicate the beginning of terminal arborization. The density of this granular precipitate rapidly increases to result in an adult-like pattern by P10.

These observations demonstrate that cortical efferents to the LGv follow a developmental time course similar to that of the corticotectal axon system (Ramirez et al., accompanying abstract), with projections to the LGv perhaps maturing slightly earlier. Both structures receive input from layer V cortical cells (Sefton et al., Brain Res., 215: 1-13, 1981). Both regions are also targets of other axon systems, e.g. the retinofugal fibers, which mature along a different developmental time-table. Comparing the maturation of multiple axon systems innervating single target structures underscores that, in addition to local cues, the cells of origin may play a major role in determining the early events of axon morphogenesis.

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373.17 DEVELOPMENT OF THE CORTICOTECTAL PROJECTION IN THE SYRIAN HAMSTER. J.J. Ramirez*, J. Hahm*, S. Jhaveri*, and G.E. Schneider. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

The maturation of projections from the visual cortex to the superior colliculus was studied with anterograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). The posterior cortex of hamster pups (day of birth=P0) was injected with either 10% WGA-HRP in a 2% dimethylsulfoxide solution or implanted with crystals of WGA-HRP in dimethylsulfoxide. Animals were labelled on consecutive days from P2 to P10, on P14, P21, and as adults. After a 30 hr survival, the hamsters were perfused and their brains processed for visualization of the HRP with tetramethyl benzidine as the chromogen. The age of the animal corresponds to the time of injection.

Although corticopontine efferents are labelled in our earliest material (P2-P3), the first labelled axons in the optic fiber layer of the superior colliculus are not detected until P4. For the next 3 days increasing numbers of corticotectal axons are present in the optic fiber layer and in the intermediate grey layer. However, a robust invasion of the target zone is not evident until P7, when a granular precipitate, indicative of terminal arborization, is observed in the superficial grey layer. By P10, the fibers have attained a pattern of innervation resembling that of adults.

Thus, corticotectal axons follow a morphogenetic progression similar to that previously described for retinofugal axons (Jhaveri, Edwards, and Schneider, Soc. Neurosci. Abstr., 9, 702, 1983). Relative to retinofugal axons, however, the developmental events of the corticotectal system are delayed by several days: cortical axons "wait" in the optic fiber layer during a period when retinal axons are actively arborizing in the superficial grey layer. This asynchrony in the development of two axon systems to the same target zone has interesting implications with regard to regulatory controls on the maturation of axonal morphology.

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373.18 GANGLIOSIDES AND DEVELOPMENTAL CORTICAL PLASTICITY: EVIDENCES FOR A ROLE OF MONOSIALOGANGLIOSIDE IN THE KITTEN VISUAL SYSTEM. G. Carmignoto*, R. Canella*, R. Siliprandi*, R. Nessi* and G. Kirschner* (SPON: S. Mazzari). Fidia Neurobiological Research Laboratories, Abano Terme, Italy.

We have previously reported that systemic monosialoganglioside (GM_1) treatment is capable of partially preventing the ocular dominance shift in striate cortex neurons in kittens subjected to either monocular deprivation (Carmignoto G. et al., J. Neurosci. Res., 12:477, 1984) or to reverse-suturing experiments (Carmignoto G. et al., Neuroscience Meeting, abs. 11, p. 1016, 1985). We now report the results obtained following continuous microperfusion of the visual cortex. A cannula micropump system containing GM_1 (10^{-3} M) or saline was implanted in the left visual cortex of 30 day-old-kittens. The day after the right eyelid was sutured for 5 days. At this latter time the eye was reopened and the ocular dominance distribution of area 17 neurons was assessed by conventional single unit recordings. Results indicate that a higher number of cells maintains a binocular response, in the hemicortex perfused with GM_1, but not with saline when compared to the non-perfused hemicortex. These data confirm that GM_1 is capable of partially preventing the loss of synaptic efficiency related to the deprived eye inputs. Moreover the development profiles of the different gangliosides of the visual cortex, including GM_1, were assessed from the fourth day after birth to adulthood. Data indicate striking developmental changes in the content and pattern of visual cortex gangliosides. GT_{1b} and GD_{1a} dramatically decrease after birth while GD_{1b} and, to a minor degree, GM_1 increase significantly reaching adult levels at 3 months of age (i.e. parallel to the occurrence of complete synaptic maturation of the visual cortex). Taken together these results suggest that GM_1 most probably plays a specific, although still poorly defined, role in the phenomenon of developmental neuronal plasticity of the visual cortex.

373.19 NEONATAL CEREBRAL HEMISPHERECTOMY BLOCKS DEVELOPMENT OF BINOCULAR DEPTH PERCEPTION IN CAT. D.A. Hovda, J.R. Villablanca and M.V. Phillips*. Mental Retardation Research Center, Departments of Anatomy and Psychiatry, School of Medicine, University of California, Los Angeles, 90024.

We studied the effect of neonatal or adult cerebral hemispherectomy on cats to see depth as measured on a visual cliff. Ten lesioned cats were studied, at least 6 months after surgery, with 6 sustaining a left hemispherectomy at a mean age of 11 days (range = 7-14) and 4 sustaining the same injury as adults. Two cats served as intact controls. The visual cliff consisted of a start box (19 X 45 X 22 cm) elevated 29 cm above a 58 X 60 cm sheet of glass covering two translucent 28 X 58 cm shelves each having a 3 X 3 cm black/white checkerboard design. The shelves were illuminated from underneath and were adjustable from 29 to 70 cm from the floor of the start box. Cats were tested, utilizing standard psychophysical staircase procedures, for both monocular and binocular thresholds. Neonatal-lesioned cats showed no binocular depth perception advantage over adult-lesioned or intact cats. Thus, neonatal-lesioned cats had binocular thresholds 114% higher than adult-lesioned animals and 135% higher than intact controls ($p<.01$). Thresholds for adult-lesioned cats were not statistically different from intact animals. Monocularly the two lesion groups were not statistically different, however both groups had higher thresholds than intact animals ($p<.01$) and adult-lesioned cats did show a slight right monocular deficit (N.S) compared to neonatal-lesioned cats. Interestingly, adult-lesioned cats made 89% of their errors to the left while neonatal-lesioned and intact cats showed no side differences. This suggest either a motor bias or a contralateral field deficit in adult-lesioned animals which is not present in neonatal-lesioned cats. These possibilities are under investigation. We conclude that neonatal-hemispherectomized animals did not develop binocular advantage for depth perception which would imply the lack of stereopsis. USPHS Grants HD 05958, HD 04612 and HD 07032.

373.20 BEHAVIORAL VALIDATION OF A PRIMATE MODEL OF HUMAN CONGENITAL APHAKIA. C. D. O'Dell*, R. G. Boothe, J. A. Gammon, and M. Tigges. Yerkes Regional Primate Research Center, Departments of Psychology, Ophthalmology, and Anatomy and Cell Biology, Emory University, Atlanta, GA 30322.

Children born with a monocular cataract are sometimes treated by surgical lensectomy of the cataractous eye followed by optical correction to a near point. This is usually accompanied by patching of the opposite eye. We have reared infant rhesus monkeys under various conditions of deprivation to model neonatal aphakia. Aphakia was produced by performing a unilateral lensectomy at about one week after birth under deep general anesthesia and sterile surgery. One week later the monkeys were fitted with a high power extended-wear lens that corrected the aphakic eye to a near point. At the time of lensectomy monkeys were also fitted with an occluder lens in the unoperated eye to continuously block light input. Monkeys of another group were continuously occluded in one eye while the other eye developed normally. Acuity was tested with a variation of forced choice preferential looking that has been used to assess acuity in infants (MacDonald et al., IOVS, 1985). The infant monkey is positioned in front of a screen with an acuity grating on the left or right side and a blank on the opposite side. An experimenter watches the monkey's eye movements and judges whether the infant monkey can see the grating based on looking preference. This is repeated for a series of gratings of different stripe widths at octave steps from 25 to 0.2 cycles/degree. Acuity estimates for a monkey were obtained monocularly and binocularly within a single testing period.

Monkeys tested within the first month after birth had acuities ranging from 1.6 to 3.2 cycles/degree regardless of the treatment condition. This is within the same range as normal monkeys tested during the first month with the same methods. During the next 5 months the acuity of the occluded eyes remained either at the birth range or decreased an octave. These results are similar to those obtained in animals with the other eye untreated. By contrast, the optically corrected aphakic eyes improved from 1 to 3 octaves by 6 months of age. These results are similar to that of monkeys with normal eyes tested at the same ages. The aphakic monkeys showed interocular acuity differences at 6 months ranging from 1 to 4 octaves. Our results from infant monkeys compare favorably to results obtained from aphakic human infants tested with similar methods.

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373.21 ONTOGENY OF RAT VISUAL EVOKED POTENTIALS. G. C. Rigdon, R. S. Dyer and W. K. Boyes. Neurophysiology Branch, Neurotoxicology Division, U.S.E.P.A., Research Triangle Park, NC 27711.

The development of flash evoked potentials (FEPs) and pattern reversal evoked potentials (PREPs) was investigated in awake, unsedated rats. Litters were crossfostered on day 1 (date of birth=day 0) and consisted of 4 males and 4 females. Pups (n=8-20) were tested daily from days 8 through 20 and every 3 or 4 days until day 41. Pups were chronically implanted with epidural recording electrodes over the visual and frontal cortex (reference) with a ground electrode.

FEPs were obtained by placing unrestrained pups in mirrored chambers, and averaging responses to 128 strobe flashes. On day 9, in some animals, a single negative wave was observed 200 msec or more following the flash. By day 11 the negative wave was present in all pups and was followed by a positive peak (P2?, >250 msec). On day 12 or 13 another negative wave (N1) appeared on the leading shoulder of the first. By day 15, the negative peak first seen on day 9 or 10 either disappeared or merged into N1. On day 14 a small positive peak (P1) with a latency of about 45 msec appeared and a negative peak (N3, latency > 275 msec) following P2 was observed. Peaks N2 and P3 were not seen in group average waveforms until after day 32 or 33. After eye opening (day 14-15), peak amplitudes increased and peak latencies decreased steadily. Latencies were near adult values by day 34.

Averaged evoked responses to 50 reversals of a black and white square wave grating were not detected until late in the third or early in the fourth postnatal week. Rats were trained to perch on a pedestal facing a monitor screen which displayed the gratings. Before the fourth week the PREP waveform consisted of two small negative peaks (around 100 msec and 220 msec respectively). Later, a positive peak (P2) developed between the two negative peaks. The amplitude of the late negative peak (N3) increased and the amplitude of the early negative peak (N2) decreased with time. The latencies of all peaks steadily decreased with time. In the middle of the fourth week another positive peak (P1) appeared on the leading edge of N2, and early in the fifth week, the waveform developed an early negative peak (N1, 45-55 msec).

By day 34 the response was similar in form to that seen in the adult (peaks N1-P1-N2-P2-N3 present). By day 37 the peak latencies and amplitudes were near adult values. (This research was supported by a National Research Council Research Associateship Award).

LIMBIC SYSTEM

374.1 DIFFERENTIAL ANTICONVULSANT EFFECTS OF CARBAMAZEPINE AS A FUNCTION OF STAGE AND TYPE OF KINDLING. R.M. Post, S.R.B. Weiss*, F. Szele*, and R. Woodward* (Spon: K.L. Zbicz). NIMH, Bethesda, MD 20892.

Kindling is the progressive development of seizures following repeated intermittent stimulation with either an electrical or a pharmacological stimulus. We have investigated the effects of carbamazepine (an anticonvulsant also used in the treatment of affective illness) on the development of electrically and pharmacologically kindled seizures and on fully developed kindled seizures. Electrical kindling of the amygdala was accomplished by once-daily stimulation for one second at 800 μamps; pharmacological kindling was induced by once-daily intraperitoneal administration of lidocaine or cocaine (65 mg/kg).

In the electrical stimulation procedure, carbamazepine was not effective in preventing the development of amygdala-kindled seizures when given either intraperitoneally (15 mg/kg), or in the diet (150 mg/day). This same dose of carbamazepine was, however, effective in suppressing fully developed amygdala-kindled seizures. In contrast, the rats given carbamazepine in their diet showed a marked suppression of the development of lidocaine-kindled seizures and a modest suppression of the development of cocaine-kindled seizures and lethality. However, carbamazepine (10-50 mg/kg) was ineffective in suppressing completed local anesthetic-kindled seizures and also increased lethality to acute high doses of cocaine.

Thus, carbamazepine blocks completed but not developing electrical-kindled seizures and, conversely, blocks developing but not completed local-anesthetic-kindled seizures. These data suggest that the biochemical and/or neuroanatomical substrates of limbic seizures differ as a function of both stage and type of kindling.

374.2 SUSCEPTIBILITY TO SEIZURES INDUCED BY PILOCARPINE IN DEVELOPING RATS. D.F. Silva*, L.S. Calderazzo-Filho*, L. Turski and E.A. Cavalheiro*. Dept. of Neurol. Neurosurg., Lab. of Exp. Neurol., Escola Paulista Med., CEP-04023 Sao Paulo, SP, Brazil and Dept. of Pharmacol., Med. Sch., PL-20-090 Lublin, Poland.

The pilocarpine (PILO) model of epilepsy offers the attractive features of allowing intractable seizures and status epilepticus to be induced rapidly after application of the drug, and spontaneous motor seizures to be observed in the long-term periods after treatment. This model of seizures resembles the functional impact of human temporal lobe epilepsy in that rats and mice undergoing a treatment with PILO display morphological alterations in the brain comparable in many aspects to those known from human pathology. It is not firmly established, whether seizures and seizure-related brain damage induced by PILO in adult rats differ from those produced by the drug during the development. The present study was therefore undertaken to complement previous investigations in adult rats by comparing behavioral, electroencephalographic (EEG) and neuropathological features of seizures produced by PILO in developing rats. PILO hydrochloride, 100-380 mg/kg, was administered systemically in 3-90 day -old Wistar rats. PILO, 100-380 mg/kg, presented characteristic array of behavioral patterns in immature rats. Hyperactivity, stratching and mild tremor with myoclonic movements of head and limbs, dominated the behavior in 3-14 day-old rats. The EEG activity in the hippocampus progressed from high-voltage spiking registered during the first week of life into spiking and discharges, which spread to cortical recordings during the second week of life. No morphological alterations were detected in the brains of 3-14 day-old rats subjected to the action of PILO, 380 mg/kg. The adult pattern of behavioral and EEG sequelae of PILO was encountered in 15-21 day-old rats. After PILO, 380 mg/kg, akinesia, tremor and head bobbing progressed to motor limbic seizures with rearing, forelimb clonus, intense salivation and falling, and status epilepticus. The lethal toxicity in rats subjected to PILO, 380 mg/kg, at the age of 15-28 days, reached about 60%. Immediately after administration of PILO, 380 mg/kg, high voltage fast activity superposed over hippocampal theta rhythm, progressed (10-20 min) into high-voltage spiking and spread to cortical records. The EEG activity became well synchronised and then developed into seizures and status epilepticus. Morphological analysis of frontal forebrain sections from 15-28 day-old rats, which underwent status epilepticus after PILO revealed widespread damage to the hippocampus, thalamus, amygdala, olfactory cortex and neocortex. The extent and distribution of brain damage in 15-28 day-old rats resembled well those detected in the brains of mature rats. The lethal toxicity rate progressively decreased and reached the mature level of about 10% in 36-43 day-old rats. The present study demonstrates age differences in the susceptibility of rats to convulsions elicited by PILO. Supported by FAPESP, CNPq and FINEP (Brazil).

374.3 EPILEPTOGENESIS IN AMYGDALA NEURONS: ACUTE AND CHRONIC MODELS. Po-Wu Gean* and Patricia Shinnick-Gallagher. Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston, TX 77550.

Experimental and clinical evidence have indicated that the amygdala is important in the initiation and spread of seizures. The present research sought to develop a brain slice of the amygdala as a model of epilepsy and to analyze new agents for use as antiepileptics. Rat brain tissue containing the basolateral amygdaloid nucleus (BLAN) was sectioned on a Vibratome (500 uM) in the coronal plane. Intracellular recordings were made using 4 M K acetate-filled microelectrodes and visually placed in BLAN. Stimulation of the stria terminalis pathway to the amygdala evokes an EPSP-IPSP sequence. In 7 neurons tested the IPSP reversed polarity at -74 mV which is close to equilibrium potential for chloride in other CNS neurons. Application of convulsants, picrotoxin (50 uM) or bicuculline (10 uM), reduced the IPSP and resulted in the development of paroxysmal depolarizing shifts with afterdischarges. Spontaneous paroxysmal depolarizing shifts were observed in higher concentrations of picrotoxin (100 uM). Excitatory amino acid antagonists, DL-2-amino-5-phosphonovalerate (DL-APV, 50 uM) and γ-D-glutamylglycine (γ-DGG, 100 uM) reversibly depressed the epileptiform burst firing. The effect of phencyclidine (PCP, 1 uM) was similar but not reversible. These results suggest that N-methyl-d-aspartate (NMDA) receptors may contribute to epileptiform activity induced by convulsants in BLAN neurons. Kindling, a chronic model of epilepsy, was produced by daily administration of a subconvulsive stimulus through an electrode implanted in the basolateral nucleus of the amygdala. A contralateral brain slice was prepared 28 days after kindling was complete. Stimulation of the stria terminalis evoked epileptiform activity in a kindled rat. DL-APV, (50 uM) as well as atropine (0.5 uM) antagonized the kindled epileptiform activity. These data suggest that chronic epileptiform activity induced in vivo is maintained in the amygdala brain slice and that NMDA and muscarinic receptors may be involved in amygdala kindling.

374.4 KINDLING-LIKE STIMULI INDUCE A REDUCTION OF INHIBITION AND EPILEPTIFORM DISCHARGES IN THE *IN VITRO* HIPPOCAMPUS. A. Stelzer*, N.T. Slater and G. ten Bruggencate. Physiology Institute, University of Munich, Pettenkoferstrasse. 12, D-8000 Munich 2, W. Germany, and Department of Physiology, Northwestern University Medical School, Chicago, IL 60611 U.S.A.

The application of repeated tetani to the Schaffer collateral-commisural fiber pathway to CA1 neurons in the guinea-pig hippocampus *in vitro* produces a progressive enhancement of synaptic efficacy associated with long-term potentiation leading to the genesis of both spontaneous and stimulation-evoked epileptiform discharges[1]. This tetanus-induced hyperexcitability was not associated with altered cellular excitability, but was synaptic in origin and dependent on the repeated activation of NMDA receptors[1]. Previous *in vitro* models of epilepsy have stressed the importance of changes in synaptic inhibition as an underlying cause for increased synaptic excitation and the genesis of paroxysmal depolarization shifts (PDSs) in hippocampal neurons. We have explored to what extent altered synaptic inhibition may contribute to the epileptiform activity induced by this *in vitro* model of kindling. Intra- and extracellular recordings were made in the stratum pyramidale of guinea-pig hippocampal slices in which the CA3 subfield was removed by dissection as previously described[1]. Tetani (5 trains at 50 Hz, 2 s, delivered at 10 s intervals) were applied to the stratum radiatum every 30 min and the orthodromic ipsps evoked by test stimuli applied to stratum radiatum, or antidromic ipsps evoked by stimulation of the alveus were examined 25 min after each preceding tetanus. Depolarizing and hyperpolarizing current pulses were also applied to test for changes in input resistance and cellular excitability. The application of kindling-like tetani produced a progessive decrease in the magnitude of both phases of the orthodromic ipsps, as well as a reduction of the monophasic antidromic ipsp. After 4 tetani ipsps were reduced to 20% or less of control values. No changes in mean resting potential, input resistance, spike accomodation or afterhyperpolarizations were associated with these marked decreases in ipsp amplitude. Spontaneous ipsps recorded with KCl-filled microelectrodes were similarly affected, being reduced in both frequency and amplitude to 10% or less after 4 tetani. This reduction of both stimulus-evoked and spontaneous ipsps was matched by the appearance of both stimulus-evoked and spontaneous PDSs when higher stimulus intensities were applied. In contrast to this reduction of ipsps induced by tetani in normal solution, the application of tetani to slices in the presence of the NMDA receptor antagonist D-APV had no effect on ipsps. Following the washout of D-APV further tetani then produced a reduction of ipsps. These results provide evidence for the hypothesis that both the reduction of inhibition and the development of epileptiform activity in this *in vitro* model of epilepsy are mediated through the activation of NMDA receptors within synaptic pathways intrinsic to the CA1 hippocampal subfield.

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[1]N.T. Slater, A. Stelzer and M. Galvan. Neuroscience Lett., 60: 25-31 (1985).

374.5 GOLGI STUDIES REVEAL DIFFERENCES IN DENDRITIC SPINES BETWEEN SEPTAL KINDLED RATS AND CONTROLS. L.A. Paul[1,*] and C.G. Wasterlain[2] (SPON: Y. Gerson). [1,2]Dept. of Neurology, School of Medicine, UCLA, Los Angeles, CA 90024, and [2]Dept. of Neurology, VAMC, Sepulveda, CA 91343.

Repeated low-intensity electrical stimulation through implanted electrodes for small amounts of time leads to progressively increasing afterdischarges and eventually elicits seizures in several species ("kindling"). Although the behavioral and electrophysiological changes associated with kindling are dramatic, no clear-cut anatomical alterations have been noted. In search of morphological correlates of the kindling phenomenon, we kindled rats through the medial septum (400 μA, 1 sec., 60Hz, AC; once per day) and stained the brains with a Golgi variant.

Horizontal brain sections from 8 rats (4 kindled, 4 controls) were cut and mounted on glass slides and the slides re-coded to eliminate experimenter bias. Photographs of well-stained spined dendrites in hippocampal region CA3, both apical and basilar, which met certain criteria were taken through the microscope at 100X by one investigator (L.P.). This resulted in a pool of dendrites of different orders (secondary, tertiary, quaternary) and different diameters.

Following the establishment of criteria based on careful viewing of these dendrites at high magnification prior to slide relabelling, the photographs were ranked according to their conformity to morphological criteria by the other investigator (C.W.). These criteria included spine shape (long, with narrow neck vs. short and stubby) and overall spine heterogeneity on a particular dendrite. Spines of a given order were analyzed separately. No differences were seen between kindled and control rats for apical shafts, secondary apical dendrites, or for basilar dendrites.

Third- and fourth-order segments from the two groups, however, were successfully distinguished. Using the Mann-Whitney test for two independent samples, we established a difference between kindled and control rats ($p < 0.01$). Kindled animals had dendritic spines which were shorter, stubbier, and more homogeneous, while control spines appeared longer, with narrow necks. Along a control dendrite, more varied spine morphologies were found.

We are continuing qualitative and quantitative analyses of this problem, using additional control groups.

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374.6 THE RETICULAR THALAMIC NUCLEUS: A PACEMAKER FOR SPINDLE RHYTHMS. M. Steriade, L. Domich, M. Deschênes and G. Oakson*. Lab. Neurophysiol.,School of Medicine,Univ.Laval,Québec,Canada G1K 7P4.

Sequences of EEG spindle waves (7-15 Hz) recur periodically (0.1-0.3 Hz) during natural sleep or barbiturate anesthesia, and are generated within the thalamus in the total absence of cerebral cortex and brainstem. After disconnection from inputs arising in the reticular thalamic nucleus (RE), spindle rhythms are abolished in thalamocortical neurons despite the fact that basic intrinsic properties are preserved in these elements (J. Neurophysiol. 1985, 54: 1473-1497). We now report that spindle rhythmicity persists in deafferented RE neurons.

Unit discharges and focal waves were extracellularly recorded in the rostral pole of RE nucleus which was completely disconnected by transections from all other thalamic nuclei. In some experiments, cortical afferents to the rostral RE pole were also severed by appropriate transections. Similar results were obtained in two types of acute experiments: brainstem-transected preparations that exhibited spontaneous spindle sequences and animals under ketamine anesthesia in which transient spindling was repeatedly precipitated by low doses of a short-acting barbiturate.

Both spindle rhythms (7-15 Hz and 0.1-0.3 Hz) were seen in focal recordings of the deafferented RE nucleus. The presence of spindling rhythmicity in the disconnected RE nucleus contrasted with total absence of spindles in cortical EEG leads and in thalamic recordings behind the transection. Oscillations within the same frequency range as that of spontaneous spindles were evoked in the deafferented RE nucleus by subcortical white matter stimulation. Quantitative group data showed that the burst parameters of disconnected RE cells were in all respects identical to those of RE neurons with intact connections (J. Physiol.Lond., in press). In the deafferented RE nucleus, spike bursts of RE neurons recurred periodically in close time-relation with simultaneously recorded focal spindle sequences. The great reduction in burst occurrence of deafferented RE cells after systemic administration of bicuculline supports the assumption that rhythmic spike bursts of RE cells are generated through intra-RE inhibitory interactions (Brain Res. 1985, 334: 165-168).

The preservation of both spindle-related rhythms in the disconnected RE nucleus, together with the abolition of spindle oscillations in thalamic nuclei after lesions of RE nucleus, demonstrate that RE nucleus is the pacemaker of spindle rhythms.

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374.7 BEHAVIORAL DYSFUNCTION FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID: NEUROANATOMICAL AND NEUROPHYSIOLOGICAL CORRELATES. N.W. Milgram. Dept. of Psychology, Scarborough Campus, Univ. Toronto, West Hill, Ont., Canada M1C 1A4.

Systemic administration of kainic acid (KA) can trigger recurrent convulsive seizures and a subsequent pattern of brain damage which closely corresponds to the brain damage observed in temporal lobe epileptics. The present research studied the long-term behavioral consequences of treatment with KA. Rats received a subcutaneous injection of 10 mg/kg, a one week recovery interval, and were then tested for activity in an open field, acquisition of a passive avoidance response, and for spatial learning ability using a water maze. There were highly significant differences between the KA group and controls on all three tasks; KA produced an increase in activity and retardation in passive avoidance and spatial learning. The groups were subsequently retested after a two month recovery period. The KA group showed poor retention of previously acquired passive avoidance, a significant deficit in the acquisition of a new passive avoidance, and significantly more activity than the controls. However, the performance on a spatial learning task was no longer different from that of the controls.

The behavioral deficits were correlated with both anatomical and neurophysiological consequences of KA. Passive avoidance and open field activity correlated with extent of damage to a ventral forebrain region which included the piriform cortex, entorhinal cortex and amygdala. Water maze performance was found to vary with efficacy of intrahippocampal synaptic transmission. This was studied by tracing field potentials triggered in Ca1 by stimulation of CA3, and potentials triggered in dentate gyrus by stimulation of angular bundle. In both pathways, field potentials were initially abolished but showed a recovery over several weeks. Recovery of spatial learning followed a similar time course.

The results suggest that KA produces both permanent behavioral deficits, which are related to degenerative processes, and more transient deficits which may be a consequence of intense epileptiform activity. This preparation may provide a useful animal model for studying behavioral deficits resulting from temporal lobe dysfunction. Relevant human neurological disorders include temporal lobe epilepsy and Alzheimer's disease.

374.8 NEUROCHEMICAL, HORMONAL AND BEHAVIORAL RESPONSES TO GRADED STRESSORS IN ISOLATE RATS. R.R. Holson*, S.F. Ali, A.C. Scallet, G.D. Newport* and W. Slikker, Jr.*. (SPON: R.E. Stull). Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Isolation rearing involves the deliberate withholding of most forms of experience. When animals reared in isolation are then exposed to unfamiliar stimuli later in life, their response is often one of fear, presumably due to the high novelty of most stimuli. To determine whether isolation rearing causes a primary increase in fear or timidity, two experiments were conducted. In both, subjects were male and female albino rats. All subjects were reared either in islation (ISOL) or in social pairs (SOC) from weaning on postnatal day (PND) 21 until testing began on PND 90. At that time, rats from each experiment were subdivided into three graded stress groups. In Experiment 1, stressors were handling, no handling, or prior electroshock. All subjects were then given four behavioral tests: open field activity, emergence latency, auditory startle response, and latency to accept food from the experimenter. In Experiment 2, the three graded stressors were a 5-minute forced swim, a 20-minute forced swim, or no swim. Subjects were decapitated at the end of the swim, blood was collected, and brains were dissected into frontal pole, caudate nucleus and nucleus accumbens. Plasma was assayed by RIA for corticosterone. Brain regions were assayed for dopamine (DA) and metabolites (DOPAC and HVA) by HPLC with electrochemical detection. In neither experiment was there an interaction between stress and rearing condition. However, a variety of rearing effects was seen. On the behavioral tests, isolates showed slowing of adaptation to the open field, a reduction in auditory startle amplitude, reduced freezing on the emergence task, and increased willingness to eat from the experimenter's hand among handled rats. Isolation caused a drop in the concentration of DOPAC and HVA in frontal pole but not caudate nucleus, as reported by Blanc et al. (1980). Plasma corticosterone levels were increased in a graded fashion by the forced swims, but there was no difference between rearing conditions. Thus, these experiments provide no evidence that isolation rearing produces a primary, global increase in fearfulness. They do suggest that such rearing may impair development of prefrontal cortex, leading to a rather general disinhibition of prepotent behavioral responses.

374.9 **INDUCTION OF HIPPOCAMPAL LONG-TERM POTENTIATION USING PHYSIOLOGICALLY PATTERNED STIMULATION.** G.M. Rose and T.V. Dunwiddie. Medical Research Service, VAMC, and Dept. of Pharmacology, UCHSC, Denver, CO 80220.

Within the last decade, much of the search for physiological substrates for memory has focussed upon an electrophysiological phenomenon called long-term potentiation (LTP). LTP is manifested as a long lasting increase in synaptic efficacy following high frequency electrical stimulation; it occurs in seconds and may last for weeks, and thus meets the temporal criteria for a mnemonic process. A problem with relating LTP to an endogenous mechanism for memory formation is that the stimulation parameters commonly used for its induction are well beyond the normal physiological firing range of hippocampal neurons. We considered the possibility that the threshold for the induction of LTP might be reduced if the pattern of stimulation more accurately reflected normal neuronal firing.

Experiments were conducted using the in vitro hippocampal slice preparation. Responses were recorded in the CA-1 pyramidal cell layer following activation of the commissural/associational afferents. Stimulus intensities were adjusted to evoke a 1-2 mv population spike. Lasting (> 30 minute) increases of the population spike could be evoked by the patterned presentation of as few as 4 stimulus pulses. The most effective pattern of stimulus presentation was a single priming pulse followed 170 ms later by a short train of 3 or more pulses at 100 Hz; control trains of 5 pulses at 25 or 100 Hz had no enduring effect. The most effective interval between the priming pulse and subsequent stimuli was approximately 140-170 ms; intervals less than 100 ms or greater than 500 ms had little long-term effect on response amplitude. A total of at least 4 stimuli (1 + 3) was necessary to elicit a lasting increase in the population spike; from this threshold, the magnitude of the change was proportional to the number of stimuli delivered following the priming pulse (up to 10). This primed burst (PB) potentiation was similar in both timecourse and duration to the LTP induced by 100 Hz/1 sec stimulation. However, unlike LTP in CA-1, PB potentiation could be induced heterosynaptically if the priming pulse and subsequent train were delivered to different dendritic fields; in these cases, only the last-stimulated input was potentiated. Antidromic activation of the pyramidal cells was not effective as a priming pulse. The potentiating effects of PB and LTP trains were not additive, suggesting that they share a common mechanism.

In summary, patterns of afferent stimulation well within the physiological range can induce lasting increases in the efficacy of hippocampal connections.

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374.10 LONG TERM POTENTIATION IN THE DENTATE GYRUS: INDUCTION BY ASYNCHRONOUS VOLLEYS IN SEPARATE AFFERENTS. J. Winson and D. Dahl The Rockefeller University, New York, N.Y. 10021

Long term potentiation (LTP) of perforant path (PP)-granule cell synaptic efficiency was first reported following trains of stimuli applied to the PP at 10-100 Hz for 2-20 sec. It was subsequently reported that LTP was most reliably elicited by stimuli applied at 400 Hz (Douglas, Brain Res., 126:361, 1977) and shorter trains of pulses at this frequency are currently utilized. The present study was undertaken to quantify the relative efficiency of 400 Hz stimulation in inducing LTP in the dentate gyrus and to ascertain how this high frequency effect might be realized in the normal animal.

In urethane-anesthetized rats, low intensity, repetitive (LTP) stimulation (5 trains of 5 pulses each, trains separated by 1 sec) were applied to the granule cells via either the medial or lateral PP. The frequencies of stimulation were either 100, 200, 300 or 400 Hz. LTP current levels were raised incrementally until LTP of the EPSP was achieved. Significant and substantial LTP was preferentially induced by 400 Hz stimulation as compared to the lower frequencies.

Similar low intensity stimulation was then applied at 200 Hz to either the medial or lateral PPs separately, to both pathways simultaneously (achieved by aligning the respective fiber volleys) or asynchronously. In the asynchronous condition, stimulation from one pathway was delayed 2.5 msec with respect to the other, thus providing a composite 400 Hz stimulus to the granule cell dendrite. LTP current levels were raised incrementally until LTP was observed. Substantial and significant LTP of both the EPSP and population spike was induced preferentially by asynchronous stimulation.

These data show a clear sensitivity of LTP to 400 Hz stimulation and further suggest that, in the normal animal, lower frequency physiological input volleys arriving asynchronously at medial and lateral PP synapses may induce LTP by activiting a 400 Hz sensitive mechanism that is capable of integrating spacially separated granule cell inputs.

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374.11 EFFECTS OF ALCOHOL ON PAIRED PULSE INHIBITION AND POTENTIATION ON CA1 OF THE RAT HIPPOCAMPUS. J.F. Ott*, B.E., Hunter and D.W. Walker. Univ. of Florida Col. of Med. and V.A. Hospital, Gainesville, FL 32610

This study was conducted as part of an ongoing project to understand the effects of feed forward (FFI) and recurrent inhibition (RI) in the CA1 of the rat hippocampus and to understand how chronic ethanol treatment (CET) affects these inhibitory mechanisms. To help dissociate FFI from RI effects, paired-pulse stimulation was delivered to CA1 in either orthodromic/orthodromic (O/O) or antidromic/orthodromic (A/O) pairs. To evaluate ethanol effects on O/O and A/O pairs, in vivo experiments were conducted in rats having CET or in sucrose pairfed 10 month old male Fischer 344 rats.

Rats were given a nutritionally complete ethanol (group E) or sucrose-containing (group S) liquid diet for 20 weeks. Ad lib lab chow and water were then given to the animals for at least 8 weeks prior to acute electrophysiological manipulations. Concentric bipolar electrodes were placed in stratum radiatum at the CA1-CA2 border for orthodromic stimulation and tin the alveus for antidromic stimulation. Glass micropipettes were positioned in stratum oriens or radiatum to record EPSP or population spike (PS) responses. To allow comparison between animals, percentages of PS maximum or EPSP at PS threshold were used rather than absolute amplitudes. Orthodromic condition pulse intensities of 25% and 75% of the PS maximum were used as were the current intensity at the PS threshold and 25%, 50% and 75% of the EPSP at PS threshold. Antidromic condition current intensities were either 25% or 75% of the antidromic PS maximum. Test pulse current intensities were either 25% of the PS maximum or 50% of EPSP at PS threshold. Interpulse intervals ranged from 20-3000 msec.

CET produces effects in CA1 with paired pulse in Fischer-344 rats similar to effects found in Long-Evans rats, as we previously reported (Rogers, et al., Soc. for Neurosci. Abstr., 10:305.7, 1983). While there are few statistically significant alterations in PS amplitude or the EPSP, there are a number of trends. With A/O stimulation, one finds group E less inhibited at IPI's longer than 30 msec than group S. With O/O stimulation, group E and group S show similar test pulse amplitudes when paired with lower amplitude conditioning pulses (25, 50 and 75% EPSP at PS threshold) at all IPS's. At greater amplitude test pulses (threshold and 25 and 75% PS maximum, group E showed similar inhibited PS amplitudes at short IPI's (20, 30 msec), but were much more potentiated than group S at IPE's longer than 30 msec.

These findings indicate that CET increases potentiation, but only in O/O paired pulses and only at longer IPI's.

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374.12 INFLUENCE OF VARIOUS DELAYS OF REINFORCEMENT UPON ACQUISITION OF OPERANT AUTOSHAPED BEHAVIOR IN 25 DAY OLD RATS. C.A. Cohen, M.E. Lipton*, and S.B. Sparber. Depts. of Psychology and Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Previous studies indicate that male Long Evans rats with limbic system lesions caused by the organometal neurotoxin trimethyltin can learn a forward, food reinforced autoshaped lever touch behavior, if the food reinforcer is delivered immediately. However, if a six second delay is interposed between the lever retraction and the food pellet delivery, the lesioned subjects do not learn the task (Cohen et al, Neurosci. Abs., 10,1205,1984). Because the hippocampus is not fully developed in 25 day old rats, we hypothesized that a similar profile would emerge when comparing their ability to learn the task with 0 and 6 sec delays of reinforcement. We also included a group which was required to associate lever presentation with delivery of the reinforcer after a 3 sec delay, to more completely characterize the effect of delay of reinforcement. Lastly, male and females maintained at normal growth rates were compared with neonatally malnourished rats (modified Slob technique, Sparber and Lichtblau, J.P.E.T., 225, 1983) so that we could determine if early undernutrition can affect performance of this task.

Rats were exposed to operant chambers equipped with retractable levers that were extended into the chambers on a random time 45 sec schedule, and withdrawn either when the animal made a lever touch or 15 sec had elapsed. When the lever was retracted a food pellet was delivered immediately or 3 or 6 sec later. All subjects were autoshaped for 10 consecutive days of 24 trials/session. A repeated measures ANOVA of the 0 sec delay indicated that all groups except the control females learned the task. The results from the 3 sec delay task showed that 6 of 20 animals including at least one subject in each group learned the task. However, with the exception of one undernourished female, no subjects learned the 6 sec delay autoshape task. It would appear therefore, that a functioning hippocampus is necessary to learn the autoshaping task with a 6 sec delay of reinforcement. The generally elevated performance seen in the undernourished females suggests that early postnatal malnutrition may induce some non-specific behavioral activation in these animals. Present studies are underway using 45 day old littermates to further investigate these effects.

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374.13 THE RETROSPLENIAL CORTEX MAY TRANSMIT LEARNING-RELATED INFORMATION FROM HIPPOCAMPUS TO CEREBELLUM. C.L. Weikart*, J.L. Bassett, E.S. Nisenbaum and T.W. Berger. Department of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Classical conditioning of the rabbit nictitating membrane (NM) response is associated with increased firing of hippocampal pyramidal neurons (Berger et al., J. Neurophysiol., 1983), and neurons of the deep cerebellar nuclei (McCormick and Thompson, Science, 1984). Lesions of the hippocampus produce severe deficits in reversal though not discrimination learning (Berger and Orr, Behav. Brain Res., 1983), whereas lesions of the deep cerebellar nuclei result in failure to develop a conditioned response during any paradigm (McCormick et al., PNAS, 1982). We will present data supporting the hypothesis that learning-related hippocampal activity affects NM behavior through multi-synaptic projections to the cerebellum via a hippocampo-subiculo-retrosplenial-ventral pontine circuit.

We have shown previously that the subiculum projects to layers I and IV of the retrosplenial cortex. Anatomical studies examined the distribution and relative strengths of subcortical efferents from the rabbit retrosplenial cortex using autoradiographic techniques. It was found that one of the two most prominent projections was to the lateral, ventral and paramedian pontine nuclei, and arises from layer V of retrosplenial. Thus, output from retrosplenial cortex can influence one of the major sources of mossy fiber input to the cerebellum.

Electrophysiological studies examined latencies to activation of neurons within the rabbit subiculum and retrosplenial after electrical stimulation of the hippocampus and subiculum, respectively. Stimulation of the CA1 pyramidal region resulted in 2-3 ms orthodromic activation of subicular neurons. Stimulation of the subiculum resulted in 3-4 ms latency excitation of layer V retrosplenial neurons. CS-evoked pyramidal cell firing in hippocampus precedes conditioned NM responding by 45 ms. Thus, hippocampal activity can influence layer V retrosplenial neurons long before onset of conditioned NM responding.

Lesion studies tested the hypothesis that interrupting the proposed circuit would produce behavioral deficits similar to those observed after hippocampectomy. Rabbits were given either aspiration lesions of the hippocampus or electrolytic lesions of the retrosplenial cortex. Other animals were given lesions of the neocortex overlying these regions. After recovery, the NM response of all animals was classically conditioned using a tone-light discrimination-reversal paradigm. Damage to either the hippocampus or the retrosplenial cortex, but not the neocortex, resulted in nearly identical, severe retardation of reversal learning. Learning of the initial discrimination was not affected. Supported by NSF (83-16464) and NIMH (MH00343).

374.14 INFLUENCE OF CONTRALATERAL HIPPOCAMPUS ON NONLINEAR PROPERTIES OF PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION. R.L. Port, R.J. Sclabassi & T.W. Berger, Departments of Psychology & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Characterization of the functional properties of the perforant path-dentate (PP-DG) circuit has revealed that dentate granule cell response is nonlinear when the frequency of PP stimulation approximates the spontaneous firing characteristics of entorhinal neurons (Robinson et al., Soc. Neurosci. Abstr., 11, 1985). Among several pathways which influence dentate granule cell excitability are commissural projections from the contralateral hilus. To determine the relative contribution of the commissural input, the experiments reported here examined the system properties of the PP-DG circuit following surgical removal of the contralateral hippocampal formation.

Adult, male albino rabbits received aspiration lesions of the contralateral hippocampal formation and/or parietal neocortex. Two weeks later, bipolar stimulating electrodes were placed in the PP and a recording electrode positioned in the granule cell layer of the DG. Two weeks after implantation, random impulse trains (4064 impulses, Poisson distribution with a mean frequency of 2.0 Hz) of electrical stimulation were applied to the PP and evoked field potentials were recorded from the DG. Stimulation intensity was adjusted to elicit a population spike 10-20% of the maximum amplitude elicited by impulses delivered once every 10 s. First, second, and third order kernels were computed using cross-correlation techniques.

Data from control subjects were consistent with previous analyses of intact subjects (Berger et al., Soc. Neurosci. Abstr., 9, 1983). Second order kernels, which reflect the modulatory influence of the preceding impulse on the excitability of the system, indicated that inter-impulse intervals of short duration (<30 ms) resulted in a virtual suppression of the population spike. At intervals between 50 and 400 ms, facilitation of spike amplitude occurred; peak facilitation (30-60%) occurred at intervals of approximately 100 ms. In contrast, second order kernels from animals with contralateral hippocampal damage revealed a prolongation of inhibitory interactions (up to intervals of 150 ms) and a degradation of facilitatory interactions (which peaked at <20% to intervals of approximately 200 ms). Third order kernels, which reflect the modulatory influence of the preceding pair of impulses, also were affected. Most notably, short intervals which produce robust inhibition in control animals, resulted in a profound facilitation of the population spike in animals with unilateral hippocampal damage. These findings suggest that the contralateral hippocampus may play a prominent role in determining granule cell excitablity in response to perforant path activation. Supported by the Whitaker Foundation and NIMH (MH00343).

374.15 NONLINEAR CHARACTERISTICS OF HIPPOCAMPAL PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION VARY AS A FUNCTION OF POPULATION SPIKE LATENCY. J.L. Eriksson*, D.A. Ciarolla*, R.J. Sclabassi and T.W. Berger (SPON: S. Wolfson). Departments of Psychology, Psychiatry and Neurological Surgery, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We recently have used random impulse train stimulation of the perforant path and systems analysis techniques to examine nonlinearities in dentate granule cell discharge in response to a wide range of inter-impulse intervals (Berger et al., Soc. Neurosci. Abstr., 9, 1983). Amplitude of the evoked population spike was used as the measure of dentate granule cell output. Using an extension of Wiener kernel analysis, results showed that when population spikes of all latencies were included in the calculations, inter-impulse intervals $\leq$ 20 ms produced inhibition of population spike amplitude, longer intervals (50-300 ms) produced a marked facilitation, and intervals of approximately 300-700 ms resulted either in a weak facilitation or inhibition depending on whether or not the preparation was anesthetized. Here we report the results of computing separate first and second kernels for population spikes of different latencies, to determine if nonlinearities vary as a function of postsynaptic cell excitability and/or the subpopulation of perforant path fibers stimulated.

Experiments were conducted using halothane anesthetized and unanesthetized, chronically implanted rabbits. A random interval train (Poisson distribution, λ=500 ms, range=1 ms-5 s) was used to determine the sequence of 4064 single shock (0.1 ms duration) stimulations of the perforant path. Stimulation intensity was that producing a population spike amplitude 10-20% of maximum in response to single impulses delivered once every 10 s. Evoked population spikes were divided into three groups based on latency ("short", "moderate" and "long"), and first and second order kernels were computed for each group.

Results showed that second order kernels were significantly different depending on the latency of population spikes included in the calculations. Short latency spikes exhibited only facilitative interactions in response to inter-impulse intervals of 1-5 ms. Moderate latency spikes were inhibited by intervals $\leq$ 20 ms, facilitated by intervals of approximately 50-300 ms, and inhibited by intervals of approximately 300-700 ms. Long latency spikes were inhibited to inter-impulse intervals as long as 100 ms in unanesthetized animals, facilitated to intervals between 100-600 ms, and never exhibited inhibition to intervals longer than 600 ms. These data demonstrate that the perforant path-dentate projection is not homogeneous, and incorporates functionally different systems. Supported by The Whitaker Foundation and NIMH (MH00343 and MH30915).

374.16 COMPARISON BETWEEN NONLINEARITIES IN HIPPOCAMPAL PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION REVEALED BY RANDOM IMPULSE TRAIN AND PAIRED IMPULSE STIMULATION. R.J. Sclabassi, J.L. Eriksson*, D.A. Ciarolla*, and T.W. Berger. Departments of Psychology and Neurological Surgery, Univ. of Pittsburgh, Pittsb., PA 15260.

Paired impulse stimulation with varying inter-impulse intervals traditionally has been used to study the dependence of dentate granule cell discharge on frequency of perforant path input. We recently have developed an alternative methodology involving random impulse train stimulation and nonlinear systems analysis. In the experiments reported here, we compared the effects of random train and paired impulse stimulation by applying both procedures to the same preparations.

Experiments were conducted using halothane anesthetized and unanesthetized, chronically implanted rabbits. For random train stimulation, a Poisson distribution with λ=500 ms and a range of 1 ms-5 s was used to determine the sequence of 4064 single shock stimulations of the perforant path. For paired impulse stimulation, inter-impulse intervals varied between 10-1200 ms (pairs delivered every 20 s). Amplitudes of evoked population spikes were used to measure granule cell output. Stimulation intensity was that producing spike amplitude 10-20% of maximum in response to single impulses (delivered every 10 s).

Results showed that systems analysis and paired impulse procedures revealed significantly different nonlinearities in perforant path-dentate synaptic transmission. Second order kernel analysis of random train data showed that, in anesthetized preparations, inter-impulse intervals $\leq$ 20 ms produced inhibition of population spike amplitude, longer intervals resulted in facilitation maximal at 90-100 ms, and intervals between 300-700 ms produced a slight inhibition (maximum 30%). Data from unanesthetized preparations showed similar trends except that the magnitude of facilitation to 90-100 ms intervals was significantly smaller, facilitation extended to intervals as great as 1000 ms, and thus no inhibition to intervals in the range of 300-700 ms (or longer) was observed. In contrast to these results, paired impulses in both anesthetized and unanesthetized animals produced near-total inhibition when intervals $\leq$ 20 ms, facilitation that was maximal at 50-60 ms (extending to 200-300 ms) and 2-3 times greater than observed in response to random trains, and pronounced inhibition (maximum 80%) to intervals ranging from 300-1000 ms. These results demonstrate that nonlinearities different from those observed using paired impulses are revealed when the effects of inter-impulse intervals are tested within a continuous sequence, and thus are evaluated against the emergent properties of the neural network containing the perforant path-dentate projection. Supported by The Whitaker Foundation and NIMH (MH00343 and MH30915).

374.17 DEVELOPMENT OF TRANSIENT CONTINUOUS AND PERMANENT DISCRETE PATTERNS OF ACETYLCHOLINESTERASE ACTIVITY IN THE HUMAN ENTORHINAL CORTEX. **Z. Kelović, I. Kostović and R.S. Nowakowski (spon.: N. Zečević)**, Dept. of Anatomy, Medical Faculty, Zagreb, Yugoslavia and Dept. of Anatomy, UMDNJ-Johnson Medical School, Piscataway NJ 08854.

The distribution of acetylcholinesterase (AChE) activity was examined in the entorhinal cortex of human fetuses and premature infants ranging in age from 22 to 28 weeks of gestation. The initial appearance of AChE activity was found at 25 weeks of gestation as a continuous band in the superficial-most portion of the marginal zone. At 28 weeks of gestation the AChE positive band in the superficial-most part of the marginal zone remains, but, in addition, there are 25-40 discrete AChE positive patches which span the distance from the superficial portion of the marginal zone to the prospective layer III and which extend longitudinally in an antero-posterior direction. Correlative analysis of adjacent Nissl-stained sections revealed that the AChE reactive vertically-oriented zones correspond to cell clusters situated at the interface between the marginal zone and the cortical plate. Also, some of the cells in these clusters are AChE positive.

Previously, we have shown (Kelović and Kostović, Anat. Rec. 199: 135A, 1981) that in the adult entorhinal cortex AChE activity is present only in discrete patches in layers I and II. This means that the initial continuous distribution of AChE activity is present only transiently. Since both the permanent (i.e., adult-like) discrete pattern of distribution of AChE activity and the transient continuous pattern of distribution are present simultaneously, the possibility that the discrete pattern forms by an interruption of the continuous pattern as a result of the ingrowth of non-AChE fiber input is eliminated. This suggests that the two types are produced by inputs arising from different areas. We hypothesize that the early-appearing continuous pattern results from the ingrowth of axons from the basal forebrain and that the late-appearing discrete pattern of AChE innervation results from the ingrowth of AChE positive axons from dopaminergic nuclei or some as yet unidentified source.
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374.18 INTRINSIC CHOLINERGIC NEURONS IN RAT HIPPOCAMPUS: IMPLICATIONS FOR STUDIES OF NEURONAL PLASTICITY. S.N. Blaker*, D.M. Armstrong, G. Bruce*, L.B. Hersh and F.H. Gage (SPON: G.M. Peterson). Department of Neurosciences, School of Medicine, University of California at San Diego, La Jolla, California 92093.

Cholinergic innervation of the hippocampus is derived primarily from magnocellular neurons in the basal forebrain. However, the recent evidence supporting the existence of intrinsic choline acetyltransferase-positive (ChAT) neurons in the hippocampus suggests an additional source of hippocampal cholinergic innervation (Levey et al. Neurosci., 13:341, 1984). The possible presence of this intrinsic innervation has significant implications for studies of intracerebral grafting and neuronal sprouting. We therefore characterized and quantified two morphologically distinct populations of cholinergic neurons in the rat hippocampus.

Adult female Sprague-Dawley rats (250g) were deeply anesthetized and perfused through the heart with 4% paraformaldehyde in 0.1% PO_4 buffer. 40μM sections were processed by the avidin-biotin immunolabeling procedure using a polyclonal antibody against human placental ChAT (Bruce et al. J. Neurochem., 45: 611, 1985). Type I cells were small (9x8μM), lightly stained, round neurons with either more than two or no discernable processes. These cells were found predominantly in caudal and temporal hippocampus. Although they were observed in all layers, they were more abundant in the stratum lacunosum-moleculare and the hilus of the fascia dentata. The cells resembled a subpopulation of small, cortical cholinergic neurons found in retrosplenial and subicular cortex dorsally and pyriform, entorhinal and subicular cortex ventrally. Type II cells were large (21x13μM), darkly stained, ovoid neurons with few detectable processes. They were observed in the columns of the fornix rostrally and above and below the dorsal hippocampal commissure caudally. They resembled the magnocellular cholinergic neurons in the midline raphe region of the medial septal nucleus.

Thus at least two anatomically separate and morphologically distinct ChAT-positive cell populations exist in the rat hippocampus. Neither population contributes significantly to the total cholinergic innervation of the intact hippocampus. However, following fimbria-fornix transection the greater number of the small, type I neurons in the ventral-caudal hippocampus may contribute to the cholinergic sprouting and increased ChAT activity reported. In addition, our own observations suggest that the large, type II neurons do sprout in response to fimbria-fornix transection.

Therefore, in studies of neuronal plasticity in the hippocampus in which reinnervation of previously denervated regions appears to arise from extrinsic pathways or from implanted cells one may have to examine carefully the origins of cholinergic fibers.

374.19 ABSENCE OF A STRAIN DIFFERENCE IN THE NUMBERS OF HIPPOCAMPAL PYRAMIDAL CELLS IN FIELD CA1 OF SPRAGUE-DAWLEY AND WISTAR RATS. B.D. Boss. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

The hippocampus is composed of two primary cell types, dentate granule neurons (which form the dentate gyrus) and pyramidal neurons (which form Ammon's horn). These two classes of neurons arise at distinctly different times during neuronal development; in rodents, the larger pyramidal neurons are generated around embryonic day 18, while a majority of the smaller granule neurons are generated during the first postnatal week. Unlike pyramidal cell genesis, which ceases in a few days, division in the dentate granule cell layer has been shown to continue for several months after birth.

I have previously reported a significant difference in initial cell numbers and patterns of growth in the dentate gyrus of Sprague-Dawley and Wistar rats (Brain Res., 338:144-150, 1985). In Sprague-Dawley rats, the number of granule cells remains more or less constant at about 1 million throughout the first year of life; in Wistar rats, on the other hand, there are about 700,000 granule cells at 1 months of age, 1 million at 4 months, and 800,000 at the end of the first year. Since granule cell proliferation continues throughout this entire period in both strains, it appears that the pattern of neurogenesis and of cell "turnover" in the dentate gyrus of the two strains is quite different.

I have now estimated the number of cells in one of the two major fields of the hippocampus (field CA1) to see if there are other differences in the initial cell populations of the two strains, since it is known that there are marked differences in their mean body weights throughout life -Wistar rats being on average about 30% smaller (see Brain Res., 338:144-150, 1985). Using standard techniques with appropriate corrections to guard against double counting, I find that there are 287,000 (± 12,000 S.E.; n=4) CA1 pyramids in Wistar rats and 327,000 (± 22,000 S.E.; n=4) in Sprague-Dawley animals. These numbers are not significantly different as judged by the Multiple Range Test of Duncan. From this finding it would appear that the strain differences found in the dentate gyrus are most probably attributable to intrinsic differences in the regulation of cell proliferation (and cell death) during the postnatal period of granule cell genesis rather than to a more general developmental difference in neurogenesis between the two strains.

374.20 CULTURE OF HIPPOCAMPAL NEURONS FROM POSTNATAL RAT PUPS. C. Rovira* and R. Dingledine (SPON: T.K. Harden), Department of Pharmacology, Univ. North Carolina, Chapel Hill, N.C. 27514.

The maturation of hippocampus begins in the embryo with the differentiation of pyramidal cells and interneurons. Granule cells of the dentate gyrus appear postnatally up to day 21 (P21). In order to obtain a culture of hippocampal neurons that had partly differentiated in vivo we plated out cells from P0 to P16 rat pups.

The procedure was adapted from a method developed by R. Baughman and J. Huettner (in press), and is based on gentle enzymatic treatment with papain. The enzymatic solution was made up in MEM and contained papain (Cooper Biomedical; 300 µg/ml), L-cysteine (160 µg/ml), glucose (3 mg/ml) and HEPES (25 mM). The chosen concentration of papain was found in preliminary experiments to give better results than 10, 30, 100 and 1000 µg/ml. This solution was incubated in the absence of tissue for 30 min at 37° under 5% CO_2 - 95% O_2 to activate the papain, then a 90 min incubation with tissue was carried out in which one third of the enzyme solution was added every 30 min. During the incubation the tissue was gently stirred at 37° under O_2 - CO_2. The pH was maintained near 7.4 throughout. The reaction was stopped by adding a papain inhibitor dissolved in MEM (fatty acid free bovine serum albumin, 100 µg/ml ; ovomucoid, 100 µg/ml; glucose, 3 mg/ml; HEPES 25 mM). The tissue was gently centrifuged, resuspended in papain inhibitor and cells were dispersed by gently trituration. The cell suspension was then washed twice with papain inhibitor and suspended in one of four plating media. Either Weymouth 705/1 or a 3:1 mixture of DMEM and Ham's F-12 was combined with 20% Nu-serum or 10% fetal calf serum plus 10% heat inactivated horse serum. Gentamycin (50 µg/ml) was added to all plating media. Cells were seeded at a density of one hippocampus (appromixately 600,000 cells) per ml onto plastic dishes that had been coated overnight with 0.01% polylysine. After three days in culture the serum was reduced to 10% Nu-serum or 10% horse serum. Cells were subsequently fed with this medium every four days.

The best results have been obtained so far when cells were plated in DMEM: F-12 with Nu-serum. Pups aged P4-P7 gave more consistent results than those of age P15. Glial cells predominated in these cultures, although a variable number of neurons were present in each plating. Neurons could be identified by their characteristic morphology in the phase microscope, by immunofluorescence staining with a monoclonal antibody against neurofilament (obtained from J. Wood), and by the presence of action potentials. Neurons survived for up to three weeks. These results demonstrate the feasibility of growing hippocampal neurons from postnatal rats. Supported by NS-17771.

374.21 NEURONS FROM POSTNATAL RAT DENTATE GYRUS IN DISSOCIATED CELL CULTURE. P. A. Trimmer and O. Steward. Dept. of Neurosurgery, Univ. of Va. Sch. of Med., Charlottesville, VA 22908.

As part of a continuing series of studies examining synaptogenesis and the growth of dendrites from granule cells of the rat dentate gyrus, we have endeavered to establish cultures of isolated dentate granule cells. In previous studies (Trimmer, P., Binder, L. and Steward, O., Anat. Rec. 211: 200A, 1985), we were able to maintain neurons dissociated from rat dentate gyrus in vitro growing on a bed layer of astroglial cells. More recently we have been able to maintain isolated neurons growing on a substratum of polylysine using a specially formulated, high potassium (24mM) medium (Burry, R., Brain Res., 261: 261-275, 1983). To prepare the cultures, hippocampi were excised from 6 day-old rats. At this age, the granule cells of the dentate gyrus are undergoing their final cell division. A cut was made parallel to the hippocampal fissure and a column of tissue containing the dentate gyrus was removed. The meninges were stripped away and the tissue was dissociated using the method of McCarthy, K.M. and de Vellis, J., (J. Cell Biol., 85: 890-902, 1980). Prior to plating on polylysine-coated chamber slides and coverslips, the cell suspension was layered over a 4% solution of bovine serum albumin and centrifuged at low speed. Within one hour after plating, a siginificant percentage of the small cells generated by this procedure began to extend 1 to 2 processes. The cell bodies and processes of these cells stained positively with antibodies to microtubule associated protein 2 (MAP2) and β-tubulin using indirect immunofluorescence. This observation confirmed that these cells are neurons since MAP2 is selectively localized in neuronal cell bodies and dendrites. Other more irregularly shaped cells stained positively with antibody to glial fibrillary acidic protein, indicating that they are astroglia. After 24 hours in culture, nearly all the neurons expressed from 1 to 3 MAP2-positive processes which were sometimes branched and extended away from the cell body for several cell diameters. The cell bodies of these small neurons were from 10 to 12 microns in size and spindle or triangular in shape. Neurons from the dentate gyrus have been maintained as isolated cells for several weeks although the proportion of astroglial cells and fibroblasts contaminating the culture gradually increases. The neurons derived from the dentate gyrus were morphologically different from neurons prepared from fetal hippocampus and grown under the same conditions. We conclude that the small neurons in our culture are dentate granule cells; we are exploring the use of electron microscopy and immunostaining with antibodies to neurotransmitters to further confirm this conclusion. Supported by NIH grants T3207199 to P.T. and NS12333 to O.S.

375.1 CHRONIC MORPHINE TREATMENT DECREASES μ-OPIOID RECEPTOR RESERVE IN SINGLE LOCUS COERULEUS NEURONS. M.J. Christie*, J.T. Williams and R.A. North. Neuropharmacology Laboratory, M.I.T., 56-245, Cambridge, MA 02139, U.S.A.

Intracellular recordings were made from locus coeruleus (LC) neurons in brain slices cut from naive rats or from rats treated chronically with morphine (16 x 75 mg pellets per rat over seven days). Outward potassium currents were measured under voltage clamp during superfusion of μ-opioids and α_2-adrenoceptor agonists. Both types of agonist activate the same potassium conductance in LC neurones. The maximum effects of Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO)(3 μM) and normorphine (30 μM) in control animals were not different from maximum effects of full α_2-agonists (noradrenaline, 30 μM; 5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline (UK14304), 1 μM). The membrane properties (action potential frequency, amplitude, duration and afterhyperpolarization; steady state conductance) of cells from morphine treated animals were indistinguishable from those from control animals. In cells from morphine treated animals, the EC50 for DAGO was about two-fold higher (control: 108±17 nM, n=8; treated: 181±36 nM, n=12); however, the maximum effect of DAGO remained the same as the maximum effect of α_2-agonists in morphine-treated animals. In contrast, the maximum response to normorphine was reduced to 65±5% (n=6) of the maximum response to normorphine in naive rats and the EC50 was shifted to the right two-fold (control: 0.94±0.25 μM, n=7; treated: 1.98±0.15 μM, n=6). The degree of tolerance therefore ranged from about six-fold at the foot of the concentration-response curve to infinite at about 65% of the maximum response in naive rats. In naive tissues, the insurmountable antagonist β-CNA (30 nM for 30 min) shifted the concentration-response curves of both normorphine and DAGO to the right and reduced their maximum effects (to 53±7% (n=5) and 78±7% (n=7) respectively). In tissues taken from morphine-treated animals, β-CNA pretreatment depressed the responses to normorphine and DAGO (maxima were 53% and 54% of the maxima observed in morphine treated animals without β-CNA). β-CNA did not change the sensitivity to α_2-agonists in either naive or morphine treated rats. Naloxone was without effect in slices from morphine treated animals nor was the affinity of the μ-receptor for naloxone changed by morphine pretreatment (pA_2=8.6). The results suggest that normorphine has lower efficacy than DAGO and is therefore more sensitive than DAGO to the loss of receptors, or to a less efficient coupling of receptor to channel, that occurs during chronic morphine treatment.

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375.2 MORPHINE AND METHADONE DEPENDENT RATS EXHIBIT DIFFERENT SENSITIVITIES TO INTERFERON ALPHA MODULATION OF THE NALOXONE PRECIPITATED WITHDRAWAL SYNDROME, by J.M. Pearl*, P.M. Dougherty*, K.J. Krajewski*, and N. Dafny (SPON. J.L. Willmore). Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, P.O. Box 20708, Houston, TX 77025.

The mechanisms underlying the establishment of physical dependence upon opiates and the expression of the associated withdrawal syndrome are as yet unknown. Among the hypotheses attempting to explain this phenomena, it has been suggested that an immune component may be important. Previous communications from our group have elaborated upon this hypothesis in that single injections of the immune modifier interferon alpha reduces the severity of precipitated withdrawal signs in morphine dependent rats. As a test for the possible clinical applications of this form of abstinence treatment, the effect of human recombinant interferon alpha (IFN, 150 IU/g body wt) upon the withdrawal from methadone, the generally accepted form of current therapy for narcotic abusers, was investigated. Sixty-six male Sprague-Dawley rats were divided into four groups. Each group received chronic drug treatment by subcutaneous pellet implants which consisted of carrier only in group one, 75 mg of morphine base in group two, 40 mg of methadone base in group three and first 75 mg morphine followed by 40 mg of methadone base in group four. Once dependence had been fully established seventy-two hours after pellet implantation, one-half of the animals in each group were injected with 1 mg/kg naloxone and scored for seven signs of abstinence behavior including wet dog shakes, teeth chattering, fecal boli, hyperactivity, exploratory behavior, diarrhea, and scream to touch. IFN was administered two hours before naloxone precipitated withdrawal in the remaining animals of each group. Analysis of variance testing was later used to determine significant differences between the various experimental groups for each behavioral sign. The results demonstrate that our method for establishment of methadone dependence by pellet implantation results in the expression of a withdrawal syndrome after seventy-two hours of exposure similar to that resulting from morphine pellet implantation. However, a difference in sensitivities to IFN modulation was observed, as the methadone dependent animals show no attenuation following IFN treatment while the morphine or morphine plus methadone treated animals demonstrate marked attenuation following this form of immune modification. Finally, since the morphine treated rats exhibit a milder withdrawal syndrome following IFN than the morphine-methadone treated animals, these observations suggest that a possible alternative treatment for narcotic addiction may be provided by immune modulation in place of methadone maintenance.

375.3 DEVELOPMENT OF PHYSICAL DEPENDENCE TO VARIOUS OPIOIDS. K.A. Steece, F.A. DeLeon-Jones*, J.M. Lee*, J.Z. Fields, & R.F. Ritzmann. Hines & West Side VA Med. Ctr. & Univ. of IL, Chgo, IL, 60612.

Several types of opioid receptors have been demonstrated and classified on the basis of their different pharmacological affinities. Among these are the mu(μ) and delta(δ) opioid receptors which preferentially interact with morphine and enkephalins, respectively. Opioids have well documented effects on catecholamine systems. Both μ and δ opioid receptor agonists have been demonstrated to alter dopaminergic synaptic transmission. Chronic adminstration of either morphine or methionine enkephalin (met-enk) results in development of physical dependence (p.d.). Data from our lab suggests there are some differences in p.d. produced by morphine and met-enk with regards to withdrawal symptoms as well as certain neurochemical systems. In particular, striatal and hypothalamic dopamine receptor binding and behavioral/physiological correlates, stereotypy and hypothermia, respectively. Chronic met-enk treatment appears to up-regulate dopamine receptors in hypothalamus which positively correlates with an increased sensitivity to apomorphine (APO)-induced hypothermia. This is similar to that observed in chronic morphine treated animals. In striatum, in contrast to morphine treatment where there is an up-regulation and increased sensitivity to the dopamine agonist APO, neither striatal dopamine binding nor APO-induced stereotypy appears different in chronic met-enk treated animals compared to their controls. Administration of 3H-met-enk (icv) suggests there are no significant differences in met-enk distribution in these areas which would appear to account for these differences. It has been demonstrated in several labs that prolonged exposure to certain opioid peptides can induce δ receptor down-regulation in cell culture and brain slice preparations. On the other hand, it is generally agreed that chronic morphine administration does not down-regulate μ or δ receptors. The difference in morphine and met-enk regulation of their respective receptors may account for some difference observed in symptoms of p.d. on these compounds. In addition, since both areas are abundant in δ receptors, there may be a difference in regulation of these receptors in striatum and hypothalamus which may account for the differences observed in the dopaminergic system of these two regions in animals treated with met-enk. Data from our lab suggests that there is no change in striatal δ receptor recognition site (no change in K_d or B_{max}) following chronic treatment with met-enk. This does not however rule out the possibility of change in regulation beyond the receptor per se, ie: desensitization or receptor /effector (n-protein) uncoupling. Experiments to determine whether chronic met-enk has an effect on modulation of opioid receptor function in striatum and hypothalamus will be reported.

375.4 ABSTINENCE-PRECIPITATING PEPTIDE IN CSF OF MORPHINE-DEPENDENT RATS. D.H. Malin, R.N. Ferebee*, L. Harter*, K.L. Thrasher*, D.H. Bruce*, P.A. Farley*, M.S. Blair*, and W.R. Mills*. University of Houston-Clear Lake, Houston, Texas 77058.

Previous studies have shown that CSF from opiate-abstinent rats precipitated an abstinence syndrome when infused into the third ventricle of opiate dependent rats. CSF from non-dependent rats failed to produce this effect. As with artificial opiate antagonists (naloxone), no abstinence syndrome could be precipitated by infusion into non-dependent rats. The current study is a preliminary characterization of the active factor in CSF from opiate abstinent rats.

Twenty-four rats were rendered highly morphine-dependent by 7 days of continuous infusion via Alzet 2MLl osmotic minipump at a rate of 1.67 mg/kg/hr s.c. Six hours after removal of the pumps, rats were anesthetized and CSF was withdrawn from the cisterna magna. CSF was vacuum filtered at 4°C through a collodion filter, 10,000 mol. wt. cut-off. 80 μl aliquots of filtrate and saline-reconstitued retentate were infused into the third ventricle of morphine-dependent rats (morphine-infused with 0.33 mg/kg/hr s.c. for seven days via 2 Alzet 2001 minipumps). Rats were observed for 30 min. on a standard checklist of opiate abstinence signs (abdominal writhes, wet-dog shakes etc.). Four rats receiving CSF filtrate exhibited 66.0 ± 11.3 (mean ± SEM) abstinence signs, while 5 rats infused with retentate exhibited 11.2 ± 4.7 signs. This difference was significant, $p<.01$.

Since the activity was associated with the CSF filtrate (< 10,000 mol. wt.), further characterization procedures were carried out upon the filtrate alone. Four rats were infused with CSF filtrate that had been heat treated (100°C for 150 secs.). They exhibited 44.0 ± 5.2 abstinence signs, not significantly different from unheated filtrate. Additional aliquots were incubated for 30 mins. at 30°C, either with or without an excess of carboxypeptidase A, a broad spectrum proteolytic enzyme. Infusion of the enzyme-treated CSF filtrate into 4 rats induced only 6.7 ± 1.2 abstinence signs, while infusion of the incubated, but non-enzyme-treated, CSF filtrate induced 53.7 ± 15.7 abstinence signs. This difference was significant, $p<.02$.

The above data is consistent with the hypothesis that the active substance in CSF from morphine abstinent rats is a peptide. Additional data will be presented regarding the approximate molecular weight of the active substance, as determined by gel filtration.

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375.5 VOLUNTARY ORAL CONSUMPTION OF MORPHINE SOLUTIONS BY WISTAR RATS
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A method for inducing physical dependence on morphine, in which rats self-administer the drug and as a consequence do not experience the experimental stressors of repeated injections and partial withdrawals, is evaluated. Adult Wistar rats from the U. of Helsinki colony were offered 0.5 mg/ml morphine sulfate in 10% sucrose as their sole source of fluid. Over 4-7 days, only 5 of 20 drank this solution; day-to-day intake fluctuated, but self-administered dose rose to around 100 mg/kg/day in 20 days. The rats which initially refused morphine were allowed to recover to their initial body weight, and then were offered increasing concentrations (0.1 to 0.4 mg/ml) of morphine in 10% sucrose. Consumption dropped sharply with the introduction of each new concentration, and the ingested dose rose to only 30 mg/kg/day. This level was sufficient to produce physical dependence, since body weight fell significantly when the morphine solution was withdrawn. A control group maintained on sucrose drank the same amount of a 0.1 mg/ml solution as the Ss which had become dehydrated when offered the 0.5 mg/ml solution, indicating that the taste alone, and not the association of taste with prior illness, was responsible for the reluctance to drink. In contrast, 20 weanling Wistar rats readily accepted increasing concentrations (0.1 to 0.5 mg/ml) of morphine in tap water. Consumption was stable, 50 mg/kg/day doses were achieved, and physical dependence was unequivocally present. These data indicate that most adult Wistar rats will drink morphine solutions only with great reluctance; apparently they detect the bitter taste of morphine even when it is presented in an otherwise highly palatable solution. However, if Wistar rats are presented with morphine in tap water shortly after weaning, their consumption is reliable and sufficient to induce physical dependence. Thus, we suggest that young rats of the Wistar strain are appropriate subjects for use in this drinking paradigm.

375.6 THE EFFECTS OF METHADONE ON RETINAL DEVELOPMENT IN NEONATAL RATS.
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Maternal opiate exposure has been shown to retard growth and neuroontogeny in newborn rats (Exp. Neurol., 56: 538, 1977). Although this developmental effect has been demonstrated in the brain, we became interested in opiate-mediated activity in the rat retina. Others have shown that the retinal layers in the rat gradually decrease in thickness after about the first postnatal week of normal development. If the opiate methadone retards maturation in the neonatal rat retina, we would expect to see thicker retinal layers in experimental animals when compared with controls. From Day 1, neonatal Sprague-Dawley rats were given daily subcutaneous injections of either 5.0 mg/kg methadone or 0.9% NaCl for 21 days. Animals were weighed daily and separated from their mother until the gross behavioral effects subsided. Eyes were processed for light microscopic morphometric analyses of the thickness of the outer and inner nuclear layers (ONL, INL), and of the entire retina (RT). No significant histological differences were detected between control and experimental retinas. Control ONL (54.4 ± 0.5 μm (SEM)) and RT (134.2 ± 2.0 μm) were significantly thicker than experimental ONL (52.5 ± 0.6 μm) and RT (127.9 ± 1.4 μm), while control INL (33.0 ± 0.5 μm) was not significantly different than opiate-treated INL (32.2 ± 0.5 μm). No significant differences were found between sexes. When neonatal body weights were compared, opiate-treated pups weighed significantly less than controls during the later time intervals. These results contradict our working hypothesis in regard to retinal thickness changes. Although there were differences detected in the ONL and RT measurements, we interpret these slight differences to be not biologically significant.

Previous work has shown that opioids interact stereospecifically, with the opiate receptor postulated to be the site of opiate action (Brain Res. Bull., 7: 25, 1981). If opiate receptors are necessary for drug-induced growth modulation to occur, then receptor distribution may indicate regions of potential endogenous opiate activity. Since opiate receptors have not yet been localized in the rat retina, this may explain why methadone treatment had an unpredictable retinal effect in this study. (Supported by NIH Grant EY02359-09).

375.7 THE EFFECTS OF PRENATAL EXPOSURE TO MORPHINE ON POSTNATAL THYROID AXIS FUNCTION IN THE RAT.
W.J. Litto and J. Rabii. Dept. of Biological Sciences and Bureau of Biological Research, Rutgers University, Piscataway, NJ 08854

We have previously shown that exposure to morphine during a critical period of prenatal development, in the rat, can produce postnatal alterations in the neuroendocrine regulation of LH and TSH secretion. Here we report further evidence concerning postnatal thyroid axis function in rats treated with morphine in utero. Time-mated female rats were injected twice daily on days 5 to 12 of gestation, with morphine sulphate (MS) or saline (SAL) vehicle. Basal plasma TSH, plasma thyroxine (T4), and pituitary TSH concentrations were determined in male and female offspring of both prenatal treatment groups, at 10, 15, 20, 25, 35, and 50 days of age. In addition, the TSH response to acute restraint stress (10 min) in 15-, 35-, and 50-day-old offspring, and the TSH response to exogenous TRH (1.0 and 20.0 μg/kg BW) administration in 50-day-old offspring were determined. The developmental profile for TSH in SAL-treated offspring was characterized by a distinct peak in TSH levels at 15 days. This peak was not present in MS-treated male offspring, and was extended by 5 days in MS-treated female offspring. In addition, 35-day-old MS-treated females had significantly lower TSH levels than their corresponding SAL controls. The developmental profile for T4 was also characterized by a significant peak at 15 days in SAL-treated offspring. This 15 day elevation was not present in MS-treated male offspring, and 10- and 20-day-old MS-treated males had significantly greater T4 levels than their SAL controls. Pituitary TSH content increased in a linear fashion from 10 to 50 days of age, in both MS- and SAL-treated offspring. Ten day-old MS-treated males and females had significantly lower, and 50-day-old MS-treated females had significantly higher, pituitary TSH levels than corresponding SAL controls. Restraint stress produced a significant inhibition of TSH release in SAL-treated offspring at all ages tested. The stress response was not present in 35- and 50-day-old MS-treated males, or in 15- and 35-day-old MS-treated females. The TSH response to exogenous TRH, for both doses tested, was similar for male offspring of both prenatal treatment groups. MS-treated females had a significantly diminished TSH response to TRH, compared to SAL controls, for both doses of TRH. In addition, only the high dose of TRH produced a significant TSH response in MS-treated females. In conclusion, prenatal exposure to morphine produces alterations in the postnatal development of the thyroid axis, as well as the response of this system to physiological stimuli. (Supported by the Busch Memorial Fund of Rutgers University.)

375.8 PRENATAL EXPOSURE TO MORPHINE ALTERS THE NOCICEPTIVE THRESHOLD AND DEVELOPMENT OF OPIATE TOLERANCE IN RATS,
by J. Aronowski*, P.M. Dougherty*, and N. Dafny (SPON: M.E. Miner), Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, PO Box 20708, Houston, Texas, 77025.

Most of the studies on opiate induced effects have concentrated on adult animals and not those induced in developing animals. The present study investigates the effect of prenatal exposure of morphine upon the baseline nociceptive threshold, opiate induced antinociception, and the development of tolerance to this effect. Pregnant mothers at days 14 of gestation were injected with morphine (i.m.) in an increasing regimen of three daily injections with dosages starting at 15 mg/kg and ending at 90 mg/kg/day by the sixth day of treatment. This protocol was sufficient to induce physical dependence in the mothers as assessed by naloxone precipitated withdrawal. Control pregnant animals received three daily injections of saline. The newborns from both groups were allowed seven weeks to develop and achieve a body weight of approximately 150 gr. No significant differences in weight development or behavior were noticeable between the experimental and control animals. The experiment began with establishing the nociceptive baseline using the tail immersion assay, with the noxious stimulus provided by 55°C water. Once the baseline response was obtained, 5 mg/kg morphine was administered and the antinociceptive effects of morphine was assessed as before. The effect of prenatal exposure to morphine on the development of tolerance was assessed following chronic exposure to morphine by increasing i.m. injection dosages three times a day across five days similar to those described above. The animals were then allowed twenty four hours to clear residual morphine from their systems and a second baseline was determined. Tolerance was then assessed by measurement of the tail immersion response following injection of 5 mg/kg morphine. These observations demonstrate that the animals treated prenatally with morphine had an elevated nociceptive baseline versus control animals. Following chronic morphine treatment, the baseline responses of both animal groups became identical which appears to indicate that both groups had become compensated to chronic morphine exposure in the same manner. Yet the effect of morphine at this time was enhanced in the prenatally exposed animals, suggesting that these rats either developed a lower degree of tolerance, or developed partial tolerance by a different means than the control animals. These results may suggest that prenatal exposure to morphine modifies the development of endogenous opiate systems.

375.9 INTERACTIONS OF IMMUNOMODIFYING AGENTS UPON OPIATE INDUCE HYPOTHERMIA INTERFERON PREVENTS THE DEVELOPMENT OF TOLERANCE TO MORPHINE HYPOTHERMIA. C. Harper*, P.M. Dougherty* and N. Dafny (SPON. F.M. Yatsu), Department of Neurobiology and Anatomy, University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas 77025.

It has recently been demonstrated that several diverse forms of immune modification can effect CNS functions associated with responses to opiates which include the expression of morphine withdrawal and its nociceptive properties. The present study elaborates upon these previous observations by investigating the influence of immune modification by alpha-interferon, cyclosporine-A, and partially immune-ablating doses of gamma-irradiation upon both acute morphine induced hypothermia and the development of tolerance to this effect. Temperatures were measured by flexible probes inserted 4 to 5 cm into the rectum of restrained male albino rats connected to a tele-thermometer multi-channel readout. After allowing an accommodation period, baseline temperatures were recorded over a 30 minute interval. Following the establishment of the baseline responses, the animals were immune modulated by ip injection of 150 IU/g body wt Hoffman-LaRoche recombinant human alpha-interferon, 15 mg/kg cyclosporine-A (Sandoz) or whole body exposure to 500 rads gamma irradiation (Cesium 137 source), then returned to the restrainers after a 90 minute recovery period and observed for temperature changes across the next 40 minutes. Two hours after the immune modulation treatments, the rats were injected with 10 mg/kg morphine ip and once again observed for temperature responses over the next 250 minutes. Tolerance was then induced in the experimental animals by continuous exposure to morphine for 72 hours from a subcutaneous morphine (75 mg) pellet implant. After this time (72 hours) the pellets were removed and the animals were allowed to clear the residual drug from their systems across the next twenty four hours, at which time the animals were again measured for baseline temperature and injected with 10 mg/kg morphine to assess the development of tolerance. The results of this study demonstrated that among the treatments employed, only irradiation exposure altered the baseline temperature. In addition, none of the treatments interferon with or prevent the normal hypothermic response to morphine in day 1 of the experiment. Finally, neither cyclosporine nor gamma-irradiation prevented the development of tolerance to morphine (10 mg/kg) induced hypothermia, however, interferon prevented the development of tolerance to morphine as the rats responded with the same degree of hypothermia after chronic exposure to the drug as on the first day of the experiment. These results further illustrate an interaction of the immune system and the CNS at the level of opiatergic physiology.

375.10 EFFECTS OF NALOXONE AND NALTREXONE ON SERUM LH LEVELS ACROSS THE LIFE SPAN AND CONTRAST WITH DURATION OF ANALGESIA BLOCKADE E. Field* and C. Kuhn (SPON: N. Cant), Department of Pharmacology, Duke Univ. Med. Ctr., Durham, N.C. 27710

Both the advent of reproductive function during puberty and its progressive decline in middle-aged and older female rats are accompanied by changes in the timing and magnitude of the (LH) surge as well as in LH pulse amplitude. Reported age-related alterations in the activity of endogenous opioid neurons which tonically inhibit LH secretion might account for such changes. To test this possibility the present study evaluated the magnitude of LH effects of opiate antagonists in immature, intact young adult and middle-aged female rats. In addition, we compared the duration of LH effects to effects on morphine-induced analgesia after opiate blockade with putative short and long-acting antagonists.

Naloxone (NAL, 2 mg/kg), naltrexone (NTX, 1 or 50 mg/kg) or saline (SAL) was injected sc in 5, 10 and 25-d.o. and 1 ½ and 10-month-old female rats and trunk blood was collected following decapitation at various times after treatment. In cycling 3-month-old and 10-month-old rats, blood samples were collected on the afternoon of diestrus I from intra-atrial cannulae prior to and at intervals after NAL (2 mg/kg, iv). For analgesia testing by tail immersion, rats were treated with SAL, NAL or NTX at various times and with MOR or SAL 30 min prior to testing.

Developmental differences in magnitude of the LH response to antagonists were observed. In 5 and 10 d.o. rats, serum LH increased 5 to 10-fold after NAL treatment ($p < .005$) and 3-fold after high or low-dose NTX ($p < .01$). NAL increased LH 3 to 4-fold in 25 d.o. rats ($p < .001$). After sexual maturation LH responsiveness to NAL was less marked and did not change with age. Interestingly, after a single or multiple (4) doses of NAL to 5 d.o. pups, LH increased at 20 min, but showed a rebound suppression at 6 hr ($p < .05$). The measured duration of LH responses to antagonist treatment was no longer than 1 hr. In contrast, blockade of analgesia by NTX lasted for at least 12 hr ($p < .05$) and by NAL for up to 2 hr ($p < .05$).

Our results confirm previously reported age-related changes in LH response to opioid receptor blockade during sexual development. We have further demonstrated that no similar decreases occur through middle age, despite reported changes in other neurochemical/neuroendocine paramaters during this period. Further studies are required to evaluate the possibility of changing opioid tone in aging constant estrus or anestrus females as has been reported in aging male rats. Two findings of this study have importance for reports of chronic antagonist effects on pubertal development. NTX or NAL-induced LH effects are relatively brief in comparison to the sustained blockade of opioid receptrs revealed by analgesia testing. In addition, both the elevation and the rebound suppression of LH persist after repeated administration of NAL

375.11 INSENSITIVITY OF THE PROLACTIN REGULATORY MECHANISM TO MU OPIATE RECEPTOR STIMULATION DURING LACTATION IN THE RAT. J. Janik, P. Callahan, L. Grandison and J. Rabii. Dept. Bio. Sci., Rutgers Univ., and Dept. Physiol. (LG), UMDNJ, Piscataway, NJ 08854.

We have previously reported that morphine sulfate (MS) administration is unable to stimulate basal release of prolactin in the lactating female model. This is in direct contrast to the significant prolactin increases produced by MS in male and cycling female rats. The purpose of this study was to further determine the sensitivity of the lactating female model to opiate stimulation and to compare it to the diestrous female rat. For this purpose, the mu agonist methadone was administered to both models. In addition the dopamine antagonist haloperidol was administered to lactating females as a means of assessing the functional activity level of the tuberoinfundibular dopaminergic (TIDA) neurons.

In these experiments, lactating female rats were used between days 4 and 10 post-partum. Non-lactating females were used on diestrous day 2 as verified by daily vaginal smears. On the day of the experiment, dams were separated from their pups for 2 hours prior to the administration of any drug. One group of lactating females and one group of diestrous females were injected with methadone (5 mg/kg, ip). Other groups of lactating and diestrous females received the opiate antagonist naloxone (2 mg/kg, ip) 10 minutes before and 15 minutes after the methadone injection. A separate group of lactating females received the dopamine antagonist haloperidol (0.1 or 0.5 mg/kg, iv).

The administration of methadone, like MS, was unable to stimulate basal levels of prolactin in the lactating female. In contrast, methadone did produce a significant increase in circulating levels of prolactin in the diestrous female. This increase was naloxone reversible. Furthermore, methadone produced a significant increase in circulating levels of GH in both models. These increases were also naloxone reversible. Haloperidol produced a significant increase in basal prolactin levels in the lactating model. This suggests that the TIDA neurons are indeed active during lactation even though two mu agonists, i.e. MS and methadone, failed to alter basal levels of prolactin in this model. Thus we suggest that the mu opiate receptor subtype may not be involved in the regulation of prolactin release during lactation. (Supported by the Busch Memorial Fund.)

375.12 DIFFERENTIAL SENSITIVITY OF THE PROLACTIN REGULATORY MECHANISM TO OPIATES DURING LACTATION IN THE RAT. P. Callahan, J. Janik, L. Grandison and J. Rabii. Dept. Bio. Sci., Rutgers Univ., and Dept. Physiol. (LG), UMDNJ, Piscataway, NJ 08854.

We have shown that the mu agonists morphine sulfate (MS) and methadone, do not produce any alteration in basal levels of prolactin in the lactating female rat. However, these opiate agonists do cause a significant increase in GH levels in this model. The purpose of this study was to further characterize the sensitivity of the lactating female model to opiate stimulation by administering endogenous opioid peptides (EOP).

Lactating female rats on day 2 post-partum were implanted with chronic cannula placed in the lateral ventricle. On the day of the experiment, dams were separated from their pups for 2 hours prior to the administration of any drug. One group received saline or the epsilon agonist beta-endorphin (10 or 20 µg, ivt). A second group received saline or the delta agonist d-ala-d-leu-enkephalin (DADLE; 5 or 10 µg, ivt). A third and fourth group received the opiate receptor antagonist naloxone (5 mg/kg, ip) 10 minutes prior to and 15 minutes after beta-endorphin (10 µg) or DADLE (10 µg). In addition, two other groups received the opiate antagonist WIN 44,441 (5 mg/kg, ip) 10 minutes prior to the opiate agonists. Naloxone is primarily a mu receptor antagonist while WIN 44,441 has been reported to effectively block the mu, delta and kappa sites.

Both beta-endorphin and DADLE produced significant increases in circulating levels of prolactin. Additionally, these same animals also showed a significant increase in circulating GH levels. Interestingly, the prolactin response to neither beta-endorphin nor DADLE was reversible by either naloxone or WIN 44,441. However, the GH increase was blocked by both antagonists. This suggests that the beta-endorphin and DADLE-induced increases in prolactin may be mediated by a receptor subtype which is not blocked by either of these antagonists. We suggest that a receptor subtype, other than the mu, is involved in the regulation of prolactin release in this model. Although the epsilon receptor may be implicated by these results, further studies are necessary to confirm this apparent conclusion. (Supported by the Busch Memorial Fund.)

375.13 INTERACTION BETWEEN STEROIDS AND OPIATES ON LH AND LHRH RELEASE
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An interaction between opiates and steroids at the level of the hypothalamo-pituitary axis is suggested by the sexual dysfunction associated with chronic narcotic use. We have systematically analyzed the effects of continuous exposure of castrated rats to morphine (M) and/or varying testosterone (T) concentrations on LH and LHRH release. Rats castrated for 2 weeks were implanted s.c. with M pellets alone (2 pellets every other day) or in combination with T-containing Silastic capsules (5mm or 30mm length) for 4 days; treatment groups were: Sham, M, T5, T30, T5 + M and T30 + M implants. Continuous opiate receptor stimulation with M pellets alone, or treatment with T5 which produced sub-physiological serum T levels of 300- 500 pg/ml, failed to affect the post-castration LH hypersecretion. However, M and T5 in combination drastically suppressed LH levels to the range detected in intact rats (< 1 ng/ml). LH release, suppressed by T30 implants to around 1 ng/ml was further reduced significantly by combined T30 + M treatment (0.4 - 0.6 ng/ml). Whether this supersensitivity of LH response to T feedback, induced by continuous M exposure, was due to altered release of hypothalamic LHRH or altered pituitary response to LHRH was assessed. Hypothalami of rats treated as described above were perifused in vitro to determine basal LHRH release rates and the LHRH response to the opiate receptor antagonist, naloxone and to K^+. The basal LHRH release rates were not altered by the addition of M treatment as compared to that from rats receiving the appropriate T treatment alone (T5 vs T5 + M and T30 vs T30 + M). Surprisingly, M treatment also did not alter the amounts of LHRH released in response to a 10 min pulse of K^+ (56 mM) or 30 min infusion of naloxone hydrochloride (1 mg/ml). Pituitary responsiveness to exogenous LHRH, in terms of LH release, was assessed in similarly treated rats fitted with intrajugular cannulae for blood withdrawal. As expected, basal LH release was drastically suppressed in M + T5 (0.5 ng/ml) and T30 rats (1.1 ng/ml) and was unchanged in M (3.2 ng/ml) or T5 groups (4.0 ng/ml). Despite these effects, LH increments in response to LHRH, administered in physiological range ($1x10^{-2}$ or $0.5x10^{-2}$ nmol), were similar in these groups. These studies show that continuous opiate receptor stimulation renders castrated rats supersensitive to the feedback effects of T on LH release. The drastically reduced LH release may not be due to either impaired LHRH secretion or reduced pituitary responsiveness to LHRH. Thus these findings are in accord with the emerging hypothesis that M treatment, in combination with subphysiological or physiological doses of T, may evoke the release of a hypothalamic factor(s) which suppresses pituitary LH release, independent of the effects on LHRH secretion, if any. (Supported by NIH HD 11362).

375.14 REDUCED MORPHINE RESPONSIVENESS ON PROESTROUS AFTERNOON IN THE RAT.
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We have demonstrated previously that a reduced responsiveness to several aspects of morphine action in the CNS is associated with the steroid-induced LH surge in rats. The present studies were undertaken to determine if a similar morphine insensitivity occurs during the afternoon proestrous LH surge. Normal cycling rats were tested for morphine-induced analgesia and LH suppression at a time prior to (1000 h) and during (1630 h) the proestrous LH surge and on the afternoon of diestrus. Tail flick latency, a measure of spinal cord mediated analgesia, was measured before and at 10 min. intervals for 2 h after treatment with morphine sulfate (MS, 5 mg/kg, s.c.). On proestrous morning, rats exhibited a maximal analgesic response to MS within 10 min and this response persisted through at least 90 min. In contrast, rats treated on proestrous afternoon exhibited an analgesic response which was slow in onset, peaked at 40 min. and dissipated rapidly thereafter. Rats treated on diestrous afternoon showed an analgesic response which was intermediate between that on proestrous morning and proestrous afternoon. Integration of the area under the time-response curve revealed that the analgesic response to morphine was equivalent on proestrous morning and diestrous afternoon but was significantly decreased on proestrous afternoon. These data indicate that associated with the LH surge on proestrous afternoon is a significant decline in the analgesic response to morphine.

To determine if the LH secretory mechanism shows a similar decrement in its sensitivity to morphine on the afternoon of proestrus, serum LH concentrations were determined 30 min. after administration of saline, 5 mg MS/kg or 10 mg MS/kg. In diestrous afternoon and proestrous morning rats, MS caused a 45% and 33% ($p < 0.05$) reduction, respectively, in serum LH levels at the 5 mg MS/kg dose and no further decrease at the higher MS dose. In contrast, at doses as high as 10 mg MS/kg no alteration in serum LH levels were observed during the proestrous afternoon LH surge. These data indicate that a marked decline in the opiate responsiveness accompanies the prostrous LH surge and suggest that the development of a refractoriness to opiates or endogenous opioid peptides may be involved in the generation of the proestrous LH surge and accompanying opioid mediated events (Supported by grants AG 02021 and HD 14075).

375.15 EARLY MATURATION OF OPIOID MODULATION OF TSH SECRETION.
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Acute administration of opiates supresses TSH secretion in adult rats. The purpose of the present study was to investigate the ontogeny of opioid induced TSH suppression to test the hypothesis that opioid control of TSH secretion matures before other neural controls. Methadone administration (2.5 mg/kg) diminishes TSH secretion in 60 day old rats. A similar effect was observed when 5 and 15 day old pups received the same treatment. This opiate action seems to be mediated by both mu and kappa opiate subtype receptors. Morphiceptin (0.5 μg), a mu selective agonist, suppressed TSH levels at 5 days of age, whereas [D-Pen 2,5]-Enkephalin (1 μg), a delta receptor agonist, was not effective. U50488 and dynorphin, purported kappa agonists, were also able to inhibit TSH secretion in 5 d.o. pups. These observations correlate well with early opioid modulation of other hormones in neonatal rats. For example, β endorphin stimulates secretion of prolaction, growth hormone, and corticosterone in 5 day old pups. Opioid induced secretion of prolactin, like TSH, is mediated by mu and kappa receptors whereas growth hormone and corticosterone effects are controlled by delta and mu receptor subtypes, respectively.

Since opioidergic control seems to be functional in the early postnatal period, we were interested in determining whether other known neural controls of TSH secretion were also mature at this time. Three neural regulators of TSH secretion were evaluated: the inhibitory action of dopamine agonists, which are thought to act at the pituitary, and the stimulatory actions of cold stress and the alpha agonist clonidine. In adult rats, cold exposure (4°C) significantly increased TSH secretion, and both basal and cold stimulated TSH levels were suppressed by apomorphine. While apomorphine inhibited the normal 7 fold rise in TSH levels subsequent to exogenous TRH administration in neonatal rats, 10 d.o. pups failed to respond to exposure to 22°C or 4°C or to clonidine (.45 mg/kg).

Our evidence suggests that opioid mechanisms are capable of controlling TSH secretion at an early age and that the effects are mediated through both mu and kappa receptor components. Furthermore, dopaminergic control of TSH secretion through pituitary mechanisms also seems to be intact early. In contrast, the purported hypothalamic adrenergic receptor mechanism through which cold exposure stimulates TSH secretion is not present in the early postnatal period.

376.1 **[^{3}H]PCP BINDING: REDUCTION IN NEOCORTEX BUT NOT IN STRIATUM FOLLOWING 6-OHDA VTA LESIONING.** D.C. Javitt*, E.L. Gardner, S.R. Zukin. Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine/Montefiore Medical Center and Bronx Psychiatric Center, Bronx, NY 10461.

The psychotropic actions of phencyclidine (PCP) are mediated via specific PCP/sigma receptors. While the regional distribution of PCP/sigma receptors has been mapped in detail, little is known about their functional localization with respect to defined neuronal pathways. To this end, we investigated the effect of dopaminergic denervation on neocortical PCP receptor density.

Male Long-Evans rats were housed in common and were treated according to one of three paradigms. In groups A and B, rats were injected in the left ventral tegmental area (VTA) with 1 ul of vehicle (0.2 mg/ml citric acid in water) or 8 mg/ml 6-hydroxy dopamine (6-OHDA), respectively. In group C, rats received bilateral 6-OHDA injections. Lesions were allowed to mature for 2 -3 weeks at which time the animals were sacrificed and the brains were dissected. Following regional dissection, tissue from cingulate cortex and striatum was assayed for [^{3}H]PCP receptor binding using a rapid filtration assay (Zukin, SR et al, Brain Res 258: 277-284, 1983). K_D and B_{max} values were calculated by Scatchard analysis. For statistical analysis, data was pooled among the lesioned and among the non-lesioned groups. Samples of nucleus accumbens were simultaneously assayed for DA, DOPAC, and HVA. DA and DA metabolite levels were found to be significantly decreased in tissue from the 6-OHDA lesioned sides ($p<.005$).

PCP binding values for denervated and non-denervated cingulate cortex and striatum are shown below:

	CINGULATE CORTEX		STRIATUM	
NON-	K_D	B_{max}	K_D	B_{max}
LESIONED	190 nM	303.6 fmol/mg prot	54 nM	39.7 fmol/mg prot
LESIONED	44 nM	94.6 fmol/mg prot	103 nM	26.2 fmol/mg prot

Lesioning of the VTA produced a significant decrease in PCP receptor density in the cingulate cortex ($p<.01$) but not in the striatum. The reduction in PCP receptor density following either vehicle or 6-OHDA VTA injection correlated directly with the reduction in DA levels in the corresponding nuclei accumbens ($p<.005$). These data suggest that PCP receptors in the neocortex may be located in part on pre-synaptic dopaminergic terminals and that PCP may mediate its psychotomimetic effects in part by modification of pre-synaptic mesocortical dopamine release.

This work was supported in part by P.H.S. research grant DA-03383 to SRZ.

376.2 CORRELATION BETWEEN LOCALIZATION OF [3H]-TCP AND NMDA BINDING SITES. J.B. Penney, W.F. Maragos, D.C.M. Chu, and A.B. Young. Dept. of Neurology and Neuroscience Program, Ann Arbor, MI, 48104.

[3H]-N-(1-[2-thienyl]cyclohexyl) 3,4-piperidine (TCP), a derivative of the dissociative anesthetic phencyclidine (PCP), binds to a sigma-opiate receptor. Behaviorally and electrophysiologically, drugs of this class inhibit noncompetitively the excitatory properties of the glutamate receptor agonist N-methyl-D-aspartate (NMDA). The dissociative anesthetics may mediate their effects by blocking a voltage-sensitive cation channel coupled to the NMDA receptor. To study the relationship between dissociative anesthetics and the NMDA receptor, we compared the regional brain distribution of [3H]-TCP binding and NMDA-sensitive glutamate binding using quantitative autoradiography.

Radiolabelling of each receptor class was carried out on alternate 20 µm slide mounted sections taken at various levels of rat and human brain. For [3H]-TCP binding, sections were preincubated for 30 minutes in cold 50 mM Tris-acetate, pH 7.4 and then incubated for 45 minutes in the same buffer with 1 mM magnesium acetate and 20 nM [3H]-TCP in the presence or absence of 20 µM PCP. [3H]-Glutamate binding was carried out as previously described (Greenamyre et al., JPET 233:254, 1985). Sections were preincubated for 30 minutes in cold 50 mM Tris-acetate, pH 7.4, and then incubated in the same buffer containing 200 nM L-[3H]-glutamate and 1 µM quisqualic acid to inhibit high affinity binding to quisqualate receptors. Nonspecific binding was determined in the presence of 1 mM glutamate. Autoradiograms were analyzed by computer-assisted microdensitometry.

A striking similarity was observed between the densities of [3H]-TCP and NMDA receptor binding. In rat, bound values for the two ligands in selected regions were highly correlated (Pearson's $r = 0.95$ $p<.001$). In rat and human, highest densities were observed in stratum radiatum of CA1 in the hippocampus. High levels of binding at both receptors were also observed in the dentate gyrus and stratum oriens of CA1, and layers 1 and 2 of somatosensory cortex. In rat, moderate levels were observed in the striatum, thalamus, amygdala, deep layers of cerebral cortex, medial geniculate nucleus and external plexiform layer of olfactory bulbs. The only area in which there were NMDA but not TCP sites was the cerebellum.

Thus, the regional localization of the sigma-opiate receptor is highly correlated with the distribution of NMDA receptors. Since the NMDA receptor subtype is significantly decreased in dementia of the Alzheimer type (DAT), studies of [3H]-TCP should be carried out in DAT. Phencyclidine derivatives could conceivably be used as PET probes in DAT and other neurodegenerative disorders. Supported by the Arbogast Foundation, Roy R. Gettel and USPHS Grant NS 15655.

376.3 REGIONAL COMPETITION PROFILES OF U50,488 FOR ^{3}H-NALOXONE BINDING IN RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY. G.F. Wooten and W.A. Geary, II. Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908.

Regional competition profiles of the κ selective benzeneacetamide U50,488 (UpJohn) versus ^{3}H-naloxone binding were examined in 22 rat brain structures using quantitative autoradiography. ^{3}H-naloxone (3.0nM, 54-57 Ci/mmole, Amersham) was used as a nonselective probe for multiple opioid binding subtypes (James and Goldstein, Mol. Pharmacol. 25:339-342, 1984); U50,488 (1-1000µM) was used to selectively demonstrate regional κ specific binding by competitive inhibition. Quantitative autoradiography of ^{3}H-naloxone was performed as previously described (Geary and Wooten, JPET 225:234-240, 1983); all data were corrected for regional differential autoabsorption of tritium (Geary and Wooten, Brain Res. 336:334-336, 1985). All experiments were performed in phosphate buffered saline (PBS). The 22 regions studied included areas reported to have different relative densities of opioid binding subtypes. U50,488 maximally displaced 100% of ^{3}H-naloxone specific binding monophasically in all regions studied. The range of all 22 regional IC_{50} values for U50,488 was 5.9-37µM with mean±SD of 20.6±9.8µM. Slopes for all 22 competition profiles with U50,488 were parallel with a mean slope 1.71. Inhibition profiles of U50,488 were also parallel to inhibition profiles of the µ-agonist sufentanil (Geary and Wooten, Soc. Neurosci. A220.6:757, 1985). Sufentanil competition yielded IC_{50} values (mean±SD) of 6.2±3.0nM with a range of 2.6-13.3nM. The mean slope value for the 22 parallel sufentanil profiles was 1.78. The potency ratio for IC_{50} values for U50,488/sufentanil was 3,300; this potency ratio was similar to the potency ratio of these compounds for analgesia (3,000-6,000). Our data showed that selective µ and κ agonists inhibited 100% of ^{3}H-naloxone binding in a monophasic and parallel fashion differing only in potency. The data were consistent with a single naloxone binding site which exhibited no selectivity for sufentanil or U50,488.

In addition, U50,488 displaced ^{3}H-naloxone total binding from cerebellar gray folia; inhibition was incomplete with a 55% plateau with 1000µM U50,488 (slope = 0.84, IC_{50} = 395µM). Total binding of ^{3}H-naloxone in cerebellar folia was unaffected by naloxone, dynophin (κ), sufentanil (µ), or nonselective agonists (levophanol, etorphine, and morphine). Thus, inhibition of cerebellar ^{3}H-naloxone total binding was unique for U50,488 and was not exhibited by opiate ligands of µ, κ, or mixed subtype specificity. This nonspecific property of U50,488 may partially account for multiphasic patterns of U50,488 inhibition seen in homogenate binding studies in Na^+ free buffers. This work was supported by grant NIH DA03787.

376.4 **LOCALIZATION OF NMDA, PCP, AND AMPA RECEPTORS: COMPARISON OF THEIR DISTRIBUTIONS AND THE EFFECTS OF SELECTIVE LESIONS.** D.T. Monaghan, L. Nguyen*, and C.W. Cotman. Dept. Psychobiology, Univ. Calif., Irvine CA 92717.

The excitatory amino acid receptors selectively activated by N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) are both found in high concentrations in the CA1 stratum radiatum of the rodent hippocampus. Synaptic transmission within this dendritic zone appears to be mediated by two acidic amino acid receptors — the NMDA receptor and a AMPA/kainate-like receptor. NMDA receptors in this region are necessary for long term potentiation and have been postulated to be located both presynaptically and postsynaptically. We have evaluated the cellular localization of these receptors by generating lesions which remove either the postsynaptic or the presynaptic elements of the Schaffer collateral pathway. We have also compared the organization of these sites to those of phencyclidine (PCP) binding sites since PCP is a non-competitive NMDA antagonist.

Male Sprague-Dawley rats were anesthetized with Nebutol and received injections of either 0.5 µg kainic acid into the lateral ventricle to remove the CA3 projection to CA1 or 2.5 µg NMDA into the dorsal hippocampus to remove the pyramidal cells in CA1. Following post-operative recovery times of 2, 4, 7, and 14 days, animals were sacrificed and autoradiograms of NMDA-sensitive L-^{3}H-glutamate binding sites and ^{3}H-AMPA binding sites were prepared as we have previously described (J. Neurosci. 5 2909-2919, and Br.Res. 324 160-164).

Qualitatively, removal of the CA3 projection to the CA1 region did not reduce either NMDA or AMPA binding sites at any time studied. This lesion did however result in a significant loss of binding sites in region CA3. Receptor loss two days following postsynaptic cell loss was only partial while significant loss of binding sites was observed at later time points. These results suggest that the majority of both NMDA and AMPA binding sites are located postsynaptically. These results also suggest that the decrease in AMPA receptors that we find following ischemia may be occuring postsynaptically (Westerberg, Monaghan, Cotman, and Weiloch, Soc. Neurosci. Abstr., these proceedings).

PCP blocks the action of NMDA. However, it is not yet known if these two sites are generally colocalized. We have compared the distribution of PCP sites to that of NMDA receptors in the rat brain. PCP sites were localized using ^{3}H-TCP as the radioligand (Sircar and Zukin, Br. Res. 344 142-145) and 1 mM ketamine as a displacer. In general, there is a striking correspondence between the distribution of PCP and NMDA sites in the rat brain. For example the lamination of PCP sites in the pariatal cortex and the hippocampus were identical. In addition, the pattern of ^{3}H-TCP binding in thalamus, hypothalamus, and caudate/putamen was strikingly similar to the distribution of NMDA sites. This work was supported by grant DAMD 17-83-C-3189 and the MacArthur Foundation.

376.5 THREE-DIMENSIONAL DISTRIBUTION AND RELATIONSHIPS OF QUENCH-CORRECTED BENZODIAZEPINE RECEPTOR SUBTYPES IN MOUSE CNS. W.F. White, S. O'Gorman* and A. Wang*, Prog. Neurosci., Harv. Med. Sch., Boston, MA 02115, Dept. Neurosci., Children's Hosp., Boston, MA 02115, Dept. Dev. Neurobiol., Salk Inst., La Jolla, CA 92037.

Benzodiazepine receptors have been classified into 3 subtypes, neuronal Type-1 and Type-2, and nonneuronal. The neuronal subtypes appear to be associated with the GABA receptor complex where they modulate GABA synaptic efficacy; the function of the nonneuronal receptors is unknown. The purpose of the present study is to determine the distribution and interrelationships of benzodiazepine receptor subtypes throughout the mammalian CNS. The brains from adult C57BL/6J mice were serially sectioned at 10 um on a cryostat in the coronal plane and thaw mounted onto subbed slides. Adjacent sections were processed for different subtypes; groups were taken at 120 um intervals from C3 to frontal pole. One hour preincubations and incubations were carried out in ice cold buffer (100 mM NaCl and 50 mM $NaPO_4$ pH 7.4). Slides were rinsed for a total of 2 min in 4 changes of buffer followed by a dip in distilled water, and dried quickly. Autoradiograms were prepared by 4 week exposures to LKB Utrofilm. Total benzodiazepine binding sites were labelled with 1 nM ^{3}H-flunitrazepam, 10 uM GABA was added to measure GABA stimulated sites, Type-1 were distinguished from Type-2 sites by the addition of 1 uM CL 218-872, nonneuronal sites were labelled in the presence of 1 uM clonazepam, and nonspecific binding was determined in the presence of 10 uM diazepam. Differential quench was measured by the binding of ^{3}H-succinimidyl proprionate. The optical density of receptor distribution was quantified at a resolution of 50 um; nonspecific binding was subtracted, and optical density was converted to specific activity. Data from the individual incubations were registered into a three-dimensional array using isodensity contours, and differential quench was corrected in each data section. Relationships between different conditions were calculated by performing mathematical functions between adjacent sections on a pixel by pixel basis. Relationships investigated were total binding minus Type-2 binding to yeild Type-1 binding, and the ratios of total binding to GABA stimulated binding, total binding to Type-2 binding, total binding to Type-1 binding, Type-1 binding to GABA stimulated binding, Type-2 binding to GABA stimulated binding, and nonneuronal binding to GABA stimulated, total, Type-1, and Type-2 binding. Each of the resulting distributions were registered with a three-dimensional atlas of the same mouse brain and the anatomical distributions of the receptor subtypes and relationships were determined. These data provide an extensive investigation of the distribution and relationships between benzodiazepine receptor subtypes.

376.6 AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-ZOLPIDEM BINDING SITES IN THE RAT AND MONKEY CNS. R. Niddam*, A. Dubois*, S. Arbilla*, B. Scatton and S.Z. Langer (SPON : S. MRAOVITCH), Biology Department, L.E.R.S., 58, rue de la Glacière, 75013 Paris, France.

Zolpidem is a novel non-benzodiazepine hypnotic with rapid onset short duration of action which interacts with central benzodiazepine (BZD) receptors. Recent binding studies with ^{3}H-zolpidem have revealed that this radioligand labels the GABA-benzodiazepine macromolecular complex with preferential affinity for the BZD_1 receptor. In order to investigate further this possibility, we have localized precisely ^{3}H-zolpidem binding sites in the rat and monkey brain and spinal cord by quantitative autoradiography.

For binding assay, brain and spinal cord sections (15 or 30 µm in thickness) from the rat and baboon were incubated for 30 min at 4°C in a 50 mM Tris-HCl buffer, pH 7.4 containing 120mM NaCl and 5mM KCl in the presence of 5nM ^{3}H-zolpidem (S.A. 60.6 Ci/mmol). Non-specific binding was defined by Ro 15-1788 (2µM).

In the rat brain, the highest levels of ^{3}H-zolpidem binding were found in the olfactory bulb (glomerular layer), islets of Calleja, cerebral cortex (especially in layer IV), ventral pallidum, inferior colliculus, medial septum, nucleus of the diagonal band of Broca, cerebellum (molecular layer), substantia nigra pars reticulata and some amygdaloid nuclei. Intermediate levels of binding were found in the CA1 field of the hippocampus (molecular layer), primary olfactory cortex, dorsal tegmental nucleus, dorsal raphe, central gray, superior colliculus, endopiriform nucleus, subthalamic nucleus and in some thalamic and hypothalamic nuclei. Low levels of ^{3}H-zolpidem binding sites were found in the caudate putamen and dentate gyrus of the hippocampus. No specific ^{3}H-ligand binding sites could be found in the spinal cord In the monkey brain, ^{3}H-zolpidem binding sites were particularly concentrated in the cerebral cortex (layer IV), cerebellum (molecular layer), subthalamic nucleus, claustrum and pallidum. Intermediate levels of binding were observed in the amygdala, some thalamic nuclei, substantia nigra and mammillary bodies. Low levels of the ^{3}H-ligand binding were detected in the caudate nucleus, putamen, dentate gyrus, nucleus accumbens, red nucleus and spinal cord.

In conclusion, the CNS distribution of ^{3}H-zolpidem binding sites in the rat and monkey is compatible with the view that zolpidem binds to BZD recognition sites. That high concentrations of the ^{3}H-ligand were present in brain areas containing BZD_1 receptors (e.g. substantia nigra, pallidum, cerebellum, cortex) whereas low or undetectable ^{3}H-ligand binding was found in CNS areas containing BZD_2 receptors (e.g. spinal cord, caudate putamen, nucleus accumbens) reinforce the contention that zolpidem preferentially labels the BZD_1 subtype.

376.7 IMMUNOCYTOCHEMICAL LOCALIZATION OF A $GABA_A$/BENZODIAZEPINE RECEPTOR COMPLEX IN THE HUMAN HIPPOCAMPAL FORMATION. C.R. Houser, R.W. Olsen, J.G. Richards and H. Möhler*. Brain Research Institute, UCLA, Los Angeles, CA 90024 and Research Department, Hoffman La-Roche, CH-4002 Basel, Switzerland.

Monoclonal antibodies to a purified $GABA_A$/benzodiazepine receptor complex from bovine cerebral cortex (Schoch, P. et al., Nature 314:168, 1985) have been used to determine the normal localization of this receptor in immunocytochemical preparations of the human hippocampal formation. The highest levels of staining were observed in the molecular layer of the dentate gyrus, where much of the immunoreactive product appeared to be localized on the dendrites of granule cells. A thin band of moderate staining was also observed in the infragranular layer whereas lower intensities of staining were present within the granule cell layer. Moderate levels of staining were present in the stratum pyramidale and stratum radiatum of the hippocampus in field CA1. The intensity of staining in these layers decreased progressively through fields CA2 and CA3. Within CA3 and the hilus of the dentate gyrus, immunoreactive product was observed predominantly along the perimeter of cell bodies and dendrites of nonpyramidal neurons, and these labeled neurons contributed to a complex dendritic field within the hilus. Nonpyramidal neurons in the stratum oriens of all subfields of the hippocampus were also outlined by immunoreactive product, and the horizontally oriented dendrites of these neurons formed a distinct band near the boundary between stratum oriens and the alveus.

Studies of $GABA_A$/benzodiazepine receptor localization in surgical specimens from the hippocampal formation of patients with temporal lobe epilepsy are in progress. Receptor localization in these tissues has reflected the loss of neurons in various regions of the hippocampus and dentate gyrus and emphasized the concomitant loss of the dendritic trees of these neurons. Loss of nonpyramidal neurons and their dendritic arborizations within the hilus has been one of the most consistent findings. The receptor localization has also focused attention on neurons remaining within regions of extensive cell loss, and such neurons and their dendrites were often heavily outlined by immunoreactive product. A prominent dendritic localization of the $GABA_A$/benzodiazepine receptor on several types of neurons was one of the most striking findings in both normal and pathological specimens of the human hippocampal formation. Supported by NIH grants NS21908 and NS22071.

376.8 CHARACTERIZATION OF ADENOSINE RECEPTORS AND ENDOGENOUS ADENOSINE IMMUNOREACTIVITY IN THE RETINA. K.M. Braas, M.A. Zarbin*, W.R. Green*, and S.H. Snyder. Dept. of Neuroscience and Wilmer Ophthalmological Institute, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

The purine nucleoside adenosine is a putative neurotransmitter or neuromodulator in the CNS. Recently, we demonstrated the immunocytochemical localization of adenosine-containing neurons throughout the rat brain (Braas et al, 1986). The retina possesses adenosine A_1 receptors mediating adenylate cyclase inhibition and selective uptake of ^{3}H-adenosine (Paes de Carvalho and de Mello, 1985; Ehinger and Perez, 1984). We have now employed immunocytochemical and autoradiographic techniques to demonstrate the localization of endogenous adenosine and adenosine receptors in the retina of rats, monkeys, and humans. The specificity and sensitivity of antisera to the adenosine derivative laevulinic acid ($O^{2'},^{3'}$-adenosine-acetal) for adenosine have been previously characterized by RIA and immunocytochemistry (Newby and Sala, 1982; Braas et al, 1986). Eyes were fixed with 2.5% glutaralde-hyde, washed, and embedded in paraffin. Tissue sections were stained immunocytochemically with antisera using the avidin-biotin-peroxidase complex technique. The rat retina contains intense adenosine immunoreactivity in the nerve fiber layer and ganglion cell layer. Diffuse staining was observed throughout the inner plexiform layer and in select cells of the inner nuclear layer. The corneal epithelium also displayed intense adenosine immunoreactivity. Similar retinal staining patterns were seen in both monkeys and humans; while both also exhibited staining in cells of the outer portion of the inner nuclear layer, human retinas also contained staining in select cells of the outer nuclear cell layer.

Autoradiographic localization of adenosine A_1 receptors was determined using ^{3}H-l-phenylisopropyladenosine (PIA) as previously described (Goodman and Snyder, 1982). Binding sites were greatly enriched over the nerve fiber layer, ganglion cell layer, and inner plexiform layer of the rat retina. In monkey retina, binding was diffuse from the nerve fiber layer to the outer plexiform layer, but was dense over the same regions as the rat retina. Binding was observed throughout the entire human retina but was enriched in regions of the inner retina, especially in the nerve fiber layer. The pharmacology of the binding (Murphy and Snyder, 1982) of ^{3}H-l-PIA to rat retinal membranes showed a Bmax of approximately 150 fmol/g wet wt. Among the adenosine analogues tested, l-PIA was the most potent in competition experiments; the rank order of analogue potencies is consistent with adenosine A_1 receptors. We are presently characterizing the localization of adenosine uptake sites using ^{3}H-nitrobenzylthioinosine.

376.9 PHARMACOLOGICAL CHARACTERIZATION OF α1-RECEPTORS COUPLED TO PHOSPHOINOSITIDE METABOLISM IN THE CILIARY PROCESS OF THE ALBINO RABBIT. P.Mallorga, S.Buisson* and M.F.Sugrue*. Lab. MSD-Chibret, BP 134, 63 203 Riom Cédex, France.

The ciliary process is the site of aqueous humor secretion into the posterior chamber of the eye. Aqueous humor secretion is known to be regulated by beta-adrenoceptors coupled to adenylate cyclase present in the ciliary epithelium. In contrast, the direct role of receptors such as muscarinic and alpha$_1$-adrenergic coupled to phosphoinositide metabolism on aqueous humor secretion is unclear.

Their presence in the ciliary process was investigated using a direct assay which involves the prelabelling of phospholipids with ^{3}H-inositol. New Zealand albino rabbit ciliary processes were preincubated with ^{3}H-inositol and the accumulation of inositol-phosphates (IPs) induced by various agonists was determined after an incubation at 37°C for 45 minutes. In the absence of agonist the fractions of IPs recovered consisted of inositol-1-phosphate (IP1) (56%), inositol-1,4-bis-phosphate (IP2) (41%) and inositol-1,4,5-trisphophate (IP3) (3%). LiCl (10 mM) increased the labelling of IPs by 100% and radioactivity was recovered as IP1 (73%), IP2 (25%) and IP3 (2%). In the presence of LiCl (basal activity), carbachol (1-10000 µM) produced a dose-dependent stimulation of IP accumulation. The Ka for carbachol stimulation was 209 µM and maximum stimulation was 910% of basal. The carbachol-induced response was sensitive to the muscarinic antagonist atropine (Ki= 2nM) but not the nicotinic antagonist mecamylamine (100 µM). Phosphatidyl inositol (PI) hydrolysis was also induced by l-norepinephrine (l-NE) (1-1000 µM) and l-phenylephrine (0.1-1000 µM) but not by clonidine (1 mM) or l-isoproterenol (1 µM). The l-NE response was dose-dependent with a Ka of 16 µM and a maximum stimulation of 482% of basal. Phenylephrine was a partial agonist with a maximum response of 283% of basal and a Ka of 1 µM. The l-NE response could be potently and competitively antagonized by the alpha$_1$-adrenoceptor antagonist prazosin (Ki=34 nM), the alpha$_2$-adrenoceptor antagonist rauwolscine being 294-fold less potent. A dispersed cell suspension containing epithelial cells was prepared from ciliary processes using enzymatic digestion with 0.1% trypsin, centrifugation and sieve filtration. Stimulation of PI hydrolysis by carbachol (10 mM) (376%) and l-NE (1 mM) (218%) was still present in the cell suspension.

Our results demonstrate the presence of muscarinic cholinergic receptors and alpha$_1$-adrenoceptors coupled with phosphoinositide metabolism in the ciliary process of the rabbit. A fraction of these receptors are localized on epithelial cells and may be involved in the direct regulation of aqueous humor secretion through calcium mediated events.

376.10 AUTORADIOGRAPHIC LOCALIZATION OF IL-1 RECEPTORS IN RAT BRAIN. W.L. Farrar[1], J.M. Hill[2], M.R. Ruff[3] and C.B. Pert[2]*. [1]Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, Division of Cancer Treatment, NCI-Frederick Cancer Research Facility, Frederick, MD 21701-1240, [2]Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA and [3]Cellular Immunology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research, NIH, Bethesda, MD 20892.

Interleukin-1 (IL-1) is a 17,500 Mr polypeptide hormone produced by macrophages, keratinocytes and brain glial cells which acts as a soluble mediator in immunological and inflammatory reactions. Although its best known effect on the central nervous system is its ability to cause fever, it has been found to influence cell growth, food intake and slow-wave sleep.

We have developed a binding assay for ^{125}I-labeled recombinant human IL-1 and show it to be highly specific. Additionally, affinity crosslinking studies indicate that the rat brain IL-1 receptor has a molecular weight of approximately 75 Kd, which is similar to the previously described recognition molecule on T cells and fibroblasts.

Using autoradiographic techniques, we have visualized the distribution of ^{125}I-IL-1 binding in sections of fresh frozen rat brain. IL-1 receptors were found to be widespread throughout the brain forming a distinctive pattern of distribution. Areas of especially dense receptors were typically neuron-rich sites of the brain such as the granule cell layer of the dentate gyrus, the pyramidal cell layer of the hippocampus and the granule cell layer of the cerebellum as well as in the hypothalamus.

The pattern of IL-1 receptor distribution revealed here indicates the presence of receptors on neuron cell bodies and the localization to numerous discrete brain areas other than those hypothalamic sites involved in temperature regulation suggests a broader role for IL-1 in brain functioning than previously recognized. IL-1, derived from local or systemic sources, may function in the brain to coordinate behavioral and neuroendocrine activities with immunological and inflammatory reactions throughout the body.

376.11 IGF-II, IL-1 RECEPTORS AND THY 1.1 ANTIGEN HAVE THE SAME PATTERN OF DISTRIBUTION IN RAT BRAIN. J.M. Hill, M.A. Lesniak and C.B. Pert* (SPON: S. Feldman). Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA and Diabetes Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892.

As part of our ongoing research into growth factors and the interrelatedness of the nervous system and the immune system we have recently determined the pattern of distribution of insulin-like growth factor-II (IGF-II) receptors, interleukin-1 (IL-1) receptors and the thymocyte surface antigen Thy 1.1 in rat brain.

The distribution of ^{125}I-IGF-II and ^{125}I-IL-1 binding to rat brain sections was determined with autoradiography and the distribution of the surface antigen Thy 1.1 was determined with radioimmunocytochemistry. The pattern of distribution of all three was found to be identical. The similarity was not limited to a common occurrence on the same brain regions but extended to a co-localization of the same cell layers within these regions. A striking feature was the presence of these surface proteins in laminated areas of the brain such as the olfactory bulb, hippocampus, and cerebellum where binding was usually most prominent in those layers composed of densely-packed neurons.

In most, but not all brain regions, the pattern made by all three factors is identical to the pattern achieved by a Nissl stain. This suggests that IGF-II receptors, IL-1 receptors and Thy 1.1 are present on the cell bodies of almost all of the neurons of the brain.

Excess nonradioactive IGF-II was shown to inhibit the binding of radiolabeled IGF-II and IL-1 but not anti-Thy 1.1. Similarly, excess nonradioactive IL-1 inhibits the binding of radiolabeled IL-1 and IGF-II but not anti-Thy 1.1. Anti-Thy 1.1 inhibits the binding of radiolabeled IGF-II and IL-1, but was not inhibited by either IGF-II or IL-1.

These data suggest that either IGF-II and IL-1 receptors and Thy 1.1 share some component or binding site, or, most probably, separate and specific receptors for IGF-II and IL-1 exist on the same cells which express Thy 1.1. Thus, mutually interactive binding effects among these surface molecules may hereby reflect a shared molecular components (e.g., N or G subunits).

Although the function of these factors in the brain are not well understood, both IGF-II and IL-1 are growth factors and Thy 1.1 may play a role in cell adhesion. Perhaps, among other things, these growth factors and Thy 1.1 function in the development and maintenance of neurons and synaptic contacts in the brain.

376.12 DISTRIBUTION AND CHARACTERISTICS OF CALCITONIN GENE-RELATED PEPTIDE BINDING SITES IN THE SPINAL CORD AND BRAIN STEM OF THE CAT. V. John Massari[1], Patricia C. Contreras[2], and Robert T. Jensen[3]. Dept. of Pharmacology, Howard Univ. Med. School Washington, DC 20059[1]; Experimental Therapeutics Branch, NINCDS, Bethesda, MD[2]; Digestive Diseases Branch, NIDDK, Bethesda, MD [3].

We have investigated the distribution and characteristics of calcitonin gene-related peptide (CGRP) binding sites throughout the central nervous system of the cat. Binding sites for CGRP were labelled on 20u slide-mounted sections using ^{125}I-CGRP as a ligand. ^{125}I-CGRP was diluted to a final concentration of 6.5 picomolar in 50mM Tris buffer containing .04% bacitracin, .002% chymostatin and .004% leupeptin to retard degradation of the ligand. Alternate sections were treated identically, except that 1 uM unlabelled CGRP was added in order to saturate specific binding sites. Sections were exposed on LKB Ultrofilm for 3 days.

The kinetics of CGRP binding was evaluated in sections of cat cerebellum. Binding of rat ^{125}I-CGRP was inhibited by unlabelled CGRP, but the slope of the inhibition curve was very broad. This result suggests that ^{125}I-CGRP labels more than 1 site or state of the receptor.

Autoradiographically labelled ^{125}I-CGRP binding sites were observed throughout the spinal cord. The distribution of binding sites was not uniform in different segments of the cord. Binding appeared to be greater in segments C_1, T_1, and S_1 than in some other cervical, thoracic, lumbar, and sacral segments. In C_1, T_1, S_1, dense binding was seen in the superficial laminae of the dorsal horn, and in lamina X. Moderate binding was seen in the ventral horn. The white matter showed light and apparently uniform binding in all funiculi. The dorsal root ganglia also showed moderate levels of ^{125}I-CGRP binding. In the medulla dense binding was observed in the hypoglossal nuclei, olivary nuclei, external cuneate nucleus, area postrema, nucleus of the solitary tract, nucleus ambiguous, dorsal cochlear nucleus and dorsal tegmental nucleus. Moderate binding was seen in the vestibular nuclei, facial nucleus, marginal nucleus of the brachium conjunctivum, locus coeruleus and motor trigeminal nucleus. At the level of the pons, dense binding occurred in the pontine nuclei, the superficial layers of the superior colliculi, the interpeduncular nucleus, the substantial nigra and the dentate gyrus. The raphe nuclei showed moderate binding as did the tegmental reticular nucleus, some areas of peri-aqueductal gray, the dorsolateral geniculate nucleus, and the cingulate gyrus.

These data suggest that CGRP may influence a wide variety of brain and spinal cord functions, possibly through its effects on more than one endogenous receptor.

376.13 LOCALIZATION OF CORTICOTROPIN RELEASING HORMONE mRNA IN THE BRAIN OF THE RAT BY *in situ* HYBRIDIZATION. D.M. Frim* and J.A. Majzoub* (SPON:E. Spindel). Howard Hughes Medical Institute, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115.

Corticotropin releasing hormone (CRH) is a 41 amino acid hypothalamic peptide believed to be involved in the hypothalamic-pituitary-adrenal response to stress. To date, its only known physiologic function is to stimulate the release of ACTH from the anterior pituitary. However, immunohistochemical techniques have localized it to many extrahypothalamic brain regions, most notably limbic system structures, raising the possibility that CRH may possess other, nonendocrine functions. Whether CRH is actually synthesized in these extrahypothalamic sites is not known. To address this question, we undertook the localization of CRH messenger RNA to discrete brain areas with a histohybridization technique. Brains were prepared from normal Sprague Dawley rats by perfusion with PBS followed by 4% paraformaldehyde, postfixed for 2 hours and then frozen at -80°C. Brains were thawed to -20°C, cut in 20 micron sections and hybridized to a [35S]-labeled rat CRH cRNA probe. Intense hybridization signal was seen in expected areas, including the paraventricular nucleus of the hypothalamus, the amygdala, and the cingulate cortex. However, we also noted equally intense signal in the primary auditory cortex, hippocampus (CA 1,2,3,4, and dentate gyrus), habenula, primary visual cortex, and cerebellum. In conclusion, CRH is synthesized in several discrete extrahypothalamic brain regions, suggesting that in addition to its neuroendocrine role, it may possess other functions, such as that of a neurotransmitter.

376.14 REGIONAL DISTRIBUTION OF CORTICOSTERONE-BINDING IN THE SONG SPARROW, MELOSPIZA MELODIA, AS DETERMINED BY TRITIUM-SENSITIVE FILM AUTORADIOGRAPHY. E. Ronchi*, J.C. Wingfield* and B.S. McEwen (Spon: D. Micco). The Rockefeller University, New York, NY 10021

As in mammals, circulating glucocorticoids of birds are involved in the neuroendocrine response to stress and environmental changes. Recent investigations of free living song sparrows have correlated fluctuations in circulating plasma levels of corticosterone (CORT) with a variety of adaptive behaviors, such as increased foraging and suppressed reproductive behavior during periods of stress. In addition, although many of the same neurohumoral pathways found in mammals are also found in birds, the question of whether the avian adrenals may be regulated without direct pituitary stimulation is still unsettled and very few studies have been undertaken in birds to determine which brain centers are involved in the adrenal steroid feedback inhibition of corticotropic responses. A problem encountered in a previous attempt to map CORT receptors in the avian brain has been the lack of information on the specific nature of receptor binding and the inadequate quantification of receptor localization. Competition with unlabelled CORT as well as chemical adrenalectomy, using metyrapone (2-methyl-1,2-di-3-pyridil-1-propanone), an inhibitor of 11-β-hydroxylation, has provided an efficient tool for mapping, using quantitative autoradiography, the specific distribution of CORT receptor binding sites in avian brain. Subjects were male Song Sparrows, Melospiza melodia, which has been extensively studied in the wild. Adult males were treated with metyrapone implants six hours before steroid injection, since preliminary experiments showed that by this time point a maximal decrease in circulating CORT plasma levels had occurred. Controls were given a subcutaneous implant of unlabelled CORT the day before sacrifice, or alternatively were injected intramuscularly with unlabelled CORT an hour before treatment with the labelled steroid. ^{3}H-CORT was administered through the jugular vein 30 minutes before sacrifice. Birds were decapitated and their brains rapidly removed, frozen on dry ice and stored at -80°C. Coronal brain sections (25μ) were cut and thaw mounted onto subbed slides. Slices and standard sections were exposed to tritium sensitive LKB film for five months. Autoradiograms were analyzed using a computer assisted densitometer. Heaviest binding occurred in the hyperstriatum accessorium, tractus septomesencephalicus, area parahippocampalis, hippocampus, neostriatum and neostriatum caudale. Binding was moderate in the ventral tuberal area of the hypothalamus. With the exception of binding in the hypothalamus, these findings agree well with a previous autoradiographic study performed in the duck by Rhees, Abel and Haak (Gen. Comp. Endo., 1972) and with investigations on mammals which have found the greatest amount of binding in the hippocampus, septum and amygdala respectively. (Supported by NH41256 and DCB-8316155).

376.15 EFFECTS OF ESTRADIOL ON HYPOTHALAMIC OCYTOCINERGIC NEURONS G.F. Jirikowski*, J.D. Caldwell*, W.E. Stumpf and C.A. Pedersen. Department of Anatomy and Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27514.

The rat hypothalamus was examined immunocytochemically for changes in oxytocin-like immunoreactivity due to estrogen treatment. In another set of experiments combined autoradiography and immunocytochemistry were used to study the topographical distribution of oxytocinergic estradiol target neurons.

Ovariectomized rats received silastic implants with estradiol or empty implants for six days. After perfusion-fixation, serial vibratome brain sections were examined with antibodies to oxytocin (OT). In control animals OT immunoreactive perikarya were found in neurons of the magnocellular hypothalamo-neurohypophysial system (HNS) and in the periventricular nucleus (pev). Neuronal processes were visible in the preoptic region, the ventromedial hypothalamus and the median eminence. In estradiol treated animals, additional immunoreactive perikarya could be observed in the septo-hypothalamic nucleus (shn), the anterior commissural nucleus (acn) and the zona incerta (zi). These latter immunoreactive neurons seem to have predominant central projections. Processes from neurons of the pev project to the subependymal layer of the IIIrd ventricle. OT-axons could also be found in the lateral septum, the preoptic region, the striatum and the medial amygdala.

Untreated ovariectomized rats were injected with ^{3}H estradiol 1 hr before sacrifice and processed for combined thaw-mount autoradiography and oxytocin immunocytochemistry. About 30% of the OT neurons in the HNS showed nuclear radiolabeling with ^{3}H estradiol. OT neurons of the acn, shn, and zi did not have radiolabeled nuclei under the conditions studied. Periventricular OT neurons were only occasionally estrogen-labeled.

The results suggest that oxytocinergic neurons of the hypothalamo-neurohypophyseal system are in part under direct genomic control of estradiol. The lack of estrogen concentration in certain OT immunoreactive neurons may indicate that estrogen receptors are absent in these neurons or low in number. Therefore estrogen effects on OT immunoreactivity in these cells may also be mediated through indirect genomic effects on associated neurons or through non-genomic mechanisms.

G.F. Jirikowski is a Fogarty Fellow. Supported by NIH grants NS09914, HD16159 and NIMH grant MH33127.

376.16 CULTURED CEREBROMICROVASCULAR SMOOTH MUSCLE AND GLIAL CELLS: PROSTAGLANDIN RECEPTORS LINKED TO ADENYLATE CYCLASE. B. Wroblewska*, O. Kempski* and M. Spatz. Lab. of Neuropathology and Neuroanatomical Sciences, NINCDS, National Institutes of Health, Bethesda, MD 20892.

Prostaglandins may play an important modulatory role in the brain, influencing through specific receptors neuronal transmission and cerebral blood flow. It is not clear, however, whether the various cerebral cellular elements have distinct receptors for each type of prostaglandin which might be associated with a specific cellular response. We have previously characterized prostaglandin receptors linked to the adenylate cyclase (AC) system in cultured cerebromicrovascular endothelium Karnushina et al (Life Sci. 32: 1427, 1983). The aim of the present study was to determine and compare the AC responses to various prostaglandins in cerebromicrovascular smooth muscle cultures with those in glial cell cultures.

The smooth muscle cell cultures were prepared from the dissociated cerebromicrovascular fraction of 2 day old rats, while glial cell cultures were dissociated from rat brains of the same age according to the methods of Spatz et al (Brain Res. 280: 387, 1983) and Frangakis and Kimmelberg (Neurochem. Res. 9: 1685, 1984), respectively. All the experiments were performed on confluent cell cultures (2nd-3rd generation). Increasing concentrations of each prostaglandin (PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGI_2 and PGD_2) were added to the incubating medium for 5 min (except PGI_2 for 2 min). The content of cAMP in the cultured cells was assayed according to the technique described by (Karnushina et al Life Sci. 32: 1427, 1983).

PGE_1 and PGE_2 in contrast to $PGF_{1\alpha}$ and $PGF_{2\alpha}$ stimulated cAMP formation in both cell types. The smooth muscle AC system showed a greater affinity for E-type prostaglandins than the AC system in glial cells. In general, the EC_{50} values for prostaglandins were one order of magnitude lower in the smooth muscle cells than in the glial cells. Most importantly, the cultured cerebromicrovascular smooth muscle and glia cells revealed different AC responses to PGI_2 and PGD_2. Formation of cAMP in the smooth muscle was stimulated by increasing concentration of PGI_2 but not by PGD_2. On the other hand, the glial cells showed a dose-dependent PGD_2 enhancement of cAMP formation, while PGI_2 failed to increase significantly the accumulation of cAMP over the basal level at any of the investigated concentrations (except at 1).

ese findings indicate that the prostaglandin receptors linked to AC are distinct in each cell type.

376.17 ROLE OF PHORBOL ESTERS IN REGIONAL CEREBRAL BLOOD FLOW REGULATION. D.F. Hanley*, G.R. Uhl*, M. Miyabe* and R.J. Traystman*. (SPON: G. Alexander). The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Phorbol esters are known to activate protein kinase C, an intracellular enzyme capable of phosphorylating membrane associated receptors. By using phorbol-12-13-dibutyrate (PDBU), we investigated the presence and function of protein kinase C on canine cerebral vessels. In vitro tissue autoradiographic studies performed on 8 u sections of canine cerebral vessels with H^3-PDBU revealed a 2 to 1 ratio of specific to nonspecific binding. Competitive displacement was demonstrated for 2 physiologically active phorbol esters but could not be demonstrated for 2 physiologically inactive phorbol derivatives.

DISPLACEABLE PHORBOL BINDING TO CANINE BASILAR ARTERY

Ligand	Displacer	Physiologic Activity	Binding (Grains /1000 u^2)
H^3-PDBU	None	-	24
H^3-PDBU	Phorbol	-	21
H^3-PDBU	Phorbol-12-Monoacetate	-	20
H^3-PDBU	DPB	+	12
H^3-PDBU	PDBU	+	8

The effect of PDBU on regional cerebral blood flow (rCBF) was then studied in vivo using radiolabelled microspheres in 6 dogs. Measurements were made during control ventriculo-cisternal CSF infusions and 5,10,15,20 and 25 minutes after infusion of .1 nM/min PDBU. For grey matter regions in contact with the perfusate, caudate nucleus, cortical watershed and cerebellum, blood flow increased from 33 ± 6 to 45 ± 7, 20 ± 2 to 27 ± 2, and 31 ± 2 to 42 ± 5 ml/min/100 gm, respectively, rCBF was unchanged for brainstem, temporal lobe or white matter regions. We conclude 1) PDBU has high affinity binding to canine cerebral vascular smooth muscle, and 2) PDBU produces an increase in rCBF when delivered intraventricularly. These data suggest a possible role for protein kinase C in the regulation of cerebral blood flow.
(Supported by NIH NS20020)

376.18 SEX DIFFERENCES IN ANDROGEN RECEPTOR BINDING IN THE BRAIN STEM NUCLEI ARE NOT CORRELATED WITH NEURONAL NUMBER. W.H.A. Yu and M.Y. McGinnis*. City Univ. of New York Med. Sch., New York, NY 10031 and Mt. Sinai Sch. Med., New York, N.Y. 10029

We have shown previously that cranial nerve nuclei in the brain stem of adult rats contain specific binding sites for androgens (J.Neurosci. 6:1302, 1986). We now report that androgen receptor binding values in the brain stem motor nuclei are significantly higher in males than in females, and that this sex difference is independent of the number of neurons in these nuclei. Sprague-Dawley rats weighing 200-250 g. were castrated 2-3 days before sacrifice. Tissues of hypoglossal and facial motor nuclei were dissected and pooled from 4-5 rats for each determination. Cytosolic androgen receptor binding was measured by a single point assay using 4nM ^{3}H-R1881 as ligand. Neuronal number in the hypoglossal and facial motor nuclei was counted from male and female rats of 4 months of age. After intracardiac perfusion with aldehyde fixative, the brain stem was blocked, embedded in paraffin, serially sectioned at 7 um, and every 11th section was stained with cresyl violet. Neurons with visible nucleoli were counted bilaterally, and the total number of neurons in each nucleus was calculated. Results, shown below, indicate that there are sex differences in receptor binding values but not in neuronal number between males and females.

	Hypoglossal nucleus		Facial nucleus	
	receptor binding (fmol/mg protein)	neuronal number	receptor binding (fmol/mg protein)	neuronal number
Males	11.2±1.6*(9)	4474±85(6)	6.0±0.6*(4)	5920±200(6)
Females	6.6±0.7 (4)	4652±110(8)	3.9±0.8 (4)	6113±241(8)

Values are mean ±SEM. Parentheses indicate the number of determinations. *$p < 0.05$, significantly different from females, Student's t-test.

CATECHOLAMINES: ELECTROPHYSIOLOGICAL STUDIES

377.1 DIFFERENTIAL EFFECTS OF HALOPERIDOL AND CLOZAPINE IN THE RAT NEOSTRIATUM AND NUCLEUS ACCUMBENS: MICROIONTOPHORETIC STUDIES. X.T. Hu* and R.Y. Wang (Spon: I. Fand), Department of Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

Prolonged treatment with classical antipsychotic drugs (APDs) such as haloperidol (HAL) and chlorpromazine induce a time-dependent depolarization inactivation of dopamine (DA) neurons in both the substantia nigra (A9) and the ventral tegmental area (A10) of the rat brain. In contrast, treatment with atypical APDs such as clozapine (CLOZ) and thioridazine selectively decrease the number of A10 DA neurons. This chronic APD-induced inactivation of A10 and A9 DA neurons could be prevented by lesions of the nucleus accumbens (NAc) and the caudate nucleus (CdN), respectively, suggesting that postsynaptic sites are important in mediating the actions of APDs on DA cells. The aim of the present study was to determine whether the atypical APD CLOZ has a preferential site of action in the NAc. For comparison, the effect of HAL on neurons in the NAc and the CdN was also investigated. Standard extracellular single unit recording and microiontophoretic techniques were used to assess the effect of CLOZ and HAL on DA-induced inhibition. In addition to spontaneously active cells, glutamate (GLUT)-activated cells were studied because, as previously reported, many neurons in the CdN and NAc have very low discharge rates or are quiescent.

Iontophoresis of DA (ionto-DA) suppressed both the spontaneous and GLUT-activated firing activites in the NAc and the CdN. Ionto-HAL prevented or reversed DA inhibition in both the NAc (n=5) and the CdN (n=6). In contrast, CLOZ showed a preferential action on NAc cells; CLOZ was effective in preventing DA-inhibition on 11 out of 16 (or 69%) NAc cells tested whereas in the CdN, CLOZ was mostly ineffective (only 1 out of 13 CdN neurons responded). In addition, preliminary results show that ionto-HAL activated both NAc and CdN cells whereas ionto-CLOZ preferentially increased the firing rate of NAc cells. Interestingly, CLOZ was particularly effective in reversing the DA effect on GLUT-induced activation on quiescent NAc neurons; CLOZ was effective on all 8 quiescent cells studied and was efficacious on only 2 out of 8 spontaneously active NAc cells. It is suggested that CLOZ is especially potent in antagonizing effects produced by tonically active A10 DA neurons which presumably rendered the NAc cells quiescent.

In conclusion, the results of the present study indicate that CLOZ preferentially acts upon A10 DA systems. This may account for the fact that CLOZ, among all APDs, has very low potential for causing extrapyramidal side effects. (Supported by USPH grants MH-41440 and MH-41696 to R.Y.W.)

377.2 DIFFERENTIAL EFFECTS OF ACUTE CLOZAPINE AND HALOPERIDOL ON THE ACTIVITY OF MESOLIMBOCORTICAL (A10) AND NIGROSTRIATAL (A9) DOPAMINE NEURONS. Timothy H. Hand, Xiu-ti Hu* and Rex Y. Wang. Department of Psychiatry and Behavioral Science, SUNY, Stony Brook, N.Y. 11794.

Recent electrophysiological experiments (Chiodo and Bunney, 1983, 1985; White and Wang, 1983) have shown that chronic administration of prototypical neuroleptic compounds such as haloperidol (HAL) inactivates A9 and A10 dopamine (DA) cells non-selectively. However, chronic treatment with atypical antipsychotic drugs (APDs) such as clozapine (CLOZ) inactivates A10 DA neurons without significantly changing the number of spontaneously active A9 DA neurons. We were interested in determining whether acute administration of the APDs HAL and CLOZ was also capable of inactivating DA units, and if so, whether the effects of acute CLOZ were specific for A10 DA neurons. We therefore examined the effects of acute CLOZ and HAL on the baseline firing rate of A9 vs A10 DA units using standard extracellular single-unit recording techniques.

CLOZ was found to increase the firing rate of A10 DA units at unexpectedly low doses. The geometric mean dose for producing at least a 20% increase in rate was 1.32 mg/kg. Higher doses of CLOZ drove 9/23 (39%) of A10 cells into an apomorphine (APO)-reversible depolarization blockade. CLOZ did not change A9 DA cell activity in the great majority of sampled cells (14/17). HAL, on the other hand, produced an APO reversible activation and depolarization inactivation in both subpopulations of DA neurons. Multiple exposures to antipsychotic drugs are therefore not necessary to inactivate DA cells. Scopolamine, a muscarinic receptor blocker, did not mimic the effects of CLOZ on A10 neurons, and it also failed to block the activating effect of HAL on A9 units. This indicates that the antimuscarinic activity of CLOZ is not involved in its actions on DA neurons. CLOZ completely reversed APO-induced suppression of A10 DA activity, but in many cases only partially reversed suppression of A9 neurons. Transections of the diencephalon, which severed the medial forebrain bundle and other feedback pathways to the DA somata, prevented the excitatory action of HAL on most A9 neurons sampled. However, this procedure left intact the activation of A10 neurons produced by HAL and CLOZ, indicating that forebrain feedback pathways are not as critical in mediating the action of APDs on A10 neurons. It is suggested that the different pharmacological and clinical properties of HAL and CLOZ may be partially explained by a differential mode of action on the A10 and A9 subpopulations of DA cells. The data also provide pharmacological evidence that the A9 and A10 DA cell groups are regulated by different mechanisms. (Supported by MH 41440 and MH 41696 to RYW and by PHHS Training Grant, 1 T32 MH1801001A1)

377.3 SIMULTANEOUS SINGLE UNIT RECORDING AND LOCAL DRUG INJECTION IN THE LOCUS COERULEUS OF FREELY MOVING CATS. B.L. Jacobs and E.D. Abercrombie. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

The traditional neuropharmacological approach to understanding neurotransmitter systems employs relatively specific receptor agonists and antagonists to manipulate the activity of particular subsets of neurons in the brain. Problems arise with this approach because systemic administration of drugs has indirect effects on neuronal activity and effects of drugs applied directly via iontophoresis or pressure ejection in acute preparations are confounded by anesthesia or immobilization. In order to avoid these problems, it is desirable to be able to investigate drug actions on identified neurons during a variety of behavioral contexts. We have developed a technique which allows the infusion of substances during electrophysiological recording of single unit activity in behaving cats. Unit activity is recorded by means of movable 32 and 64 um diameter insulated nichrome wires implanted in two bundles of six each above the LC (P 4.0 L 2.8 H -1.9). A movable 22g stainless steel guide cannula is interposed between the two electrode bundles. This cannula terminates 1.0 mm above the recording site. A 28g stainless steel injector is constructed so that when placed within the guide cannula it protrudes to a depth equal to that of the microelectrode bundles with approximately a 500 um lateral displacement. The injector is connected to a 1.0 ul syringe via PE 20 tubing and is prefilled with drug. Drug infusion is regulated by a remotely operated syringe pump. After collecting baseline unit activity measures, the drug is infused in a volume of 0.1 ul over 1 min. LC neurons are identified as noradrenergic by their long duration action potential, slow baseline firing rate and complete suppression during REM sleep. Neurochemical identity is further confirmed by the complete suppression of activity within 20 min of a systemic injection of alpha-2 agonist clonidine (25ug/kg i.p.). When 1.0 ug of clonidine is infused locally, unit activity is significantly decreased within 1 min and is completely suppressed within 5 min. In contrast, sterile saline infusion has no effect. Given the high density of opioid receptors found in LC and the conflicting evidence regarding the physiological effect of opioid receptor agonists on LC neurons, the effect of infusion of 1.0 ug morphine on LC units was examined. This produced a significant suppression (76% below quiet waking baseline) of unit activity within 15 min. This suppression persisted for the entirety of the 60 min observation period. Thus, we have demonstrated direct drug effects in the absence of general behavioral or physiological changes which accompany systemic drug injection. (Supported by NIMH Grants MH09256/MH23433 and Air Force Grant AFOSR85-0034).

377.4 MORPHINE INCREASES LOCUS COERULEUS NORADRENERGIC NEURONAL ACTIVITY IN FREELY MOVING CATS AND IN MIDBRAIN SLICES IN VITRO. T.J. Trulson, K. Arasteh and M.E. Trulson. Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

The locus coeruleus (LC) contains high concentrations of opiate receptors, as well as enkephalin-like immunoreactivity. Morphine administration to anesthetized rats suppresses the spontaneous activity of LC neurons. Furthermore, a recent study showed that morphine suppresses the in vitro activity of LC neurons recorded in brain slices obtained from anesthetized rats. However, it is well-known that anesthesia alters the response of CNS neurons to psychoactive drugs (Trends Pharmacol. Sci. 5, 1984, 287-289). Therefore, we examined the effects of morphine on the activity on noradrenergic (NE) LC neurons in freely moving cats. Cats were implanted for macro- and micro-electrodes as previously described (Brain Res. 163, 1979, 135-150). After recovery from surgery the cats were placed in recording chambers and allowed to adapt to the novel setting. They then received i.p. doses of morphine sulfate (0.5, 1.0, 2.5 or 5.0 mg/kg) and unit activity was monitored for 8 hours. Morphine produced a dose-dependent increase in LC unit activity from approximately 15% at the lowest dose tested to more than 50% at the highest dose ($P<0.01$ ANOVA). When the cats were first anesthetized with chloral hydrate (350 mg/kg, i.p.) and then administered morphine, different results were obtained. First, the anesthetic produced a significant increase in LC unit activity (+150%) above the quiet waking baseline (1.61 ± 0.24 spikes/-sec.). Administration of morphine (2.5 mg/kg, i.p.) to chloral hydrate anesthetized cats produced a significant depression of unit activity from the elevated baseline due to the anesthetic. This depression of unit activity in chloral hydrate anesthetized cats by morphine was reversible by administration of naloxone (1 mg/kg, i.p.). It is important to note, however, that the above results in freely moving cats may be due to the behavioral state of the cats and/or activation of afferent inputs to the LC by morphine. Therefore, we examined the effects of morphine on the activity of NE-containing LC neurons recorded from mouse brain slices in vitro. Brain slices were prepared using standard procedures as previously described (Eur. J. Pharmacol., 114, 1985, 105-109). Morphine produced a dose-dependent increase in the activity of NE-containing LC neurons recorded in vitro. The response was not changed in a low calcium/high magnesium medium, indicating that the observed effects are the result of a direct action of morphine on LC neurons. When the mice were anesthetized with chloral hydrate prior to preparation of tissue slices, morphine produced a suppression of LC activity. These studies suggest that previous data showing a decrease in LC unit activity following incubation with morphine in vitro are due to the use of anesthesia.

377.5 RHYTHMIC ACTIVITY AND ADRENERGIC RESPONSES IN LOCUS COERULEUS NEURONS FROM YOUNG RATS. K.C. Marshall and J.T. Williams. Neuropharmacology Laboratory, M.I.T., Cambridge, MA 02139.

The possible role of the locus coeruleus (LC) in influencing development of other brain regions, and reports of changes in neurotransmitter responsiveness of LC neurons during development have led us to study brain slices from young rats (1-4 weeks). Intracellular recordings were made from LC neurons in submerged slices (300 µm) maintained at 37 °C. Drugs were applied by superfusion. The majority of LC neurons from 1-4 week animals exhibited rhythmic variations in membrane potential, 5-15 mV in amplitude and at a frequency of 0.3-3 Hz. The slowest frequencies were observed in slices taken from the youngest animals. Such rhythmic activity is seldom seen in LC neurons in slices from adult rats. Spontaneous action potentials were usually generated at the top of the depolarizing phase of these rhythms. Hyperpolarization with current abolished the action potentials but not the slow rhythmic activity. Superfusion with 11.5 mM $MgCl_2$ blocked all spontaneous activity. The frequency of the slow rhythm was increased by phenylephrine and muscarine and slowed by noradrenaline (NA) and cocaine. The full action potentials recorded in cells from young (8-12 days) rats were of greater duration (1.7-2.2 ms) than in cells from adults (1.2-1.6 ms). In slices from these young rats phenylephrine (100 µM) increased the frequency of spontaneous action potentials or gave depolarizations of 3-5 mV in cells hyperpolarized to below threshold. In slices from adult rats phenylephrine (10-100 µM) has no effect or slows the firing rate. The depolarization induced by phenylephrine was observed in 11.5 mM $MgCl_2$ and thus is a direct action on the cell. The action of NA (10-100 µM) following treatment with cocaine (10 µM) gave a strong hyperpolarization (15-25 mV), as in adults. However in cells from 1-2 week animals, 30-100 µM NA gave complex responses in which an initial strong hyperpolarization was reduced by 25-40% shortly after its onset. On washing out the NA, the cells again hyperpolarized and then slowly recovered to the control membrane potential in 3-5 min. After treatment with prazosin (500 nM) NA (30-100 µM) gave a simple hyperpolarizing response, with a much faster recovery to control potential after washout. This pattern was less evident on the cells from 3-4 week animals, though hyperpolarizing responses to NA were still larger and of shorter duration in some cells. Our results indicate differences in electrophysiological properties of LC neurons to those from adults and a greater α_1-adrenoceptor responsiveness in cells from 1-2 week old animals than in older animals.

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377.6 EFFECTS OF NOREPINEPHRINE ON RAT HIPPOCAMPAL NEURONS IN DISSOCIATED CELL CULTURE. P. A. Rosenberg, D. M. Barnes, and M. A. Dichter. Department of Neurology, Children's Hospital, Boston, Mass. 02115.

We are interested in identifying the target cells for norepinephrine (NE) in cerebral cortex and in characterizing the effects of NE on these target cells. Hippocampi were obtained from E20-21 day rat embryos and were maintained in dissociated cell culture using methods previously described (Frosch, Barnes, and Dichter, Soc. Neurosci. Abstr., 9: 680). Penetrations with microelectrodes were generally restricted to neurons with somal diameters greater than 18 um, and NE or the beta agonist isoproterenol (ISO) at 1-10 x 10^{-9}M were applied by pressure perfusion from pipets with tip diameters of 5 um. A total of 101 cells were successfully impaled and monitored for direct or indirect responses to NE or ISO. As in neocortical cultures, no direct membrane responses to NE or ISO were observed (Rosenberg, Schweitzer, and Dichter, Brain Res., 344: 369-372). The slow afterhyperpolarization observed in the hippocampal slice preparation was only rarely present in these cultures, and was not encountered during these experiments. For assaying for indirect responses, observable at the impaled cell by an increase in synaptic activity, the drug pipet was brought successively to one cell after another in the field surrounding the impaled neuron, testing approximately 10 cells per impaled cell. Twenty-three detector cells showed one or more responses when drug was applied to cells in the surround. The response was typically a barrage of epsps and spikes, and an exclusively ipsp response was never seen; the response was long-lasting, ranging from one to three minutes; marked tachyphylaxis was seen with little or no response evoked by second application of drug. By keeping track of the phase contrast morphology of the responding cells, it became clear that a morphologically distinct population of neurons seemed to be underlying the indirect response to NE or ISO. The distinctive features of these presumed responders was their small size (9-10 um diameter) and flat appearance in phase contrast, explaining why they had not previously been penetrated with microelectrodes. Definitive identification of these small cells as NE targets must await direct recording from them. Identification of these small cells with respect to their in vivo counterpart is also uncertain.

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377.7 **DIFFERENTIAL EFFECTS OF NOREPINEPHRINE ON HIPPOCAMPAL NEURONS** K. Pang[1] and G.M. Rose[1,2] [1]Dept. of Pharmacology, UCHSC, and [2]Medical Research, VAMC, Denver, CO 80262.

The central noradrenergic system has long been postulated to play a modulatory role in learning and memory. Since the hippocampus is also important in these functions, the actions of norepinephrine (NE) were examined in this brain region. Previous electrophysiological studies found that NE produced beta receptor mediated excitatory and alpha receptor mediated inhibitory effects in the hippocampus, with inhibitory actions predominating. In these experiments, the actions of NE were examined only upon presumed hippocampal pyramidal neurons. However, the hippocampus is composed of a mixed population of neurons: complex-spike, theta and granule cells. There is good evidence that complex-spike neurons are pyramidal neurons, while theta neurons are interneurons; granule cells are the primary cell type of the dentate gyrus. In the present study, the actions of NE were examined on all three hippocampal cell types.

Recordings were made from the hippocampal region of anesthetized rats using standard extracellular recording methods. Hippocampal cells were identified as complex-spike (duration $\geq$ 0.6 ms) or theta (duration $\leq$ 0.4 ms) neurons based on their unfiltered action potential durations. Theta neurons and dentate granule cells, which have similar action potential durations, were identified by their response to afferent stimulation. Dentate granule cells fired only a single action potential in response to perforant path stimulation, no matter how intense the stimulus, while theta neurons fired multiple action potentials. Recording electrode location was verified by monitoring the response evoked by stimulation of the commissural or entorhinal afferents to the hippocampal formation. Drugs were locally applied from multibarreled micropipettes by pressure microejection.

NE caused differential effects on the spontaneous firing of hippocampal complex-spike and theta neurons. Complex-spike neurons were inhibited by NE, a response which was demonstrated to be mediated by an alpha-1 receptor. In addition, activation of alpha-2 and beta receptors using selective agonists excited complex-spike cells. Theta neurons were excited by NE, an effect which was also shown to be mediated by alpha-2 and beta receptors. Although theta neurons and dentate granule cells have similar electrophysiological characteristics, NE had different effects on these two cell types. Whereas theta neurons were excited by NE, dentate granule cells were inhibited. As was the case for complex-spike neurons, the inhibitory action of NE was mediated by an alpha-1 receptor.

These results demonstrate that NE produces different responses on hippocampal complex-spike, theta and granule cells through interactions with distinct receptor populations. This work was supported by the Veterans Administration Medical Research Service.

377.8 NORADRENERGIC RESPONSES IN RAT HIPPOCAMPUS: EVIDENCE FOR INHIBITORY RESPONSES MEDIATED BY α_1-RECEPTORS. Michelle Mynlieff* and Thomas V. Dunwiddie. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Previous studies of evoked potentials in the CA1 region of rat hippocampus have shown a biphasic response to norepinephrine. Low doses of norepinephrine produce an increase in population spike size mediated via β-adrenergic receptors, whereas higher doses of norepinephrine decrease the size of the population spike. This latter response is thought to be mediated via α-receptors, since it can be antagonized by phentolamine, but the receptor subtype involved is unclear. The goal of this study was to determine whether the inhibition of the population spike is mediated through the α_1- or the α_2-adrenergic receptors.

Evoked potentials in the CA1 region were examined in the in vitro hippocampal slice preparation following stimulation of the Shaffer and commissural afferents to this region. Preliminary experiments demonstrated that 1 μM timolol was sufficient to block the β-adrenergic component of the response, so all slices were pretreated with this concentration of timolol in order to eliminate the complicating effects of the β response. The inhibitory response to norepinephrine (25 μM) and timolol was antagonized 38-72% by phentolamine (0.5-5 μM). Idazoxan (0.1-1 μM), a selective α_2 antagonist, was unable to block the response to norepinephrine. Prazosin, a selective α_1 antagonist, antagonized the inhibitory response by 59% at 1 μM. Both clonidine (1-50 μM) and phenylephrine(5-50 μM) were found to mimic the effect of norepinephrine in the hippocampus, producing a decrease in population spike size of 20-50% and 43-72% respectively. The concentrations of norepinephrine, phenylephrine, and clonidine required to inhibit the population spike response are consistent with their affinities for α_1-adrenergic receptors, but not for the α_2 receptor subtype. These results indicate that the inhibitory effect of norepinephrine on evoked responses in the hippocampus is mediated through the α_1-adrenergic receptors.
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377.9 RECURRENT FACILITATION OF LOCUS COERULEUS NEURONS IN THE RAT IS MEDIATED BY α_1-ADRENOCEPTORS. T.Sakaguchi*, S.Nakamura and F.Kimura* (SPON. T.Tsumoto). Dept. of Neurophysiology, Inst. of Higher Nervous Activity, Osaka University Medical School, Kita-ku, Osaka 530 JAPAN.

The nucleus locus coeruleus (LC) in the rat is a dense cluster of noradrenaline(NA)-containing neurons. NA released from the terminals of axon collaterals and possibly from dendro-dendritic synapses is known to inhibit spontaneous activity of LC neurons by activating α_2-adrenoceotors located on the soma-dendritic membranes (Aghajanian et al., Brain Res. 136: 570, 1977). In addition to α_2-adrenoceptors, a recent study has revealed the presence of α_1-adrenoceptors in the LC (Jones et al., J.comp.Neurol., 231: 190, 1985). Based on these findings, the present experiments were carried out to elucidate the physiological role of α_1-adrenoceptors in the LC. Young (1 to 14 days old) and adult (more than 7 weeks old) rats were anesthetized with urethane (1.3 g/kg, i.p.), and single unit activity was recorded extracellularly in the LC. Electrical stimulation of the dorsal noradrenergic bundle (DNB) arising in the LC caused both inhibition and excitation of LC neurons. The excitatory responses occurred predominantly while LC neurons were not firing, and the effects of DNB stimulation upon LC neurons, in turn, became principally inhibitory at relatively high frequencies of spontaneous firing. The excitation caused by DNB stimulation was partially attenuated or completely blocked by the α_1-antagonist BE 2254 (generously supplied by Dr. E.Hofferber, Beiersdorf AG). These results suggested the presence of α_1-adrenoceptors which were responsible for the recurrent facilitation of LC neurons. Although all LC units examined were inhibited by iontophoretically applied NA at a concentration of 100 mM, NA at concentrations of 1 and 10 mM frequently caused excitation of LC neurons. The excitation by NA occurred more frequently in LC neurons of young rats, which were not spontaneously active or firing at low frequencies. Taken together, the present experiments suggest that in addition to the negative feedback mechanism mediated by α_2-adrenoceptors, the electrical activity of LC neurons is regulated by the positive feedback mechanism mediated by α_1-adrenoceptors, and that these two mechanisms operate in an activity-dependent fashion.

377.10 INCREASES IN LOCUS COERULEUS (LC) SPONTANEOUS ACTIVITY BY CARBACOL (CAR) ARE ASSOCIATED WITH DISRUPTION OF LC SENSORY RESPONSES. Edward F. Aulisi and Rita J. Valentino, Department of Pharmacology, George Washington University Medical Center, Washington, DC 20037

The anatomical and physiological characteristics of the noradrenergic nucleus, LC, have prompted investigations into the function of these neurons. LC cells are spontaneously active and their discharge characteristics appear to be related to states of arousal. In addition to tonic activity, LC neurons are responsive to sensory stimuli. The sensory responsiveness of LC cells has been, in part, a basis for hypotheses of LC function in arousal and behavioral vigilance. To further elucidate the role of the LC in different behavioral states the relationship between tonic and phasic LC activity was investigated. In the present study the muscarinic agonist, CAR, was used as a tool to examine this relationship. Single unit LC activity was recorded in halothane-anesthetized rats before and after intracerebroventricular (ICV) administration of CAR (0.1 μg or 0.5 μg) or saline (SAL, 0.5 μl). Sensory-evoked activity was recorded during the repeated presentation of 80-100 stimuli to the sciatic nerve (0.1 Hz). Spontaneous activity was recorded for 3 min before and 3 min after ICV administration of CAR or SAL. Only CAR, 0.5 μg, significantly increased LC spontaneous discharge rates. LC discharge rate after 0.5 μg CAR was 651 $\pm$ 128% of the pre-drug rate (n=8). The response of LC cells to sciatic nerve stimulation was characterized by a brief period of excitation followed by a period of relative inhibition. CAR, 0.5 μg, altered this pattern by increasing the background discharge activity and the number of discharges in the inhibitory component, while it decreased or had no effect on discharge activity during the excitatory component of the sensory response. SAL and 0.1 μg CAR did not appear to dramatically alter this pattern. When the response to sciatic nerve stimulation was plotted as normalized cumulative histograms it appeared that the temporal distribution of discharges during sensory stimulation was disrupted by 0.5 μg CAR such that there was a decreased signal to noise in the sensory response. To determine whether the disruption of LC sensory response was related to increases in tonic activity, a measure of the change in LC sensory responses produced by CAR or SAL was plotted as a function of the change in spontaneous activity. The results demonstrated a strong positive correlation (0.79) between these two measures suggesting that increased LC spontaneous discharge rate produced by CAR is associated with, and possibly responsible for, disruption of LC sensory responsiveness. The results suggest that activation of tonic LC activity will often be accompanied by disruption of phasic activity. Such changes in sensory responsiveness of LC neurons may have important behavioral consequences. Supported by PHS Grants MH 40008 and DA 03695.

377.11 ELECTROPHYSIOLOGICAL EFFECTS OF NOREPINEPHRINE PERFUSION ON CEREBELLAR GRAFTS IN OCULO: RECEPTOR SPECIFICITY. M.R. Palmer, A.-C. Granholm, A Seiger*, L. Olson* and B.J. Hoffer. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO, USA 80262 and Dept. of Histology, Karolinska Institute, Stockholm, S104 01 Sweden.

Previous investigations have shown that norepinephrine (NE) applied locally by microiontophoresis causes beta-mediated depressions of the spontaneous firing rates of cerebellar Purkinje neurons in situ. More recently, Basile and Dunwiddie found that NE causes alpha-mediated depressions of Purkinje neurons when perfused in the in vitro cerebellar slice. To resolve this discrepancy, a cerebellar preparation was utilized which: 1) permits both local and perfusion application of NE, and 2) has an intact NE neuronal input. Thus, the present study investigated the receptor specificity of the electrophysiological effects of NE administration to intraocular cerebellar grafts. Fetal cerebellar anlage (E 14-15) were homologously transplanted to the anterior eye chamber of adult recipient rats and allowed to mature for at least 5 weeks. Spontaneous activity was recorded extracellularly from single Purkinje neurons in the brain grafts under urethane anesthesia. Perfusions of 5-10 uM NE caused excitations as well as biphasic responses consisting of an initial inhibition followed by a prominent excitation. NE doses of 30 uM and higher primarily caused inhibitions which, in some cases, were preceeded by excitations. In general, the excitations caused by lower doses of NE were blocked by 5 uM timolol, a specific beta adrenergic antagonist. In contrast, the inhibitions caused by higher doses of NE were antagonized by 50 uM phentolamine, an alpha adrenergic antagonist. We are currently conducting similar pharmacological protocols with NE applied locally by microiontophoresis from multibarrel micropipettes. Our results to date suggest, however, that the inhibitory effects of perfused NE in cerebellar grafts in oculo may be produced by alpha-adrenergic mechanisms while the excitations appear to be beta-mediated. (Supported by USPHS grants AA-05915 and ES-02011 and by Swedish Medical Research Council grants 14X-06555 and 14X-03185. Dr. Granholm is an American-Scandinavian Foundation postdoctoral fellow.)

377.12 EFFECTS OF ENDOGENOUS MONOAMINES ON LATERAL HYPOTHALAMIC NEURONAL RESPONSES TO IONTOPHORETICALLY APPLIED ACETYLCHOLINE AND SYSTEMATIC CHANGES IN OSMOTIC AND BLOOD PRESSURE. F.M. Sessler, J.T. Cheng and B.D. Waterhouse, *Dept. Cell Biology and Anatomy, U.T.H.S.C., Dallas, Texas 75235.*

Considerable evidence suggests that the lateral hypothalamus (LH) participates in systemic fluid regulation through the integration of osmotic and cardiovascular afferent information. Recently it has been proposed that endogenous monoamine activity is needed to maintain the integrity of these functions. The present studies were conducted to determine the effects of norepinephrine (NE) and serotonin (5HT) on LH neuronal responses to osmotic and blood pressure (BP) changes, as well as to acetylcholine (ACh) iontophoresis. Extracellular activity of single LH neurons was recorded from halothane anesthetized male Long-Evans rats. The carotid artery, femoral artery and vein were cannulated to allow for blood pressure measurements and fluid injections. LH neuronal responses to iontophoretic pulses of ACh (10-50 nA; 10-50s duration) or injection of 0.3-1 M NaCl (0.1 ml; i.c.) or phenylephrine (5-10 μg; i.v.) were examined before, during and after NE or 5HT microiontophoresis (5-35 nA). In 29 out of 78 cells tested, NaCl injection caused either an increase (n=9) or a decrease (n=10) in spontaneous firing rate. In 16 of these neurons, the response to NaCl was potentiated by microiontophoretic application of NE. In 3 cases, NE administration caused enhancement of an otherwise subthreshold response to NaCl. The response to NaCl was unchanged (n=11) or antagonized during NE iontophoresis (n=2) in the remaining cases. Fourteen of the cells that were responsive to NaCl and NE were also sensitive to 5HT. The response to NaCl was potentiated by iontophoretically applied 5HT in 6 cases and antagonized (n=1) or unchanged (n=7) in 8 others. The effect of 5HT on NaCl responses was similar to that of NE for the same cell. All cells responding to drug-induced changes in BP (n=9) were sensitive to NE or 5HT administration. Iontophoretic NE potentiated the BP response in 2 of 9 cells tested. ACh caused an increase in spontaneous firing rate in 21 of 92 LH cells examined. In 8 of 12 neurons cholinergic responses were potentiated by NE iontophoresis. In 4 of these cases, otherwise subthreshold doses of ACh caused marked increases in cell firing during the period of NE administration. ACh-induced excitation was antagonized by NE in 4 neurons. In 4 of 8 cells studied, 5HT exerted a consistent suppressant effect on ACh excitation at doses which caused little depression of spontaneous discharge. In summary, these findings suggest that NE and 5HT may change the detection level or modulate the amplitude of LH neuronal responses to physiologically significant afferent signals. As such, these monoamines may play a strategic role in the homeostatic control of body fluid. *(Supported by NS18081, AFOSR-85-0155 and the Klingenstein Foundation.)*

377.13 ALTERATIONS OF EXTRACELLULAR POTASSIUM ($[K^+]_o$) DYNAMICS AND "RESTING" LEVELS IN THE STRIATAL DOPAMINE (DA) TERMINAL REGION AFTER ACUTE AND CHRONIC ADMINISTRATION OF ANTIPSYCHOTIC DRUGS (AD). J. O. Schenk*, B. Mogahhadam*, B. S. Bunney, and R. N. Adams (SPON: C. Hughes). Neuropsychopharm. Res. Unit, Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. of Chem., Univ. of Kansas, Lawrence, KS 66045.

Systemic administration of AD's results in alterations of DA cell functioning. Acute treatment increases DA cell firing rates, the number of spontaneously active DA cells, and DA synthesis and metabolism. Repeated administration of AD's results in a cessation of DA cell firing with synthesis and metabolism returning to near control levels. Evidence suggests that the cessation in DA cell firing is due to depolarization inactivation (DI). The site of action mediating DI is thought to be the terminal regions since cutting the feedback pathways prevents and reverses DI. Intracellular studies suggest that DI is due to an alteration of a Ca^{2+}-dependent K^+ current. These findings raise a number of questions. Does a gross perturbation in $[K^+]_o$ occur after treatment with AD's? What effect could the changes have on the excitability of DA terminals and other cells nearby? Could changes in $[K^+]_o$ be involved in DI or the AD side effect observed in humans, tardive dyskinesia?

We describe preliminary results suggesting that the buffering of $[K^+]_o$ levels in the striatum of the rat is altered by acute and chronic treatment with the AD, haloperidol. $[K^+]_o$ levels were measured in vivo with 2-20 um diameter valinomycin-based glass ion selective microelectrodes (ISM). The measurements were made in the anterior striatum. Resting levels of $[K^+]_o$ in non-treated controls were 1.9 (±0.08 SEM, N=16) mM. Acute administration of AD increased this value by 1.1 mM. The time course of the observed change paralleled the time course of the electrophysiological changes. Chronic treatment with AD's results in a reduction of $[K^+]_o$ by 0.5 mM. Anatomical control areas (corpus callosum and occipital cortex) did not show these changes. When K^+ was injected close to the ISM an increase in $[K^+]_o$ occurred with clearance times (in non-treated controls) following closely those observed by Gardener-Medwin (J. of Physiol. 335: 393). However, the clearance of $[K^+]_o$ in AD treated animals took much longer. These data suggest two ways in which AD's can exert influences in the DA system through K^+ mediated events: (1) by altering the excitability of DA terminals and other neurons tonically (the magnitude of $[K^+]_o$ changes observed here have altered excitability in other systems) and (2) by changing the response time of neurons dependent on efficient reestablishment of $[K^+]_o$ after an action potential.

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378.1 **ANTIPARKINSONIAN EFFECT OF L-DOPA REQUIRES AN INTRANEURONAL L-DOPA DECARBOXYLASE IN THE NIGROSTRIATAL PATHWAY.** C.C. Chiueh, V.K. Weise*, K.F. Bankiewicz*, I.J. Kopin and S.P. Burns*, Clinical Brain Imaging Section, NIMH; NINCDS, Bethesda, MD, and Vanderbilt University Medical Center, Nashville, TN.

In the 1983 Neuroscience meeting, we reported that MPTP causes a selective lesion in the substantia nigra compacta neurons and produces a persistent extrapyramidal motor dysfunction. L-DOPA is effective in reversing symptomatically the parkinsonian syndrome and the duration of effect lasts for about two hours. Subsequent studies indicated that outward signs of parkinsonism (rigidity and freezing episodes) were observed only in monkeys with more than 80% damage in the nigrostriatal neurons. The therapeutic efficacy of L-DOPA was diminished and even disappeared completely when MPTP inflicts a greater than 95% lesion of the nigrostriatal neurons. It is known that therapeutic effects of L-DOPA for the treatment of Parkinson's patients decreases along with the progress of deterioration of this disease.

In the present study, we used this primate model of parkinsonism to investigate the mechanism for a decrease in L-DOPA's efficacy for the treatment of severe cases of Parkinson's disease. Three severely lesioned monkeys were obtained after administration of MPTP (2 to 2.5 mg/kg, i.v. or 0.6 to 0.8 mg/kg, i.a.) and kept alive for at least two weeks by feeding a liquid diet thru a nasogastric tube. The MPTP-induced damage to the nigrostriatal neurons was assessed by measuring dopamine and its biosynthetic enzymes. Within 24 hours after the administration of MPTP, the severely lesioned monkeys became immobile, rigid, adipsic and aphagic. These animals showed signs of postural tremor and ulcers in the gastrointestinal tract. No spontaneous or symptomatic recovery following L-DOPA therapy (Sinemet 100/10 mg) which was completely different from those of mildly affected animals. The striatal dopamine content and the activity of its biosynthetic enzymes (e.g., tyrosine hydroxylase and L-DOPA decarboxylase) of the severely lesioned monkeys were decreased uniformly by more than 98%. Despite a 80-90% lesion in the nigrostriatal neuron of mildly affected monkeys, the L-DOPA decarboxylase activity decreased by only 40 to 60%.

These results support the notion of a discrete distribution of L-DOPA decarboxylase in the nigrostriatal neurons. L-DOPA may require this intraneuronal decarboxylase to acquire its therapeutic effects for the treatment of Parkinson's disease.

378.2 **IMAGING OF MPP^+-INDUCED DAMAGE TO THE NIGROSTRIATAL DOPAMINERGIC NEURONS.** I. Namura*, P. Douillet*, C.J. Sun*, S.P. Markey*, R.M. Cohen* and C.C. Chiueh, (SPON: J.G. Kenimer), Clinical Brain Imaging Section, LCM, NIMH, Bethesda, MD 20892.

MPP^+ is a oxidative metabolite of MPTP which is trapped in the monkey brain for a long half-life. The oxidation process of MPTP appears to be responsible for its neurotoxic effects since MAO-B inhibitors block the formation of MPP^+ and also prevent MPTP's neurotoxic effects. MPP^+ appears to be reactive and produces a Paraquat-like action, e.g. cataract formation, pulmonary damage, cationic surfactant activity (bactericide), and some herbicidal effect (trade name: Cyperquat).

In the 1985 Neuroscience meeting, we reported that intranigral injection of MPP^+ caused a dose dependent depletion of striatal dopamine in rats. In the present study, we used radioactive pre- and post-synaptic ligands in addition to ^{45}Ca to visualize autoradiographically MPP^+ effects on dopaminergic systems.

Intranigral administration of 8 ug MPP^+ (2µl/10 min) caused 98% depletion of striatal dopamine. When MPP^+ was injected into the raphe nucleus and locus coeruleus, toxicity was again observed in serotonergic and noradrenergic areas of the forebrain, but to a lesser degree, 50% and 60-70% respectively. In each instance a localized cytotoxic lesion was observed histologically either by thionin staining or ^{45}Ca imaging.

Autoradiographic procedure was performed, two weeks after an intranigral injection of MPP^+. 3-^{14}C-L-DOPA (50 nC/g, 30 min after 25 mg/kg carbidopa) was injected thru the tail vein. Rats were sacrificed at various intervals thereafter. In controls, ^{14}C imaging revealed dopamine-rich areas, e.g. caudate nucleus, nucleus accumbens, olfactory tubercle, paraventricular nucleus, preoptic nucleus and median eminence. The ^{14}C imaging was decreased significantly in the striatum of the nigral lesioned rats. The striatal ^{14}C density was only two times higher than that of non-dopaminergic areas. This was due to an accumulation of ^{14}C-3-methoxyl-DOPA in the brain as detected by a HPLC procedure. High background activities diminished also the potential of using radioactive L-DOPA or L-Fluoro-DOPA for imaging of brain noradrenaline. Receptor supersensitivity was not observed one month after MPP^+ treatment.

In conclusion, intracerebral administration of MPP^+ produced lesions not only in the substantia nigra compacta but also in raphe nucleus and locus coeruleus areas. Despite a high background activity, ^{14}C-L-DOPA is useful in imaging of damage to brain dopaminergic but not noradrenergic pathways.

378.3 **ANTIBODY PROBES FOR THE DETECTION OF MPTP-LIKE NEUROTOXINS.** S.P. Markey*, C.J. Markey*, J.P. Bacon*, J.M. Savitt*, D.S. Hanselman*, and J.N. Johannessen (SPON: H.L. Frenk). Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered as a by-product in illicit drug synthesis. Its ability to produce a permanent parkinsonian syndrome in man similar to idiopathic Parkinson's disease has prompted speculation as to whether related compounds might be found in the environment or as a result of biological processes in the brains of Parkinson's disease patients. In order to address this question, antibodies to MPTP and its principal brain metabolite MPP^+ have been raised and their specificity demonstrated.

Amino-substituted MPTP and MPP^+ analogues were synthesized beginning from 4-phenylpyridine by nitration and separation of the 2', 3', and 4'-isomers. These were converted into their respective MPTP analogues by methylation with dimethylsulfate and reduction with $NaBH_4$ in the presence of Pd/C. The amino-MPP^+ analogues were prepared in several steps from their respective nitro-pyridine precursors - reduction with $SnCl_2$, acetylation, methylation, and hydrolysis. Each analogue was diazotized and linked to bovine serum albumin or keyhole limpet haemocyanin.

Sixteen female New Zealand white rabbits were injected subcutaneously with emulsions of BSA-antigens at two week intervals and bled after 2-4 months. Maximum titers were obtained from sera from the second and third bleeds which had detectable activity at dilutions of 10^5-10^6. Sera were characterized by specificity in recognition of MPTP or MPP^+. No differences were seen in antibodies raised to 3'- vs 4'-diazolinked antigens. Assays were performed using an ELISA similar to that described for paraquat (Niewola, Z., et al., Int. J. Immunopharmac. 5: 211, 1983).

The suitability of the antibodies for the detection of MPTP and MPP^+-like compounds in brain was tested by treating mice with MPTP, sacrificing the mice after 15, 30, or 60 min, and assaying extracts of caudate with the ELISA procedure. Extracts of untreated mouse brain did not inhibit antibody-antigen interaction at the highest concentrations tested, the equivalent of 5 mg of tissue/well; whereas at 15 min, extract equivalents of 5-50 µg were readily detected by MPTP-sensitive antibodies.

C.J.M., J.M.S., and D.S.H. were supported by a grant from the National Parkinson Foundation.

378.4 **NON-SEROTONERGIC CONVERSION OF MPTP TO MPP^+.** J.P. Bacon*, J.M. Savitt*, and J.N. Johannessen (SPON: Anne E. Snow). Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a by-product of illicit opiate synthesis, selectively destroys dopaminergic nigrostriatal neurons in several species and produces parkinsonism in monkey and man. MPTP's neurotoxicity is dependent upon its oxidation to MPP^+ by monoamine oxidase type B (MAO-B). Exactly where this conversion occurs remains an unsolved piece of the MPTP toxicity puzzle. Histochemical evidence reveals that MAO-B is localized in astrocytes and serotonergic (5-HT) neurons, but the relative contribution of these neural elements to the formation of MPP^+ and subsequent neurotoxicity has not been determined. It has been proposed (R.-S. Shen et al., FEBS Letters 189, Sept. 1985) that the oxidation occurs to a significant degree in 5-HT terminals. Since a selective in vivo glial toxin is nonexistent, we have used selective 5-HT neurotoxins (5,6- and 5,7-dihydroxytryptamine; 5,7-DHT) to ablate 5-HT terminals. If the in vivo conversion of MPTP to MPP^+ in 5-HT terminals is a requirement for MPTP toxicity, then the ablation of 5-HT terminals should prevent MPTP-induced dopamine (DA) depletion.

Adult male C57BL6 mice (Charles River) were given intracerebroventricular (ICV) injections of saline, 50 µg 5,6 or 5,7-DHT, or were untreated. The 5,7-DHT group was pretreated with catecholamine uptake blockers. One week later half of each group received MPTP i.p. and the other half saline. The MPTP dose was 2 x 30 mg/kg (free base) for the 5,7-DHT experiment and 3 x 30 mg/kg for the 5,6-DHT experiment. One week later the mice were sacrificed and the caudates were analyzed by HPLC-EC for DA, 5-HT, and their metabolites.

ICV treatment with saline, 5,6- or 5,7-DHT did not in itself produce any DA depletion relative to untreated controls. The 5-HT reductions in these groups were 0, 35, and 50%, respectively. MPTP treatment alone resulted in a striatal DA depletion of more than 50%, while animals pretreated with 5,6- or 5,7-DHT showed DA losses of 67 and 66%. Thus, 5-HT depletion did not attenuate the toxicity of MPTP. Furthermore, no correlation existed between the 5-HT levels and the degree of DA depletion group. Further experiments are necessary to produce a greater 5-HT depletion in order to conclusively state that 5-HT terminals are not necessary for the metabolism-dependent toxicity of MPTP.

378.5 NEW METABOLITES OF MPTP IN MOUSE BRAIN: 1-METHYL-4-PHENYL-2-PYRIDONE AND 4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE. S.C. Yang and S.P. Markey (SPON: T.J. Sobotka). Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a by-product in the synthesis of an illicit opiate, is a neurotoxin which can induce parkinsonism in man and primates. It has been shown that the toxic effect of MPTP is dependent on its oxidative metabolism to the quaternary ion 1-methyl-4-phenylpyridinium (MPP^+) in brain. However, primates and rodents show marked differences in sensitivity to the neurotoxicity of MPTP. In addition to the fact that primates contain more neuromelanin in brain than rodents, there are some differences in the metabolism of MPTP in the two species (Johannessen, J.N. et al., Life Sci. 36:219, 1985). In order to explore these differences, radiolabeled MPTP ($^{14}C_6$-phenyl) was synthesized from $^{14}C_6$-bromobenzene (50 mCi/mmol). Adult male mice (C57BL/6) were injected i.p. with either ^{14}C labeled (10 µCi per mouse) or unlabeled MPTP (60 mg/kg). After 30 minutes, the mice were sacrificed by spinal dislocation and decapitation. Brains were homogenized and the extracts analyzed. With HPLC-radiochemical detection and gas chromatography-mass spectrometry, we have identified two new metabolites in mouse brain in addition to MPP^+ and MPTP. They are 1-methyl-4-phenyl-2-pyridone and 4-phenyl-1,2,3,6-tetrahydropyridine (PTP). Apparently, the former comes from MPP^+ by oxidation and the latter from MPTP by demethylation. Both the pyridone metabolite and PTP have been previously reported from in vitro liver metabolism studies (Baker, J. et al., Biochem. Biophys. Res. Commun. 125:484, 1984 and Cashman, J. and Ziegler, D.M., Mol. Pharm. 29:163, 1986). The action of compounds which either inhibit or enhance the neurotoxicity of MPTP in mice will be studied with respect to their effect on MPTP metabolism.

378.6 THE ROLE OF MONOAMINE OXIDASE-B (MAO-B) IN THE DOPAMINERGIC NEUROTOXICITY INDUCED BY 1-METHYL-4-(2'METHYLPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE (2'CH_3-MPTP). R.E. Heikkila, S.K. Youngster*, P.K. Sonsalla, B.A. Sieber*, D.M. Placenti*, M.V. Kindt* and R.C. Duvoisin. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The nigrostriatal dopaminergic toxicity induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) depends on at least two distinct processes: 1) the oxidative conversion of MPTP to its pyridinium ion, 1-methyl-4-phenylpyridinium (MPP^+), the first step of which is catalyzed by the enzyme monoamine oxidase-B (MAO-B), and 2) the active transport of MPP^+ into the dopaminergic neuron by the dopamine (DA) transport system. We have recently synthesized an analog of MPTP, namely 2'CH_3-MPTP, which is a considerably more potent dopaminergic neurotoxin in mice than is MPTP (Youngster et al., Eur. J. Pharmacol., 1986).

As one might expect of an MPTP-like compound, we have found that 2'CH_3-MPTP is oxidized by MAO-B and that its metabolite 2'CH_3-MPP^+ is an excellent substrate for the DA transport system. The results of kinetic studies showed that the K_m values of MPTP and 2'CH_3-MPTP for MAO-B were similar but that the V_{max} for 2'CH_3-MPTP oxidation was approximately 3-fold greater than that for MPTP oxidation. Both the K_m values and the V_{max} values obtained for 2'CH_3-MPP^+ uptake into neostriatal synaptosomes were similar to those obtained with MPP^+, and in fact were similar to the values obtained with ^3H-DA itself. The pretreatment of mice with DA uptake inhibitors, at doses which prevent MPTP-induced neurotoxicity, also protect against 2'CH_3-MPTP-induced neurotoxicity. However, the pretreatment of mice with selective doses of MAO-B inhibitors, doses which prevent MPTP-induced neurotoxicity, has only a minimal protective effect on 2'CH_3-MPTP-induced neurotoxicity. To determine reasons for this relative lack of protection by MAO-B inhibitors, we have pretreated mice with various doses of MAO-B inhibitors (deprenyl, MDL 72145, and AGN 1135) and measured the in vitro oxidation of MPTP and 2'CH_3-MPTP. In brain mitochondrial preparations obtained from mice pretreated with the MAO inhibitors at doses selective for the inhibition of MAO-B (e.g. 2.5 mg/kg), MPTP oxidation was inhibited by about 90%. Although 2'CH_3-MPTP oxidation was inhibited by about 80%, there was a substantial amount of oxidation product still formed. We believe that this residual oxidation of 2'CH_3-MPTP may be of sufficient magnitude to cause neurotoxicity. Other possibilities however, cannot be excluded. These observations with 2'CH_3-MPTP may be relevant to clinical studies in which MAO-B inhibitors will be given to Parkinson patients in an attempt to halt the progression of their disease. These studies are based, in part, on the premise that idiopathic Parkinsonism may be due to an MPTP-like toxin.

378.7 MITOCHONDRIAL AND METABOLIC TOXICITY OF 1-METHYL-4-(2'METHYLPHENYL)-1,2,3,6-TETRAHYDROPYRIDINE (2'CH_3-MPTP). M.V. Kindt*, W.J. Nicklas and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity is believed to be caused by the major metabolite of MPTP in the CNS, 1-methyl-4-phenylpyridine (MPP^+), the formation of which is catalyzed by monoamine oxidase-B (MAO-B). MPP^+, but not MPTP itself, inhibits the oxidation of NADH-linked substrates by intact mitochondria. The incubation of mouse brain striatal slices with MPP^+ or MPTP results in metabolic changes consistent with inhibited mitochondrial respiration and increased anaerobic glycolysis, e.g., increased lactate formation. Just as MPTP-induced neurotoxicity in vivo is prevented by pretreatment with the selective MAO-B inhibitors deprenyl and MDL 72145, MPTP-induced lactate formation is blocked completely when neostriatal tissue slices are prepared from mice treated with either inhibitor.

We have synthesized a methylated analog of MPTP, 1-methyl-4-(2'methylphenyl)-1,2,3,6-tetrahydropyridine (2'CH_3-MPTP), which is better than MPTP as a substrate for MAO-B and which is more potent than MPTP in producing nigrostriatal toxicity in mice (Youngster et al., Eur. J. Pharmacol., 1986). 2'CH_3-MPTP is also more potent than MPTP in causing increased lactate formation in vitro. The corresponding pyridinium ion, 2'CH_3-MPP^+, like MPP^+, inhibits the mitochondrial oxidation of NADH-linked substrates and promotes lactate formation in neostriatal tissue slices. However, unlike the case for MPTP, pretreatment with low doses of selective MAO-B inhibitors does not completely prevent 2'CH_3-MPTP toxicity in vivo, 2'CH_3-MPTP oxidation by brain mitochondrial preparations or 2'CH_3-MPTP-induced lactate formation in neostriatal tissue slices. Pretreatment of mice with higher, non-selective doses of MAO inhibitors produces a dose-dependent inhibition of 2'CH_3-MPTP oxidation by mitochondrial preparations, a corresponding decrease in 2'CH_3-MPTP-induced lactate formation and an attenuation of 2'CH_3-MPTP-induced neurotoxicity. It would appear as if 2'CH_3-MPTP-induced lactate formation is caused by its corresponding pyridinium ion 2'CH_3-MPP^+ formed by the oxidation of 2'CH_3-MPTP. We hypothesize that the inhibition of NADH-linked substrate oxidation and consequent metabolic disturbances may play important roles in the mechanism by which MPTP and 2'CH_3-MPTP produce neurotoxicity.

378.8 AN EVALUATION OF THE DOPAMINERGIC NEUROTOXICITY OF SEVERAL ANALOGS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP). S.K. Youngster*, P.K. Sonsalla and R.E. Heikkila (SPON: C. Vander Wende). Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The peripheral administration of MPTP to experimental animals including mice and monkeys leads to a relatively selective destruction of the dopaminergic nigrostriatal pathway. Changes observed after MPTP administration include large decrements in the neostriatal content of dopamine (DA) and its major metabolites, a diminished capacity of neostriatal synaptosomes to accumulate ^3H-DA, a large decrease in the neostriatal activity of tyrosine hydroxylase, and a loss of nerve cells in the zona compacta of the substantia nigra. The enzyme monoamine oxidase-B (MAO-B) transforms MPTP into the 1-methyl-4-phenyl-2,3-dihydropyridinium ion ($MPDP^+$) which is spontaneously transformed to the 1-methyl-4-phenylpyridinium ion (MPP^+). The treatment of experimental animals with inhibitors of MAO-B prior to MPTP treatment results in a greatly diminished level of MPP^+ in the brain and also prevents MPTP-induced neurotoxicity. These observations have led to the conclusion that the MAO-B catalyzed formation of MPP^+ from MPTP is a necessary step in the neurotoxic process.

We have synthesized several structural analogs of MPTP and have determined their capacity to be oxidized by MAO-B and their capacity to produce nigrostriatal dopaminergic neurotoxicity in C57 black mice. Several of the analogs were not substrates for MAO-B and furthermore, were not neurotoxic in vivo. Several other analogs were oxidized by MAO-B, most to a lesser extent than was MPTP. Of the compounds that were substrates for MAO-B, only three, in addition to MPTP itself, produced dopaminergic neurotoxicity. These three neurotoxic compounds were 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (2'CH_3-MPTP), 1-methyl-4-cyclohexyl-1,2,3,6-tetrahydropyridine (MCTP) and 1-methyl-4-(3'-methoxyphenyl)-1,2,3,6-tetrahydropyridine (3'OCH_3-MPTP). The four compounds, including MPTP, had a neurotoxic potency which parallelled their capacity to be oxidized by MAO-B (2'CH_3-MPTP > MPTP > MCTP > 3'OCH_3-MPTP). Several compounds were found to be oxidized by MAO-B but did not produce nigrostriatal dopaminergic neurotoxicity. It should be emphasized, however, that all neurotoxic compounds were good substrates. These data, taken together with the data of others, indicate that the oxidation of an MPTP analog by MAO-B is a necessary but not sufficient requirement for the production of dopaminergic toxicity. Other requirements for producing MPTP-like neurotoxicity also would include the capacity of the pyridinium ion to be transported by the dopaminergic transport system as well as other, as yet unknown, considerations.

378.9 **EFFECT ON DOPAMINE TRANSMISSION OF MPTP AND MPP^+ INFUSED THROUGH A PUSH-PULL CANNULA INTO THE CAUDATE NUCLEUS OF MALE RATS.** G.D.Chang and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

Administration of N-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) causes severe damage to the nigrostriatal neurons and consequent development of Parkinsonism-like syndromes in human, monkey and mouse. To understand the mechanisms underlying the neurotoxicity of MPTP, we have examined the acute effect of MPTP and N-methylphenylpyridine (MPP^+) in vitro (Brain Res. 368:134, 1986) and herein we report their effects in vivo.

Push-pull cannulae of either 22 or 24 gauge were aimed at the caudate nucleus of adult male rats according to the deGroot's Atlas. Seven to fourteen days after implantations, modified Krebs-Ringer Phosphate buffer (KRP) (pH 7.4), was infused through the inner cannula at a flow rate of 10-14 µl/min. Perfusates of 15 min intervals were then subjected to HPLC-EC analysis of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA). MPTP and MPP^+ in KRP at concentrations of 10^{-6} M, 10^{-5} M and 10^{-4} M were administered directly into the caudate nucleus for 15 min each 90 min apart. With 24 gauge cannula, DOPAC output became significantly lower 45 min following 10^{-6} M MPP^+ (30.04 ± 9.79 vs. 51.98 ± 12.93 pg/min from the interval before MPP^+ infusion or 77.27 ± 20.81 pg/min from the corresponding interval of control animals). HVA output was also decreased by MPP^+ infusions, however, only at higher concentrations; 29.41 ± 1.95, 90 min following 10^{-5} M vs. 49.06 ± 8.54 pg/min of controls. In respect of DA release only 1/5 and 3/5 animals showed significant increase in DA release following 10^{-5} M and 10^{-4}M MPP^+, respectively. With the larger cannula a similar trend of changes in DOPAC and HVA output were found following MPP^+ infusions but of larger variability. However, better responses in DA release were elicited by MPP^+ with 22 gauge cannula: 1/5, 3/5 and 4/5 animals responded to the infusions of 10^{-6} M, 10^{-5} M and 10^{-4} M MPP^+ respectively with marked increases in DA output. Interestingly, MPTP was effective in decreasing DOPAC output only at 10^{-4} M and ineffective in stimulating DA release at all doses tested. In addition, neither drugs had significant effect on 5-HIAA output.

In conclusion, our results confirm in vivo that MPP^+ serves as the active metabolite of MPTP in the caudate nucleus and suggest a major inhibitory effect of MPP^+ on monoamine oxidase rather than on DA re-uptake activity at low concentrations. Furthermore, MPP^+ also acts to stimulate DA release directly on the secretory process at high concentrations.

378.10 COMPARATIVE EFFECTS OF THE NEUROTOXINS 6-HYDROXYDOPAMINE AND DSP-4 ON CENTRAL NORADRENALINE AND ADRENALINE-CONTAINING NEURONS. R. Fêty*, L. Lambás-Señas* and B. Renaud. Laboratoire de Neuropharmacologie, UA CNRS 1196, Faculté de Pharmacie, Université Claude Bernard, Lyon, France.

In order to find a "pharmacological tool" allowing to study separately central noradrenergic (NA) or adrenergic (A) neurons of the rat, we compared the responses of these two neuronal populations after administration of one of the two following neurotoxins: 6-hydroxydopamine (6-OHDA) or DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine).

Five or 21 days after 6-OHDA or DSP-4 administration, we measured the changes in noradrenaline and adrenaline levels occuring i) in brain microregions rich in NA and/or A cell bodies (locus coeruleus (LC), A1-C1, A2 and C2 areas) and ii) in microregions rich in NA and/or A terminals (tractus intermediolateralis (TIML) of the spinal cord, nucleus paraventricularis, n. periventricularis and n. dorso-medialis of the hypothalamus).

The intraventricular administration of 6-OHDA (250µg) induced 5 and/or 21 days later a significant decrease in noradrenaline levels (-40% to -91%) in all the microregions analysed. A significant decrease in adrenaline levels was also found, but the variations were smaller (-22% to -59%) and present only in 6 of the 8 microregions analysed.

The intraperitoneal administration of DSP-4 (50mg/kg) induced a significant decrease in noradrenaline levels (-21% to -75%) in 7 of the 8 microregions studied. Conversely, no significant change in adrenaline levels was found 5 and 21 days after DSP-4 injection except for the TIML which exhibited a decrease (-31%) only at 21 days.

These results show that 6-OHDA exerts its neurotoxic action on both NA and A neurons. Thus, this neurotoxin does not allow to study separately the two neuronal populations. Conversely, the results obtained after DSP-4 administration suggest that the A neurons could be much less sensitive to this neurotoxin than the NA neurons. Additional studies are needed to determine if DSP-4 could be used as a NA neurotoxin in conditions sparing totally the A neurons.

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378.11 CENTRAL NOREPINEPHRINE DEPLETION OF DSP4: COMPARISON OF TWO METHODS. J.D. Steketee and A.C. Swann. Department of Psychiatry and Behavioral Sciences, University of Texas Medical School and Graduate School of Biomedical Sciences, Houston, TX. 77030.

Norepinephrine (NE) can be selectively depleted peripherally and centrally by N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4). Previous research has shown that 50 mg/kg DSP4 (I.P.) depleted approximately 90 percent of cortical, hippocampal, and cerebellar NE in rats (1). Our research suggests that I.P. injections of DSP4 leads more typically to depletion of only 70 percent of cortical NE (2). One possible reason for the differences in depletion levels could be the instability of DSP4, which cyclizes in solution. Once cyclized, DSP4 cannot cross the blood brain barrier. Since we require central depletion in the range of 90 percent, we have examined the efficacy of intracerebral DSP4.

Male Sprague-Dawley rats received either unilateral intraventricular (IVT, 300 or 400 µg) or unilateral locus coeruleus (L.C. 4 or 8 µg) infusions of DSP4. Rats were sacrificied 8 or 14 days after DSP4 infusions. The right and left cortex, cerebellum, hippocampus, striatum and brain stem were dissected from each brain. NE and DA were measured using HPLC. Desmethylimipramine (DMI) and dihydroalprenolol (DHA) receptor binding were also assayed.

Preliminary results suggest that intraventricular infusion of DSP4 is an ineffective method for complete central noradrenergic depletion. NE depletion ranged from 24 percent in the brain stem to 80 percent in the cerebellum and hippocampus, with the cortex having 60 percent depletion for rats sacrificed 2 weeks after receiving 300 µg DSP4. The mortality rate for rats receiving 400 µg DSP4 IVT was greater than 50 percent. These data will be compared to DSP4 lesions of the LC in terms of central NE depletion.

(1) Jonsson, G. European J. Pharmacol. 72:173-188, 1981.
(2) Swann, A.C. Brain Res. 321:323-326, 1984.
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378.12 NEUROCHEMICAL CHARACTERIZATION OF THE NEUROTOXIN 5-AMINO-2, 4-DIHYDROXY-α-METHYLPHENYLETHYLAMINE (5-ADMP) ON NORADRENERGIC, DOPAMINERGIC AND 5-HYDROXYTRYPTAMINERGIC NEURONS IN THE RAT HYPOTHALAMUS

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One of the major means of studying catecholaminergic neurons in the brain has been to observe the functional impairments that result from destruction of these neurons by neurochemical lesions. 6-Hydroxydopamine, depending upon the route of administration, dose and pretreatment regiment, can cause the destruction of either noradrenergic or dopaminergic neurons, but doses necessary to produce total depletion of norepinephrine (NE) can also cause loss of dopamine (DA) and/or extensive non-specific neuronal damage. The more recently developed neurotoxin DSP4 destroys noradrenergic neurons projecting from the locus coeruleus to extrahypothalamic regions following systemic administration, but has little effect on subcoeruleus neurons terminating in the hypothalamus (Lookingland et al., Brain Res. 365:228, 1986). The purpose of the present study was to examine the effects of 5-ADMP, a compound reported to selectively deplete NE in the whole mouse brain (Lin et al., Soc. Neurosci. Abst. 10: 1199, 1984), on levels of amines and their metabolites in the rat hypothalamus.

Following a single injection of 5-ADMP (100 ug/rat, i.c.v.) the concentrations of NE throughout the hypothalamus were markedly reduced by 4 h and this depletion was maintained for at least 28 days. Seven days after 5-ADMP administration the greatest decrease in NE occurred within periventricular regions of the hypothalamus, and this was partially prevented by pretreatment with the NE uptake inhibitor desipramine. In contrast, 5-ADMP failed to alter the concentrations of DA, dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (serotonin; 5HT) or 5-hydroxyindoleacetic acid (5HIAA) in any hypothalamic region. Multiple injections of 5-ADMP (100 ug/rat; every 48 h x 3) did not enhance NE depletion 7 days after the last injection, but did decrease DA and 5-HT concentrations in some regions. These results indicate that 5-ADMP is a relatively selective neurotoxin for NE neurons that produces a rapid and long-lasting depletion of NE. 5-ADMP is particularly effective within the periventricular hypothalamus and should be a useful tool in determining the physiological role of NE neurons in these regions. (Supported by USPHS Grant NS15911 and The Upjohn Co.)

378.13 EFFECTIVENESS OF AMINO ACID PRECURSORS IN NOREPINEPHRINE (NE) DEPLETED MICE. C.J. Gibson. Dept. of Pathology, U.W.O., London, Ont. N6A 5C1.

The effectiveness of two amino acid precursors in restoring cortical and hippocampal norepinephrine (NE) turnover in DSP-4 treated CD-1 mice was compared by measuring NE and its major metabolite, MHPG. The noradrenergic neurotoxin, DSP-4 (generously provided by Astra Labs, Sweden) specifically depletes NE in the terminal regions of the locus coeruleus, the noradrenergic brainstem nucleus. Levels of NE in cortex and hippocampus were reduced by 73 to 87% two weeks after a single intraperitoneal injection of DSP-4 (50 mpk) and MHPG by 40 to 60%. The dietary amino acid precursor, tyrosine, was administered as the ethyl ester HCl (250 mpk) to saline or DSP-4 pretreated CD-1 male mice (5/group) 1 hour prior to sacrifice. NE levels were unchanged in control mice in both regions; MHPG was significantly increased in cortex after tyrosine injection (from 30±4 ng/gm to 47±4 ng/gm, $\bar{x}$±S.D.). In DSP-4 pretreated mice, MHPG levels were significantly increased by 188%; from 17±3 ng/gm in DSP-4 control cortex to 32±8 ng/gm in tyrosine plus DSP-4 treated mice and from 16±2 ng/gm in DSP-4 hippocampi to 30±7 ng/gm.

The synthetic NE precursor, DL-threo-DOPS, which simply requires decarboxylation to form NE, was given (200 mpk, ip) to saline or DSP-4 pretreated mice (5/group) 1 hour before sacrifice. In saline control mice NE levels were unchanged in both regions. MHPG was significantly doubled or tripled in both cortex (36±8 ng/gm to 122±25 ng/gm) and hippocampus (52±9 ng/gm to 166±46 ng/gm). In DSP-4 pretreated mice NE levels did not change, but again MHPG was increased by 400% after DOPS administration (cortex, 16±4 ng/gm to 66±10 ng/gm and hippocampus, 17±7 ng/gm to 83±9 ng/gm).

Both precursors were effective in increasing NE turnover as measured by MHPG level in NE-depleted cortex and hippocampus, DL-threo-DOPS to a greater extent than the physiological precursor, tyrosine. The effects of both precursors were greatest in DSP-4 treated mice, suggesting an activation of both synthetic enzymes, tyrosine hydroxylase and amino acid decarboxylase in remaining terminals.

Supported by Ontario Mental Health Foundation and CJG by an OMHF Scholarship.

MOLECULAR BIOLOGY OF GENE EXPRESSION AND NUCLEIC ACIDS VI

379.1 LOCALIZATION OF "BRAIN SPECIFIC" mRNAs IN MONKEY CORTEX BY IN SITU HYBRIDIZATION: EVIDENCE FOR CORTICAL HETEROGENEITY. C.C.G.Naus, G.H.Travis*, J.G.Sutcliffe*, R.J.Milner*, J.H.Morrison, F.E.Bloom. Division of Preclinical Neuroscience and Endocrinology, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Neocortex is characterized by a systematic neuronal diversity that is correlated with its lamination, allowing certain cells to be identified and categorized according to their morphology. These morphological characteristics that allow different types of neurons to be discriminated presumably arise from an underlying heterogeneity of structural and metabolic proteins. These differences must a priori arise from expression of different genes coding for different proteins, and thus should be reflected in the mRNA present in a given cell. Since specific mRNAs can be detected and localized in tissue sections by in situ hybridization, we used this approach to analyse several "brain specific" clones (i.e. present in brain, absent in liver) selected from a monkey cortex cDNA library (see Travis et al., this issue). Based on Northern blot analysis, two different populations of clones were used; one set was present in cortex and absent in cerebellum, while the second set consisted of randomly selected "brain specifics". The selected cDNAs were subcloned into an SP6 in vitro transcription vector system and anti-sense 35-S-labeled RNA probes were used for in situ hybridization on sections of monkey neocortex and cerebellum. Different probes detected different patterns of mRNA distribution, and certain of the probes exhibited a high degree of regional selectivity. One "brain specific" clone that was relatively abundant in all cortical regions by Northern blot analysis, produced a consistent laminar pattern in each region, with hybridization being most intense in layers 5 and 6, least in layer 4, and intermediate in layers 2 and 3. This probe displayed significant regional heterogeneity. For example, in the temporal lobe, visual association cortex (inferior temporal gyrus) contained more labeled cells than auditory association cortex (superior temporal gyrus). Also, in the occipital lobe, the density in the primary visual cortex was significantly greater than in the peristriate visual association cortex. Another clone, which represented a low abundance mRNA species shown by Northern blot analysis to be absent in cerebellum, hybridized more intensely to cells in the superficial cortical layers but displayed little regional heterogeneity. The observed differences in the neuroanatomical distribution of several "brain specific" mRNAs reveals an underlying molecular heterogeneity in neuronal populations which, we believe, will provide valuable insight into the understanding of the cellular organization of neocortex.

Supported by Huntington Society of Canada, NIH grants AG05131 and AA06420, and McNeil Laboratories.

379.2 HIGH RESOLUTION IN SITU HYBRIDIZATION HISTOCHEMISTRY OF SOMATOSTATIN NEURONS. F. Baldino, Jr., J. P. Card and M. E. Lewis. Central Research and Development Department, E. I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware 19898.

The tetradecapeptide somatostatin is widely distributed throughout a number of functionally distinct areas of the central nervous system. Several molecular forms of somatostatin exist within the rat CNS which are derived from a single gene product. Previous studies in our laboratory have used in situ hybridization techniques to describe the regional localization of somatostatin mRNA in rat hypothalamus. The purpose of this study was to identify the neuroanatomical distribution of individual neurons containing specific mRNA coding for preprosomatostatin.

In situ hyubridization histochemistry was performed as previously described by our laboratories. A 39 base synthetic oligodeoxynucleotide probe, complimentary to the 3' coding region of somatostatin mRNA, was used to detect specific transcripts coding for rat preprosomatostatin. Probes were labeled on the 3' end with [^{125}I] dCTP using peviously published methods. The hybridization signal was detected by liquid emulsion autoradiography. Alternate 25 µm sections were processed for immunocytochemistry using the avidin-biotin method with antibodies directed against the C terminus of somatostatin.

Localization of mRNA coding for preprosomatostatin was characterized by an extremely dense accumulation of silver grains in the neuronal cytoplasm with little or no label over the nucleus. The intense cytoplasmic labeling permitted identification of even the smallest somatostatinergic neurons and thereby allowed direct comparison of the neuroanatomical distribution of both somatostatin and somatostatin mRNA. These data demonstrate a marked concordance of mRNA localization and somatostatin immunoreactivity in all areas previously reported to contain somatostatin neurons. Individual neurons were observed in neocortex, entorhinal cortex, amygdala, anterior preoptic hypothalamus, paraventricular hypothalamus, medial basal hypothalamus, entopeduncular nucleus, lateral habenula, hippocampus, cerebellum, and brainstem. Thus, single cell resolution of this relatively rare transcript, coupled with immunodetection of somatostatin, should provide the basis for further studies on the regulation of somatostatin expression in the CNS.

379.3 RADIOIODINATED SYNTHETIC OLIGONUCLEOTIDES PERMIT RAPID, HIGH-RESOLUTION IN SITU HYBRIDIZATION HISTOCHEMISTRY.
M. E. Lewis, R. Arentzen* and F. Baldino, Jr. E. I. du Pont de Nemours and Co., Central Research and Development Department and Biomed. Prod. Department, Experimental Station, Wilmington, Delaware 19898

In situ hybridization histochemical studies are typically carried out with [^{32}P]-labelled probes, which provide relatively rapid information at the structural level, or with [^{3}H]-labelled probes, which provide cellular resolution but require prolonged exposure times. We and other investigators have employed synthetic oligonucleotide probes to circumvent the disadvantages of nick-translated probes obtained from cloned DNA. The synthetic probes are typically 5' end-labelled with [^{32}P], but can also be 3' end-labelled with multiple [^{3}H]deoxynucleotides via terminal deoxynucleotidyl transferase to improve anatomical resolution. Alternately, multiple nucleotides can be incorporated into an oligonucleotide probe by using a message-sense oligonucleotide as a template and the Klenow fragment of DNA polymerase I to catalyze the extension of a primer across the template. Although both labelling methods result in the incorporation of multiple [^{3}H]nucleotides in the synthetic probes, the specific activity of the probes is still relatively low, and prolonged exposure to emulsion is required. Our approach to this problem has been to increase the specific activity of the probes by incorporating multiple [^{125}I]nucleotides by either the primer extension or 3'-tailing methods. For the primer extension reaction, a 24-mer, complementary to part of the glycoprotein coding region of the mRNA for the rat vasopressin precursor, was used. This oligonucleotide was partially extended using a 17-mer message-sense template complementary on the 3' end to the last 9 nucleotides on the 3' end of the 24-mer. The reaction mixture contained Klenow fragment, dATP, dGTP, and [^{125}I]dCTP (2900 Ci/mmol; DuPont NEN), and the extended primer was separated by gel electrophoresis and purified on NENSORB -20 (DuPont NEN). For the 3'-tailing reaction, the above 24-mer was incubated with terminal transferase and [^{125}I]dCTP and then purified on NENSORB -20. When either radioiodinated probe was hybridized to brain tissue sections from paraformaldehyde-perfused rats, a 2-3 day exposure of the emulsion-coated sections was sufficient to produce a very high density of silver grains over magnocellular neurons of the paraventricular and supraoptic nuclei. Silver grains were largely confined to the perikaryon, with low background labelling. These results compare favorably to those obtained with [^{32}P]- or [^{3}H]-labelled probes. Given the ease of the 3'-tailing method, [^{125}I]-labelled oligonucleotides appear to be especially useful probes for in situ hybridization histochemistry.

379.4 IN SITU HYBRIDIZATION HISTOCHEMISTRY OF CALMODULIN mRNA IN RAT BRAIN.
J. M. Roberts-Lewis, F. Baldino, Jr., R. Krause*, M.-F. Chesselet, B. Weiss, and M. E. Lewis. Division of Neuropsychopharmacology, Department of Pharmacology, Medical College of Pennsylvania, E.P.P.I., Philadelphia, Pennsylvania 19129 and E. I. du Pont de Nemours and Company, Central Research and Development Department, Wilmington, Delaware 19898.

Calmodulin (CaM) is an acidic, calcium binding protein that influences a number of important calcium-dependent cellular processes in virtually every eukaryotic cell. CaM is particularly enriched in brain, where it is associated with sites that are actively involved in neurotransmission, such as postsynaptic membranes, postsynaptic densities, synaptic vesicles, and coated vesicles. CaM activiates a number of calcium-dependent enzymes found in brain, such as adenylate cyclase, phosphodiesterase, protein kinase, and ATPase. Moreover, CaM appears to be involved in the synthesis and release of many neurotransmitters, and is sensitive to alterations in central neurotransmitter activity, as well. Despite the ubiquity of CaM, several studies have shown that its distribution in brain is heterogeneous, with particularly high levels of activity in select nuclei regions throughout the brain. To determine whether the differential abundance of the protein is potentially related to differences in gene expression, we examined the distribution of apparent CaM mRNA in brain by in situ hybridization histochemistry. The hybridization probe was a 30 base synthetic oligonucleotide complementary to part of the 3-coding region of CaM mRNA. This probe was radiolabelled with [^{125}I]dCTP by terminal deoxynucleotidyl transferase, hybridized to fixed rat brain tissue sections, and detected by film or emulsion autoradiography. As a control, some sections were pretreated with RNase A, while other sections were exposed to increasing temperatures in 0.5X SSC to determine the specificity of hybridization. The resulting melting curve was obtained by densitometric scanning of the sections with a Leitz/DADS image analysis system. Preliminary results suggest a differential abundance of apparent CaM mRNA in brain, consistent with reports showing a heterogeneous distribution of CaM. Control studies in striatum showed the hybridization signal to be RNase-sensitive and to melt off with a steep, mono-phasic profile, indicating that the probe is selective for a single mRNA species.

379.5 IN SITU HYBRIDIZATION ANALYSIS OF OSMOTIC STIMULUS-INDUCED CHANGES IN rRNA IN RAT SUPRAOPTIC NUCLEUS.
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The hypothalamo-neurohypophysial system, which produces vasopressin and oxytocin, has served as a major model system for studying the mechanisms of the biosynthesis, transport, and secretion of peptides. In order to investigate humoral effects on the regulation of gene expression we stimulated peptide synthesis by salt-loading rats, and used in situ hybridization with H^{3} labeled DNA complementary to ribosomal RNA (a portion of 45S fragment, Chikaraishi et al., Nuc. Acids Res., 11: 6437-6452, 1983). We analyzed separately vasopressin-producing neurons (predominantly in the ventral part of rat supraoptic nucleus, SON) and oxytocin-producing neurons (dorsal part of SON)--hyperosmolarity is known to stimulate synthesis of both hormones.

Fresh-frozen brain blocks from ovariectomized Long-Evans rats were prepared. Besides the control group, rats had been challenged with 2% NaCl water as their drinking solution for 1, 4, or 14 days. Sections 6 micra thick, prepared in a cryostat, were fixed by alcohol:acetic acid. Prehybridization, hybridization, wash, and autoradiographic steps were done as reported (McCabe et al., J. Histochem. Cytochem., 34: 45-50, 1986). Grain counts per cell as well as cell area were analyzed with a computer (Bioquant II).

Hybridizable ribosomal RNA was localized in the neuronal soma as well as the nucleolus. Cell area was significantly increased following salt-loading, and statistically significant increases in numbers of grains per cell were seen in the ventral portion of SON after 1 or 4 days of salt-loading. These changes were greater than after 14 days, and changes in the ventral SON tended to be greater than changes in dorsal SON. This in situ hybridization result only reflects quantitative levels of hybridizable ribosomal RNA, but is consistent with the notion that the hyperosmotic stimulus had a substantial effect on the expression of rDNA.

379.6 IN SITU HYBRIDIZATION OF SUBSTANCE P mRNA IN RAT BRAIN USING SYNTHETIC OLIGODEOXYNUCLEOTIDE PROBES BASED ON THE NUCLEOTIDE SEQUENCE OF THE RAT SUBSTANCE P mRNA.
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The putative neurotransmitter Substance P (SP) is an undecapeptide produced after proteolytic processing of a larger precursor. The sequence of the bovine precursor has been reported (H. Nawa, et al., Nature 306, 32, 1983). Using a synthetic oligodeoxynucleotide probe complementary to the bovine SP mRNA, we screened a rat brain λgt11 cDNA library. Screening of approximately 5 x 10^{5} clones at low stringency resulted in 6 putative clones which yielded two positives upon additional screening. The inserts from each of these clones were subcloned into the EcoRI site of pBR322. These pBR322 recombinants were prepared for BAL31 exonuclease "shotgun" cloning into M13-mp18. Sequencing was performed using the Sanger dideoxy-termination method. Although numerous differences were detected when comparing the bovine and rat cDNA sequences that encode the SP precursors, a high degree of conservation existed in the neurotransmitter coding portions. Only 1 nucleotide difference was found in the 33-nucleotide portion encoding the SP peptide and no differences were found in the region encoding the related tachykinin Substance K.

Based on this rat sequence, we synthesized an oligodeoxynucleotide probe (33-mer) complementary to the SP peptide coding region. The ^{32}P-radiolabeled probe was used to localize neuronal perikarya containing SP mRNA by in situ hybridization. Sections (50 μm) were cut on a vibratone after PLP fixation of the brain and the sections were hybridized with the SP oligodeoxynucleotide probe. Positive hybridization signals were found in various brain structures but were most prominent in caudate, preoptic area, arcuate nucleus, and medial habenula. These loci correspond to immunocytochemically localized SP-containing perikarya. Comparison of SP message and SP peptide at the regional and cellular level now allows assessment of how treatments (drugs, lesions, etc.) differentially affect the regulation of SP-containing neuronal systems.

379.7 IN SITU HYBRIDIZATION OF NEUROPEPTIDE TYROSINE (NPY)-mRNA IN HUMAN CEREBRAL CORTEX USING RNA PROBES. G.Terenghi, J.M.Polak, *E.O'Brien, C.O.Inyama, °J.Dixon, +G.Yasargil, @V.Chan-Palay. (SPON: European Neuroscience Association) Depts of Histochemistry & *Haematology, Hammersmith Hospital, London, W12, UK, °Dept. Biochemistry, Purdue University, West Lafayette, Ind. 47907, USA, +Neurosurgery Dept. and @Neurology Inst., University Hosp., Zurich, 8091, Switzerland.

Neuropeptide tyrosine (NPY) is found in brain in concentrations exceeding that of any other peptide and is present in many non-pyramidal cerebral cortical neurons. The distribution of mRNA encoding NPY in neurons of normal human cerebral cortex was studied by in situ hybridization techniques. A NPY-cDNA clone (300 base pair) was subcloned in the vector pSP62-PL. The resulting construct was used in conjunction with SP6 polymerase and ^{32}P-CTP to prepare a radiolabelled antisense mRNA probe. The ability of this probe to hybridize to mRNA was confirmed by Northern blot analysis. Biopsies of normal cerebral cortex were taken from the overlying cortex of eleven patients operated for removal of tumours or for intractable epilepsy, according to protocols consistent with NIH guidelines for the use of human tissues. Tissues were fixed according to a variety of formulae utilising paraformaldehyde either briefly or for several hours. Two samples of cerebral cortex were perfused with fixatives in situ . 30 µm sections were cut on a cryostat. Following overnight incubation with ^{32}P-labelled antisense probe (2 ng/section), the sections were exposed for 3 days before being processed for autoradiography.

Hybridization to NPY-mRNA was observed in all samples, discretely localised to neuronal cell bodies in the deep cortical layers, particularly laminae IV-VI and to small cell bodies in the underlying white matter. The sizes and locations of the cells showing mRNA hybridization was comparable to that of NPY-immunoreactive neurons as shown in adjacent sections from the same tissue blocks, similarly fixed but processed for immunocytochemistry using NPY-specific antibodies and the peroxidase antiperoxidase method. There was a discrepancy between the smaller number of neurons expressing NPY-mRNA detected by in situ hybridization and the larger number immunostained for NPY. This may reflect varied metabolic states of the neurons and/or the fact that transcription for NPY is not a continuous event for all cells whereas the immunocytochemical techniques demonstrate existant pools of NPY-peptide.

This study clearly demonstrates the consistent localisation of NPY gene transcription and mature neuropeptide expression in normal human cortical neurons.

379.8 **LOCALIZATION OF NEUROPEPTIDE Y mRNA BY *IN SITU* HYBRIDIZATION.** D.R. Gehlert, B.M. Chronwall, M.P. Schafer* and T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS and Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892.

Neuropeptide Y (NPY) is a centrally active member of a family of structurally related peptides which includes polypeptide YY (PYY) and pancreatic polypeptide (PP). Previous immunohistochemical studies have demonstrated the presence of NPY in a number of brain regions which are involved in the central processing of autonomic information, a proposed role for NPY in the CNS. NPY also appears to coexist with catecholamines in a number of brain regions and is coreleased with norepinephrine following sympathetic stimulation (Lundberg et al., Neurosci. Lett., 52: 175, 1984). In order to examine the distribution of NPY mRNA in the brain we have synthesized a 28 base oligodeoxynucleotide probe complementary to bases 208-236 of the NPY mRNA sequence using the β-cyanoethyl phosphoramidite chemistry on a Vega Coder 300 DNA synthesizer. The probe was purified using reverse phase HPLC. The region and size of the probe were selected to minimize the interaction with PPY and PP mRNA (Minth et al., PNAS 81:4577, 1984). The probe was radiolabeled using terminal deoxynucleotidyl transferase and [^{35}S]-dCTP or [^{125}I]-dCTP. The labeled probe was then purified by gel electrophoresis or NENsorb® columns. Ten micron sections of fixed rat or mouse brain were prepared for *in situ* hybridization by immersion in 95% ethanol at −70°C after which the sections were rehydrated through a series of graded alcohols. The slides were then transfered into 0.2 N hydrocholoric acid followed by 2XSSC at 70°C. The probe (500,000 cpm/section) was applied to the section in a mixture containing 50% formamide, tRNA, Denhardt's solution and salmon sperm DNA in 2XSSC. After an 18 hour hybridization period, the slides were rinsed in decreasing ionic strength buffers, 0.5XSSC at 45°C and a series of graded alcohols. Some slides were then processed for simulataneous immunohistochemistry using PAP or immunofluorescence techniques. Autoradiograms were generated by dipping the labeled sections in NTB-2 emulsion (1:1) and subsequently exposing them for one week. Dense clusters of autoradiographic grains, indicating the presence of NPY mRNA:cDNA hybrids, were detected in a number of brain regions where NPY containing cell bodies had previously been reported. In the arcuate nucleus of the hypothalamus, grains were detected directly over cell bodies which stained for NPY. In the cortex, scattered cell bodies containing both NPY mRNA and NPY immunoreactivity were also detected. The use of *in situ* hybridization to detect NPY mRNA represents a powerful tool to study the transcriptional regulation of this neuropeptide.

379.9 ASSAY OF TYROSINE HYDROXYLASE TRANSCRIPTS IN RAT BRAIN AND ADRENAL GLAND. G. Pasinetti, C. Zarow, S.A. Johnson*, and C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089.

In situ hybridization can quantitate regional mRNA gene expression and may provide a marker for specific neurotransmitter systems at the cellular level. A rat tyrosine hydroxylase (TH) cDNA clone 300nt (Lewis at al, J. Biol. Chem. 258:14632, 1983) was recloned into the transcription vector, pGEM-1. This vector allows for synthesis from either strand of the cDNA using either SP6 or T7 phage RNA polymerases. In control experiments, the sense and the antisense strand were verified by hybridization of labeled single strand RNA probes to gel blots of rat brain poly(A+)RNA. In our experiments a ^{35}S single strand RNA TH probe (2x10^9 DPM/ug RNA) was used. A computerized image analysis/grain counting densitometry system was used to quantify the grain density over neurons. The sense strand was used to estimate nonspecific background hybridization rather than using the extent of labeling over the neuropil (where little RNA is localized).

The rat substantia nigra (A-9 region), a brain area enriched in dopaminergic neurons, was hybridized with ^{35}S labeled TH probes. The neuron area (identified by Nissl staining) which was occupied by silver grains was measured in 105 neurons from 4 sections. 7.68 ±0.75 % of the neuron area was occupied by grains after hybridization with the antisense strand, while 0.77 ±0.06 % of the neuron area was occupied by grains with the sense strand (80 neurons in 4 sections; mean ±SEM). As expected labeled neurons in the A9 region were localized in the zona compacta, where the dopaminergic cell bodies are primarily localized. Grain density over the hippocampus CA-3 region and cerebral cortex was also analyzed in sections hybridized with antisense TH strand: 0.49 ±0.08% and 0.33 ±0.06% respectively of the cell neuron area was occupied by grains, confirming hybridization specificity, since TH containing neurons are not found in these regions.

Also, we compared TH mRNA synthesis in adrenal gland of control and reserpine treated rats. Northern blots of total adrenal RNA indicate a 2 fold increase in TH sequence with acute reserpine treatment (10 mg./kg. i.p. 3 days before sacrifice) and a 4 fold increase with chronic reserpine treatment (5 mg./kg. i.p., 5 days, sacrifice 3 days after last injection). These findings together with the in situ results allow for quantification of TH gene expression in central nervous system structures at the cellular level following treatments presumed to regulate TH synthesis. Supported by NIH grant AG-05142 to CEF.

379.10 PRESENCE OF BETA-NERVE GROWTH FACTOR mRNA IN HUMAN CORTEX FROM BOTH CONTROL AND ALZHEIMER'S DISEASE PATIENTS. M.S. Cole*[1], J.K. Glenn*[2], P.D. Coleman[2] and M.K. Fahnestock*[1]. [1] Molecular Biology Dept., SRI International, Menlo Park, CA, 94025; [2] Dept. Neurobiology & Anatomy, Univ. of Rochester Medical Center, Rochester, NY, 14642.

Nerve Growth Factor (NGF) is a protein necessary for the development and survival of sympathetic and some sensory neurons. NGF has also been found in the CNS of rodent and rabbit, and a defect in the NGF system has been postulated to play a role in certain neurological diseases. We are investigating the presence and distribution of NGF mRNA in human brain and its possible alteration in Alzheimer's disease (AD). We report here the identification of ß-NGF mRNA in human frontal cortex.

Tissue samples were obtained two to six hours postmortem and poly(A)$^+$ mRNA purified by standard procedures. The poly(A)$^+$ mRNA from both control and AD cases appears undegraded on denaturing as well as native agarose gels.

Northern blots of RNA samples from human frontal cortex were hybridized to a single-stranded murine ß-NGF probe generated from an M13 phage template (obtained from L.F. Reichardt, Univ. of Calif., San Francisco). A single band comigrating with mouse ß-NGF mRNA and measuring 1.35 kb in comparison to RNA molecular weight standards is evident in human samples. The hybridizing band is present in poly(A)$^+$ but not poly(A)$^-$ samples.

Samples from both control and AD cases exhibit a ß-NGF mRNA signal. Neither the size of the transcript nor its presence in the poly(A)$^+$ fraction appear altered in AD as compared to control tissue. The concentration of ß-NGF mRNA in human cortex is roughly of the order of one part in 5 x 10^8 of the total poly(A)$^+$ RNA in both AD and control tissue. Although we have not ruled out subtle differences in ß-NGF mRNA concentrations, we can conclude, on the basis of samples analyzed so far, that gross changes are not found in the content of ß-NGF mRNA in human frontal cortex in AD.

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379.11 Development of Abnormal Calmodulin Gene Expression and Related Activities in Genetically Dystrophic Chicken Muscle. M.S. Hudecki, H.R. Thacore,* P.K. Kibler,* P.J. Davis,* F.B. Davis,* S.D. Blas,* J. Galindo,* C.C. Gregorio,* C.M. Pollina.* Depts. of Biological Sci., Microbiology and Medicine, SUNY at Buffalo, Buffalo, NY 14260 and Veterans Administration Medical Center, Buffalo, NY 14215.

The mechanism by which calcium (Ca) is abnormally sequestered in genetically dystrophic chicken and its significance in pathogenesis is presently unknown. The accumulation of Ca by affected skeletal muscle is associated with elevated muscle calmodulin (CaM) content and increased expression of the CaM gene (Hudecki et al., Biochem Biophys Res Commun, in press). Recent evidence from our lab has indicated in 60 day-old animals that dystrophic (D) muscle sarcoplasmic reticulum (SR) Ca-ATPase activity, in contrast to that in normal (N) muscle, is resistant in vitro to CaM. The current studies were carried out to define the emergence of resistance of Ca-ATPase activity to CaM in affected birds of varying age. Crude SR membranes were prepared from pectoralis muscle of N and D animals at 13,20,34, and 48 days ex ovo by the method of Hanna et al. (Biochim Biophys Acta 643:41, 1981). Ca-ATPase activity was measured by ATP hydrolysis (P. Davis and S. Blas, Biochem Biophys Res Commun 99:1073, 1981) with and without 12 μM free Ca. Enzyme stimulation by exogenous bovine brain CaM, 20 ng/mg membrane, was the difference in enzyme in the presence and absence of added CaM and was termed ΔCaM; basal activity was that without added CaM.

	Ca-ATPase Activity (μmoles Pi/mg/60')			
	N-Basal	N-ΔCaM	D-Basal	D-ΔCaM
13 days	29.9	8.5	40.3	4.6
20 days	55.1	11.2	39.8	-0.9
34 days	65.9	12.9	57.7	-9.1
48 days	41.7	15.9	34.0	-6.3

At 13 days, CaM-responsiveness of D enzyme is less than N; at 20 days, CaM-resistance of D enzyme is evident. Concurrent studies of muscle CaM mRNA are underway. The pathogenesis of elevated skeletal muscle Ca in the avian model of muscular dystrophy is postulated to reside at the level of SR Ca-ATPase and its resistance to CaM; secondary changes follow in muscle concentrations of CaM mRNA and CaM itself. In the present studies we have shown the emergence of CaM resistance of the enzyme progressively in chicks of age 13-20 days ex ovo. Thus, the progressive appearance of the dystrophic syndrome in this model parallels the changing sensititvity of SR Ca-ATPase to its activator protein.

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379.12 DOUBLE MUTANT COMBINATIONS WITH QUAKING: NEW APPROACH TO AN ENIGMATIC MUTATION. M.K. Wolf, M.E. Pomeroy*, and S. Billings-Gagliardi, Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605

Unlike the other well-studied CNS hypomyelination mutations, qk does not seem to have a single myelin protein defect that could represent a primary effect of the mutant gene. Furthermore, abnormal qk morphology seems unrelated to the reported CNS abnormalities in both protein and lipid chemistry. We have sought more information about the qk locus by making double mutant mice expressing both the qk and shi or qk and jp mutations, using B6C3-hybrid based stocks. The qk*shi double mutant is one of five possible behaviorally affected genotypes produced by mating unaffected qk/+*shi/+ double heterozygotes. qk/qk*shi/shi animals are recognized by their distinctive ultrastructural phenotype in P-18 to P-26 optic nerve and cerebellar white matter. This is confirmed by control matings, which generate the other four possible genotypes but not qk/qk*shi/shi. The qk*jp double mutant is produced by three cycles of crossing T^{2J}+/+qk males, first to Tajp/++ carrier females wild type at the qk locus and then to their +qk*Tajp/++ and qk/qk*Tajp/++ descendants. The qk/qk*Tajp/Y genotype is identified both by the marker genes and by its distinctive ultrastructure. qk*shi CNS (P-21 optic nerve) has less than 5% the normal number of myelinated axons, markedly fewer than either single mutant. Those myelin sheaths present are usually thin. Otherwise, qk*shi resembles P-21 shi rather than qk: sheaths only partially surround axons and show no compaction with major dense line (MDL) formation, and there are prominent bundles of oligodendrocyte microprocesses but none of the redundant myelin whorls characteristic of qk. Thus the interaction of qk and shi appears to be quantitatively synergistic, but qualitatively favors shi. In qk*jp CNS, on the other hand, the number of myelinated axons is similar to jp. Myelinated axons are clustered, as in jp, and have MDL. Some are of normal thickness, others are thin, and still others show the whorls and tangles associated with qk. The white matter contains numerous macrophage-like cells filled with heterogeneous, often membraneous, material. Criteria for identification of these cells are not absolute; however, many of them resemble cells seen in qk while only a few are similar to classic "jimpy cells" at this age. The phenotype of qk*jp therefore seems to resemble jp in amount of myelin but not in white matter structure. In the two qk double mutants, the morphological features of qk are expressed in the presence of jp, but not shi. This suggests that in certain features, qk is epistatic to jp, while shi is epistatic to both qk and jp. Supported by NS-11425 (Javits Award).

379.13 INTERACTIONS OF SHI AND JP ALLELES: COMPARISON OF ALL FOUR DOUBLE MUTANTS. S. Billings-Gagliardi, M.E. Pomeroy*, and M.K. Wolf. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605

The shi and jp mutations, which produce two different syndromes of CNS hypomyelination, have been mapped to chromosomes 18 and X respectively. At each locus, there are two presumed alleles (shi and shi^{msd}: jp and jp^{msd}) which produce similar but not identical phenotypes. Surprisingly, the morphological phenotype of a double mutant mouse expressing both shi and jp (shi*jp) is sharply different from that of the corresponding shi^{msd}*jp^{msd} double mutant. To seek explanation, we have studied the CNS structure of the other two possible double mutants, shi*jp^{msd} and shi^{msd}*jp. Double mutants produced from B6C3-hybrid based stocks are identified by CNS morphology (P-19 to P-25 optic nerve and cerebellar white matter) clearly distinguishable from both single mutant ancestors. Based on TEM comparison of number of myelinated axons and amount of myelin/sheath, the double mutants can be ranked (1) shi*jp (most), (2) shi*jp^{msd}, (3) shi^{msd}*jp, 4) shi^{msd}*jp^{msd} (least). Morphologically normal oligodendrocytes are more numerous in the shi than in the shi^{msd} combinations, and tend to occur as cohorts rather than individually. In all four double mutants myelinated axons are grouped in clusters, as in jp and jp^{msd}. However, the lipid-filled cells so characteristic of both jp and jp^{msd} are absent in shi*jp but are numerous in the other three combinations. The parallel arrays of oligodendrocyte microprocesses prominent in both shi and shi^{msd} are equally prominent in shi*jp and are also present in much smaller numbers in shi*jp^{msd}, but only in the patches of CNS which contain the clusters of myelinated axons; they have not been observed in either shi^{msd}*jp or shi^{msd}*jp^{msd}. Most myelin sheaths in all four combinations are of the P-21 shi and shi^{msd} type lacking major dense line (MDL) and with abnormally dense intraperiod line. Only shi*jp sheaths have occasional regions of normal or interrupted MDL. Thus while the morphology of shi*jp suggests partial reciprocal intergenic suppresion, the other three combinations suggest different degrees of additive or synergistic gene interactions. At both the shi and jp loci, the rank order of "severity" of the two alleles is reversed in the double mutants. Alone, only shi^{msd} has MDL at P-21, but in combination with jp, only shi has MDL at that age. Alone, jp^{msd} has about twice as much myelin as jp, but in combination with either shi or shi^{msd}, jp has more myelin than jp^{msd}. shi has a deletion impinging on the MBP gene, whereas shi^{msd} is probably not a deletion. Our evidence suggests that jp and jp^{msd} will also prove to represent different types of DNA alterations. Supported by NS-11425 (Javits Award).

379.14 EXPRESSION OF THE GENE ENCODING GLUTAMIC ACID DECARBOXYLASE (GAD) IN MUTANT MOUSE CEREBELLA STUDIED BY IN SITU HYBRIDIZATION. C.W. Wuenschell*[1], A. Messer[4], and A.J. Tobin[1,2,3] (SPON: M. Gordon). [1]Department of Biology, [2]Molecular Biology Institute, [3]Brain Research Institute, University of California, Los Angeles, CA 90024; and Wadsworth Laboratories, New York State Department of Health, Albany, NY 12201.

In the normal mouse cerebellum, expression of the GAD gene is characteristic of the differentiated phenotype of four types of neurons: Purkinje, Golgi II, stellate and basket neurons. Expression of the GAD gene in each of these cell types could result from the unfolding of a program of gene expression established early in cerebellar development and dependent thereafter only upon factors intrinsic to the cells themselves. Alternatively, expression of the GAD gene in one or more of these neuron types could be triggered later in development by influences from surrounding cells. Cerebellar mutants in which cell types are lost or misarranged offer an approach to the study of this issue.

We have previously shown that GAD mRNA is detectable in the four GABAergic neuron types of normal mouse cerebellum by in situ hybridization with ^{35}S-labeled antisense GAD RNA. We have now examined the cellular distribution of GAD mRNA in the Lurcher mouse. Our preliminary results suggest that all of the normally GABAergic cell types which are present in the 21 day old Lurcher cerebellum do contain GAD mRNA. When hybridized with antisense GAD RNA, the molecular layer contained both labeled and unlabeled cells. Larger labeled cells appeared both scattered within the granular layer as well as at the boundary of the molecular and granular layers. Granule neurons and cells in the deep white matter were not labeled. No cells were specifically labeled following hybridization with sense GAD RNA. We are undertaking studies with hybridization probes specific for either Purkinje or Golgi II cells in order to clarify the identity of the larger labeled cells.

We have also begun to study the expresion of the GAD gene in several other murine cerebellar mutants. Preliminary results show that GAD mRNA is present in specific cerebellar neurons of both 21-day staggerer mice and of 14-day weaver mice.

This work was supported by NIH grants to AJT (#NS 20356 and NS 22256) and to AM (#NS 17633). CWW was supported in part by a USPHS Training Grant in Genetic Mechanisms (#GM 7104).

379.15 EXTENSIVE POSTMORTEM STABILITY OF RNA FROM RAT AND HUMAN BRAIN. S.A.Johnson*,D.G.Morgan and C.E.Finch. Andrus Gerontology Center and Dept. of Biol. Sci., Univ. Southern California. Los Angeles, CA 90089-0191.

Since it is impossible to obtain human brain tissue immediately after death, the postmortem stability of human brain RNA is an important concern for those who wish to study the molecular genetics of human brain neurodegenerative disease.The post-mortem stability of brain RNA for up to 48 hours was measured in rat and human samples. Whole rat brains, cooled at a rate similar to human brains awaiting autopsy, were collected at intervals from 0 to 48 hours after death and frozen. These samples were compared with freshly obtained, unfrozen rat brains. Two characteristics of these RNA populations were measured: (1) the recovery or yield of RNA/gram tissue (quantity) and (2) the integrity or extent of degradation (quality). Total RNA yields were similar after all post-mortem delays. Hybridization of [^{32}P]labeled cDNA probes (chick brain B-Tubulin, Mouse Thy-1, hamster scrapie prion) to nitrocellulose filter blots of electrophoretically separated rat brain RNA demonstrated the integrity of specific rat brain mRNAs during the post-mortem period. Similarly, in vitro translation of these same rat total RNA samples produced high molecular weight translation products with no differences between long and short post-mortem times.

Human cerebral cortex RNA was prepared by the same methods as those used for rat brain from a neurosurgical sample and 4 other donors with post-mortem intervals from 7 to 36 hrs. Typically, the yield of total RNA from human brain was 400-500 ug/g vs 800-1000 ug/g tissue for rat brain. When analyzed by RNA gel blot hybridization studies, human cortical RNA appeared slightly degraded, on the basis of B-Tubulin size distribution. However, the degree of apparent RNA degradation was not related to the post-mortem interval. In vitro translation products of human cortical RNA revealed high molecular weight peptides at all post-mortem intervals, but slightly less [^{35}S]incorporation into these bands was found at the longer post-mortem intervals relative to the shorter times. Previously, others have shown that in vitro translation products from human brain total RNA were not noticebly influenced by postmortem interval (Morrison and Griffin, Anal. Biochem. 113:318-324, 1981). The present data confirm these results, and extend them to direct measures of RNA yield and integrity. Together, these results demonstrate an extensive stability of brain RNA during the post-mortem period that encourages aggressive use of molecular genetic techniques for the study of human neurodegenerative disease. SAJ was supported by training grant AG-00093 and DGM was supported by the John Douglas French Foundation.

379.16 IDENTIFICATION OF A MUTATION IN THE GLUCOCEREBROSIDASE GENE IN NEURONOPATHIC GAUCHER DISEASE. S. Tsuji*[1], P.V. Choudary*[2], B.M. Martin*[1], J.A. Barranger*[2], EH. Kolodny*[3], BK.Stubblefield*[1], JA.Mayor*[1], SL.Winfield*[1], D. Merkle-Lehman*[1], M. LaMarca*[1], and E.I. Ginns*[1](SPON:K.A. Sigvardt) 1 Molecular Neurogenetics Unit, Clin. Neuroscience Br., NIMH, Bethesda, MD 20892; 2 Molecular and Medical Genetics Sec., Lab. Molecular Genetics, NINCDS, Bethesda, MD 20892; and 3 E.K. Shriver Center, Waltham, MA 02254 and Neurology, Mass. Gen. Hosp., Boston, MA 02114.

Gaucher disease, the most common sphingolipidosis, is caused by the deficiency of lysosomal glucocerebrosidase (EC.3.2.1.45). The chromosomal locus for this enzyme has been mapped to 1q21, adjacent to a region of chromosome 1 (1qh) abundant in structural polymorphism. On the basis of clinical signs and symptoms, this disease has been divided into three major phenotypes: type 1 (non-neuronopathic), type 2 (acute neuronopathic) and type 3 (chronic neuronopathic). In order to provide a more accurate description of the mutation in the gene, genomic DNA of a normal control and a type 2 patient have been cloned into lambda phage. The insert DNA encoding glucocerebrosidase in both cases is 12 kb in length. Each insert had all exons coding for glucocerebrosidase. Comparison of nucleotide sequences of all exons and splicing junctions in the normal and type 2 genes revealed only a single base substitution (T→C) located in the coding sequence of the type 2 Gaucher disease gene. On southern blot hybridization analysis it was demonstrated that all neuronopathic (three type 2 and four type 3)patients examined had at least one allele having this mutation, while none of eight type1 patients or eleven normals had this mutant allele. This result suggests that the frequent occurrence of this mutation is highly specific for neuronopathic Gaucher disease.

379.17 REGENERATION OF ACETYLCHOLINESTERASE IN RAT BRAIN FOLLOWING TREATMENT WITH N-METHYL-N-NITROSOUREA, A DNA ALKYLATING AGENT. S. Okoyama* and P.L. McGeer (SPON: D.D. Greenwood). Kinsmen Laboratory of Neurological Research, Dept of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada

N-Methyl-N-nitrosourea (MNU) is a representative of a class of mutagens which act by alkylating DNA, especially in transcriptionally active regions. We hypothesized that neuronal DNA damage might be revealed by reduced production of an active enzyme such as acetylcholinesterase (AChE). Accordingly, we studied AChE in rats treated with diisopropyl fluorophosphate (DFP) alone or followed by MNU and have found that the NMU significantly reduces the amount of biochemically measurable or histochemically demonstratable AChE in a number of brain regions.

For biochemical work, male Wistar rats were given 1.5 mg/kg DFP intramuscularly followed 2 hrs later by a single intravenous dose of 100 mg/kg MNU, or 3 doses of 10 or 50 mg/kg MNU at 2 hrs, 1 day and 2 days after the DFP. Control rats were given DFP alone. Rats were sacrified at 3, 7 or 14 days following the DFP. AChE and protein were measured by standard methods on homogenates of 10 brain regions (olfactory bulb, frontal cortex, parietal cortex, thalamus, pons/medulla, cerebellum, midbrain, hypothalamus, hippocampus and striatum) from one hemishere; the other hemisphere was fixed and used for AChE histochemistry. Protein levels were comparable in all groups but AChE activities were significantly less (20-77% reductions) in the frontal and parietal cortices, hippocampus and striatum in the DFP/MNU groups than in the DFP group at all doses and time intervals studied. The other 6 brain regions showed no significant effect except in rats given 100 mg/kg MNU where the olfactory bulb showed a 31% reduction at 7 days and the hypothalamus a 32% reduction at 14 days.

Histochemistry on the other hemispheres indicated much less AChE fiber staining in the DFP/MNU rats than in those treated with DFP alone. Similar histochemistry was also done on rats receiving the same drug dosages but also a second dose of DFP 6 hrs before sacrifice on day 14. The number of AChE-positive cell bodies was less in the DFP/NMU than in the DFP rats.

Since the initial DFP treatment presumably induces active synthesis of AChE, these data are consistent with the hypothesis that actively transcribing sections of the DNA are particularly affected by the mutagen and that the resultant neuronal DNA damage may result in reduced levels of particular enzymes.

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379.18 MESSENGER RNA DEPENDENT EXPRESSION OF GFAP IN THE ALZHEIMER'S DISEASE BRAIN. E.M. Sajdel-Sulkowska, R.E. Majocha*, M. Salim*, C.E. Fulwiler, H.J. Manz*, S.B. Zain* and C.A. Marotta. Harvard Med. Sch., McLean Hosp., Massachusetts General Hosp., Boston, MA 02114, Univ. of Rochester Med. Sch., Rochester, NY 14642, and Georgetown Univ. Sch. of Med., Washington, DC 20007.

Previously, we demonstrated that messenger RNA extracted from the postmortem Alzheimer's disease (AD) brain can be used to examine translation products and to prepare recombinant DNA libraries (Marotta et al, Prog. Brain Res., 1986, in press). However, we and others have noticed that the content and yield of RNA is diminished in affected brain regions of both biopsied and autopsied specimens, presumably due to a combination of transcriptional and degradative factors. The present study was performed to rule out the possibility that AD RNA undergoes exclusively non-specific losses which may indicate only generalized pre- or postmortem processing. If decreased RNA were related to the disease process, then the losses would be confined to affected cells. If this were the case, astroglial specific mRNA, such as the one for GFAP, would be expected to remain constant or to increase. We found that cytoskeletal protein prepared from the postmortem AD brain was rich in two forms of GFAP whose identity was confirmed by immunoblotting with anti-serum to GFAP. Poly(A+) RNA from both control and AD samples stimulated protein synthesis in the rabbit reticulocyte system, with the control being more active. A prominent protein of 50,000 mol. wt. was identified as GFAP by immunoprecipitation. Although AD mRNA was of lower specific activity than control, the amount of GFAP synthesized by the former was approximately twice that of the control. An increase in AD brain GFAP mRNA activity was independent of age, cause of death and postmortem interval. 2-D PAGE revealed that the newly synthesized GFAP often appeared as a doublet. In order to examine the source of potential heterogeneity, cloning experiments were carried out. cDNA was prepared from poly(A+) RNA isolated from a postmortem AD brain and cloned into the lambda gt11 expression vector system. The positive clones are being identified by screening with antiserum to GFAP. Results indicate that the loss of AD brain RNA is associated with neurons. Thus, mRNAs that are not exclusively neuron-specific may appear to remain at nearly normal levels or may be elevated due to the contribution from glia. The measured levels of these mRNAs in AD brain RNA preparations must be interpreted with caution. We conclude that a model to explain the increased proliferation of astrocytes in AD must take into account high levels of synthesis of glial specific protein rather than, or in addition to, a postulated decreased degradation of glial constituents. Supported by grants from the American Health Assistance Foundation, the McKnight Foundation, and NIH grants AG02126, AG04522 and AG05134.

380.1 NEUROGENIC INFLUENCE ON CEREBRAL MICROVESSELS IN THE RAT HIPPOCAMPAL SLICE. T. Smock, A. Topple and R. Cach*. Behavioral Neuroscience Program, Department of Psychology, University of Colorado, Boulder, CO 80309.

Study of the physiology of cerebral vasculature is limited to cerebral blood flow measurements and experiments on pial vessels and other superficial vessels in the whole animal. Large cerebral arteries have been studied in isolation and recently cerebral microvessels have also been studied in vitro (Dacey and Duling, Am. J. Physiol. 243:H598). Lacking is an in vitro preparation that permits investigation of such vessels in the context of their neural and glial environment. We adapted the rat hippocampal slice, a standard preparation for neurophysiology, to the purpose of evaluating the neural control of brain microvessels.

131 slices were incubated in a brain slice chamber under standard conditions. Time-lapse photomicrographs were taken of individual vessels (avg. lumenal diameter=17 μm, range 5-40 μm). 51% of the slices contained vessels that responded to electrical stimulation. Single pulses were uniformly ineffective in eliciting a vascular response but brief trains of pulses produced vasoconstriction that outlasted the stimulus duration. On occasion, periods of rhythmic contractions ensued after the stimulus. Frequently, there was a delay of 1-20 seconds between stimulus termination and vascular response. Vessels did not respond to subsequent trains presented in rapid succession, but would respond repetitively (up to 50 trials) if a refractory period of 1-5 minutes was allowed. Optimal stimulation conditions for vessels that responded repetitively were a 300 msec train of 10 v., 0.5 msec pulses delivered at 100 Hz. These conditions also produced long-term potentiation of the perforant path to dentate psp.

Tetrodotoxin (TTX, 1 μM) was applied to block propagated neuronal action potentials. Complete blockade of the evoked dentate field potential was obtained in 20 min. After 20 min in TTX, vessels ceased to respond to the electrical stimulus (n=8). However, vascular responses could still be obtained in the presence of TTX if the stimulus strength was increased or if the electrode was placed close (less than 250 microns) to the vessel in question (n=12), presumably reflecting vasoconstriction due to direct activation of vascular smooth muscle.

Brain microvessels receive contacts from projection fibres and local circuit neurons. Some of these contacts have been shown to contain vasoactive transmitters. The TTX-sensitive vasoconstriction that we have observed could be due to activation of these neurovascular contacts or possibly to some non-synaptic event, such as build-up of extracellular potassium, that ensues upon repetitive neuronal discharge.

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380.2 VASOCONSTRICTION AND NEURONAL HYPEREXCITABILITY IN THE HYPOXIC HIPPOCAMPAL SLICE. A. Topple and T. Smock. Behavioral Neuroscience Program, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Anoxia and hypoxia produce neuronal hyperexcitability in vivo and in vitro (Sloper, Johnson and Powell, Br. Res. 198:204, 1980); Schiff and Somjen, Br. Res. 337:337, 1985). Cerebral vessels also respond to anoxic and hypoxic conditions in the intact animal. We employed the rat hippocampal slice preparation to examine neural and vascular consequences of transient hypoxia under circumstances in which CO_2 concentration, pH and ionic environment were controlled experimentally.

26 slices were incubated at the liquid-carbogen interface in a brain slice chamber under standard conditions. Hypoxia was induced by alternating between 95% O_2/5% CO_2 gas and 95% N_2/CO_2 gas introduced into the bath. The mean duration of hypoxia was 64(±26) sec. Extracellular field potentials were recorded from the granule cell body layer of the dentate gyrus following single pulse DC current to the perforant path. Time lapse photomicrographs were taken of individual microvessels (5-40 μm in diameter). Measurements of bath pH during repeated episodes of normal to hypoxic conditions showed no significant change in pH. During transient reduction in tissue O_2 tension a significant increase in neuronal excitability was observed, as shown by a 60% (±6 S.E.M.) increase in postsynaptic population spike amplitude (n=11). There was also a 43% (±6 S.E.M.) decrease in EPSP voltage. Vessel diameters were measured from photographs at intervals corresponding to 10-20 micron segments. During hypoxia local regions of each vessel (n=8) displayed constriction with regard to the baseline period. The mean reduction in diameter was 25% (±6 S.E.M.), compared to vessels not exposed to hypoxia (n=7, significant difference 0.01 level, 1-tailed t-test). The constriction was uniformly restricted to a portion of the vessel with adjacent regions not responding.

The transient hypoxic neural excitability and the vascular response may be direct and independent consequences of lowered O_2 tension on each of the cell types. However, the possibility that the neural excitability itself has a vascular consequence cannot be overlooked. Though the bath composition of salts, CO_2 and hydronium ion was held constant in our study, local changes of these parameters in the microenvironment of vessels and neurons may have occurred.

Supported by grants from the Epilepsy Foundation of America, a grant from the Colorado Heart Association and NSF #BNS-8520622.

380.3 TRANSIENT HYPEREXCITABILITY WITH RETURN OF SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES DURING MODERATE HYPOXIA. S.J. Schiff and P.G. Aitken. Division of Neurosurgery and Department of Physiology, Duke University Medical Center, Durham, NC 27710.

Evoked potentials were recorded from the CA1 region of hippocampal tissue slices during a hypoxic insult sufficient to suppress synaptic transmission within 2-6 minutes. We here report the transient return, during hypoxia, of synaptic transmission accompanied by neural hyperexcitability.

Transverse hippocampal slices 400μm thick were prepared from isolated hippocampi with a tissue chopper or from sagitally hemisected brains with a vibratome. Slices were maintained at a gas-fluid interface; the fluid phase consisted of 35-36°C artificial cerebrospinal fluid (K^+,3.5mM; Ca^{2+},1.2mM; Mg^{2+},1.2mM) equilibrated with 95% O_2 - 5% CO_2 and flowing at 1.6-2.2 ml/min. The gas phase consisted of 95% O_2 - 5% CO_2 flowing at 225 ml/min, except during hypoxia when it was replaced with 95% air - 5% CO_2 flowing at the same rate. Stratum radiatum was stimulated with 100-500μs pulses at current intensities sufficient to evoke half-maximal to maximal amplitude orthodromic population spikes from stratum pyramidale. In some experiments, intracellular recordings from CA1 pyramidal cell somata were obtained.

In all slices, synaptic transmission disappeared 2-6 min into hypoxia. In approximately 80% of slices, synaptic transmission transiently returned 1-2 min later and remained for 20-220 sec before again disappearing. The return of synaptic transmission was accompanied by marked hyperexcitability evidenced by multiple population spikes being evoked by each stimulus pulse, occasionally with a marked increase in the amplitude of the population spikes. In approximately 80% of slices, the hypoxia induced spreading depression (SD), with a sustained negative shift of the extracellular potential. The occurrence of SD was not correlated with transient hyperexcitability, but in those slices where the two phenomena occurred, SD abruptly terminated the transient hyperexcitability. Approximately 30% of SD episodes were preceded by spontaneous paroxysmal synchronous discharge of the CA1 zone as reported previously (Soc. Neurosci. Abstr. 11:433, 1985). Intracellular recordings during hypoxia suggested that the brief periods of hyperexcitability are the result of cellular depolarization plus enhanced ephaptic interactions between cells. If these phenomena occur in situ, they could play a role in hypoxic or ischemic seizure activity.

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380.4 DELAY OF HYPOXIA-INDUCED SPREADING DEPRESSION BY GANGLIOSIDES. M. Balestrino. Dept. of Physiology, Duke University Medical Center, Durham, NC 27710.

We have previously shown (Balestrino and Somjen, Brain Res., in press) that delay of hypoxia-induced spreading depression (SD) by chlorpromazine protects brain slices against irreversible damage caused by hypoxia. This delay is protective because it shortens the time during which intracellular calcium concentration is raised as a result of the ionic shifts during SD. GM_1 ganglioside has been shown (Janigro et al., J. Neurosci. Res. 12:499-509, 1984) to delay SD in CA3 during hypoxia. The present study investigates a possible similar effect by the GM_1-derived AGF_2 ganglioside.

Rat hippocampal slices were maintained in an interface-type chamber at 35°-36°C. Extracellular potential from stratum pyramidale of CA1 was monitored using a DC-coupled amplifier, and in some slices impulse transmission from Schaffer collaterals to CA1 was also monitored. Slices were aerated with 95%O_2, 5%CO_2. Rats were injected i.p. with either GM_1 or AGF_2 gangliosides (30 mg/Kg for 3 days), and 0.5μM of the same drug were dissolved in the artificial cerebrospinal fluid (ACSF) in the chamber. In an attempt to maximize ganglioside incorporation into cell membranes, slices were exposed to the ACSF in the chamber for 4 hours before hypoxia was induced. Slices from saline-injected rats incubated for 4 hours in drug-free ACSF were used as controls. All animals were 33-36 days old at the time of sacrifice. Hypoxia time was always 9 minutes. It was induced by substituting O_2 with N_2 in the gas phase.

The onset of SD from the time of oxygen withdrawal ("SD latency") was (mean ± standard deviation) 159±74 seconds in the control group, 227±65 seconds in GM_1-treated slices, and 280±112 seconds in the AGF_2-treated slices ($p<0.05$, 1-way analysis of variance).

When recovery of synaptic transmission upon reoxygenation was monitored, all slices in which SD latency was longer than 260 seconds (5 out of a total of 11) showed such a recovery, whereas no slice in which SD latency was shorter (6 out of 11) recovered ($p<0.01$, chi-square test).

These results show that AGF_2 ganglioside is effective in delaying hypoxia-induced SD, and further confirm that delay of SD during hypoxia is a determinant of protection against hypoxic damage.

Supported by USPHS grant NS 18670 to Dr. G. Somjen. I thank Dr. G. Toffano of Fidia Research Laboratories for the gift of GM_1 and AGF_2 gangliosides.

380.5 TOXICITY OF INTRACELLULAR CALCIUM: AN INVERTEBRATE MODEL. L.S. Jones, M. Balestrino, and D.V. Lewis (SPON:W.A. Wilson). Depts. of Peds.(Neur.) and Physiology, Duke Univ., Durham, NC 27710.

Ca^{2+} entry into cells (e.g. during hypoxia) may be a determinant of cell death. Strong support for this hypothesis exists, but direct evidence is still missing. We report here on the toxic effects of elevating $[Ca]_i$ in invertebrate neurons.

The nonbursting, silent medial pleural cells from Aplysia californica were impaled with KCl-filled recording electrodes, voltage clamped at -30 mV (see Jones et al., Brain Res., in press) and iontophoretically injected with either Ca^{2+} or Mg^{2+}. Only cells with resting membrane potentials (Vm) more negative than -40 mV with all electrodes in place were used. Ca^{2+} and Mg^{2+} were injected repeatedly into different cells by passing current for 20 seconds. The first injection was a 50 nA current, the second was 100 nA, and subsequent injections were increased by 100 nA increments. Membrane conductance (g) and Vm were measured before each injection. Results for the two groups (Ca^{2+}, n=4; Mg^{2+}, n=4) were remarkably distinct. Cells receiving Ca^{2+} became hyperpolarized initially, then (after the 4th-5th injection) Vm dropped abruptly to -10 to -20 mV and direct electrical excitability was lost. Mg^{2+} cells, however, became somewhat hyperpolarized and stayed that way after many more, larger injections. The Vm for 3 of 4 Mg^{2+}-injected cells remained more negative than -50 mV even after cumulative injections of 140 mM Mg^{2+}. The Vm's of Ca^{2+} injected cells were all -30 mV or worse by the time 70 mM of cumulative Ca^{2+} had been injected; in 3 of 4 cells the Vm was more positive than -30 mV following only 30 mM of cumulative Ca^{2+}. Increases in g paralleled the loss of Vm.

The profound depolarization, increased membrane g and loss of electrical excitability were not reversible and might be equated with cell death. Lack of damage by Mg^{2+} at the same doses strongly argues against a nonspecific divalent cation effect. The need for repeated Ca^{2+} injections before damage was seen points to protective mechanisms against excessive elevations in $[Ca]_i$. Invertebrate systems may be useful models for studying mechanisms of Ca^{2+} toxicity.

This investigation was supported by a research grant from the Epilepsy Foundation of America and USPHS (#NS 18670).

380.6 THE PROTECTIVE EFFECT OF THERAPEUTIC ULTRASOUND ON THE HYPOXIC BRAIN E.W. Peterson, G.V. Forester*, O.Z. Roy* and J.R. Scott* Division of Electrical Engineering, National Research Council of Canada, Ottawa,Ontario,Canada. K1A OR6.

In earlier studies from our laboratory, there has been evidence that ultrasound at therapeutic levels improves the contractility of hypoxic myocardium. The present study concerns the direct application of therapeutic levels of ultrasound to the cat's brain and its effect on function during induced hypoxia.

Experiments were carried out on 9 adult male cats. They were anesthetized, curarized and then the calvarium was removed to extensively expose the dura over the convexities. The animals were artificially ventilated by means of an endotracheal tube which facilitated the rapid change of inspired gases in order to produce acute hypoxic episodes as desired. Systemic arterial blood gases were measured serially. These demonstrated that under normoxia, the range was (pO_2 180-220 mmHg) and during transient global hypoxia, the pO_2 fell to 21-30 mmHg. The visual evoked potentials (VEPs) were used as a measure of pathway functions and were recorded by averaging differentially between the visual cortex and the frontal region. Ultrasound (1W/cm² SATA at 1 MHz) was applied directly to the brain through a gas-free aqueous coupling system, such that there was no direct pressure upon the dura.

As expected, the hypoxia had a deleterious effect on the VEPs affecting both the primary fast and secondary slow components of the wave form. When the brain was treated with ultrasound before, during and after the hypoxic episodes, there was clearly a prevention of these changes. Ultrasound applied during the normoxic state had no effect on the VEPs.

These results suggest that there is a direct effect of ultrasound on cerebral cell function by possibly facilitating delivery or utilization of the limited oxygen supplied to the brain. Because some heating of the tissue resulted from the application of ultrasound, additional control experiments were performed using whole body heating. These revealed that simple temperature elevation could not mimic the protective effect seen with ultrasound. It is suggested by these results that ultrasound may have a therapeutic effect on brain tissue subject to hypoxia, and would be capable of sustaining cerebral function to a significant degree during induced or accidental hypoxia.

380.7 POTASSIUM ION HOMEOSTASIS IN TURTLE BRAIN DURING ANOXIA, GLYCOLYTIC INHIBITION AND ISCHEMIA. Feng Zi-Cai, T.J. Sick and M. Rosenthal, Department of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Studies of brain anoxia and anaerobic metabolism in mammals are made difficult by that tissue's dependence upon energy derived from oxidative metabolism. Degenerative changes occur so rapidly that within a few minutes, transmembrane ion gradients are lost, energy sources are depleted and recovery becomes questionable. To understand anaerobic metabolism and the capability of brain to compensate for anoxia or ischemia, an animal model is needed that has a very high anaerobic capacity. Turtle brain is ideal in this regard. In these studies, reflection spectrophotometry was used to monitor reduction/oxidation changes of cytochrome oxidase, polarographic microelectrodes monitored brain tissue oxygen tension and hydrogen clearance (blood flow) and ion-selective microelectrodes recorded the activity of extracellular potassium ion (K^+o) in intact brains of turtles anesthetized with pentobarbital. During N_2 inspiration, cytochrome oxidase became fully reduced and tPO_2 fell to zero indicating that oxygen storage did not prevent attainment of complete anoxia. However, K^+o rose only 3-5 mM during 24 hours of anoxia indicating that ion homeostasis was not completely sacrificed (in rat, K^+ rose to over 70 mM after only a few minutes of anoxia). Restoration of brain oxygenation after anoxia in turtle produced full recovery of K^+o. In contrast, ion homeostasis was lost within 30 min of ischemia in turtle brain and, within 60-90 minutes, recovery of K^+o and brain blood flow was not evident when carotid ligatures were removed. To determine whether energy failure or the loss of perfusion was responsible for these changes, turtles were rendered anoxic after glycolysis was inhibited by superfusion of the brain with iodoacetic acid (IAA). IAA superfusion itself had little effect on K^+o but, combined with anoxia, IAA quickly produced loss of ion homeostasis. During combined anoxia with IAA, K^+o remained elevated and brain blood flow progressively declined. However, recovery of K^+o and blood flow occurred if brain was reoxygenated within 8-10 hours. No recovery occurred after 15 hours of IAA and anoxia. These data demonstrate that ion homeostasis can be maintained by anaerobic glycolysis during anoxia in turtle brain and suggest that irreversible effects of ischemia are not produced by loss of ion homeostasis. These data also suggest that continuance of some fluid perfusion of turtle brain markedly enhances the potential for recovery and that blood stasis during ischemia may affect the brain vasculature in a manner that precludes reperfusion and reoxygenation and thereby precludes recovery of ion homeostasis. (supported by the National Parkinson Foundation through a grant from the Envor Armington Fund).

380.8 RELATIONSHIP BETWEEN BRAIN O2 CONSUMPTION AND CYTOCHROME C OXIDASE REDOX STATE DURING HYPOXIC HYPOXIA IN THE CAT. P.A.Lee*, A.L.Sylvia F.F.Jöbsis-VanderVliet*, and C.A. Piantadosi*, Depts. of Physiology and Medicine, Duke University Medical Center, Durham, NC 27710.

Systemic hypoxia initiates compensatory responses which serve to maintain O2 delivery to hypoxia-sensitive tissues such as the brain. Since most of the oxygen delivered to tissues is utilized by mitochondrial cytochrome a,a_3 during respiration, changes in the oxidation level of this enzyme will reflect changes in tissue O2 availability. Non-invasive reflectance multiwavelength near infrared spectrophotometry was used to evaluate changes in cytochrome a,a_3 oxidation level, regional blood volume and tissue hemoglobin color (indicates relative O2 saturation) in the intact brain cortex of six pentobarbital anesthetized, ventilated cats during graded 10 minute periods of alveolar hypoxia (21% control, 12%, 9% and 7% O2). The NIR optical responses were compared with conventional measurements of cerebral oxygen delivery and utilization. Total and regional cerebral blood flow (CBF) with each O2 gas mixture was measured using radiolabeled microspheres (15μm; 141Ce, 113Sn, 103Ru, 95Nb) injected into the left ventricle. Blood flow was computed by the reference sample method. Arteriovenous O2 difference (A-VDO2) was calculated from the arterial and sagittal venous O2 contents determined after each injection. CMRO2 for each condition was calculated as CBF x (A-VDO2).

The most severe level of hypoxia tested (7%O2) decreased mean arterial and venous PO2 from 109 ± 6 torr to 23 ± 2 torr and from 38 ± 4 torr to 18 ± 1 torr, respectively. Slight decreases in arterial and venous PCO2 were also observed with each hypoxic gas mixture yet there was no significant difference in mean arterial blood pressure. Total CBF increased over 500% with 7%O2 relative to normoxic control values. No significant change in CMRO2 occurred during hypoxia indicating that the increase in CBF maintained sufficient O2 delivery to the brain to meet its metabolic requirements. However cytochrome a,a_3 oxidation level decreased from 80% with room air to near 30% with 7%O2 (where 100% and 0% oxidation are the redox states during ventilation with 100%O2 and 100%N2, respectively). As expected, the NIR tissue hemoglobin signals reflected the decreased O2 content of the blood. A linear relation (r=0.997) between the amount of oxidized cytochrome a,a_3 in the cortex determined by the NIR method and the sagittal venous oxygen content was found. If the cerebrovenous O2 content reflects tissue O2 concentration in hypoxia, then stable oxygen consumption (CMRO2) of the brain during hypoxia may be explained by a "cushioning effect". Since the rate of oxygen uptake with respect to time ($-dO2/dt$) is proportional to the product of [O2] times [reduced cytochrome a,a_3] (Chance, B. Fed. Proc. 16: 671-680, 1957), the decreases in tissue O2 concentration in the brain are compensated for in part by the increasing reduction level of cytochrome a,a_3.

380.9 MITOCHONDRIAL AND PLASMA MEMBRANE POTENTIALS DURING ANOXIA AND NORMOXIA. P.E. Nielsen* and G.E. Gibson, Cornell Univ. Med. College at Burke Rehabilitation Center, White Plains, NY 10605

Impairment of synaptosomal calcium uptake during hypoxia and anoxia may lead to altered neurotransmitter metabolism. Indirect pharmacological studies suggest that the decrease in calcium uptake is due to a reduction in the mitochondrial membrane potential. The present study examined this possibility by directly estimating the plasma and mitochondrial membrane potentials. Synaptosomes were preincubated for 15 min at 37° C with ^{86}Rb (a marker of the plasma membrane potential), ^{3}H-tetraphenylphosphonium (a marker of the plasma and mitochondrial membrane potentials) and ^{14}C-sucrose (a marker of the extracellular space). Samples were then placed on ice, flushed with either 100% oxygen or 100% nitrogen for 10 min, returned to the 37°C incubator and then stopped by centrifugation through an oil:dinonylphthalate mixture.

Samples were incubated for varying times under nitrogen or oxygen. Under oxygen, the plasma membrane potential was 58.8 ± 2.0, 58.8 ± 1.5 and 52.6 ± 1.8 at 5,10 and 20 min, respectively; the potential diminished 7 mV after only 5 min of anoxia and remained depressed. Under oxygen, the mitochondrial membrane potential was 139.0 ± 1.5, 140.0 ± 1.1 and 147.4 ± 2.0 mV at 5, 10 and 20 min, respectively. The magnitude of the anoxic-induced decline (-38 mV) in the mitochondrial membrane potential was greater than that of the plasma potential, occurred by 5 min and remained depressed.

These results were extended by examining the effects of either depolarizing concentrations of K^+ (60 mM) or a mitochondrial uncoupler (valinomycin) for 5 min before the end of a 20 min incubation with either oxygen or nitrogen. During normoxia, K^+ reduced the plasma potential by 48 mV to 4.6 ± 1.5 mV and valinomycin decreased it to 30.6 ± 3.0 mV. Anoxia diminished the resting membrane potential by 9 mV to 43.7 ± 2.7 mV, but had no further influence during K^+ depolarization or in the presence of valinomycin. During normoxia, the mitochondrial membrane potential (147.4 ± 2.0 mV) declined slightly with K^+ depolarization to 129.7 ± 2.1 mV and was greatly reduced with valinomycin to 19.4 ± 10.1 mV. Anoxia reduced the mitochondrial membrane potential by 44 mV under resting conditions and by 48 mV with K^+ depolarization, but did not reduce the valinomycin group further. These findings support the hypothesis that the decreased synaptosomal calcium uptake during hypoxia or anoxia is due to a partial collapse of the mitochondrial membrane potential.

380.10 MYELOPEROXIDASE ACTIVITY IN ISCHEMIC CEREBRAL INFARCTION T.H.Liu*,Y.O.Luk*,C.Y.Hsu, G.Konat* and E.L.Hogan. Dept. of Neurology, Med. Univ. S.C. Charleston, SC 29425

Polymorphonuclear cell (PMN) infiltration is believed to contribute to ischemic injury. The role of PMN in ischemic cerebral infarction has yet to be defined. Using a model of focal cerebral infarction in the rat (Chen ST, Hsu CY, Hogan EL et al, Stroke, 1986, in press) we studied the time course of post-ischemic PMN infiltration. The extent of PMN infiltration in the ischemic cerebral cortex was determined by spectrophotometric assay of myeloperoxidase (MPO)(Bradley, PP et al, J Invest Dermatol, 78:206,1982), a marker enzyme of PMN. Standard curves for MPO activity were derived from peritoneal PMN suspension (1.5 - 50 x 10^5 cells/ml) following glycogen challenge. Supernatant of cortex homogenate in phosphate buffer containing hexadecyltrimethylammonium bromide does not interfere with MPO assay. Cortex from sham-operated rats showed no detectable MPO activity (less than 2 unit/100 gm wet weight or less than 7.5 x 10^4 cells/hemisphere). Focal ischemia which caused a consistent cortical infarction in the right middle cerebral artery territory resulted in the appearance of MPO activity in the same region. MPO activity was barely detectable 6 hr after ischemia when ischemic infarction was evident by triphenyl tetrazolium chloride stain. Post-ischemic MPO activity was 6.3+0.5 units/100 gm wet weight at 24 hour; 18.5+1.9 at 36 hour; 33.3+10.0 at 48 hour; 15.4+2.1 at 60 hr; 12.1+2.1 at 72 hr; and 4.9+2.4 at 168 hr. The development of PMN infiltration only after the ischemic infarction was apparent, suggest that PMN infiltration probably does not play an initial role in infarct formation. Further studies are needed to determine whether PMN contributes to other late-onset processes such as breakdown of the blood brain barrier and vasogenic edema (supported by NS-11066, NS-12044, NS-00792 and MUSC Research Fund).

380.11 GRADED FOCAL ISCHEMIC INSULTS: EXPLORATION OF AN ANIMAL STROKE MODEL FOR THERAPEUTIC TRIALS Y.O. Luk*, M. Wong*, C.Y. Hsu, E.L.Hogan (SPON: J.B. Pickett). Depts. of Neurology and Anatomy, Med. Univ. S.C., Charleston, S.C. 29425

An ideal stroke model for assessing the therapeutic efficacy of new drugs should involve an ischemic insult of moderate severity with a demonstrable lesion which may be significantly reduced in magnitude with drug treatment. Severe ischemia may cause irreversible and massive damages not amenable to any pharmacological intervention. Mild ischemia may produce only minimal injury leaving little opportunity for detecting therapeutic efficacy. The end point for the trial effect should be quantitative and objective. Searching for a stroke model fulfilling these criteria, we studied the effect of graded ischemic insults on infarct size using a model of focal cerebral infarction in the rat (Chen ST, Hsu CY, Hogan EL et al, Stroke, 1986, in press). Ligation of the right middle cerebral artery (MCA), and the right common carotid artery (CCA) and temporary clipping of the left CCA resulted in a consistent cortical infarction in the right MCA territory. The infarct volume was quantitatively determined by computer-assisted morphometric measurement of infarct area in serially sectioned brain samples. The graded ischemic insult was induced by varying the duration of the left CCA clipping. The duration of clipping and resultant infarct volumes were: 90 min, 171.8+3.1 mm^3 (mean + SEM, N=13); 75 min., 99+4.9 (N=11); 60 min., 117+2.7 (N=19); 45 min., 99.5+4.6 (N=11); 30 min., 42.1+2.7 (N=11); 0 min., 35.2+3.3 (N=9). These results indicate that infarct volume was not necessarily proportional to the degree of ischemic insult based on the duration of left CCA clipping. For example there was no significant difference in infarct volume at 45, 60 and 75 min. durations. The clipping time of 45 min. was adopted for our ongoing screening of therapeutic agents because of its greater likelihood of responding to therapy with reduced infarct volume. (Supported by NS-11066, NS-12044, NS-00792 and MUSC Research Fund.)

380.12 EFFECTS OF (a) CHRONIC THIAMINE DEPRIVATION OR (b) PYRITHIAMINE (ANTAGONIST) TREATMENT ON REGIONAL CNS TRANSKETOLASE ACTIVITIES IN THE RAT. R.F. Butterworth, H. Fournier* and J.F. Giguère*. Lab. of Neurochemistry, Clinical Research Center, Hôpital St-Luc (University of Montreal), 1058 St. Denis St., Montreal, Qué. H2X 3J4, CANADA.

Thiamine deficiency in man and experimental animals results in nystagmus, ataxia and memory impairment and in selective damage to certain structures of the CNS. The thiamine pyrophosphate (TPP)-dependent enzyme, transketolase (EC 2.2.1.1.) was measured in groups of presymptomatic, symptomatic and thiamine-treated (reversed) animals in 9 discrete brain regions including structures known ultimately to show signs of neuropathological damage. Results obtained from rats made thiamine deficient by chronic dietary deprivation were compared to those obtained following treatment with pyrithiamine.

Chronic thiamine deprivation in the rat resulted in 6-8 weeks in ataxia, loss of righting reflex and in lesions of the lateral vestibular nucleus of the pons. Transketolase (TK) activities in whole blood hemolysates from symptomatic thiamine-deprived rats were reduced to below 10% of normal values and the relative degree of saturation of TK in blood by the cofactor TPP (the so-called "TPP Effect") was significantly elevated. TK was selectively reduced by 25% in lateral vestibular nucleus prior to the onset of neurological symptoms. Further deprivation led to generalized reductions of TK in all brain structures. Measurement of TK in the presence of added TPP did not lead to complete reversal of TK activities suggesting loss of apoenzyme. Administration of thiamine to symptomatic thiamine-deprived rats resulted in reversal of neurological symptoms, reversal of the abnormal "TPP Effect" in blood hemolysates and reversal of TK deficits in non-vulnerable brain structures such as cerebral cortex and striatum. However, neither blood TK nor TK activities in lateral vestibular nucleus were normalised following thiamine treatment.

Daily pyrithiamine administration to rats results, within 3 weeks, in loss of righting reflex, convulsions and widespread neuropathological damage (pons, thalamus, hypothalamus, cerebellum). TK activities were significantly reduced in blood and in all brain structures prior to the onset of neurological symptoms. In contrast to chronic thiamine deprivation, pyrithiamine treatment leads to:

(i) reversible reduction of TK in blood hemolysates,
(ii) irreversible TK deficits in both vulnerable and non-vulnerable brain structures following onset of neurological symptoms.

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380.13 EFFECTS OF AMPHETAMINE ON CEREBRAL METABOLISM FOLLOWING BRAIN DAMAGE AS REVEALED BY QUANTITATIVE CYTOCHROME OXIDASE HISTOCHEMISTRY. R.L. Sutton, M.J. Chen*, D.A. Hovda and D.M. Feeney, Departments of Psychology and Physiology, UNM, Albuquerque, NM 87131 and Mental Retardation Res. Center, UCLA, Los Angeles, CA 90024.

We have reported that the pervasive depression of glycolytic metabolism which occurs following unilateral brain injury can be ameliorated by amphetamine (AMP) treatment (1). Since cytochrome oxidase (CYO) is the terminal enzyme in the metabolic pathway and is more efficient for energy production than glycolytic enzymes, this enzyme marker provides an efficacious index of cerebral oxidative metabolism (COM). To determine the effects of brain damage and/or drug treatment on COM, normal rats (groups N-AMP and N-SAL) and rats with right sensorimotor cortex ablation (groups A-AMP and A-SAL) were given an injection of AMP (2 mg/kg) or saline (24 hr postinjury). Twenty-four hr after drug or saline treatment animals were sacrificed and 40 micron coronal sections of brain tissue were stained for CYO activity using the method of Wong-Riley (2). Spot optical density readings were taken in 10 structures: Nucleus Accumbens (ACB), Caudate-Putamen (CPU), Globus Pallidus (GP), Subthalamic Nucleus (CL), Substantia Nigra (SN), Red Nucleus (NR), Superior Peduncle (BC), Locus Coeruleus (LC), Dorsal Tegmental Nucleus (NTD), and the Cerebellum (Cb). Comparison of data from the N-SAL and A-SAL groups revealed that injury produced a marked bilateral reduction of COM in all structures except for the NTD and the right SN. The level of CYO activity after brain injury was improved by AMP as the A-AMP animals showed higher COM bilaterally in the GP, CL, NR, LC, and Cb as well as in the contralateral ACB and ipsilateral CPU compared to A-SAL rats. Finally, COM was reduced bilaterally in N-AMP rats compared to N-SAL rats in all structures but the CL and, the level of CYO activity was lower (bilaterally) in the SN, NR, LC, NTD and Cb of N-AMP animals than it was for A-AMP rats. This depression of CYO activity in N-AMP rats may reflect an immediate postintoxication effect of AMP on COM. These overall results support the hypothesis that a generalized, remote functional depression of COM occurs following brain injury and that AMP can, at least in part, return COM toward normal levels.

1. Feeney, et al., Physiol. Psych., 13, 197-203, 1985.
2. Wong-Riley, M. Brain Res., 171, 11-28, 1979.

380.14 DEHYDRATION-INDUCED INCREASES IN PLASMA-DERIVED U-^{14}C-PALMITATE INCORPORATION INTO BRAIN OF AWAKE LONG-EVANS OR BRATTLEBORO RATS. J.M. Gnaedinger and S.I. Rapoport, (Spon: A. Noronha), Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Water-deprivation of Long-Evans rats (LE) induces changes in brain cell structure, blood flow and local glucose utilization which are similar to changes in Brattleboro rats (DI) with access to water. Brattleboro rats are unable to produce a functional vasopressin, and are therefore slightly dehydrated even with free access to water. The most pronounced changes in glucose utilization are seen in the regions outside the blood-brain barrier (e.g. posterior pituitary, subfornical organ), having neural connections with the hypothalamic regions of brain. However, the supraoptic and paraventricular hypothalamic nuclei also are affected. We previously measured incorporation of plasma palmitate into non-barrier regions of water-provided and water-deprived Long-Evans (LE) and homozygous Brattleboro (DI) rats (Gnaedinger and Rapoport, Soc. Neurosci. Abst. 11:378, 1985). We now report rates of incorporation of plasma palmitate, Jpalm, in brain regions within the blood-brain barrier.

Male, 3-4 month-old, LE and DI rats were either provided with water ad-libidum or deprived of water. U-^{14}C-palmitate was injected iv as a bolus, and animals were killed 4 h later. After freezing at -50°C, brains were cut into 20 micron sections and placed against Kodak SB-5 film for 5 weeks. Films were densitometrically scanned and optical densities were converted to Jpalm, using integrated plasma specific activity of palmitate. Bonferroni t-statistics for multiple comparisons were used to analyze data.

Regional increases in Jpalm were seen throughout the brain in water-deprived LE animals and in water-provided and water-deprived DI rats as compared to hydrated LE rats. Water-deprived LE rats showed an average 72% increase in Jpalm, whereas water-provided and water-deprived DI rats had increases of 63% and 80% of hydrated LE-control, respectively. The severity of dehydration, as assessed by plasma osmolality, roughly correlated with the increase in Jpalm in both LE and DI strains. As vasopressin was present in the LE rats, the results indicate that plasma hypertonicity is the independent factor which increases Jpalm generally throughout the brain of LE and DI rats. The effect may be mediated by an increased brain pool of palmitate, caused by the two-fold increase in plasma palmitate in water-deprived LE and DI rats, and water-provided DI rats.

380.15 THE BEHAVIORAL AND HEMATOLOGICAL EFFECTS OF NIMODIPINE AND VERAPAMIL ON SODIUM NITRITE-INDUCED METHEMOGLOBINEMIA. J.M. Fahey*, R.L. Isaacson and L. Glazer*. Department of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, N.Y. 13901.

In rodents, the administration of sodium nitrite ($NaNO_2$) has been found to decrease motor activity (Behroozi et al., Israel J. Med. Sci. 8:1007,1972), to increase aggression (Greuner, Pharmacol. Biochem. Behav. 2:267-269,1974), and to produce deficits in the acquisition of an inhibitory avoidance response (Martinez et al., Psychopharmacol.60:221-228,1979). This is thought to be due to the formation of $NaNO_2$ - induced methemoglobinemia which impairs the oxygen-carrying capacity of erythrocytes. The behavioral and hematological effects of two calcium slow channel antagonists on $NaNO_2$-induced methemoglobinemia were examined in the present study.

Eighty male Long-Evans Hooded rats, 45-55 days of age, were non-systematically separated into four groups. Equimolar intraperitoneal (i.p.) injections of either nimodipine (5 mg/kg) or verapamil (5.45 mg/kg) were administered 2 or 24 hr before i.p. injections of $NaNO_2$ (75 mg/kg). A fifth group received only $NaNO_2$. The animals in these five groups were randomly selected to be tested either behaviorally or biochemically.

Motor abilities were measured by performance on a rotorod. Untreated animals were trained for three days on the apparatus until they reached criterion performance (30 sec on the rod rotating at 14 rpm). On the next day the animals were tested after the administration of drugs and 10 min of swimming in 21°C water. Pilot studies indicated the greatest performance deficits induced by $NaNO_2$ occur after oxygen-debt exertions. Blood was obtained from other, non-behaviorally tested etherized animals by cardiac puncture following drug administration. The levels of methemoglobin (MHb) were determined as a percentage of total Hb using the technique of Evelyn and Malloy (J. Biol. Chem. 126:655-662,1938).

After swimming, both nimodipine and verapamil-treated animals were impaired in rotorod performance relative to the non-drug group in both the 2 and 24 hr pretreatment groups. The amount of MHb in the blood did not parallel the behavioral effects. Nimodipine and verapamil reduced the MHb levels at both time periods relative to the animals that only received $NaNO_2$.

This suggests that the slow calcium channel blockers reduce the formation of MHb. However, our results do not indicate a strong association between MHb levels and the impairment of motor activity. The impairment in rotorod performance following the administration of the calcium antagonists coupled with the exercise of swimming remain to be explained. Since previous unpublished studies have shown that swimming did not alter MHb blood levels in $NaNO_2$-treated animals, future experiments should examine whether it is the exercise or stress components of the swimming procedure that produce the calcium channel blockers-induced impairment.

380.16 REGIONAL CEREBRAL AND NEURAL LOBE BLOOD FLOW DURING INSULIN-INDUCED HYPOGLYCEMIA IN UNANESTHETIZED RATS. B.R. Hollinger*, R.B. Page, R.M. Bryan, Jr., (SPON: R.A.W. Lehman), Dept. of Surgery (Neurosurgery), Anatomy, and Physiology. The M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033.

The effects of hypoglycemia on regional cerebral (rCBF) and neural lobe (NLBF) - blood flow were studied in unanesthetized rats. Rats were anesthetized with halothane (1.5%) and nitrous oxide (70%) in a balance of oxygen and surgically prepared with catheters in the left femoral artery and vein. Following surgery, the rats were restrained with a plaster hip cast and allowed 4 hours to recover from anesthesia. Body temperature was monitored rectally and maintained with a heat lamp. The rats were divided into 3 groups consisting of 1) a normoglycemic group which received only saline, 2) hypoglycemic group A, which was given 15 units of insulin I.V./kg of body weight 30 minutes prior to flow measurement, and 3) hypoglycemic group B which was given 15 units of Insulin I.V./kg body weight 90 minutes before flow was measured and 5 additional units/kg body weight 30 minutes prior to flow measurement. Regional cerebral blood flow (rCBF) was measured using ^{14}C-iodoantipyrine. Mean plasma glucose was 8.76 umol/ml in the control group, 2.63 umol/ml in the hypoglycemic group A, and 1.51 umol/ml in the hypoglycemic group B. Mean $PaCO_2$ was 38, 43 and 44 mmHg for the normoglycemic control group, hypoglycemic groups A and B respectively. Plasma epinephrine increased 3-fold and norepinephrine 2-fold over control in both of the hypoglycemic groups. rCBF was corrected for changes in flow due to an increase in $PaCO_2$ in the hypoglycemic groups. rCBF significantly increased in the olfactory bulb, caudate-putamen, and thalamus in the hypoglycemic group A. In the hypoglycemic group B, rCBF increased significantly in all brain regions measured with the exception of the neural lobe which decreased. The increase in rCBF ranged from 38% in the hypothalamus to 139% in the thalamus. Neural lobe flow significantly decreased by 31%. A plot of rCBF as a function of plasma glucose indicated that rCBF was not changed from the normoglycemic rates until glucose dropped below approximately 2 umol/ml. Below 2 umol/ml rCBF changed dramatically. It is interesting to note that the neural lobe is the only region not protected by a blood brain barrier and thus may be sensitive to changes in vasoactive agents in blood such as epinephrine and norepinephrine. (AHA Grant-in-Aid with funds contributed from the Palm Beach County Chapter, FL and PHS Grants NS19341 and NS15926).

380.17 **CEREBRAL METABOLITES IN EDEMATOUS WHITE MATTER OF THERMAL VERSUS MECHANICAL BRAIN INJURY.** P.A. Tornheim, K.R. Wagner and K. Meininger.* Depts. of Anat. & Cell Biol. and Neurol., Univ. of Cincinnati Coll. Med., V.A. Med. Center, Cincinnati, OH 45267.

A cerebral cold injury, used frequently as a model for brain trauma, causes a spread of protein-rich (vasogenic) edema into the white matter. With this insult, high-energy phosphate concentrations are not depleted in the edema territory (Sutton et al., J.Neurosurg. 53:470). In the present study, we compared metabolite concentrations in edematous white matter near a mechanically-induced cerebral contusion to those in white matter neighboring a cold lesion.

Physiologically-monitored anesthetized cats received intravenous Evans Blue dye (EB), and were subjected to one of the following: a) a cold lesion of the left suprasylvian gyrus; b) impact to the left side of the skull that caused acceleration and rotation of the head; or c) sham injury. At 2-3 hours after trauma, brain metabolite concentrations were fixed by in situ freezing. Head-injured animals were selected for study based on the presence of left cerebral contusion. Visible staining with EB-tagged serum protein was used to determine the location of vasogenic edema. In cold-injured and head-injured cats, white matter samples were taken both within and peripheral to the vasogenic edema territory on the left side. Samples were measured for ATP, ADP, AMP, phosphocreatine, lactate, glycogen and glucose concentrations by enzymatic-fluorometric techniques; magnitude of edema was measured with an organic density gradient. Metabolite concentrations were corrected for dilution with edema.

In hemispheres with a cold injury, metabolite concentrations were unchanged in white matter without edema. In the vasogenic edema territory of these animals, lactic acid was elevated but there was no measurable change in high-energy phosphate concentrations. Cats with a contusion of mechanical injury had a mild increase in lactic acid without changes in high-energy phosphates in nonedematous white matter peripheral to cerebral contusion; by contrast, white matter with the vasogenic edema of contusion had a marked increase in lactic acid and significant decreases in ATP and phosphocreatine.

These findings suggest that white matter containing the vasogenic edema of mechanical trauma suffers more severe metabolic perturbation than that peripheral to a thermal insult. We speculate that pathophysiologic events occurring within minutes after mechanical injury can cause acute metabolic perturbations (Wagner et al., J. Neurosurg. 63:88-96) that are exaggerated by the spread of vasogenic edema from contusion.

This study was supported by NIH Grant NS 17975.

BRAIN METABOLISM IV

381.1 **FURTHER EVIDENCE OF NEGLIGIBLE INFLUENCE OF GLUCOSE-6-PHOSPHATASE ACTIVITY ON DEOXYGLUCOSE METHOD.** K.Mori*, G.Lucignani*, K.Schmidt*, T.Jay, E.Palombo*, T.Nelson*, C.Kennedy and L.Sokoloff. Lab. Cereb. Metabolism, NIMH, Bethesda, MD 20892

The deoxyglucose (DG) method for measurement of local cerebral glucose utilization assumes that products of DG phosphorylation are essentially trapped in the tissues, not forever, but for the duration of the experimental period (Sokoloff et al, J.Neurochem. 28:897, 1977). Its application indicated no detectable loss of product at 45 min., a small loss at 60 min., and progressively greater losses at 90 and 120 min. following an I.V. pulse of [^{14}C]DG (ibid.; Sokoloff, Adv.Neurochem. 4:1, 1982). The loss, when it occurs, is presumably due to G6Pase. Fishman and Karnovsky (J.Neurochem., 46:371, 1986) have explained the lag in appearance of G6Pase activity by finding that hydrolysis of DG6P by G6Pase in brain is rate-limited by the diffusion of DG6P from the cytosol, where it is formed, across the endoplasmic reticular membrane to the site where G6Pase resides. The original DG model was revised to include this new information, and an equation was derived to define total tissue ^{14}C concentration, $C_i^*(T)$, in terms of time, T, history of arterial plasma DG concentration, $C_p^*(t)$, and 5 rate constants including one for diffusion of DG6P across the endoplasmic reticular membrane and another for its hydrolysis by G6Pase. Programmed I.V.infusions of [^{14}C]DG were administered to 45 conscious rats to achieve and maintain constant arterial plasma concentrations; 5 animals were killed at each of 9 time points between 2.5 and 120 min. Time courses of arterial [^{14}C]DG concentrations were measured by radioassay, and local ^{14}C concentrations in 23 cerebral structures at the kill time were determined by quantitative autoradiography. The equation was fit to the measured data by a non-linear least-squares routine to obtain the best-fitting rate constants. The results demonstrate that the rate constant for diffusion of DG6P across the endoplasmic reticular membrane is indeed rate-limiting; it equals only 0.001 per min., less than 1/50 the rate constant for the phosphorylation of DG by hexokinase. The data were also analyzed by the graphical evaluation technique of Patlak et al.(J.Cereb. Blood Flow Metab., 3:1, 1983) in which $C_i^*(T)/C_p^*(T)$, the apparent distribution space, is plotted against $\int_0^T C_p^*(t)dt / C_p^*(T)$. This plot predicts that following an equilibration period there is a linear relationship as long as there is no loss of product. The results demonstrate that after the initial 15 min. of equilibration there is such linearity up to 60 min. and then a small and progressively increasing deviation from linearity at 90-120 min. These results confirm the negligible influence of G6Pase activity on the DG method during the prescribed experimental period of 45 minutes.

381.2 **CONTAMINANTS IN INADEQUATELY PURIFIED GLUCOSE AND INCOMPLETE RECOVERY OF METABOLITES ARE RESPONSIBLE FOR THE ERRONEOUS CONCLUSION OF HIGH GLUCOSE-6-PHOSPHATASE ACTIVITY IN RAT BRAIN.** G.Dienel, T.Nelson*, N.Cruz*, T.Jay, L.Sokoloff, Lab of Cerebral Metabolism, NIMH, Bethesda, MD 20892

Huang and Veech (J.Biol.Chem.257,11358,1982; Trans.Am.Soc. Neurochem.15,189,1984) claimed to have demonstrated a high rate of glucose-6-phosphatase (G-6-Pase) activity in rat brain on the basis of measurement of selective detritiation of [2-^{3}H, U-^{14}C]glucose. This method is critically dependent on the isolation of pure glucose from the tissue. They provided no evidence for such purity nor for complete recovery of glucose metabolites, both of which are required for valid calculation of the glucose metabolic rate. Their results could not be confirmed by Nelson et al (Science, 229,60,1985) and by Dienel et al (Soc.Neurosci. Abstr.11:2,1123,1985) who used more extensive glucose purification procedures. In this study, the "glucose" fraction was isolated from extracts of freeze-blown brains by the procedures of Huang and Veech. In brief, the brain extracts were applied to tandem Dowex-1-formate/Dowex-1-borate columns which were washed with water, and the glucose fraction was eluted from the borate column with 10 ml of 4 N formic acid. The eluate was dried at 75°C, and the boric acid was removed by addition of methanol and drying. At least 7 labeled contaminants could be resolved by 2-dimensional thin layer chromatography of the borate column eluate. Neutral amino acids were present in the borate column eluate; 84-93% of radiolabeled glutamine, alanine, and leucine, and 62% of glucosamine present in or added to the neutralized extract could be recovered in this glucose fraction. Furthermore, detritiated but ^{14}C-labeled acidic compounds were also found in the borate column eluate. These contaminants co-purified with glucose through a Dowex 50 column step and were co-eluted with gluconic acid or G-6-P from a Dowex-1-formate column after the glucose was derivatized by glucose oxidase or hexokinase to separate it from other neutral contaminants. Thus, both of Huang and Veech's "glucose purification procedures" (1982,1984) isolated impure glucose. They also provided an environment for detritiation of the labeled glucose and for side reactions that converted glucose and contaminants to other ^{14}C-labeled compounds. [^{14}C]Contaminants in the "glucose" fraction artifactually lower the recovery of ^{14}C in the glucose metabolite fraction and artifactually increase the apparent [^{14}C]glucose specific activity, thereby depressing the computed rate of phosphorylation of [^{14}C]glucose relative to that of [^{3}H]glucose. In properly purified glucose fractions, there was no detectable selective detritiation of [2-^{3}H,U-^{14}C]glucose and no evidence for high G-6-Pase activity in brain.

381.3 STRATEGIES FOR THREE-DIMENSIONAL RENDERING OF REGIONAL BRAIN ACTIVITY FROM SERIAL ^{14}C 2-DEOXYGLUCOSE AUTORADIOGRAPHS. W.K. Smith, D.S. Schlusselberg and D.J. Woodward. *Dept. of Cell Biology and Anatomy, Univ. of Texas Hlth. Sci. Ctr. at Dallas, Texas 75235.*

Ongoing studies in this laboratory have explored the general problem of three-dimensional reconstruction of neuroanatomical objects from serial sections using computer graphics techniques. The initial development of the **CARP** system (**C**omputer **A**ided **R**econstruction **P**ackage) was fueled by an intense interest in cell topography and neuronal connectivity in brain stem nuclei. It became apparent that the hierarchical database structure and computer graphics and image processing techniques utilized to study brain stem nuclei could also be applied to other areas of neuroanatomical research. We report here the implementation of a general set of strategies for the three-dimensional display of regional brain activity from serial ^{14}C 2-deoxyglucose (2-DG) autoradiographs.

In experiments on rats, tracer amounts of 2-DG are injected intraperitoneally, the animal subjected to a drug injection or behavioral procedure, and sacrificed. The brains are quickly removed and frozen, and 20μ thick coronal sections are made using a cryostat. The sections are placed on cover slips and rapidly dried before being covered with X-ray film to make a contact print of activity. Each piece of X-ray film is also exposed to a set of radioactive standards with known activities and tissue equivalents.

The film is placed on a light table below a video camera. A mechanical iris vignettes each image to provide a stable video black level. Each autoradiographic slice is video digitized into an Ikonas (Adage 3000) frame buffer. Microcode routines have been written to perform video averaging, lighting correction and geometric interpolation on the digitized images. Sequential slices are aligned by storing the previous section in the green image plane, video digitizing the new section into the red and superimposing the two planes in real-time. The most recently calibrated standards are recorded as attributes of each digitized slice along with the pixel intensity data.

Regional brain activity in each section can be quantitated by tracing around the region of interest and initiating a fill operation which computes area, mean activity and other statistics. Alternatively, graphical templates from an atlas or histological preparation can be mapped onto the autoradiographic slice and used to direct the analysis.

The filled regions from adjacent sections corresponding to a three-dimensional object can be displayed according to various strategies. Activity topography maps from arbitrary viewpoints in space can be constructed from the region fill data. Solid surface models can be color coded to represent the different levels of functional activity. New volume imaging techniques can be used to create color-coded clouds of regional activity. A volume representation of the autoradiographic data allows easy selection of arbitrary clipping planes for editing and display. Pictures created using these modalities can also contain models rendered by more conventional three-dimensional display techniques. These methods allow a more detailed correlational analysis of glucose uptake and local anatomical organization.

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381.4 CROSSCORRELATION ALIGNMENT OF ASYMMETRIC RAT BRAIN CORONAL AUTORADIOGRAPH IMAGES. L.S. Hibbard, Departments of Radiology and Anesthesia, The. M.S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania 17033

Three-dimensional (3D) reconstruction of the brain from quantitative autoradiography provides a mechanism for the direct comparison of different metabolic states over the entire brain. Accurate reconstructions depend on serial image registration, and it was demonstrated earlier that coronal section alignment can be effected by the principal axes transformation (Hibbard and Hawkins, Am. J. Physiol. 247:E412-E429, 1984). This method, the superposition of image centroids and principal axes, works well in the forebrain, but can break down in the hindbrain. There, the tissue sections are normally less symmetric than in the forebrain, and sometimes irregular due to the separation of, or loss of structures not mechanically attached to each other within a given section. In addition, the first and second principal moments tend to be closer in value in the hindbrain than in the forebrain, with a correspondingly less reliable determination of the image alignment. Finally, to complicate automated alignment methods, image misalignment has rotational and translational components which are mutually-dependent and which must be refined iteratively. Drawing on experience with an unrelated 3D reconstruction project, (Hibbard, et al., Comput. Biol. Med., in press, 1986), these problems have been overcome using crosscorrelation techniques. The crosscorrelation of two images is a function in which the location of the maximum defines the relative translation needed to superimpose one image onto the other. Translational misalignment is adjusted by determining the crosscorrelation of high pass-filtered coronal images. Rotational misalignment is determined from the crosscorrelation of polar-resampled, gradient-operated coronal images. Refinement of the principal axes alignment then, consists of alternate cycles of rotational and translational adjustments, the progress of which is reflected in the value of the crosscorrelation maximum which tends monotonically upward toward an asymptotic limit. Misalignment errors are modelled by calculations of the crosscorrelation on aligned images from various brain regions purposely misaligned by defined amounts. Crosscorrelation produces accurate and objective alignments for those irregular or asymmetric sections which could only be obtained previously by manual means. (Work supported by NSF grant BNS-8506479.)

381.5 THREE DIMENSIONAL RECONSTRUCTION AND IMAGE ANALYSIS OF BRAIN USING OCTREE REPRESENTATION. S.B. Berger, L.W. Tucker* and D.J. Reis. Laboratory of Neurobiology, Cornell Univ. Med. College, New York, N.Y. 10021.

Computer-based three-dimensional (3D) reconstruction of serial sections is a powerful tool in neuroanatomy capable of revealing functional and anatomical relationships not readily apparent in 2D views. Computer-graphics display operations include resectioning in alternate planes to reveal interior structural relationships, shading to reveal 3D shape and coloring for the display of functional maps. From a quantitative perspective, the reconstructed brain models prove to be useful for correlation of spatial maps, which are derived from different imaging and histochemical modalities (e.g., immunocytochemistry, autoradiography, PET, CT, MRI), and are independent of traditional, discrete, anatomical regions.

A major problem of this technique is the substantial computational and storage demand for reconstruction, manipulation, display and analysis, due to the extremely large number of data elements (2 million or more) which compose a brain model of even modest complexity. To decrease the computational effort, we have chosen to employ octree-encoding which involves hierarchical subdivision of the volume into cubes of varying sizes, such that large homogeneous regions (e.g. background) can be stored as a small number of large blocks. In addition to decreasing storage requirements, the hierarchical structure permits the use of highly efficient algorithms, many of which are linear in complexity, and provides a uniform representation of neuroanatomical features. It is therefore possible to simultaneously display and quantitatively compare spatial distributions from, for instance, autoradiography and neuronal mapping studies.

We have designed and implemented algorithms for serial-section reconstruction, display and analysis of octree-encoded brain models. Analysis of the algorithms, using the rat brain as an example, confirms that the octree structure is both compact and computationally efficient. The increase in efficiency translates directly into increased system performance and decreased expense, which provides a tool for quantitative analysis of morphology and function to the individual investigator.

381.6 EVALUATION OF COMPLEX PATTERNS OF BRAIN METABOLISM ASSOCIATED WITH ANESTHESIA. D.W.Davis, L.S.Hibbard, and R.A.Hawkins Departments of Anesthesia, Physiology and Radiology, M.S.Hershey Med.Ctr., Penn State Univ., Hershey, PA 17033

Autoradiographic techniques are widely used to provide detailed regional information about brain energy metabolism, blood flow, blood-brain barrier transport, protein synthesis and receptor distribution. Three-dimensional (3D) reconstructions of serial, aligned autoradiographs typically display a complex pattern of changes over the entire brain, regardless of the nature of the experiment. Many intriging questions may yield answers if a method for extracting meaningful data from these complex, 3D patterns were available. For example, would comparisons across treatment groups for a variety of anesthetic agents lead to observations about the general mechanisms of anesthesia? We are applying several statistical methods to the analysis of groups of 3D metabolic maps in a search for correlated, or variable, metabolic behavior among brain regions. Data were used from experiments in which rats were anesthetized with various intravenous anesthetics and brain energy metabolism was measured by [6-14C]glucose. Brains were prepared for autoradiography, scanned and converted to 3D metabolic maps as previously described (Hibbard and Hawkins, Am. J. Physiol. 247:E412,1984). Pairwise comparisons of experimental and control results by subtraction of 3D maps produce serial sets of difference images which seem to best display gross changes which occur over large portions of the brain. Comparisons among more than two 3D maps involve the calculation of maps of the variance and principal component images. High-valued features in the variance images correspond to regions exhibiting the greatest variation in response to the experimental stimulus. The areas with low variance correspond to regions with similar response (or a correlated lack of response) to the treatment; such 3D maps show entire structures behaving in a coordinated fashion. Likewise, principal component analysis, based on the inter-experiment covariances, extracts those regions of brain which have the least-correlated responses in the experiment. The analysis of variance maps and the principal component maps can be examined for correlations with neuroanatomical structure by comparison with a computer stereotaxic atlas developed in this laboratory (Hibbard, et al., Soc. Neurosci Abst.11:1090,1985).This work was supported in part by NIH grant NS16737 and NSF grant BNS-8506479.

381.7 TOPOGRAPHY OF BASAL GLUCOSE UTILIZATION IN THE HIPPOCAMPUS DETERMINED WITH (1-^{14}C)-GLUCOSE AND HIGH RESOLUTION AUTORADIOGRAPHY. W.E. Stumpf, G.E. Duncan* and G.R. Breese. Dept. of Anatomy and Biological Sciences Research Center, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

The distinct stratified histology and connectivity of the hippocampus invite analysis of functional activity within this region using autoradiographic determination of local glucose utilization. Previous work from our laboratory demonstrated dramatically different patterns in the uptake and retention of radioactivity within the hippocampus, 30 min after injection of (1-^{14}C)-glucose or (^{14}C)-2-DG (Duncan et al. Neuroscience 17:99-106, 1986). Much higher levels of radioactive metabolites accumulated from (1-^{14}C)-glucose, compared to (^{14}C)-2-DG, in the granular and molecular layers of the dentate gyrus and in the CA-1 pyramidal cell field. Accumulation of high levels of ^{14}C from (1-^{14}C)-glucose, relative to (^{14}C)-2-DG, could result from exceptionally low rates of loss of CO_2 from these regions or a high rate of intracellular transport of radioactive metabolites into the dentate granular cells and CA-1 pyramidal cells, from regions distal to these cell bodies. If such factors are responsible for the differences in the hippocampal labeling patterns 30 min after injection of (1-^{14}C)-glucose or (^{14}C)-2-DG, the relative amount of radioactivity in the various hippocampal regions should be different at shorter intervals after injection of (1-^{14}C)-glucose. Therefore, in the present study, a detailed anatomical evaluation was made, with high resolution autoradiography, of the uptake and retention of radioactivity from (1-^{14}C)-glucose in the dorsal hippocampus, 10 and 30 min after i.v. injection of this compound. Virtually identical topographic patterns of radioactivity within the hippocampus were observed in rats after the 10 min or 30 min survival periods, but more radioactivity was present in all regions at 30 min compared to 10 min. For both survival times, the highest accumulation of ^{14}C occurred in the granule cell layer of the dentate gyrus and in the pyramidal cell layer. Relatively high retention of radioactivity was also found in the molecular layer of the dentate gyrus and in the stratum lacunosum-moleculare. The stratum radiatum contained the lowest levels of radioactivity among grey matter regions of the hippocampus. The present findings indicate that an exceptionally low rate of loss of $^{14}CO_2$, or intracellular transport of ^{14}C-labeled metabolites into cell bodies, do not account for the relatively high levels of radioactivity found in the CA-1 pyramidal cell layer and in the dentate gyrus, 30 min after administration of (1-^{14}C)-glucose, since the relative distribution of radioactivity was nearly identical for the 10 min and 30 min survival periods. Supported by PHS grants NS09914, MH-33127, MH-36294 and HD-03110.

381.8 THE EFFECTS OF RAPHE NUCLEI STIMULATION ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN CONSCIOUS RATS. A. Cudennec*, D. Duverger*, E.T. MacKenzie*, B. Scatton and A. Serrano* (SPON: E. Hamel). Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo, 31, avenue P.V. Couturier, 92220-Bagneux, France.

The influence of the serotonergic system on integrated functional activity in the brain, as studied by the quantitative 2-deoxyglucose (2-DG) technique, is relatively controversial. For instance, the serotonergic agonist, quipazine, has been reported to effect widespread increases or decreases in local cerebral glucose utilization (LCGU). To obviate the problems related to the specificity of pharmacological agents we have examined the effects of direct electrical stimulation of two rostral (dorsal and median) raphé nuclei on LCGU.

One week prior to the definitive experiments, rats were anesthetized with chloral hydrate, placed in a stereotaxic frame with non-perforating ear bars and electrodes were introduced into either the dorsal or the median raphé nucleus. At the time of the 2-DG study, femoral catheters were inserted and a loosely fitting abdominal cast was applied. Two hours after recovery from anesthesia, the dorsal(n=7) or median (n=5) raphé nucleus was stimulated (~200µA, 19 Hz, 2 msec) for 45 min. The [14-C]-2-DG was administered 5 min after beginning the stimulation. In parallel, sham, non-stimulated rats were studied (n=7).

Stimulation of the dorsal raphé nucleus induced a number of behavioural changes; vibrissal movements, nystagmus, stereotypic movements (especially of the jaws). A moderate rise in mean arterial pressure (approximately 20 mm Hg) was seen in stimulated rats in which an arterial hypocapnia was also noted. Overall, 56 brain regions out of 119 examined showed a significant increase in LCGU following dorsal raphé stimulation; 27 structures were increased following median raphé stimulation. No significant decrease was ever noted. In some structures, LCGU doubled with dorsal raphé stimulation (the effects of median stimulation were consistently less marked). Striking increases in LCGU were observed in discrete thalamic nuclei (9 out of 18) and in very limited cortical regions which correspond to the frontoparietal somatosensory and frontal motor cortices. The increase in focal cortical LCGU was both laminar (layers II, IV and VI) and columnar in nature. Almost all the relays of the extrapyramidal system were activated by raphé stimulation (eg. globus pallidus, red nucleus, substantia nigra) with the signal exception of the caudate nucleus.

These findings reinforce the view that excitation of ascending serotonergic pathways causes a neuroanatomically defined activation (not inhibition) of brain functional activity, as reflected by glucose use. Some thalamocortical and extrapyramidal systems are markedly activated by electrical stimulation of the rostral raphé nuclei.

381.9 THE EFFECTS OF AGING ON CEREBRAL GLUCOSE METABOLISM IN F_1 HYBRID PIGMENTED RATS. Richard W. Johnson*, Diana L. Dow-Edwards, Laurel A. Freed*, and Arnold S. Lippa*. (SPON: K. Toizumi) Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Health Sciences Center at Brooklyn and Natrix Research Laboratories Inc., City College of New York.

The normal aging process is associated with a generalized decrease in cerebral functional activity and in the development of cognitive impairment. It has been demonstrated that there are decreases in cerebral glucose metabolism in components of the sensory, motor, and limbic systems. In addition, brain structures that are implicated in learning and memory, have been found to be affected. Although several studies of regional glucose metabolism have been carried out in the rat, there is no general consensus as to whether aging decreases all sites equally and whether pigmented rats show similar trends. Therefore we have employed the quantitative 2-deoxyglucose method of Sokoloff to young and aged pigmented rats.

5 seven month and 6 twenty five month F_1 hybrid pigmented rats, purchased from Jackson Laboratory, Bar Harbour, Maine, were housed under standard laboratory conditions for 4 weeks. Following an overnight fast, animals were anesthetized with a mixture of halothane-N_2O-O_2 for the placement of venous and arterial catheters. Loosely fitting abdominopelvic plaster casts were applied and the animals were allowed to awaken. After 2 hours, 125uCi/kg of (^{14}C)-2-deoxyglucose was administered through the venous catheter. Timed arterial blood samples were taken over the next 45 minutes. The animals were killed with a lethal injection of sodium pentobarbital. The brains were removed and processed for autoradiography. Local cerebral glucose utilization was calculated using tissue concetrations of (^{14}C) and the plasma curve for glucose and deoxyglucose. The results will be compared to previous studies of glucose utilization in aged rats.

381.10 CHARACTERIZATION OF REGIONAL GLUCOSE UTILIZATION AFTER EXPERIMENTAL CONCUSSION IN THE RAT. M.L. Giebel,* B.G. Lyeth, C.E. Dixon, H.H. Stonnington,* H.F. Young,* and R.L. Hayes. Richard Roland Reynolds Neurosurgical Research Laboratories, Division of Neurosurgery and Department of Rehabilitation Medicine, Medical College of Virginia, Richmond, VA 23298-0001.

This research used the method of Sokoloff (J. Neurochem. 28:897, 1977) to examine changes in the uptake of (^{14}C) deoxyglucose (2DG) and rates of local glucose utilization in brain regions after concussive brain injury in the rat.

Prior to experimentation, Sprague-Dawley rats were habituated to Plexiglas restrainer tubes in one-hour sessions over six days. On the day of the experiment, femoral arteries and vein were cannulated under ketamine and methoxyflurane anesthesia. All incisions were liberally infused with 1% lidocaine. Animals were placed in restrainer tubes and allowed to recover from anesthesia for three hours in a sound and light attenuation chamber. Rats were anesthetized with methoxyflurane five minutes prior to a 1.5 atm concussive injury (n=4) or sham injury (n=4). HRP was injected (100 mg/kg, in saline, i.v.) at three minutes post-injury to assess blood-brain barrier integrity. Five minutes post-injury or sham, DG was injected (15 µCi/kg, i.v.). Timed plasma samples were taken to calculate LGU. Forty-five minutes following DG injection, animals were killed by an overdose of sodium pentobarbital (2.0 ml, i.v.) and perfused. Brains were removed, frozen, and sectioned for DG autoradiographic analysis by standard techniques. Alternate serial sections were collected for HRP visualization and hemotoxylin and eosin staining.

Preliminary data indicated increased glucose utilization after trauma in the basal forebrain (11%), hippocampus (40%), amygdala (20%), interpeduncular nucleus (39%), ventral tegmental nuclei (VTN) (29%), and the area surrounding the VTN (22%). All other structures sampled indicated decreases in glucose utilization. These data are consistent with previous studies in the cat (Science 233:301-303, 1984) suggesting that some of the acute responses to concussive injury may be related to increased functional activity of certain brain regions.

This research was supported by NIH Grants #NS 21458 and #NS 12587 and the Richard Roland Reynolds Neurosurgical Research Laboratories.

381.11 REGIONAL CEREBRAL GLUCOSE UTILIZATION DURING MILD HYPOXIA IN UNANESTHETIZED FREELY-MOVING RATS. C.MacNeill*, KA.Keefer and R.M.Bryan, Depts. of Surgery (Neurosurgery) and Physiology, M.S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

Previous studies have indicated that cerebral glucose utilization increases during severe hypoxic hypoxia (PaO_2 25 Torr). We have tested the hypothesis that mild hypoxia (PaO_2=42 Torr), like severe hypoxia, stimulates glucose metabolism in brain.

Rats were prepared with chronic arterial and venous catheters and placed in an individual chamber for 4 days to recover from surgery. The catheters were accessible to the investigators by passing them through the top of the chamber. Hypoxia was induced by filling the chambers with 11% O_2 and 89% N_2. Rats subjected to 2 min and 17 min of hypoxia were compared to normoxic control rats. rCMRgl was measured using (6-C^{14}) glucose. Physiological parameters for the three groups were as follows:

	Control	2 min Hypoxia	17 min Hypoxia
PaO_2 (Torr)	88	42	39
$PaCO_2$ (Torr)	38	31	28
pHa	7.40	7.51	7.51
MABP (Torr)	102	94	84
Brain lactate (umol/g)	1.23	3.01	2.58

The brain to plasma glucose ratio (0.22), necessary for calculating rCMRgl, was unchanged in the hypoxic groups. After 2 min of hypoxia, rCMRgl increased above controls in all 17 brain regions studied, of which 12 regions were statistically significant ($p < .05$). The increase ranged from 5% in the pyramidal tract, superior colliculus, and inferior colliculus to 22% in the caudate-putamen, hypothalamus and thalamus. After 17 min of hypoxia, no brain region was significantly different from the controls. We conclude that mild hypoxia, like severe hypoxia, increases rCMRgl, however, the increase is only transitory with rCMRgl returning to a control rate after 17 min. (AHA Grant-in-Aid with funds contributed from the Palm Beach County Chapter, FL and PHS Grants NS19341).

381.12 CEREBRAL GLUCOSE UTILIZATION DURING CHRONIC HYPERGLYCEMIA. R.B.Duckrow, D.C.Beard* and R.M.Bryan. Departments of Medicine, (Neurology) and Surgery (Neurosurgery), The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

During chronic hyperglycemia or starvation ketone bodies can supplement glucose as a substrate for brain energy metabolism. Ketone body utilization is limited only by their concentration in the blood and their transport across the blood-brain barrier. If one assumes that brain energy consumption remains constant during chronic hyperglycemia, the rate of glucose utilization should drop by an amount equal to the rate of ketone utilization. To test this hypothesis, rats were rendered chronically hyperglycemic by intravenous injection of streptozotocin (60 mg/kg). After 21-24 days, regional cerebral glucose utilization (rCMRgl) was measured in 17 brain regions of awake-restrained rats using [6-C^{14}]glucose. Regional glucose influx, necessary for calculating rCMRgl, was measured in chronically hyperglycemic rats using a constant intravenous injection method. Glucose influx increased during hyperglycemia. However, the transport capacity (Tmax) remained unchanged. Cerebral glucose utilization decreased by an average of 14% as compared to similarly prepared normoglycemic rats. The decrease was significant by Student's test in 13 of 17 brain regions. If one assumed a 30% decrease in glucose transport capacity (Tmax) during hyperglycemia, as measured by Gjedde and Crone (Science 214:456, 1981), the calculated rCMRgl would still be lower by 10%, with all brain regions showing a decrease during hyperglycemia (10 of 17 significant). Using a similar animal model, R.A.Hawkins et al. (Am.J.Physiol. 250:E169, 1986) have shown that ketone bodies can account for the remaining 10% of brain energy requirements after 28 days of hyperglycemia. Of interest, regional cerebral blood flow decreases by 14% during chronic hyperglycemia (c.f. Duckrow et al. Stroke 17:129, 1986). This would suggest that the coupling of brain blood flow and metabolic rate may be governed more by glucose use than oxygen consumption.

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381.13 IN VIVO GLUCOSE METABOLISM OF EXPERIMENTAL RAT GLIOSARCOMA: ^{19}F 2-FDG NMR IN VIVO ZEUGMATOGRAPHIC STUDY IN RAT BRAIN. I.L. Kwee*, T. Nakada, and T. Nagashima*. Neurochem. Res. Lab, VA Med. Ctr., Martinez, CA 94553, Dept. of Neurology, Univ. of Calif., Davis, CA 95616, Brain Tumor Res. Lab., Univ. of Calif., SF, CA 94143.

Using ^{19}F NMR spectroscopy, we have previously shown the in vivo metabolism of 2-fluoro-2-deoxy-D-glucose (2-FDG) in the pentose monophosphate shunt (PMS) and aldose reductase sorbitol (ARS) pathways non-invasively in rat brain [1,2]. In this study, we investigated the in vivo glucose metabolism of experimental brain tumor using rat gliosarcoma cell implantation.

Cultured rat gliosarcoma cells were implanted stereotactically to brains of Sprague-Dawley rats (75-100g) ca. 3mm beneath the surface the cortices [3]. Each rat was studied two to three weeks post-implantation using a Nicolet NMR System NT-200 spectrometer operating at 4.69T. 2-FDG (Behring Diagnostics, Jalla, CA) dissolved in sterile water was injected intravenously (300mg/kg). To minimize metabolic activities during data acquisition, the animal was sacrificed two hours post 2-FDG infusion just prior to placement in the NMR probe which contained a one turn-oval surface coil (1.8x2.0cm) custom designed to center over the rat calvarium. Following shimming on tissue water hydrogen (proton line width ca. 40Hz), the ^{19}F NMR signals were obtained at 188.2 MHz with a spectrowidth of 4,000 Hz. A total of twenty two files were collected at increasing equally incremented valued of the observed pulse duration. The data sets were processed using the standard two dimensional Fourier transformation method. Immediately after the study, the brain was removed and fixed. Coronal section of each brain was studied histologically to correlate with the NMR data.

This study indicates the feasibility to study glucose metabolism in brain tumor in situ using 2-FDG metabolism as a model of glucose metabolism in the PMS and ARS.

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381.14 Phencyclidine Effects on Regional Cerebral Glucose Utilization in the Rat. K. Tanimoto*, C.A. Tamminga, S. Kuo*, P.C. Contreras, T.L. O'Donohue, T.N. Chase (Spon: W. Carpenter). Maryland Psychiatric Research Center, Univ. of Md., Balto., MD 21228, NINCDS, National Institutes of Health, Bethesda, MD 20205

Phencyclidine (PCP) is a psychotomimetic in man. Its potent behavioral effects in animals have been proposed as a model of schizophrenic psychosis in humans. Receptors for PCP exist in mammalian CNS, predominantly in hippocampus and cortex. The actions of PCP may also extend to other transmitter systems, like the sigma opiod receptor, and alter CNS biochemistry in diverse sites. In an attempt to localize the areas in CNS susceptible to modification by PCP, we studied local cerebral glucose utilization with PCP in the rat using ^{14}C-2-deoxy-D-glucose. Male Sprague-Dawley rats were cannulated in the femoral artery and vein under halothane anesthesia, and restrained with a partial body cast. Two hours later, ^{14}C-2-deoxy-D-glucose (2DG) (125 µCi/kg) was administered intravenously over 10 seconds and blood samples were collected over the next 45 minutes. Temperature was maintained, while glucose and hematocrit were monitored. Animals were sacrified 45 minutes after the 2DG, and the brain processed for autoradiography. Densitometric readings were taken in over 30 brain regions on 20 micron brain tissue slices. Glucose utilization values were calculated according to the method of Sokoloff (J. Neurochem. 28:897, 1977). Results suggest a PCP-induced increase in glucose utilization in cingulate cortex, subiculum, hippocampus, and some thalamic nuclei (see Table). But relatively little PCP-induced changes are evident in frontal cortical areas, auditory tracts (including medial geniculate), and hypothalamus. These areas coincide somewhat, but not entirely with the distribution of PCP receptors. Purportedly, these are the areas in the CNS which mediate the unique PCP-induced behaviors in animals and may be involved with psychosis in schizophrenia.

Brain Region	Saline (N=3)	PCP (N=3)	% Increase
Prefrontal Cortex	132 ± 4	131 ± 26	0%
M.Geniculate	120 ± 11	138 ± 20	15%
Cingulate	124 ± 8	231 ± 60	86%
Subiculum	97 ± 8	166 ± 36	71%
Hippocampus	81 ± 11	108 ± 24	33%

µmol glucose/100 gm tissue/min

381.15 THC ALTERS BRAIN 2-DEOXYGLUCOSE METABOLIC ACTIVITY. J. Margulies* and R. P. Hammer, Jr. Depts. of Biochemistry & Biophysics and Anatomy & Reproductive Biology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822.

The effect of marijuana and its major psychoactive component, delta-9-tetrahydrocannabinol (THC) on brain function has been known for some time. However, the brain regions affected and the precise mechanism of drug action on neurons has only recently been investigated. We have examined the effect of acute THC administration on 2-deoxyglucose (2DG) uptake in a number of brain regions. Male Sprague-Dawley rats (220-250g) were anesthetized with ketamine and intracardiac cannulae were surgically implanted. The animals recovered overnight and were then prepared for THC and 2DG administration. THC was solubilized in 5% propylene glycol and 1% Tween 80. A final THC dose of 2mg/kg was injected via cannula followed in 10 minutes by a pulse of [^{3}H]2DG (0.5mCi/250g). Animals were restrained and quiet during the entire THC/2DG exposure period, though behavioral manifestations of THC exposure, e.g., abrupt startle response and unprovoked squeeling, were evident. Forty-five minutes after 2DG administration animals were decapitated, brains were frozen and sectioned. Tissue sections on coverslips were then exposed to tritium-sensitive X-ray film in cassettes for 8 weeks. Sham-operated control animals received the same dose of vehicle without THC, and were treated identically. After film development, autoradiographs were quantitatively analysed using a computer-assisted densitometry system. Values were normalized between brains and an optical density ratio of gray matter-to-white matter was calculated for various regions throughout the brain. Some brain regions, including auditory cortex, medial geniculate nucleus, mammillary nucleus, inferior colliculus, interpeduncular nucleus, and vestibular nuclei, maintain high levels of 2DG labeling in THC-exposed animals. Other brain regions, notably the hippocampus, appear to be differentially affected by THC. 2DG labeling in the stratum lacunosum-moleculare of hippocampus (particularly ventral hippocampus) appears to be enhanced in the THC group relative to the control group. Quantitative data on these and other brain regions will be presented. Thus, THC administration, even at this relatively low dose level in rats, differentially affects metabolic activity across brain regions. Hippocampal and other limbic brain structures may be most affected. These results are in agreement with previous work showing specific physiologic effects of THC on hippocampal cell activity (Deadwyler et al., Soc. Neurosci. Abstr. 11:852). Such effects on brain function may provide a basis for THC-induced behavioral alteration. (Supported by PHS Grant DA 03885)

381.16 **A 2-DEOXYGLUCOSE STUDY OF FEMALE AND MALE RATS NEONATALLY TREATED WITH MSG** Laurel A. Freed*, Diana L. Dow-Edwards and Thomas H. Milhorat. (Spon:M. E. Friedlander) Lab. of Cerebral Metabolism, Dept. of Neurosurgery, SUNY Health Sciences Center at Brooklyn, NY, 11203.

The neurotoxicity of monosodium glutamate (MSG) and the syndrome it produces when perinatally administered has been an area of intense investigation. MSG has been shown to produce lesions of the inner layer of the retina, cingulate gyrus, arcuate nucleus of the hypothalamus, and other structures of the circumventricular organ system. MSG induced neurotoxicity develops over time and as adults, the experimental rats exhibit behavioral deficits and endocrinopathies. In light of these severe abnormalities of neonatally MSG-treated rats and recent advances in the techniques of quantitative autoradiography, the ^{14}C-Deoxyglucose (DG) method of Sokoloff et al., (J. Neurochem., 28:897-916, 1977) was employed to investigate the local cerebral glucose metabolism of the adult rat neonatally exposed to MSG.

Newborn Sprague-Dawley female and male rats were culled to 8 per dam and randomly assigned to a control or experimental group. The pups were injected subcutaneously with saline or Monosodium-L-Glutamate (Sigma, St. Louis, MO) on days 1 and 3 at 2mg/kg of body weight, and on days 5, 7, 9 at 4mg/kg of body weight. At 90 days of age the DG method was performed. Under halothane, N_2O and O_2 anesthesia, catheters were inserted into the femoral artery and vein of each rat, a loose fitting abdominopelvic cast was applied, and then the animals were allowed to recover for two hours. ^{14}C-labelled 2DG was injected into the venous catheter and timed arterial samples were collected until 45 minutes when the animals were killed with sodium pentobarbital. The brains were removed, frozen in chilled isopentane and later sectioned for autoradiography. Local cerebral glucose utilization was determined from autoradiographic analysis of tissue ^{14}C concentration and the integrated arterial curve for 2DG and glucose. A Sargent-Welch Densichron and a computerized image analysis system were used to evaluate the local concentrations of ^{14}C in the areas of interest. Of the structures examined thus far, only the site of termination of the arcuate nucleus fibers, the median eminence is metabolically depressed by neonatal MSG treatment.

381.17 PHARMACOLOGICAL CHARACTERIZATION OF DOPAMINERGIC EFFECT IN BRAIN GLUCOSE METABOLISM: THE "2-DEOXYGLUCOSE DOPAMINERGIC FINGERPRINTS". J.M. Palacios and K.H. Wiederhold*. Preclinical Research, Sandoz LTD., Basle, Switzerland.

We have used a modification of the 2-^{14}C-deoxyglucose (2DG) method of Sokoloff et al. (1977) to study brain regional glucose uptake (GU) after manipulation of dopamine (DA) neurotransmission. The goal was to examine if the administration of direct or indirect DA receptor agonists or antagonists resulted in a characteristic pattern of GU modification or 2DG "fingerprint". The effects of typical and atypical neuroleptics were examined first. A "neuroleptic 2DG fingerprint", characterized by a marked increase in GU in the nucleus of the lateral habenula was produced in a specific and dose-dependent manner by all the neuroleptics examined. The order of activity and selectivity showed that the modification of GU was mediated by DA-receptors of the D_2 class and reflected interruption of DA neurotransmission as lesions of the nigrostriatal pathway, depletion of DA stores or inhibition of DA biosynthesis resulted in a similar "2DG fingerprint". The effects of DA receptor agonists of several chemical classes, were examined next. The "DA agonist 2DG fingerprint" was characterized by marked GU increases in cortical areas, some thalamic nuclei and the substantia nigra reticulata. GU in lateral habenula nucleus was decreased by many, but not all DA agonists. The effects were dose-dependent and reflected the activity of these compounds on DA-D_2 receptors, as selective D_1 agonists were without effects. Indirect DA agonists such as amphetamine, L-DOPA or DA-uptake inhibitors also produced a "DA agonist fingerprint". Partial and presynaptic DA agonists, such as transdihydrolisuride (TDHL), 3PPP or CF-25397, produced a "neuroleptic fingerprint" rather than an "agonist" one. The sensitivity state of DA receptor plays also an important role as shown by studies using selective D_1 and D_2 agonists and partial agonists such as TDHL and CF-25397, in animals with denervated DA receptors. In contrast with results in intact animals, in denervated animals, D_1 agonist administration resulted in GU modifications similar to those seen with D_2 agonists. Furthermore, partial agonists induced in the lesioned animal. In conclusion the administration of drugs acting on brain DA receptors results in two characteristic patterns of modification of brain regional GU or "fingerprints". These patterns exhibit the characteristics of a receptor mediated phenomenon. The sensitivity state of DA receptors has important effects in the pharmacological characteristics of the 2DG response. Thus, the 2DG method offers a new way to study in vivo brain effects of DAergic drugs.

381.18 DIFFERENTIAL EFFECTS OF D1 AND D2 DOPAMINE RECEPTOR SUBTYPE-SPECIFIC ANTAGONISTS ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT BRAIN. S.W. Shaver*, P. Ramm, and R.J. Beninger. Dept. of Psychology, Trent Univ., Peterborough, Ont., Canada, K9J 7B8.

Two subtypes of CNS dopamine receptors have been reported. D1 receptors may enhance adenylate cyclase activity while D2 receptors may inhibit adenylate cyclase or may not affect its activity. We examined brain glucose metabolism in male Long-Evans rats (260-325 g, n=4/gp) following administration of one of three dopamine receptor subtype-specific antagonists. Rats received injections of *cis*-flupenthixol (0.5 mg/kg), metoclopramide (10 mg/kg), SCH 23390 (1.0 mg/kg), or saline. Quantitative 2-[14C]deoxyglucose autoradiography was used to show local cerebral glucose utilization (LCGU) and relative functional activity (RFA). RFA values were obtained by normalizing LCGU values to the brain mean glucose utilization value.

Elevation of RFA was observed in the lateral habenula (LHb) following administration of each receptor blocking agent.

The LHb exhibited increased LCGU under *cis*-flupenthixol. The dorsal raphe nucleus (DR) exhibited decreased LCGU when data from the *cis*-flupenthixol (D1 and D2 antagonism) and SCH 23390 (D1 antagonism) groups were combined. Decreased LCGU was not observed in the DR when the metoclopramide (D2 antagonism) and *cis*-flupenthixol groups were combined. Thus, the observed decrease DR LCGU may be mediated via D1 receptor blockade.

These results converge with a previous report (Ramm et al., Can. J. Physiol. Pharmacol. 62:1530-1533, 1984) suggesting that the functional status of the LHb is regulated by dopaminergic systems, and that functional activity in the LHb may exert an influence upon the DR. Furthermore, the influence of the LHb upon the DR may be mediated via D1 receptors.

381.19 BEHAVIORAL AND CEREBRAL METABOLIC EFFECTS OF THE DOPAMINERGIC AGONIST BROMOCRIPTINE IN THE AWAKE RAT IN RELATION TO AGE. G.L.Ricchieri*, H.W.Holloway* and S.I.Rapoport (SPON:D.L.Robinson) Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892.

The dopaminergic system within the brain possesses D1 receptors (adenylate cyclase-linked) and D2 receptors (non adenylate cyclase-linked) at pre- and post-synaptic sites (Kebabian and Calne, 1979). Bromocriptine (BROMO), a dopaminergic agonist, is a weak antagonist at D1 sites, but is a potent agonist at D2 sites (Markstein, 1981). Presynaptic and postsynaptic activation at these sites results in hypomotility and stereotyped behavior, respectively (Costall and Naylor, 1981). In 3 mo old rats, BROMO causes biphasic behavioral responses which correspond to cerebral metabolic responses as measured with the 14C-2-deoxy-D-glucose (DG) technique (Pizzolato et al, 1985).

We measured local cerebral glucose utilization (LCGU) in response to BROMO in rats at different ages, by using the quantitative DG method of Sokoloff (1977). Awake, male Fischer-344 rats, 3, 12 or 24 mo old, were injected with BROMO (1, 5 or 20 mg/kg i.p.) or vehicle (1% lactic acid), 2 or 4 h prior to the injection of DG. Behavior (hypomotility, or hypermotility and stereotypy) was rated at fixed intervals after drug administration (Pizzolato et al, 1985). At 2 h after BROMO 20 mg/kg, hypomotility occurred in relation to a significantly ($p<0.05$) reduced LCGU in 17% of 65 brain regions examined. At 4 h stereotypic behavior and hypermotility were observed, and were greater in 24 mo than in 3 mo old rats after 20 mg/kg of BROMO. LCGU after 20 mg/kg BROMO was significantly elevated at 24 mo in 23% of brain regions, particularly in cortical motor areas, extrapyramidal areas (basal ganglia, subthalamic nucleus), and cerebellar vermis and deep nuclei. After BROMO 1 mg/kg, hypomotility and significant declines of LCGU occurred in 15% of the regions examined at 2 and 4 h, in 3 mo but not in 24 mo old rats.

Persistent hypomotility and decline of LCGU with the lower dose of BROMO in 3 mo old rats support the postulated action of BROMO on presynaptic dopaminergic receptor. The greater LCGU and stereotypy observed in the 24 mo as compared to 3 mo old rats, in response to high dose BROMO, may be a consequence of reduced presynaptic release of dopamine resulting in postsynaptic supersensitivity.

381.20 SIGMA AGONIST ACTIONS ON BRAIN LOCAL CEREBRAL GLUCOSE UTILIZATION BY +SKF-10,047. M. Dam*, A. D. Weissman and E. D. London (Spon. G. Shaw), Neuropharmacology Laboratory, NIDA/Addiction Research Center, Baltimore, MD 21224.

The prototype sigma receptor agonist N-allyl-normetazocine (+SKF-10,047), provides a valuable tool to elucidate a neurochemical system in the brain that may mediate a wide range of psychotomimetic effects. Recent work by Largent, B. L. et al., (J. PET., in press, 1986) and our laboratory have confirmed the presence of specific sigma receptors localized to discrete areas of the brain. In order to study the effects on brain metabolism mediated by these receptors, +SKF-10,047 (5 mg/kg, i.v.) was administered to rats and local cerebral glucose utilization (LCGU) was measured quantitatively 15 min later using the 2-deoxy-D-[1-^{14}C]glucose (DG) method (Sokoloff, L. et. al, J. Neurochem. 28:897, 1977).

+SKF-10,047 elicited a number of sterotypic behaviors, including increased sniffing and head weaving. Consistent with the appearance of these behaviors was an increase glucose utilization in the substantia nigra. Ataxia was also present in these animals, and this behavior was associated with an increase in LCGU in regions of the cerebellar cortex, vestibular nucleus and the red nucleus. Unlike the extrapyramidal motor system, the pyramidal motor areas, including the motor cortex and its specific thalamic projections, did not have significantly increased LCGU.

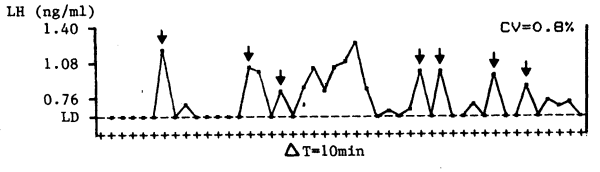
+SKF-10,047 decreased LCGU in specific sensory regions, including the cochlear and medial geniculate nuclei of the auditory system, and layers of the somatosensory cortex. In contrast, specific parts of the limbic system, such as the parasubiculum, subiculum and claustrum were stimulated. In the hippocampus, LCGU was decreased in CA1 and CA2 regions of Ammon's horn, but CA3 and the dentate gyrus remained unchanged.

The effects on LCGU following the administration of the benzomorphan, +SKF-10,047, present a pattern of activity that largely follows the distribution of sigma receptors in the brain. In this respect, the inferred receptor mediated action of this sigma agonist appears to be a decrease in cellular activity which may be related to its dysphoric effects in man (Keats, A.S. and Telford, J., Adv. Chem. Ser. 45:170, 1964). The increased metabolic activity in the extrapyramidal system and in parts of the limbic system are in contrast to the depression of LCGU in sensory and some limbic areas. The possibility exists that the hypermetabolic effects may be related to stimulation of another population of receptors which mediate the actions of phencyclidine.

NEUROENDOCRINE CONTROL: PITUITARY VI

382.1 PULSATILE LH RELEASE IN HYPOGONADAL MALE MICE WITH PREOPTIC AREA TRANSPLANTS. G.J. Kokoris, M. Ferin*, A.J. Silverman, and M.J. Gibson. Div. Endocrinol., Mt. Sinai Sch. of Med., New York, N.Y. 10029 and Columbia Univ., Coll. Phys. and Surg., Depts. Anatomy & Cell Biol. and Ob-Gyn., New York, N.Y. 10032.

Congenitally hypogonadal (hpg) male mice bearing preoptic area (POA) grafts in the IIIrd ventricle show significant elevations in pituitary luteinizing hormone (LH) and correction of their hypogonadism. In responding animals, immunoreactive GNRH neurons within the graft innervate the median eminence of the host. In normal mice, testicular function is related to episodic LH stimulation. Pulsatile LH secretion is itself regulated by phasic release of GNRH into the portal plexus. To assess whether the gonadal recovery in hpg hosts results from pulsatile secretion of GNRH from transplanted neurons, we compared the pattern of variation in plasma LH levels in normal mice and in hpg males with POA grafts. 12 hpg males with significantly elevated testes weights (range: 20.2-143.7mg; untreated hpg-7.5mg; normal-207.7) were castrated 3 months after graft surgery. Normal males (n=8) of the same strain and length of castration served as controls. A month after castration, an indwelling cannula was placed in the jugular vein. Sequential blood samples were collected (ΔT=10min) for 4-7hrs. Plasma samples (25µl) were assayed for LH using the NIADDK kit with RP-2 as standard. The assay limit of detectability varied from 8-15pg/tube. The mean intra-assay coefficient of variation (CV) was 4.5%. Elevations in plasma LH were considered pulses if they met 3 criteria: 1) a pulse peak amplitude greater than 2x the CV of the assay in which samples were measured; 2) attainment of peak amplitude within 1 sample interval; 3) a decline in pulse amplitude 2x the CV within 3 sample intervals of the pulse peak.



Pulsatile LH secretion was seen in 5 of 12 hosts (Fig.). Although individual animals showed wide variation in pulsatility, pulse frequency (0.6-1.3/hr) did not differ from that of controls. However, mean pulse amplitude in grafted hpg castrates was significantly lower ($p<.05$) than in control males. These data suggest that grafted GNRH neurons can support a functionally normal pattern of pulsatile LH release.

382.2 EFFECT OF MEDIAL PREOPTIC IMPLANTS OF ANTI-ESTROGENS ON LH SURGES IN OVARIECTOMIZED, ESTROGEN-TREATED FEMALE RATS. S.L. Petersen* and C.A.Barraclough (SPON: R. Holland). Dept of Physiology, School of Med. University of Maryland, Baltimore, MD 21201.

The occurence of proestrous preovulatory LH surges requires prior exposure of the hypothalamo-pituitary axis to estrogen during a specific time interval between diestrous day 2 and proestrus. Spontaneous LH surges also occur in short-term ovariectomized (OVX) rats (7 days) following estrogen treatment. Considerable evidence suggests that these positive feedback actions of estrogen are exerted within the CNS, although the specific site of action is unknown. One possible site of action of estrogen is the medial preoptic nucleus (MPN), an area which contains both LHRH cell bodies and axon terminals and receptors for various neurotransmitters known to be involved in the surge release of LHRH. We reasoned that by depriving this specific region of estrogen (MPN) in an otherwise completely estrogenized OVX rat, we could evaluate the importance of estrogen in modulating LHRH release via its effects on neurons within the MPN.

Rats were OVX and 2 weeks later (Day 0) 28-gauge stainless steel microcannulae were implanted bilaterally into the MPN. The microcannulae were empty or contained one of the following anti-estrogens: LY117018, CI 628 or keoxifene. One hour later, all females received s.c. Silastic capsules containing estrogen concentrations previously shown to produce blood levels of 30-35 pg/ml. On Day 2, blood samples were obtained at 1630 h and these LH values were compared to LH concentrations obtained in sequentially bled control rats (OVX + estrogen x 2 days; LH levels were measured at 1000h and hourly from 1200-1600h). LH levels and the proportion of females exhibiting LH surges were compared among groups.

Females with MPN cannulae containing any of the antiestrogens had significantly lower LH levels than those with blank cannulae. In addition, 88% of females implanted with blank cannulae exhibited afternoon LH surges compared with only 10% of CI 628-treated, 12% of keoxifene-treated and none of the LY117018-treated females. Most rats in which the cannulae were misplaced into regions outside the MPN had LH surges.

The results suggest that the preoptic region is one site at which estrogen acts to affect the surge release of LHRH. Studies currently are determining the mechanisms by which these anti-estrogens alter LHRH release when placed into the MPN. Supported by NIH Grant 02138.

382.3 IDENTIFICATION OF SOME RECEPTOR SPECIES MEDIATING EFFECTS OF DORSAL RAPHE STIMULATION ON RELEASE OF LH IN OVARIECTOMIZED RATS WITH AND WITHOUT ESTROGEN.
J.H. Johnson and C.S. Kitts. Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298.

Inhibition of LH release in ovariectomized (OVX) rats by stimulation of the dorsal raphe nucleus (DRN) in anesthetized animals involves serotonin (5HT; Arendash, Endocrinol 102:1199). We reported reversal of this effect in unanesthetized rats by priming with estradiol benzoate (EB; J Neurosci Res 15:253). In the present studies we explore the species of receptors mediating these responses to electrical stimulation of DRN, a nucleus comprised largely of 5HT neurons, using receptor antagonists with different binding properties. Ketanserin (KET) shows a differential affinity for $5HT_2$ (versus $5HT_1$) receptors, whereas methysergide (METH) and metergoline (MTG) bind to both $5HT_1$ and $5HT_2$ species. Naltrexone (NAL) is an opiate receptor antagonist, and phenoxybenzamine (PBZ) binds to α-adrenergic receptors. Rats OVX 4 weeks or more were implanted with bipolar electrodes in DRN and with a cannula in the external jugular vein. Blood samples were collected from unanesthetized rats at 10 min intervals and plasma was assayed for LH by RIA. Mean LH (ng/ml) in 7 prestimulation samples and in 7 samples collected during stimulation were compared for each treatment group by paired t test. In separate groups of rats EB (5 μg/100g) was administered sc on the 2 consecutive days preceding the experiment, and the more stable release of LH was compared in 4 prestimulation and 4 stimulation samples. Receptor antagonists were administered in the dosages and at times prior to collection of the first prestimulation blood sample as listed: KET (2.5 mg/kg ip, 60 min); METH (10 mg/kg ip, 70); MTG (1 mg/kg ip, 60); PBZ (20 mg/kg ip, 90); NAL (4 mg/kg iv, 10). In unprimed animals the inhibitory effect of stimulation of DRN on LH release was prevented by KET, METH and PBZ, but not by MTG or NAL. In estrogen-primed animals the LH-releasing effect of stimulation of DRN was antagonized by NAL, METH and MTG, but not by KET or PBZ. These results suggest that stimulation of DRN effects release of LH by dual mechanisms: a stimulation mediated by $5HT_1$ and opiate receptors; and inhibition via $5HT_2$ and α-adrenergic systems. Estrogen appears to modulate the relative effectiveness of these influences. (Supported by NIH grant HD 12165).

382.4 RESPONSES OF MEDIAL PREOPTIC AND MEDIAL SEPTAL (MPO-MS) NEURONS TO ENDOGENOUS AND EXOGENOUS NOREPINEPHRINE (NE).
Y.I. Kim, C.A. Dudley and R.L. Moss. Dept. Physiol., UTHSCD, Dallas, TX. 75235

The effects of A1- and A6-noradrenergic cell group stimulation on the activity of MPO-MS neurons were examined using extracellular recording techniques in ovariectomized, estrogen-progesterone primed Sprague-Dawley rats anesthetized with urethane. The effects of exogenously applied NE were also tested and related to the A1- or A6-stimulation-induced effects. NE (500 mM-HCl in dist. water, 4-30 nA) was applied iontophoretically from one barrel of a multibarrel assembly or ejected by pressure from barrels containing 50 mM or 0.5 mM NE-HCl (dissolved in artificial CSF, 0.5-23 psi). A1-region stmulation evoked orthodromic responses from 47 of 111 neurons: 30 neurons were excited (A1-OD+), 16 neurons were inhibited (A1-OD-), and 1 neuron showed a complex response. Stimulation of A6-region evoked orthodromic responses from 23 of 105 neurons: 4 neurons excited (A6-OD+), 18 neurons were inhibited (A6-OD-), and 1 neuron showed a complex response. Responses to exogenous NE of neurons, responding or non-reponding (NR) to A1- and/or A6-stimulation, are summarized below.

	500 mM NE iontophoresed			50 mM NE pressure-ejected			0.5 mM NE pressure-ejected		
	↑	↓	→	↑	↓	→	↑	↓	→
A1-OD+	8	7(1)*	3	1	5(2)*	4	5	1	5
A1-OD-	0	7	1	0	5	2	1	4	2
A6-OD+	0	0	1	0	0	2	0	0	2
A6-OD-	3(3)**	7	1	0	6	2	3(2)**	2	4
NR	6	15	25	1	10	23	10	3	26

Note: ↑ :excitation, ↓ :inhibition, → :no response

( )* number of neurons which showed orthodromic inhibition in response to A6-stimulation.

( )**number of neurons which showed orthodromic excitation in response to A1-stimulation.

The results demonstrate that 1) A1-noradrenergic input to MPO-MS neurons is predominantly excitatory whereas A6-noradrenergic input is predominantly inhibitory; 2) exogenous NE can mimick the inhibitory effects induced by A1- or A6-stimulation regardless of drug application method, although in the case of pressure ejection the higher concentration of NE was more reliable than the lower concentration; and 3) the excitatory effects induced by A1-stimulation tend to be mimicked by the lower concentration of NE and partially by iontophoretically applied NE. Since iontophoretically applied NE or pressure-ejected NE of a higher concentration tends to inhibit the activity of many NR neurons as well as neurons responding to A1- or A6-stimulation, the inability of NE applied as above to mimick some of the A1-stimulation-induced excitatory effects might be due to non-specific activation of different noradrenergic receptor population as a result of pharmacological concentrations achieved. Supported by NIH grant NS-10434.

382.5 LHRH RELEASE FROM NERVE TERMINALS IN VITRO: DESENSITIZATION TO NOREPINEPHRINE STIMULATION AND EVIDENCE FOR NON-GENOMIC EFFECTS OF ESTRADIOL.
C. Masotto* and A. Negro-Vilar (SPON: W.Wetsel). Reproductive Neuroendocrinology Section, Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

Norepinephrine (NE) is an important central neurotransmitter involved in the regulation of LHRH. The interaction between NE and LHRH is modulated by gonadal steroids, particularly estradiol. Using an in vitro system, we have demonstrated that NE acts through an α-adrenergic receptor to enhance LHRH release from median eminence (ME) nerve terminals, and that prostaglandin E_2 is an important intracellular mediator of the action of NE. Changes in circulating estrogen levels can result in marked alterations in neuronal sensitivity to NE and, indeed, the steroidal milieu of the donor animals determines the degree of responsiveness in vitro to the stimulatory effects of NE. We have applied this in vitro system to evaluate the NE-LHRH-Estrogen interaction, asking two specific questions: (1) Are there direct, non-genomic effects of estradiol (E_2) on LHRH nerve terminals? and (2) Is it possible to observe desensitization to the NE response in vitro and, if so, what are the effects of E_2 on this phenomenon? ME fragments (containing only nerve fibers and terminals and no cell bodies) were obtained from adult rats 1 week after ovariectomy and incubated in a Krebs Ringer Buffer (KRB) for several hours. NE was added (10μM) in each of three separate 30 min incubation periods, separated by 90 min recovery periods. In certain groups, 17β-E_2 (10^{-7}M) was added to the KRB buffer throughout the entire incubation period. Release of LHRH and PGE_2 was evaluated by RIA. NE increased LHRH release during the first period from ME's incubated in KRB or KRB+E_2. The degree of this stimulation was not affected by estrogen. During the second and third test periods, a refractory situation appeared in the tissue incubated in KRB since NE was no longer able to stimulate LHRH release. On the other hand, the ME's incubated with KRB+E_2 recovered the capacity to respond to NE during the third test period. NE consistently released PGE_2 in each test period, an effect not influenced by the presence of E_2. Addition of exogenous PGE_2 (0.5μM) at three equivalent test periods resulted in similar increments of LHRH release, regardless of the test period, or of the presence of E_2. The results indicate that desensitization of the NE effect on LHRH may be observed in vitro, and that the site of action is distal to the activation of PGE_2 secretion. Furthermore, the data suggests that, since the response to PGE_2 remains unaltered, an inhibitory factor and/or mechanism affecting LHRH release may play a role in the desensitization process. The observed effects of E_2 in vitro in a nerve terminal preparation devoid of cell bodies suggests some non-nuclear effects of the steroid, perhaps affecting membrane events not mediated by the E_2 receptor.

382.6 EFFECTS OF ACUTE HYPOPHYSEAL STALK TRANSECTION ON ANTERIOR PITUITARY HORMONE SECRETION.
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After chronic stalk transection, secretion of pituitary hormones declines, with the notable exception of prolactin, which rises promptly after removal of the tonic inhibitory control. The effects of acute transection, on the other hand, are not well known. During the course of experiments in which the stalk was cut in preparation for portal blood collection, we observed that LH rises acutely after the stalk is severed. The present experiments were designed to corroborate and expand this observation. Adult male rats were anesthesized with Althesin (Saffan, 5 ml/kg, i.p.) or ketamine-xylazine (30:1, 1 ml/kg; i.m.) and the pituitary stalk was exposed by parapharyngeal surgery. A plastic cannula was placed into the right atrium for periodic blood withdrawal (0.25-0.5 ml). Heparinized saline and heparinized, washed blood cells were used for replacement at appropriate intervals. After pituitary stalk transection (PST), a rise in plasma levels of several pituitary hormones was observed, but the dynamics and magnitude of the change differed markedly between hormones. LH rose significantly between 1-2 h after PST, peaked at 3-5 h and declined to basal levels by 7 h. FSH showed a more modest and sluggish increase, with significant increments at 3-6 h. Prolactin levels were elevated at 10 min and remained elevated throughout the sampling period. The increase in GH and TSH was apparent within 30 min. If PST was performed in orchidectomized rather than in intact animals a decrease, instead of an increase, in LH and FSH was observed. To further evaluate the possible mechanisms mediating the rise in LH, an additional experiment was performed in which an LHRH antagonist ([D-pGlu^1, D-Phe^2, D-Trp^{3,6}]-LHRH) was given at a dose of 100 μg i.v., 120 and 5 min before PST in intact male rats. LH levels prior to PST and throughout the experimental period in control animals declined to very low values. In the presence of the antagonist, PST caused a modest but significant rise in LH levels that lasted for the 4 h bleeding period. Exogenous LHRH (10 ng,iv) given to antagonist-treated rats failed to increase LH levels. The results indicate that acute PST causes a rapid and differential release of several pituitary hormones. Although the exact mechanism(s) mediating these effects remains to be determined, it is possible that some of the changes are due to the removal of peptides, amines or other factors present in portal blood which exert direct inhibitory effects on pituitary hormone secretion.

382.7 DETECTION OF SPECTRAL NON-STATIONARITIES OF EXTRA CELLULAR FIELD POTENTIALS IN THE RAT BRAIN RELATED TO STAGES OF THE ESTROUS CYCLE. C.C. Turbes, G.T. Schneider* and R.J. Morgan*. Department of Anatomy, Creighton University Sch. of Med., Omaha, NE 68178

Substantial evidence has been reviewed showing that the release of gonadotropins and other peptides from the hypothalamus and the adenohypophysis via the blood stream show changes in the extracellular slow wave field potentials (SWFP) at 1Hz to 50Hz of certain regions of the female rat brain.

Autoregressive spectral filters were developed to analyze the analog data. Special attention is given to the hypothalamic and limbic regions of the brain and the spectral non-stationarities that relate to the stages of the estrous cycle. The frequency bands of 1Hz to 50Hz of the autoregressive filter data show different patterns in each of the ongoing spectral arrays that relate to the stages of the estrous cycle. Comparisons of the autoregressive filter spectral arrays and coherence and partial coherence data show the greatest linearity between 1Hz and 20 Hz during proestrous and estrous.

382.8 CHARACTERIZATION OF SOME BIOLOGICAL PROPERTIES OF THE PUTATIVE LH RELEASE INHIBITORY FACTOR OF RAT HYPOTHALAMUS. J.-C. Hwan and M.E. Freeman*, Dept. Biol. Sci., Fla. State Univ., Tallahassee, Fla. 32306.

We have purified a novel peptide, luteinizing hormone release inhibitory factor (LHRIF), from the rat hypothalamus that can inhibit LHRH-stimulated LH release from dispersed rat anterior pituitary cells (APC) (Hwan and Freeman Endocrinology, in press). In this study, we report further *in vivo* effects of LHRIF on LH release. In addition, we report production and use of monoclonal antibodies specific for LHRIF.

Cycling rats were implanted with an intra-arterial cannula under light anesthesia. At 1130h on the day of proestrus, rats were treated with pentobarbital (35 mg/kg), followed at 1400h with LHRH (500 ng/rat) given alone or with LHRIF (100 µg/rat), injected intra-arterially. Blood samples were taken at 10 min intervals up to 30 min then at one-hour intervals from one to 3 hrs. Serum was assayed for LH concentration by radioimmunoassay. The inhibitory activity of LHRIF was apparent by 30 min after treatment. That is, while LHRH alone induced a rapid rise in blood LH levels, LHRIF actually diminished LH release below pretreatment levels.

Monoclonal antibodies to LHRIF were produced by *in vitro* immunization of mouse spleen cells with less than 1 nmole of partially purified LHRIF. Fusion of the sensitized spleen cells with mouse plasmacytoma was carried out with polyethylene glycol (PEG 1540, Baker). Hybridoma supernatants were screened by ELISA. Ascites fluid was also obtained by injection of cells from active clones into Pristane-primed mice. The anti-LHRIF hybridoma clones secreted immunoglobulin M (IgM). The antibodies from Ascites fluids also contained IgG_{2a} and IgG_{2b}. The monoclonal antibody neutralized the inhibitory effect of LHRIF on LHRH-stimulated LH release *in vitro*. The isolated immunoglobulins were used to prepare affinity columns by coupling to CNBr-activated Sepharose 4B. Large-scale purification of LHRIF from crude acid extract of rat hypothalamus can thus be performed in a single step. The 0.1 N HCL eluate contained the inhibitory activity and could be shown to be homogeneous and have the same mobility on SDS polyacrylamide gel as LHRIF purified by HPLC. Using these monoclonal antibodies and goat anti-mouse immunoglobulin conjugated with Fluorescein as the second antibody, axons in the rat hypothalamus but not other areas of the rat brain were stained. These studies provide further evidence for a possible LHRIF of hypothalamic origin. Supported by The Ford Foundation and NSF.

382.9 INHIBITORY ACTION OF ETHANOL ON PULSATILE RELEASE OF LUTEINIZING HORMONE IN MALE RATS. C. R. Pohl* and R. A. Gutlinger* (SPON: A. L. Humphrey). Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Ethanol (EtOH) is known to inhibit luteinizing hormone (LH) secretion in a variety of experimental models. This inhibitory action appears to be exerted primarily within the CNS, rather than at the level of the pituitary gland. Because the control of LH is dominated by an LH-releasing hormone (LHRH) pulse generator, the present study was undertaken to examine the actions of EtOH on this control system by measuring changes in pulsatile LH release following EtOH administration. Blood samples were collected at 6-min intervals for 4 hrs from adult male, castrated rats bearing chronic jugular catheters. The first half of the 4-hr period (intra-animal control period) was preceded by an ip injection of normal saline. The second half (experimental period) was preceded by a similar injection of saline or of EtOH at doses of 1000, 1500, or 2000 mg/kg BW. These doses produce peak blood EtOH concentrations of 63, 102, and 170 mg/100 ml, respectively (Cicero et al., 1982). Pulsatile LH release was analyzed using the PULSAR algorithm of Merriam and Wachter. Sampling periods which were preceded by normal saline injections (first or second half of the 4-hr period) had similar values for each parameter of pulsatile LH release. Injections of EtOH prior to the experimental period resulted in dose-related changes, as follows (Means ± SEM; interpulse intervals have been calculated only where 2 or more pulses were observed):

EtOH Dose (mg/kg BW)	Frequency (Pulses Per 2 Hours)	Interpulse Interval (Min)	Amplitude (ng/ml)	Integrated LH (ng/ml)
0 (Saline)	5.1 ± 0.4	26.4 ± 2.7	3.62 ± 0.42	3.61 ± 0.41
1000	3.6 ± 0.5	27.1 ± 4.2	3.56 ± 0.49	2.82 ± 0.33
1500	2.7 ± 0.4	60.8 ± 10.2	2.98 ± 0.50	2.11 ± 0.41
2000	1.8 ± 0.5	71.5 ± 23.1	2.22 ± 0.35	1.68 ± 0.26

In a further study, acute EtOH treatment *in vitro* (800 mg/100 ml) had no effect on the response of hemipituitaries to an ED_{50} concentration of LHRH.

These results suggest that EtOH inhibits LH release by reducing both the frequency and amplitude of LHRH discharges. (Supported by grant AA05913 from the NIAAA.)

382.10 DISRUPTION OF THE CENTRAL NORADRENERGIC SYSTEM DOES NOT ATTENUATE THE SUPPRESSION OF PULSATILE LH SECRETION CAUSED BY INTRAVENTRICULAR VASOACTIVE INTESTINAL PEPTIDE INFUSION IN OVARIECTOMIZED RATS. M.J. Alexander, D.K. Clifton*, A.M. Matsumoto*, and R.A. Steiner, Departments of Ob-Gyn, and Physiology and Biophysics, University of Washington, Seattle, WA 98195.

We have previously shown that intraventricular (ivt) infusion of vasoactive intestinal peptide (VIP) in ovariectomized (OVX) rats causes a pronounced inhibition of pulsatile luteinizing hormone (LH) secretion and that this effect is not a result of reduced pituitary responsiveness to gonadotropin-releasing hormone (GnRH) (Endocrinology 117:2134, 1985). Since there is increasing evidence for an interaction between VIP- and norepinephrine-secreting neuronal systems in the brain, we tested the hypothesis that disruption of the central noradrenergic system will attenuate VIP-induced suppression of pulsatile LH release.

We used 6-hydroxydopamine (6-OHDA), a catecholamine-specific neurotoxin, to disrupt the function of noradrenergic neurons. Seven days prior to blood sampling, we administered 30 µg 6-OHDA, or vehicle, ivt to long-term OVX rats (each bearing a chronic third-ventricular cannula). This treatment reduced hypothalamic norepinephrine concentrations to 35% of control values. On the day of the bleed, serial 300 µl blood samples were taken from conscious, unrestrained animals at 5 min intervals for a total of 4 h. Each sample was replaced with an equal volume of a blood replacement mixture. Immediately following a control bleeding period of 2 h, VIP (in saline) was administered ivt to each animal for 2 h (at a dose of 1.8 nmol/18 µl/h). The changes in pulsatile LH secretion caused by VIP infusion were virtually identical in the 6-OHDA- and vehicle-pretreated groups (n=5 for both):

	LH Pulse Frequency (/2h)		Mean LH (ng RP2/ml)	
	Control	VIP	Control	VIP
6-OHDA	4.6 ± 0.5	0.6 ± 0.4	7.3 ± 0.6	2.5 ± 0.3
Vehicle	4.4 ± 0.4	0.6 ± 0.4	6.8 ± 0.5	2.4 ± 0.3

These results indicate that a substantial disruption of noradrenergic innervation does not attenuate VIP-induced suppression of pulsatile LH secretion. Conclusion: The central noradrenergic system does not appear to play a major role in VIP-mediated inhibition of pulsatile LH release. (Supported by NIH Grant HD-12629.)

382.11 CHARACTERIZATION OF LHRH IMMUNOREACTIVITY IN MAMMALIAN PITUITARY NEURAL LOBE BY HPLC.

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LHRH-immunoreactive (LHRH-I) fibers have been shown by immunocytochemistry to extend into the pituitary neural lobe in bats, ferrets, monkeys and humans (Anthony et al., Cell Tissue Res. 236:5, 1984). This projection is minimally represented in rats, in which the vast majority of fibers entering the tuberal region of the hypothalamus appear to terminate in the external zone of the median eminence (ME). In bats virtually all LHRH-I fibers directed toward the ME appear to pass through the internal zone and terminate within the neural lobe. In ferrets and primates, LHRH-I fibers appear to terminate in both the ME and neural lobe. The present study was designed to verify the authenticity of neural lobe LHRH in bats, ferrets and humans by HPLC.

Hypothalamic blocks and neural lobes from 16 adult bats (Myotis lucifugus) and 8 adult ferrets (Mustela furo) were extracted and partially purified with C-18 cartridges. In bats, LHRH immunoreactivity determined by RIA (antiserum R42, Dr. T. Nett) was 58.3 pg/neural lobe and 454.4 pg/hypothalamus; in ferrets, 216.1 pg/neural lobe and 3414 pg/hypothalamus.

On reverse-phase HPLC (C18 column; TFA/acetonitrile gradient), synthetic LHRH added to bat hypothalamic extract eluted as a single peak of LHRH immunoreactivity. In this system, 85-90% of LHRH immunoreactivity extracted from both bat neural lobe and hypothalamus eluted at similar retention times. Similar results were obtained when ferret and human neural lobe extracts were subjected to HPLC. This physicochemical evidence strongly suggests that the fibers identified by immunocytochemistry contain authentic mammalian LHRH. Thus, the neural lobe may be a site of secretion of this neuropeptide in some mammalian species.

382.12 A DETAILED MORPHOMETRIC STUDY OF GONADOTROPHS IN MALE RATS AFTER ELIMINATION OF TESTICULAR NEGATIVE FEEDBACK ON THE BRAIN-PITUITARY AXIS.

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We performed detailed morphometric analyses on immunocytochemically stained LH and FSH cells in normal male rats and in male rats which were decapitated 1, 7, 35 or 90 days after castration (CX). Serum LH and FSH concentrations were measured by radioimmunoassay. In rats killed 1 day after CX, serum LH and FSH concentrations increased 10- and 4-fold, respectively compared to those of the intact controls. These increases in serum gonadotrophin concentrations were not accompanied by any changes in the percentage of pars distalis cells which contained LH or FSH, or in the mean cross-sectional area, the volume density or the numerical density of LH or FSH cells. By 7 days post CX, the percentages of LH and FSH cells and their volume and numerical densities had increased significantly, but the mean cross sectional area and the percentages of cells which had diameters >18μm were unchanged. By 35 days post-CX, the numbers of LH and FSH cells were only slightly higher than those observed in day 7 post-CX, but the mean cross-sectional area and the percentage of gonadotrophs whose diameters >18 μm had increased significantly. Also, there was a slight increase in the frequency of finding signet-ring cells. The numbers and size of gonadotrophs did not increase significantly between 35 and 90 days post-CX, but by the latter time, there was a tremendous increase in the number of signet-ring cells. In all groups of castrated rats, like in the controls, virtually all FSH cells also contained LH. The percentage of LH cells which also contained FSH increased from 88.6% in the controls to 95% in the rats that had been castrated for 90 days.

The results indicate that 1) the dramatic increases in serum LH and FSH concentrations within 24h after CX in male rats occur without a concomittant increase in the numbers or size of the cells and 2) in the post-CX changes in gonadotrophs in male rats, hyperplasia clearly antedates hypertrophy.

Supported by grants from the NIH(HD11011) and the College of Medicine, University of Lagos, Lagos, Nigeria.

382.13 TWO TYPES OF SECRETORY GRANULES IN GONADOTROPHS: DISCRIMINATION BY THE SIMULTANEOUS EM IMMUNOCYTOCHEMICAL LOCALIZATION OF SEROTONIN WITH β-FSH AND β-LH.

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Gonadotrophs of the anterior pituitary gland contain luteinizing hormone (LH), follicle stimulating hormone (FSH), and serotonin (5-HT). Since LH and FSH can differentially be released from the gland, the question arises as to whether or not there are different types of gonadotroph. We have investigated this question using electron microscopic immunocytochemistry. Mouse pituitary glands were fixed by perfusion with a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde. Non-osmicated tissue was embedded in LR White. Sites of immunoreactivity were localized using monoclonal or polyclonal antibodies to β-LH, a rabbit polyclonal antiserum to β-FSH, and a rat monoclonal antibody to 5-HT in association with species-specific secondary antisera coupled to colloidal gold. As a control, somatotrophs were also demonstrated using a rabbit antiserum to human growth hormone. The selection of gold particles of different sizes made possible the simultaneous localization of two antigens in the same tissue section. All gonadotrophs were found to contain both β-LH and β-FSH. No subsets of gonadotroph were found that contained one and not the other. On the other hand, while all secretory granules of gonadotrophs had β-LH immunoreactivity, only about 40% had β-FSH. Increasing the concentration of anti-β-FSH serum did not change this proportion. 5-HT immunoreactivity was found in 9% of the secretory granules of gonadotrophs; however, virtually none of the β-FSH-immnoreactive granules contained 5-HT. Although, the proportion of granules containing 5-HT was low, the incidence of double labeling with antisera to 5-HT and β-FSH was far less than predicted on the basis of chance ($p<0.001$). No immunoreactivity of β-LH, β-FSH, or 5-HT was ever found in somatotrophs. These observations suggest that there is only a single type of gonadotroph in the murine pituitary gland; however, within these cells there appears to be a heterogeneous population of secretory granules. All granules contain LH but only some contain FSH and none of those that contain both LH and FSH also contain 5-HT. Supported by NIH grants NS 12969, AM19743.

382.14 A GONADAL SEX STEROID, 17α,20β-DIHYDROXY-4-PREGNEN-3-ONE, ACTS AS A PHEROMONE TO RAPIDLY INCREASE SERUM GONADOTROPIN AND MILT VOLUME IN MALE GOLDFISH *Carassius auratus*.

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When added to aquarium water at low concentrations, 17α,20β-dihydroxy-4-pregnen-3-one (17,20P) rapidly induces a dramatic increase in milt volume of male goldfish. A pheromonal action of 17,20P on milt volume increase is suggested by the fact that the response to 17,20P is inhibited by olfactory tract section. Furthermore, the ability of injected gonadotropin to increase milt volume suggests that the "pheromonal" effect of 17,20P may be mediated by endogenous gonadotropin (GtH) release. To examine this possibility, mature male goldfish were either hypophysectomized (HYPOX), sham operated, or only handled on Day 1. On Day 2, milt was stripped from all males at 17:00 hrs and 17,20P added to the aquaria (150pg/ml, $5X10^{-6}$M) at 21:00 hrs. At 09:00 hrs on Day 3, milt volume in HYPOX fish (Median= 0.0 μl; n=20) was significantly ($p<0.01$) smaller than in Sham (Median= 79.0 μl; n=20) or Control fish (Median= 49.5 μl; n=20). Following milt removal, each treatment group was divided, half being placed in a spawning situation with prostaglandin-treated females, and half being placed in all-male groups. Two hours after spawning had begun, milt was again removed from all males. Participation in spawning activity elevated milt levels in Control and Sham fish ($p<0.01$) but not in HYPOX fish despite the fact that 8 of 10 HYPOX fish showed vigorous courtship and spawning activity. In a second experiment, addition of 17,20P to aquaria water was found to rapidly stimulate GtH secretion in mature male goldfish. Groups of mature fish were blood sampled under anesthesia following exposure to 150pg/ml of 17,20P for periods of 15 min, 30 min, 1 hr or 2 hr. Control fish were exposed to 100μl of ethanol for 1 hr. Serum GtH contrations were determined by radioimmunoassay using antisera to carp GtH. Exposure to 17,20P for as little as 15 min was effective in elliciting a significant threefold increase ($p<0.01$) in GtH as compared to ethanol exposed controls. Similar elevations in GtH were observed after exposure to 17,20P for 30 min, 1 hr and 2 hr. Following blood sampling, all fish were transfered to fresh flow-through aquaria and milt sampled 7-8 hrs after exposure to 17,20P. The expressable milt volume of all four groups exposed to 17,20P were significantly greater ($p<0.01$) than unstimulated controls. Results from a number of preliminary experiments indicate that (1) exposure to 17,20P increases milt volume within 4 hours, (2) blood GtH levels do not increase in female goldfish exposed to 17,20P, (3) anterior telencephalon lesions which disrupt olfactory input and impair male sexual behavior also block the increase in GtH induced by exposure to 17,20P. As 17,20P is known to increase in the blood of female goldfish undergoing oocyte final maturation, our findings that water-born 17,20P elevates GtH levels and milt volumes in the male suggest that this steroid may function as a primer pheromone to synchronize an increase in male fertility with the time of ovulation. (Supported by the Alberta Heritage Foundation for Medical Research)

382.15 DAMAGE TO THE ARCUATE NUCLEUS DECREASES LUTEINIZING HORMONE (LH) PULSE FREQUENCY IN CASTRATED MALE RATS. C. L. Sisk and A. A. Nunez. Neuroscience Program and Department of Psychology, Michigan State University, E. Lansing, MI 48824

Evidence from ablation and electrophysiological experiments suggests that the arcuate nucleus may generate periodic neural signals for the pulsatile release of LH-releasing hormone (LHRH) from the hypothalamus (Ann. Rev. Phys. 44: 583, 1982; Neuroendo. 35: 63, 1982). This study examined the effect of electrolytic lesions of the arcuate nucleus on pulsatile LH secretion in male rats. All surgeries were performed under anesthesia. Albino rats were castrated at 60 days of age, and 30 days later received either bilateral electrolytic lesions aimed at the arcuate nucleus or no surgery. Following a one week recovery period, all rats were implanted with an indwelling jugular cannula. Two days after cannulation, 0.3 ml blood samples were obtained at 5 min intervals for a 4 hr period. Blood was replaced with an equal volume of rat red blood cells suspended in a plasma protein fraction. Rats with lesions were then perfused transcardially with 0.9% saline followed by 10% formaldehyde. Coronal sections were cut through the hypothalamus, stained with cresyl violet, and microscopically examined to determine the location and extent of the lesions. Plasma concentrations of LH were determined by radioimmunoassay. Hormone pulses were defined as a set of 2 or more elevated LH values in which the coefficient of variation computed on the LH values nadir to nadir was $\geq$3x that of the assay. The frequency of LH pulses in castrated rats without hypothalamic lesions was 1.9 $\pm$ 0.53 pulses/hr. There were no cases in which the lesions resulted in complete destruction of the arcuate nucleus. Some rats sustained partial damage to the arcuate; average LH pulse frequency was 0.89 $\pm$ 0.31 pulses/hr in this group. Other rats had lesions which damaged the posterior hypothalamus and mammillary area, but completely spared the arcuate nucleus; in these animals, LH pulse frequency was 2.0 $\pm$ 0.35 pulses/hr. Mean LH levels were similar in unoperated rats and in rats with no arcuate damage, but were reduced by about one third in rats with partial lesions of the arcuate nucleus. These results are consistent with the hypothesis that the arcuate nucleus is involved in the generation of neural signals for the pulsatile release of LHRH from the hypothalamus. Supported by MH-37877 to A.A.N. and HD-21588 to C.L.S.

382.16 IN VIVO MEASUREMENTS OF CATECHOLAMINES AND LUTEINIZING HORMONE RELEASING HORMONE (LHRH) FROM THE STALK-MEDIAN EMINENCE (S-ME) IN UNANESTHETIZED OVARIECTOMIZED RHESUS MONKEYS. C. Krook*, G. Watanabe*, G.A. Davis and E. Terasawa. Wis. Reg. Primate Research Center, 1223 Capitol Court, Madison, Wisconsin 53715.

Previously, we have reported a method for the measurement of in vivo release of LHRH in conscious monkeys using push-pull cannula inserted into the S-ME. The present study reports a further application of this method to collect samples for simultaneous measurements of the neuropeptides and catecholamines. This system also allows repeated sampling of specimens from various locations of the S-ME in unanesthetized rhesus monkeys. Several months prior to the experiment 10 ovariectomized monkeys were implanted with a cranial pedestal under halothane anesthesia. Using X-ray ventriculography, the center of the pedestal was placed above the infundibular recess at the midline level and was used for a reference point. Three days before the perfusion, a push-pull cannula composed of an outer cannula (22 ga) with stylet was inserted into the S-ME with a hydraulic microdrive unit (Narishige M-95B, Tokyo) under ketamine anesthesia. This unit allowed positioning of the cannula along the X,Y,Z coordinates. Cannula placement was verified by X-ray. The animal was then placed in a primate chair. The animals were well adapted to the chair, fully conscious and able to take food and water. Three days later sample collection was begun and continued for 12-18 hrs. The stylet was replaced with an inner cannula (28 ga), and modified Krebs-Ringer solution was perfused through the inner cannula and perfusate was collected from the outer cannula at 20 µl/min by two identically calibrated peristaltic pumps. Ten minute fractions were collected continuously on ice, they were aliquoted to two vials (150 µl and 50 µl) and stored at -40°C. LHRH in samples treated with Bacitracin were measured by RIA, and catecholamines and their metabolites in samples treated with perchloric acid were measured by HPLC with electrochemical detection. Results are: 1) As we reported previously, LHRH release was pulsatile, 2) with our HPLC system norepinephrine (NE), dopamine (DA), dihydroxyphenyl glycol (DOPEG), methoxy hydroxyphenyl glycol (MOPEG), dihydroxybenyl acetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) were detected in perfusates, although contents and their quantities varied among animals and cannula location in the S-ME, 3) application of amphetamine 10^{-6}M through the push cannula stimulates the release of NE and DA as well as their metabolites, and 4) application of NE 10^{-6} through the push cannula resulted in an increase in MOPEG and DOPEG, while the application of DA 10^{-6}M resulted in an increase in DOPAC and HVA. Altogether, our method presented here is a useful tool for neuroendocrine research in vivo in nonhuman primates. (Supported by grants RR-00167, HD-11355 and HD-15433.)

382.17 INTERACTIONS BETWEEN CATECHOLAMINE AND OPIOID SYSTEMS ON NEUROSECRETION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) IN VITRO. M.J. Gallagher, R.W. Clough, R.E. Watson, G.E. Hoffman, S.J. Weigand and C.D. Sladek. (SPON: K.M. Skoog) Depts. of Neurobiol. and Anat. and Neurology, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Phasic gonadotropin secretion, characteristic of the female estrous cycle, occurs as a result of cyclic increases in the pulse amplitude and frequency of LHRH neurosecretion into the median eminence capillary plexus. Previous studies have demonstrated that the neurochemical control of LHRH secretion in vitro involves norepinephrine (NE). Since it has been demonstrated that phasic gonadotropin secretion in females can be acutely inhibited by opiate compounds, such as beta-endorphin, dynorphin, and met-enkephalin (m-ENK), the present studies were designed to determine if opioid systems interact with NE on the neurosecretion of LHRH in vitro. Additionally, we examined the immunocytochemical distribution of m-ENK in the preoptic area of the hypothalamus (POA) as this region exhibits neuromorphological sexual dimorphisms and is essential in the neural control of phasic gonadotropin secretion in female rats. Study #1: Female rats were decapitated, brains were excised and POA-mediobasal hypothalamus (POA-MBH) explants were placed into individual 500 µl incubation chambers. Six microchambers were perifused simultaneously with warmed and oxygenated culture medium at a flow rate of 20 µl/minute. Effluents of the chambers were collected in 15 minute fractions and LHRH concentration was measured by specific radioimmunoassay. The POA-MBH explants were exposed to medium containing the opiate antagonist naloxone (Nal), NE, or a combination of Nal and NE, in pulses of 30 minute duration. Nal alone had no observable effect on LHRH neurosecretion and NE had a slight but significant stimulatory effect, as previously reported (Clough et al, SN Abstracts 11:379.4, 1985). In the presence of Nal, in contrast, NE had a dramatic stimulatory effect on LHRH neurosecretion in vitro. Study #2: Male and female pairs of rats were sacrificed by perfusion and their brains prepared for the immunocytochemical localization of m-ENK using the avidin-biotin technique. A striking sex difference was observed in the density of immunoreactive (ir) m-ENK fibers situated in the periventricular POA (pePOA). Females exhibit a dense plexus of ir fibers in the pePOA while the male pePOA does not show evidence of fiber ir above that present in the surrounding medial POA. Results of Study #1 suggest that opiate systems partially inhibit the stimulatory effects of NE on the neurosecretion of LHRH in vitro. Further studies are required to determine if the m-ENK sexual dimorphism in the pePOA represents a neural substrate through which the opiate systems and NE can interact in the control of LHRH neurosecretion. Supported by PHS grants MH-09136 and HD-18418.

382.18 EVIDENCE FOR DUAL SITES OF ACTION FOR NEUROPEPTIDE Y IN STIMULATION OF LUTEINIZING HORMONE RELEASE. W.R. Crowley, A. Hassid*, and S.P. Kalra, Dept. of Pharmacology, University of Tennessee, Memphis, TN 38163 and Dept. of OB/GYN, University of Florida, College of Medicine, Gainesville, FL 32610.

Recent evidence indictes that central administration of the 36 amino acid neuropeptide Y (NPY) decreases LH secretion in ovariectomized, hormonally untreated rats but increases LH release when given to ovariectomized rats pretreated with ovarian hormones. The mechanisms by which NPY affects LH secretion are unknown. The present studies were designed to investigate whether NPY alters the release of LH-releasing hormone (LHRH) from medial basal hypothalamus (MBH) and whether NPY acts directly at the pituitary gland. In the first experiment, individual MBH fragments (arcuate nucleus plus median eminence) were incubated in Krebs-Ringer-bicarbonate-glucose medium for a 15 min preincubation, followed by a 30 min "basal" period and a second 30 min "stimulation" period with NPY (10^{-6}M) or control. In tissues from ovariectomized rats, NPY had no effect on LHRH release. However, in tissues from rats treated with estradiol or estradiol/progesterone, NPY produced a significant stimulation of LHRH release. In a second set of experiments, elevated KCl (28 mM or 56 mM) was included in the second 30 min. In tissues from ovariectomized, hormonally untreated rats, elevated KCl produced a dose-dependent increase in the release of LHRH. NPY (10^{-6}M) had no effect on basal release but significantly enhanced the release induced by 28 mM KCl. The KCl-induced stimulation of LHRH was reduced using MBH from rats treated for 2 days with a low dose of estradiol. However, this inhibition of LHRH release was reversed by NPY (10^{-6}M) in vitro. A third study tested the effects of NPY alone or in combination with LHRH on the release of LH from anterior pituitry cells. Anterior pituitary glands from ovariectomized rats were minced and the cells dispersed by collagenase/hyaluronidase. The dispersed cells were cultured for 72 h, and were exposed during a 3 h incubation to LHRH, NPY, or to the two peptides together. LHRH produced a dose dependent increase in LH release, while NPY by itself had no effect. However, NPY (10^{-9}M, 10^{-8}M) significantly enhanced the release of LH in response to low concentrations of LHRH. The same results were obtained using hemipituitary fragments. These results suggest that NPY can act both centrally, to enhance the release of LHRH from the hypothalamus, and also at the level of the pituitary gland, to augment the response to LHRH.

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382.19 CHOLECYSTOKININ INHIBITS K+ STIMULATED LHRH RELEASE FROM MALE AND FEMALE RAT HYPOTHALAMI IN VITRO. P.E. Micevych, A.M. Babcock*, D.W. Matt* and J.K.H. Lu*. Dept. of Anatomy and the Lab. of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Recently we and others have reported that tissue levels of cholecystokinin (CCK) are sexually differentiated in several distinct hypothalamic and limbic system areas. Indeed, the number of CCK cell bodies is greater in male rats in the medial amygdala, bed nucleus stria terminalis and medial preoptic nucleus. These areas constitute an interconnected, sexually dimorphic, steriod sensitive system that has been implicated in the regulation of gonadotropic secretion.

Intracerebroventricular injections of cholecystokinin (CCK) has been reported to inhibit LH release in ovariectomized (OVX) rats. In addition, CCK implants into the medial preoptic area in OVX, estrogen-primed rats stimulate LH release. CCK does not directly alter pituitary LH release, suggesting that the effects are mediated by a regulation of LHRH within the hypothalamus. Since the release of LHRH is regulated by gonadal steroids and CCK appears to modulate estrogen-dependent events (Bloch et al., Neurosci. Abstr., 12, '86), we investigated the effects of CCK on the release of LHRH from hypothalami of male, OVX female, and estrodiol benzoate treated (50 µg) OVX female rats.

Following decapitation, hypothalami were dissected and placed into separate superfusion chambers. Each hypothalamus was superfused with an oxygenated Krebs-Ringer-Hepes buffer (KRH) containing dextrose and bacitracin at a flow rate of 300 µl/min. Twelve 10 min fractions were collected following a 60 min washout period. In the sixth fraction, 50mM KCl was used to stimulate LHRH release. In the male hypothalami, the basal efflux of LHRH (26.8 ± 3.4 pg/10 min) was not altered by KRH containing 10^{-6}M sulphated CCK-8 (24.3 ± 1.9 pg/10 min). However, CCK attenuated K+ stimulated release of LHRH by 40% from male hypothalami as determined by area under the curve analysis ($p<.03$). Similarly, CCK did not alter the basal efflux of LHRH from OVX and OVX estrogen-treated rat hypothalami. The K+ release of LHRH from female hypothalami was dramatically inhibited by CCK with the most profound inhibition observed in hypothalami from OVX females. These findings indicate that CCK inhibits K+ stimulated LHRH release from male and female hypothalami and this effect is enhanced by OVX. These results are consistent with the hypothesis that estrogen modulates the function of the CCK system. Additionally, we have demonstrated that estrogen inhibits CCK binding in the hypothalamus. Thus, CCK may play a role in the inhibition of LHRH in both male and female rats and estrogen may modulate this inhibition.

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382.20 REDUCED HYPOTHALAMIC SEROTONIN SYNTHESIS AND SERUM LH LEVELS IN STREPTOZOTOCIN-DIABETIC MALE RATS. T.S. King, D.H. Rohrbach*, A.L. Miller and W.W. Morgan. Dept. Cellular & Structural Biology and Psychiatry, Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284

Infertility is a common sequela to experimentally-induced diabetes mellitus in the male rat as well as to diabetes in the human male. This has been attributed both to alterations in hypothalamo-pituitary and pituitary-testicular activity. Commonly, circulating LH and testosterone levels are decreased which undoubtedly account for the reported reduction in or absence of spermatogenesis and reduction in accessory sex organ weights. Considering the role of serotonin (5-hydroxytryptamine:5HT) in the hypothalamic regulation of LH secretion, we sought to determine the effects of streptozotocin (STZ)-induced diabetes on hypothalamic 5HT synthesis relative to the effects of STZ-induced diabetes on circulating levels of LH, FSH and PRL. Half of a group of adult male Sprague-Dawley rats were injected i.p. with 16 $mg \cdot kg^{-1}$ of STZ. The other half received vehicle only. The diabetic group were hyperglycemic throughout the study period (488.7±21.2 $mg \cdot dl^{-1}$ in diabetic rats vs. 125.1±39.3 $mg \cdot dl^{-1}$ in control rats: $\bar{X}$ ± SEM). A third group of rats served as semi-starved controls, weight-matched by total calorie restriction diet to diabetic rats. All of the rats were injected i.p. with 200 $mg \cdot kg^{-1}$ of NSD-1015 thirty minutes prior to sacrifice. Accumulation of 5-hydroxytryptophan in various hypothalamic areas was then assayed by LCED as a relative index for the rate of 5HT synthesis. Serum LH, FSH and PRL were assayed by RIA. Our results showed a 50% decrease in 5HT synthesis in the pre-optic area-anterior hypothalamus (POA-AH) and mediobasal hypothalamus-median eminence (MBH-ME) 16 weeks after STZ treatment. We also found that 5HT synthesis was inhibited in the POA-AH, but not MBH-ME, 4 weeks after STZ treatment, indicating region-specificity in the early hypothalamic response to STZ-induced diabetes. Serum LH levels were decreased both 4 and 16 weeks after STZ treatment. No changes either in hypothalamic 5HT synthesis or in serum LH levels were observed 1 week after STZ treatment. Serum FSH and PRL levels remained unaffected by STZ treatment throughout the study period. The lack of an effect of diabetes on FSH in contrast to LH may represent further evidence for the hypothesis of differential regulatory mechanisms governing the secretion of gonadotrophins and further emphasizes the pathophysiological specificity (i.e., not a generalized metabolic disturbance effect) of diabetes-induced male reproductive neuroendocrinopathy. Our study suggests a potential mechanism for such pathological interaction, namely, diabetic inhibition of at least one region-specific neurotransmitter system integral to hypothalamic regulation of LH secretion. Supported by NIH grant RR07187 (TSK), JDF grant 184418 (DHR) and NIH grant HD 10202 (Neuroendocrine Core).

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM III

383.1 LOCALIZED CALCIUM TRANSIENTS IN CEREBELLAR PURKINJE CELL DENDRITES IN GUINEA PIG BRAIN SLICES. W.N. Ross and R. Werman*, Department of Neurobiology, Hebrew University, Jerusalem, Israel.

Calcium transients were optically detected at many positions on Purkinje cells by measuring absorbance changes at 640±30 nm with 10x10 photodiode array from cells which had been injected with arsenazo III. With this method we could directly examine non-uniformities in calcium entry in different regions of the dendritic field. The calcium indicator and Lucifer Yellow were simultaneously iontophoresed into the somata of cells in sagitally cut cerebellar slices about 200 um thick. After the dyes diffused throughout the dendrites and axon, the cell was stimulated intrasomatically through the same electrode or by activating climbing fiber input with bipolar electrodes on the white matter. Each element of the array detected changes from an area of 25x25 to 40x40 μm^2 in the plane of the slice. In a typical experiment absorbance changes were measured by averaging 50 to 100 trials which were digitized with 1 msec time resolution. Signals were mapped onto positions on the cell using photographs of the Lucifer Yellow fluorescence.

When intrasomatically stimulated absorbance changes were usually detected all over the dendritic field of the cells. The rise time of the transients corresponded to the time of the slow calcium spikes with no absorbance increase during the burst of sodium dependent spikes. Absorbance transients also persisted in TTX. These experiments, together with the properties of the dye, show that we were detecting calcium transients due to voltage dependent entry of calcium into the cell.

In some experiments the rise time of the transients was essentially identical at all positions. In other experiments the rise times in different regions were separated by as much as 13 msec, too long to be due to a propagation delay. This suggested that the excitation of the dendritic field was non-uniform. Also, in other experiments, calcium transients were confined to only a part of the dendritic field even though the Lucifer Yellow fluorescence suggested that the coinjected arsenazo III was uniformly distributed.

Calcium transients were also associated with the slow depolarizing after-potential which often followed intracellular stimulation. The distribution of these transients was sometimes different from the distribution of transients due to the stimulated action potentials. We could also detect calcium transients from climbing fiber stimulation. The distribution of these synaptically evoked transients was usually confined to the dendritic field near the soma.

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383.2 HISTOCHEMICAL LOCALIZATION OF 5' NUCLEOTIDASE IN THE CEREBELLUM OF THE REELER MUTANT MOUSE. Leonard M. Eisenman. Department of Anatomy, Jefferson Medical College, Philadelphia, PA 19107.

Many different experimental procedures have been employed to demonstrate the heterogeneity of the cerebellar cortex in the normal animal. One of the initial demonstrations utilized a histochemical staining procedure for the enzyme 5' nucleotidase in the cerebellum of the mouse (Scott, 1963). The distribution of this enzyme was seen to be localized in parasagittal bands within the molecular layer. A more recent study confirmed this finding in the normal mouse and in addition examined the distribution of this enzyme in a number of neurological mutants, including homozygous weaver, nervous and Purkinje cell degeneration mice (Hess, et al., 1983). Parasagittal banding of the 5' nucleotidase was observed in the weaver, but not in the nervous or Purkinje cell degeneration mice. The latter two mutants have greatly depleted Purkinje cell (PC) populations, suggesting a possible link between groups of PC dendrites and the enzyme.

We have examined the distribution of this enzyme, 5' nucleotidase, in the adult homozygous reeler mutant mouse (rl/rl), in which the majority of PCs are ecotopicly located in the white matter of the cerebellum. The results indicate that there is differential staining of the cerebellum with most of the more heavily stained regions associated with clusters or parts of clusters of PCs. Both normally positioned PCs and ectopicly located PC clusters demonstrate differential staining. This is most evident in the vermal region of the cerebellum. The intensity of staining is generally much lower in the hemisphere. In vermal areas where differential staining is evident, a band pattern continues through a number of rostrocaudal sections but is difficult to follow for any great distance.

These results in rl/rl support the findings of Hess et al. (1983) in suggesting an association between the localization of 5' nucleotidase and certain populations of PCs. In addition, they also demonstrate that heterogeneity with regard to this enzyme is also evident in ectopic PCs. This heterogeneity appears to have some features in common with olivocerebellar projection in this mutant as studied with anterograde tracings technique.

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383.3 AUTORADIOGRAPHIC TRACING STUDY OF THE OLIVO-CEREBELLAR PROJECTION IN THE ADULT STAGGERER MUTANT MOUSE. Gene J. Blatt and Leonard M. Eisenman. The Salk Institute, La Jolla, CA 92037 and The Daniel Baugh Institute, Dept. of Anatomy, Jefferson Medical College, Philadelpha, PA 19107.

One of the most fascinating, yet still unexplained, phenomena in developmental neurobiology is how afferent fibers find and contact appropriate target cells. In some systems such as the olivocerebellar projection, the afferents organize in a very precise manner. In the normal animal, climbing fibers (CF) from particular regions of the inferior olivary complex (IO) distribute into specific orthogonally-oriented compartmental zones in the cerebellar cortex. The climbing fibers terminate on the soma and primary dendrites of their main target cell, the Purkinje cell (PC). It is not known what factors are responsible for such a precise distribution of these olivocerebellar afferents. There are many possibilities such as chemical factors, timing of arrival of afferent and target, recognition factors and target cell position and number. What would the distribution of CF afferents be like in an animal that has (1) a severe reduction of PCs, and (2) many target cells (i.e., PCs) in ectopic positions? We examined such a problem in the adult homozygous staggerer mouse (sg/sg). The staggerer is characterized by a cerebellum of less than one-third normal size, lacks most cerebellar granule cells, and has only 10-25% of the normal complement of PCs, many of which are ectopic. In addition, the staggerer inferior olivary complex contains only approximately 40% of the normal number of cells.

Using the ventral (retropharygeal) approach to the IO, we injected 8-10 µCi of tritiated leucine in specific regions of the IO in five sg/sg mice. As is observed in the normal mouse, CFs in sg/sg terminate in a series of orthogonal zones. Also, the projection appeared to be contralateral and had a similar topographic organization to that seen in the normal mouse: (1) the caudal-intermediate medial accessory olive projects to the vermis, (2) the principal olive and dorsal accessory olive projects to the paravermis, and (3) portions of the intermediate-rostral medial accessory olive and principal olive projects to the hemisphere. Similar results were previously seen in two other varieties of neurological mutants, the reeler and weaver which also have a reduced number and ectopic PCs. Thus in three mutants with varying degrees of PC abnormalities, the results indicate that final target cell position does not play a critical role in olivocerebellar distribution. Rather, as long as some target cells remain (as few as 10-25% in sg/sg), CFs are able to find target cells in the correct cerebellar region.

383.4 DISTRIBUTION OF MUSCARINIC RECEPTORS IN DEVELOPING AND MUTANT RODENT CEREBELLUM. A. Neustadt*, A. Frostholm and A. Rotter. Department of Pharmacology, University of California, Irvine, CA 92717.

Previous autoradiographic studies, using [^{3}H] propylbenzilylcholine mustard (PrBCM) to label muscarinic acetylcholine receptors, showed them to be localized exclusively over cerebellar lobules 9 and 10 (Rotter, A. Brain Res. Rev. 1:167, 1979). The present studies using [^{3}H] quinuclidinyl benzilate (QNB), show moderate levels of grain density throughout all cerebellar lobules of both rats and mice. Within the adult rat cerebellum [^{3}H] QNB binding sites appear to be highest in the granule and Purkinje cell layers, with a distinctly higher level of binding in the Purkinje cell layer. Adult mouse cerebellum shows a uniform region of moderate to high binding over the granule cell layer, with no notable increase over the Purkinje cell layer. Particularly high levels of [^{3}H] QNB binding were observed in lobules 9 and 10 (uvula and nodulus) of the rat cerebellum, a distinction not observed in mouse. No labeling was observed over the white matter. In developing mouse, at postnatal day 1, moderate to dense levels of receptors were observed throughout the entire cerebellum. By day 3, a reduction in grain density over the external granule cell layer became observable; by day 5 the internal granule cell layer, in addition to all other cerebellar structures, was clearly of higher density than the external layer. At days 9-11 the white matter and the thin molecular layer between the internal and external granule cell layers, were clearly identifiable; both were of low to moderate grain density. In 13-17 day mice, autoradiographic grains became increasingly localized over the internal granule cell layer, with only low density visible over the molecular layer. Moderate labeling over all regions of white matter was maintained. The adult distributions were attained between 21 and 30 days of age. Older animals (80 to 100 days) showed a marked decrease in grain density, with only low levels of [^{3}H] QNB labeling observed over the granule cell layer. Muscarinic receptor distribution in neurological mouse mutants suggested no significant decrease in receptor levels. Purkinje cell degeneration (pcd), a mutant in which there is an almost complete degeneration of Purkinje cells, displays essentially equal levels of grain density to the control littermate. Weaver (wv) a mutant in which granule cells are unable to migrate and which subsequently degenerate, actually displays higher levels of binding in most lobules than its control littermate. Although muscarinic receptors appear to be associated with the granule cell layer in adult mice, our developmental and mutant studies suggest that they are not located on granule cells per se. While a variety of postsynaptic locations may be hypothesized, the possibility of a presynaptic localization on afferent mossy fibers cannot be excluded.

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383.5 THE EFFECTS OF DORSAL RAPHE STIMULATION ON GRANULE CELL SPONTANEOUS ACTIVITY IN THE RAT CEREBELLUM. D.L. Armstrong, C. McNair, M. Hay, D.M. Terrian, and N. Velez. Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, TX 78285.

Anatomical studies have indicated that serotonergic efferents of the brain stem raphe nuclei contribute to the mossy fiber system innervation of the cerebellar granule layer, however, the physiological characterization of this synaptic input has not been defined. The present study was designed to determine the effects of raphe stimulation on granule cell unit activity. Stimulating electrodes positioned within the dorsal raphe (DR) were used to apply single square-wave pulses (0.1-0.5 ms duration) or pulse trains (50 Hz) in urethane anesthesized rats. Unit recording was carried out with micropipettes filled with 2 M NaCl saturated with fast green.

Of the 87 granule cells sampled 13% responded consistently to stimulation with the primary effect being a decrease in firing rate. Threshold stimulus intensity ranged from 50 to 100 uA and duration of effect ranged from 60 to 500 msec. After sampling various regions it was found that the probability of finding responsive cells was greatest in the lateral vermis and medial lateral hemispheres.

To verify that the observed responses were indeed being mediated via a direct serotonergic pathway recordings were also carried out using multibarrel pipettes. Iontophoretic application of 0.5 M 5-HT creatinine sulfate produced a decrease in the firing rate of cells that were similarly affected by DR stimulation. The 5-HT receptor blockers, cyproheptadine HCl and methysergide maleate were not effective in blocking stimulation-induced effects. The low affinity of these agents for 5-HT$_1$ receptors thought to mediate serotonin's inhibitory effects is one possible explanation for this finding and additional serotonergic agents are being tested.

Although the percentage of responsive cells is not robust the results of this study demonstrate that DR stimulation does modulate granule cell activity. The findings support the anatomical evidence of serotonergic mossy fiber terminals and the recent demonstration of a high affinity 5-HT uptake mechanism in isolated cerebellar glomeruli (Terrian et al, Brain Res Bull 14, 1985).

383.6 CLIMBING FIBER ORGANIZATION OF CRUS II AND THE PARAFLOCCULUS OF CAT CEREBELLUM. Lee T. Robertson, S.A. Elias*, and M.E. Cummins*. Neurological Sciences Institute, Portland, OR 97209.

This study examines the climbing fiber organization of Crus II and the underlying paraflocculus as part of a long-term project to determine how the body surface is represented in the cerebellar cortex. Previous work by Welker and associates on the mossy fiber input identified only a large representation of the face in Crus II, which was described as a fractured patchy-mosaic. Little information is available on the organization of the climbing fiber representation for either Crus II or the paraflocculus.

The data were obtained from 8 cats anesthetized with sodium pentobarbital. Extracellular recordings were made of individual Purkinje cells and the climbing fiber responses were elicited by cutaneous (punctate force of or less than 2.0 g) and deep (higher punctate forces or movements of the limb) tactile stimulation of the body surface. Receptive fields were carefully delineated with von Frey hairs. Histological reconstructions were made for each electrode penetration.

In Crus II, 430 Purkinje cells were isolated, of which only 25% had climbing fiber responses that were elicited by tactile stimulation. The responsive units were mainly ipsilateral, with 51% representing the face, 44% representing the forelimb, and 2% representing the hindlimb. No representations of the proximal or axial body areas were encountered. Most responsive units were interspersed amongst unresponsive units, although small patches of face or forelimb representations were occasionally encountered. Most responses (80%) of the face representation were evoked by low threshold stimulation, whereas 85% of the responses representing the forelimb required deep stimulation. The receptive fields of the face were usually small and limited to areas such as the chin, lip, vibrissae, nose, or cornea; almost all the forelimb receptive fields were distal.

In the paraflocculus, 186 cells were isolated and 22% had climbing fiber responses elicited by tactile stimulation. Most (82%) of the responses represented the ipsilateral, distal, forelimb; 15% represented the hindlimb; and 3% represented the face. The responses were about equally elicited by cutaneous and deep stimulation and were found intermingled among unresponsive units.

These data indicate in Crus II a climbing fiber representation of several body areas but less dense than has been reported for the mossy fiber system. Also provided, is the first evidence of a modest forelimb representation in the paraflocculus. (Supported by NIH grant NS 18242)

383.7 DISCRETE RECEPTIVE FIELDS AND PHYSIOLOGICAL PROPERTIES OF NEURONS WITHIN THE BASILAR PONS: CORRELATION BETWEEN DIRECT PERIPHERAL AND CORTICAL INPUTS

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Recent studies in our laboratory have demonstrated a convergence of peripheral (dorsal column nuclei) and cerebral cortical inputs to the basilar pontine nuclei (BPN) in the rat. In the current series of electrophysiological experiments we have mapped the receptive fields of BPN neurons and investigated certain of their physiological properties. Bipolar concentric stimulating electrodes were implanted in discrete areas of motor, sensory, visual and auditory cortices as well as under the skin of the forepaws, hindpaws and the vibrissal pads. The basilar pons was exposed by a ventral surgical approach under halothane anesthesia and the recording electrode positioned within the BPN ipsilateral to the cortical stimulation sites. Conventional electrophysiological recording and stimulation apparatus was used including a computer for building on-line histograms.

Data from 262 BPN neurons (37 rats) were analyzed and a partial map of BPN receptive fields has been constructed. It appears that the peripheral sensory input to BPN neurons is somatotopically organized within the medial portion of the caudal pons such that information from the hindlimb terminates most ventrally followed by forelimb and face areas more dorsally. The receptive field of each neuron was confined to small areas of skin in the case of cutaneous stimulation, or single joints following activation of deep receptors. These BPN neurons were identified as projection neurons by antidromic stimulation of cerebellum. Many of these same neurons also responded to stimulation of single or multiple areas of the cerebral cortex. Moreover, electrical stimulation of visual and auditory cortices activated cells in rostral areas of the pons.

With regard to the physiological properties of BPN neurons, it was observed that BPN neurons followed high frequency (30 Hz) stimulation. A response profile of such neurons to graded intensities and frequencies of stimulation has been compiled. Inspection of post stimulus time histograms following stimulation of selected locations within the cerebral cortex revealed an excitatory epoch of 5 - 10 msecs. followed by an inhibitory phase ranging in duration from 50 - 80 msecs. The inhibition persisted at stimulation intensities below the threshold for excitation. These observations suggest that active inhibition of BPN neurons arises subsequent to cerebral cortex stimulation. Such a suggestion is supported by previous work from our laboratory which has demonstrated that a population of GABAergic neurons resides within the BPN and that certain afferent projections to the BPN are GABAergic. Whether the inhibition observed in the present study is derived from an intra- or extrapontine circuit remains to be determined.

These data indicate that BPN neurons have discrete, somatotopically organized peripheral sensory inputs, thought to originate largely from the dorsal column nuclei. Convergence of these peripheral inputs with descending cortical afferents, as well as the combination of excitatory and inhibitory inputs suggest that BPN neurons may be involved in complex sensorimotor integrative functions. *Grants DA-2888, NS-12644, Biol. Hum. Found.*

383.8 ANALYSIS OF THE DYNAMICS OF ACTIVITY IN ENSEMBLES OF NEURONS RECORDED SIMULTANEOUSLY IN CEREBELLAR CORTEX. G.J.Carman, B.Rasnow*, and J.M.Bower, Divisions of Biology and Physics, Caltech, Pasadena, CA, 91125.

Simultaneous extracellular recordings made from groups of single neurons provides the opportunity to investigate both the distributed spatial and temporal representations of sensory information as well as the dynamical behavior of these neurons. However, conventional analysis techniques for neural spike train data most of which are based on point process statistics, are inherently unsuitable for studing neural dynamics. Accordingly, it is necessary to develop new analysis procedures.

We report here the results of an approach that applies dynamical methods to data obtained by recording simultaneously from multiple cerebellar Purkinje cells using methods previously described (Bower and Linas, Soc. Neurosci. Abstr., 9:607, 1983). This analysis is based on representing the activities of the N single neurons at a time t as a state $S(t)=(n_1,n_2,n_3,\ldots,n_N)$ where $n_i=1$ or 0 depending on whether cell i fired at time t or not. Using this convention, the instantaneous activity of N neurons has a geometrical interpretation as a single point in an N dimensional state space. The time series $\{S(t_1),S(t_2),S(t_3),\ldots\}$ can then be analyzed to determine the subset of the 2^N possible states which are occupied, as well as the subset of a combinatorially large number of cycles or trajectories which are followed. To aid in visualization, we are using the technique of simulated annealing (c.f., Carman and Van Essen, Soc. Neurosci. Abstr., 11:1243, 1985) to map the occupied regions of the N-dimensional space onto a space of reduced dimensions while preserving specific topological aspects of the original space (e.g., the Hamming distance within a neighborhood).

Preliminary results from this analysis technique reveal clear differences in the global activity of the same ensemble of Purkinje cells under different physiological conditions. We are analyzing these results to determine the functional significance of these changes in network activity.

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383.9 SEX STEROIDS MODULATE MOTOR-CORRELATED INCREASES IN CEREBELLAR DISCHARGE. Mohammad Sadeq*, Sheryl S. Smith and John K. Chapin (SPON: L.B. Hersh). *Dept. of Cell Biology and Anatomy, UTHSC, Dallas, TX 75235.*

In previous studies, we have shown that the sex steroids, 17β estradiol (E_2) and progesterone (P), exert opposite effects on neuronal excitability in the urethane-anesthetized, ovariectomized rat; E_2 increases the glutamate (GLUT) response of cerebellar Purkinje (P) cells, while P decreases GLUT but increases GABA responses of these neurons. In the present study, we utilized a behavioral paradigm employing treadmill locomotion in order to test sex steroid neuromodulatory effects on a naturally evoked excitation of Purkinje cells in awake behaving animals over the various stages of the estrous cycle. Adult, cycling female rats implanted with headstage and microdrive units were trained to walk on a computer-controlled treadmill apparatus (10 sec on every 20 sec for 2 h) during recording of single Purkinje neurons in the vermal area of the anterior cerebellum. Vigorous increases in the firing rate of individual units were found to be correlated with movement of specific limbs in particular stages of the step-swing cycle during treadmill locomotion. Both spontaneous and motor-evoked discharge of individual P neurons were monitored before and after i.p. injection of either E_2 (300 ng/kg) or P (50 μgs). (Vehicle: 0.01% propylene glycol-saline, pH 7.4). The percent increase in firing rate during locomotion versus rest was determined as a measure of the evoked/spontaneous discharge ratio. Drug-induced changes in this ratio indicate modulatory rather than simple excitatory or inhibitory effects. For the 6 neurons tested, E_2 augmented the movement evoked discharge by a mean 117% over pre-E_2 control levels for the same neurons. Nevertheless, both the spontaneous and motor-evoked discharges of these cells were increased. Initial onset for this effect was 15 min, with a peak response noted at 30-35 min post-steroid. By 60-90 min, a partial recovery of evoked/spontaneous ratio was noted (50% decrease from the peak at 30-35 min post-steroid), although absolute increases (400-1000%) in both parameters were still observed, indicating long-term effects on neuronal activity. These effects were independent of the stage of the estrous cycle. In contrast, P decreased absolute firing rates of P cells during stationary and locomotor phases, by 30% and 50%, respectively. The evoked/spontaneous ratio was decreased to an even greater degree ($\bar{X}$=70%). Latency for this effect was 9 - 12 min, with recovery to control levels of response seen at 30 min post-steroid. A second injection of P at this time reduced the evoked/spontaneous ratio by a mean 65%, accompanied by minimal increases in absolute spontaneous and evoked discharge rates. Recovery to control levels of response was observed by 45 min post-steroid. This response was typical of 6 out of 6 cells tested on estrus and 5 of 6 cells tested on diestrus 1. Cells tested on proestrus or diestrus 2, when E_2 levels are increasing, were not modulated by P using the above paradigm. These results strengthen the hypothesis that circulating sex steroids may alter neuronal responsiveness during behavior: E_2 increasing and P decreasing excitatory evoked discharge relative to spontaneous discharge, effects consistent with steroid effects in the anesthetized rat. However, in the case of P, this neuromodulatory effect is dependent upon the prevailing steroidal milieu. *(Supported by MH09010 to SSS and BNS-841979 to JKC)*

383.10 NEURONAL RESPONSES IN THE GOLDFISH CEREBELLUM TO ELECTRICAL STIMULATION OF THE OPTIC AND VESTIBULAR NERVES, AND TO FULL-FIELD OPTOKINETIC ROTATIONS. J.F. McGurk and W. Graf. The Rockefeller University, New York, NY 10021.

Single cell activity was recorded in the corpus cerebelli of the goldfish following electrical stimulation of the optic and vestibular nerves with single shocks and trains of pulses, and during optokinetic stimulation with a three-axis planetarium projector. Post-stimulus time histograms of 56 cells in the dorsal and ventral corpus cerebelli revealed convergence of visual and vestibular inputs to various degrees following electrical stimulation of the optic and vestibular nerves. 28% of the cells responded to stimulation of the optic nerve and both the contralateral and ipsilateral vestibular nerves, while another 28% responded to stimulation of the optic nerve and either the contralateral or ipsilateral vestibular nerve. 21% were activated by optic nerve stimulation only. The remainder (23%) received input from one or both vestibular nerves only. The responses varied in latency, pattern and duration. Cells exhibited periods of increased or decreased firing frequency beginning 16 to 120 ms after stimulation and extending from 32 to 1600 ms.

Cells in the dorsal and ventral corpus cerebelli exhibited responses to full-field optokinetic rotations. The planetarium was either oriented vertically (horizontal increment motion), or horizontally for stimuli in vertical planes (22.5 deg intervals measured off the midsagittal plane). Response types of spatial tuning were defined according to the planetarium orientation which produced the deepest modulation (preferred axis). Three rough cell classes could be distinguished. Preferred axis orientations were either vertical, or around 45 deg (between 22.5-67.5 deg) and around 135 deg (between 112.5-157.5 deg) in the horizontal plane. Although rotations about the preferred axis produced strong responses, rotations about the other axes elicited weak responses. Some cells displayed bursts of increased firing and pauses during optokinetic stimulation, while others responded with a uniform increase during rotation in one direction and a decrease in firing for rotation in the opposite direction. Neuronal responses were also speed sensitive. Some cells were modulated optimally by rotations of 1 deg/sec while others had more pronounced responses at speeds of 2 or 3.5 deg/sec. The spatial orientation of the three response types bears some resemblance to the spatial orientation of the semicircular canals and the extraocular muscles. However, no quantitative measurements on these parameters are available for the goldfish.

In summary, the cerebellum of the goldfish is one site of interaction for visual and vestibular inputs indicating its involvement in oculomotor control and other vestibular reflexes.

Supported by NIH grant EY04613.

383.11 ACTIVITY OF CEREBELLAR CORTICAL NEURONS DURING GRASPING OF OBJECTS OF DIFFERENT WEIGHTS AND TEXTURES. Eny Espinoza and Allan M. Smith. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, CANADA, H3C 3J7.

The force used by human subjects to lift and hold an object between the thumb and forefinger has been shown by Westling and Johansson (1984) to vary as a function of the object texture and weight. We have replicated this observation in monkeys trained to lift and hold an object of variable weight and texture. When weights of either 15 or 65 g were used or when surfaces composed of fine emery paper or polished aluminium provided different coefficients of friction, the prehensile forces employed to lift and maintain the object at a fixed height were scaled in approximately equal gradations over the 4 conditions. These changes in both the dynamic and static grip force were accompanied by comensurate changes in the EMG activity of forearm muscles. To date 24/46 cells from culmen-simplex region of the cerebellar cortex have demonstrated activity changes at some time during the grasping and lifting of an object with 2 surface textures and 2 different weights. Of these, 8 neurons were identified by the presence of climbing fiber potentials as Purkinje cells whereas the remaining 21 were unidentified. Fifteen neurons had changes in discharge limited to the dynamic application of grip force whereas 9 other units demonstrated dynamic-static discharge patterns with sustained increases or decreases in activity related to both the dynamic and static grasping. The discharge of some neurons (N = 9) was affected only by the surface texture, others (N = 8) were affected only by the object weight and a third group (N = 7) was affected by both parameters. Cells with proprioceptive and cutaneous receptive fields as well as units with mixed properties have been found, but to date, these properties do not appear to determine whether a neuron will show greater modulation of activity with texture or weight. Nevertheless, the cerebellar cortex would appear to be directly involved in adjusting grip forces to compensate for object slippage between the fingers.

This research was supported by the Medical Research Council of Canada Group in Neurological Sciences.

383.12 TECHNIQUE FOR EVALUATION OF PURKINJE CELL ACTIVITY DURING CLOSED-LOOP VISUALLY GUIDED MOVEMENT REQUIRING CONTINUAL FINE MOVEMENT ERROR CORRECTION. D.C. Tam, T.J. Ebner and C.K. Knox. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455.

One hypothesis on the role of the cerebellum in movement control stresses the importance of this structure in error detection and correction. The present study describes a technique for evaluating Purkinje cell activity during arm movements in which performance errors are systematically introduced throughout the task. Rhesus monkeys were trained to perform a visually guided, 2-dimensional, voluntary arm movement, moving a low-friction, two-joint manipulandum over the surface of a horizontal video screen on which a cross-shaped cursor was displayed corresponding to the manipulandum position. The task required the animal to position the cursor inside a square box displayed on the screen for a random period of time, then the box jumped to a new "target" location. The monkey was required to position the cursor projected from the manipulandum to a new target position, and maintain the cursor inside that target box for a short duration for a juice reward. The visual feedback between the hand position and the cursor position was altered by imposing two types of displacement errors between the cursor and hand position. The first introduced delayed feedback in which the cursor position lagged the actual hand position in time (50 msec). The second introduced a gain change feedback in which the displacement of the cursor was scaled by a gain factor (1.2 or 0.8) relative to the manipulandum displacement. When these changes in visual feedback were introduced in random order with different target locations, fine movement corrections were elicited as the animal attempted to match the actual hand trajectory with the visually directed trajectory. Oscillatory movements were often elicited under the delayed feedback and/or gain change conditions. Chronic extracellular recordings of identified Purkinje cells suggested that the simple spike discharge was different for some cells during the delayed or gain change feedback when compared to control movements. Oscillatory simple spike discharge was also common during the different feedback conditions. These findings suggest a role for Purkinje cells in visually guided movement error correction elicited by altered visual feedback. This technique of systematically introducing performance errors should be useful for studying the cerebellum's role in error detection and correction. Supported by NIH grant NS-18338.

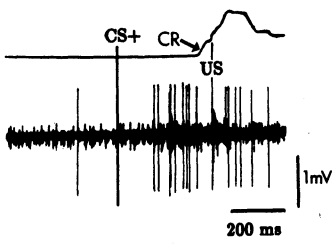
383.13 PURKINJE CELL CLIMBING FIBER AFFERENT MODULATION DURING MULTI-ARTICULAR VOLUNTARY ARM MOVEMENT IN THE MONKEY. J.J. Wang*, J.H. Kim, and T.J. Ebner. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455.

Recently we reported significant modulation of climbing fiber afferent discharge during normal and perturbed locomotion in the decerebrated cat walking on the treadmill. These positive findings led us to evaluate climbing fiber activity during a visually guided multi-articular voluntary movement in the intact animal. In this study two female Rhesus monkeys were trained to position a cursor moving with a two joint manipulandum to targets presented on a horizontally placed video sdreen. Purkinje cell activity was recorded extracellularly (tungsten metal electrodes, 3-12 megohms) in the ipsilateral cerebellar cortex using chronic unit recording techniques. EMG activity of forearm and shoulder muscles (triceps, biceps, latissimus dorsi and deltoid) were recorded during the movement using chronically implanted electrodes. Position of the hand during the movement was monitored by two linear potentiometers placed at the manipulandum's joints. Two different experimental paradigms were used. The first paradigm consisted of moving the cursor from a start box to a target box. In the second paradigm the target box was repositioned just before or after the animal captured the target box, requiring that the animal alter the ongoing movement to capture the repositioned target box. Histograms of simple and complex spikes, averages of the EMG activity and hand position were constructed. Among 73 cells studied 44 cells showed a statistically significant increase in the probability of complex spike occurrence (Fisher's Exact Test, $p < 0.05$). Two types of response patterns were observed. In the majority of cells (37/44) the probability of complex spike occurrence increased when the movement trajectory was redirected. In one-half of the cells (22/44) complex spike activity increased during the initial portion of the movement. In a few cells (8/44) the increased activity was observed during movement toward the initial target as well as when redirecting the trajectory. In conclusion the modulation of climbing fiber activity during locomotion is a common feature of a multi-joint voluntary movement, even when unexpected somatosensory input is absent. Presumably the climbing fiber afferent system plays an active role during movements, especially during the redirection of visually guided movements. Supported by NIH grant NS-18338.

383.14 CEREBELLAR PURKINJE CELL ACTIVITY RELATED TO THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE. Neil E. Berthier & John W. Moore. Dept. Psychol., Univ. of Mass., Amherst, MA 01003.

Lesions of the cerebellum disrupt the acquisition and performance of the classically conditioned nictitating membrane (NM) response (e.g., McCormick et al., Science, 223:296-299, 1984; Yeo et al., Exp. Brain Res., 60:87-98, 1985). Hemispheral lobule VI (HVI) of cerebellar cortex has been particularly implicated since HVI receives somatosensory information from the face as well as visual and auditory input, stimulus modalities typically used as conditioned stimuli in NM conditioning. Yeo et al. (Exp. Brain Res., 60:99-113, 1985) have shown that lesions of HVI disrupt conditioned responding. The lesion results support theories of cerebellar learning such as those of Marr and Albus. In the present study we extracellularly recorded from Purkinje cells of HVI. We used a differential conditioning paradigm in order to have robust evidence of CR related activity. Rabbits were trained with auditory CSs and stimulation of the periocular region as the US. The CSs were sinusoids of 600 and 1200 Hz, with one being paired with the US (the CS+) and the other being presented alone (the CS-). Rabbits typically made about 70% CRs to the CS+ and less than 40% CRs to the CS- at the time of recording. If the unit responded soley to auditory stimuli it should respond to both the CS+ and CS- regardless of whether CRs were made. If, however, the unit was CR-related it should respond only when CRs or URs were made, regardless of which tones were presented.

Many PCs responded to the US, the most frequently observed response being a burst of simple spikes. PCs in HVI showed a variety of responses to CSs that were related to CRs. The most frequently observed response was an increase in simple spikes correlated with CRs as illustrated in the inset. CR-related activity antedated CRs by 20-200 ms. A smaller proportion of cells exhibited inhibition of simple spike activity that antedated CRs. Although complex spike activity was not generally related to the US or to CRs, some observed PCs responded with complex spikes 100 ms before CRs.



(Supported by NSF BNS 85-06989 and AFOSR 83-0215.)

383.15 NORMAL SCALING OF POSTURAL RESPONSES IN CEREBELLAR PATIENTS.
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The contribution of the cerebellum to the organization of automatic postural movements was investigated by examining the effects of stimulus size and speed and the influence of prior experience on postural muscle activation patterns and resulting kinematics. Fifteen normal subjects and seventeen ataxic patients with anterior lobe (n=7), vermal (n=3) or generalized (n=7) cerebellar degeneration were tested.

Postural response scaling was examined by exposing standing subjects to backward surface translations of four different velocities (constant amplitude) and five different amplitudes (constant velocity). Stimulus anticipation effects were examined by comparing responses to random and blocked postural stimuli. The effect of stimulus size and speed on body sway, joint angles, surface forces and on the latencies, patterns, burst durations and integrated areas of surface EMG activity in six trunk and leg muscles were determined. Integrated EMG activity was normalized and the early (first 75 msec), middle (second 75 msec) and late (last 350 msec) components analyzed separately.

Response latencies and temporal sequencing of muscle activation were not affected by cerebellar disease. Initial EMG latencies (Gastrocnemius) were not significantly different in the cerebellar patients (106 + 20 msec) and normal subjects (100 + 9 msec). The cerebellar patients had normal distal-to-proximal muscle activation patterns consisting of 25 msec intersegmental latencies. Cerebellar EMG burst durations (150-200 msec), however, were approximately twice those of normals.

Despite normal timing of muscle activation, scaling of EMG burst size to stimulus characteristics was abnormal in postural unstable cerebellar patients. The slope of the relation between stimulus velocity and initial EMG burst size were reduced or zero and the slope of the relation between stimulus amplitude and total EMG size were steeper than normal. Exaggerated responses included activation of antagonist muscles, tibialis and quadriceps, normally recruited only for very large stimulus amplitudes. These scaling abnormalities resulted in sway over-corrections, particularly for the smallest (1.2 cm) perturbations.

Nevertheless, the cerebellar patients were able to normally scale responses to anticipated stimulus amplitudes. Therefore, midline cerebellar structures may be more involved in scaling automatic postural responses to concurrent stimulus characteristics (especially velocity) than to anticipated stimulus characteristics. (Supported by NIH grants NS12661 and NS06926).

SPINAL CORD AND BRAINSTEM IV

384.1 MECHANICAL PROPERTIES OF THE CAT INFERIOR OBLIQUE MUSCLE WITH RESPECT TO THE ANATOMY OF THE NERVE AND NUCLEUS.
P.J. Sorg, S.J. Goldberg, and J.R. McClung. Department of Anatomy, Medical College of Virginia-VCU, Richmond, Virginia 23298

The inferior oblique (IO) nerve has a relatively long course through the orbit and naturally divides into 2 and occasionally 3 branches as it enters the muscle. Measurements of twitch tension, maximal tetanic tension, twitch contraction time, twitch half decay time, fusion frequency, and fatigue were made with a strain gauge in response to stimulation of the whole IO nerve. These results were compared to those obtained through selective stimulation of the medial and lateral branches of the same nerve. Retrograde labeling of the IO nerve was then done by placing the cut end of the whole nerve or one of its branches in a horseradish peroxidase (HRP) filled tube. The modified Mesulam technique using tetramethylbenzidine (TMB) as a chromogen was used. Any anatomical organization of the IO nerve or nucleus could then be observed with respect to the muscle's mechanical parameters.

Preliminary studies on 6 inferior oblique nerves yielded the mechanical results in the table below. The means are presented with the ranges in parentheses.

	Whole Nerve	Lateral Branch	Medial Branch
Twitch Tension	3.8 gr. (2.3-6.4)	2.1 gr. (0.9-4.0)	1.6 gr. (1.0-2.8)
Max. Tetanic Tension	30.4 gr. (24-41)	16.2 gr. (8.0-32)	16.2 gr. (7.3-25)
Twitch Cont. Time	6.8 msec. (5.9-7.5)	8.3 msec. (6.3-9.5)	6.4 msec. (5.1-7.4)
Twitch Half Decay Time	10.9 msec. (8.8-14)	15.0 msec. (10.2-19)	9.5 msec. (6.7-14.1)
Fusion Frequency	270 Hz (230-280)	180 Hz (150-210)	262 Hz (250-270)

The part of the muscle innervated by the medial branch tended to show greater fatigue after 2 minutes than either the lateral branch or the whole nerve.

A very preliminary anatomical analysis of the IO nucleus has shown no definitive difference in motoneuron location within the nucleus with respect to the nerve branch labeled. Examination of cell body number and size with regard to specific nerve branches is also underway.

(This research was supported by NSF grant BNS-8507610.)

384.2 FATIGUE OF LATERAL RECTUS, RETRACTOR BULBI AND SPLIT LATERAL RECTUS-RETRACTOR BULBI MOTOR UNITS IN THE CAT.
S.M. Gurahian and S.J. Goldberg. Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298

The cat abducens nerve contains axons of lateral rectus (LR) and retractor bulbi (RB) motor units as well as axons that project to both LR and RB muscles (split LR-RB). The present study was designed to examine the fatigue properties of these three types of motor units.

The muscle fibers of a motor unit are morphologically and histochemically uniform (Edstrom et al., J. Neurol. Neurosurg. Psychiat., 31:424-433, 1968). The first aim of this project was to compare the fatigue properties of the separate muscle components of split LR-RB motor units to determine the physiological uniformity of muscle fibers within a single motor unit.

In extremity muscle, motor units have been categorized on the basis of their twitch contraction time and fatigue resistance (Burke et al., Science, 74:709-712, 1971). The second aim of this project was to determine if "pure" LR and RB motor units may also be differentiated on the basis of fatigue.

Stimulation of the VIth nerve was utilized to antidromically identify LR, RB and split LR-RB motoneuron axons in the region of the VI nucleus. The LR and RB muscles were attached to separate strain gauges and set so that maximum twitch tension was observed. Motor unit fatigue was studied by applying an intraaxonal stimulus consisting of a 500 msec duration train at 100-150 hz. This was repeated at a rate of 1/sec. for 2 min. Twitch tension, twitch time, fusion frequency, and tetanic tension were also studied.

Preliminary results show that 1) the fatigue properties of the LR component of split LR-RB motor units are different than the fatigue properties of the RB component, 2) all RB motor units are fatigable, 3) LR motor units demonstrate a continuum of heterogeneous fatigue resistances. This preliminary evidence suggests that the muscle fibers of a motor unit need not be physiologically uniform and that lateral rectus motor units apparently do not divide into discrete groups based on fatigue resistance.

(This research was supported by NSF grant BNS-8507610.)

384.3 MORPHOLOGICAL RELATIONSHIPS AMONG EDL, TA, AND ST MOTONEURON POOLS REVEALED BY RETROGRADE TRANSPORT OF FLUORESCENT TRACERS. J.E. Hoover* and R.G. Durkovic (SPON: S. Nord). Dept. of Physiol., SUNY Health Science Ctr. at Syracuse, Syracuse, NY 13210.

Reflexes of certain flexor muscles [semitendinosis (ST) and tibialis anterior (TA)] have been shown to be activated by superficial peroneal (SP) nerve stimulation and to exhibit classically conditioned facilitation in spinal cat. In contrast, another physiological flexor [extensor digitorium longus (EDL)] often exhibits primarily inhibition to SP stimulation and does not show conditioned behavior (Durkovic, Neurosci. Lett. 39:155, 1983). In an attempt to formulate a morphological-functional hypothesis to explain these behavioral results, we simultaneously used three different fluorescent dyes to determine the relative spinal locations of these muscles' motoneurons.

The following compounds were used as fluorachromes: 10% solution of bisbenzimide (Bb), 2% solution of nuclear yellow (NY), and 10% solution of propidium iodide (PI) in 1% DMSO. In anesthetized cats, tracers were applied either by intramuscular injection (Bb, NY) or by soaking the nerve (PI). Most experiments were performed bilaterally. Data were collected following a three-day recovery period.

EDL motoneurons were distributed between rostral L-6 and caudal L-7. The TA motor nucleus had approximately the same rostro-caudal distribution. In general, the ST motor-cell column was displaced somewhat caudal to TA and EDL, extending from L-6 to caudal S-1. Both EDL and TA occupied a position in the lateral portion of lamina IX. Their somas exhibited an overlap, varying among animals, from approximately 50 to 100% with the EDL somas either intermingling with or dorsal to those of TA. On the other hand, the ST motor nucleus occupied a more ventromedial position in lamina IX. Good evidence of bilateral symmetry was found.

These data demonstrate that the simultaneous labeling of different neuronal populations with fluorescent tracers is of great advantage for resolving the morphological relationships among adjacent and overlapping motor nuclei. This technique proved particularly helpful for investigating EDL and TA cell columns which have been thought to share a common spinal representation area (Romanes, 1951). Furthermore, since the degree of intermingling of these two motoneuron pools varies from animal to animal, this morphological variance may be related to the range of responses elicited in EDL by SP stimulation. Supported by NSF Grant No. BNS 8415917.

384.4 FLEXOR MUSCLE PRIMARY AFFERENT SPINAL PROJECTIONS AND MOTONEURON POOL TOPOGRAPHY: AN HRP STUDY. J.C. Leahy* and R.G. Durkovic (SPON: M.M. Mozell), Dept. of Physiology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

As part of a study concerned with flexion reflexes in spinal cat (Durkovic, Neurosci. Lett. 39:155, 1983), we studied the topographical organization of the motoneuron pools and the primary afferent projections in the lumbosacral cord of 3 flexor muscles [semitendinosus (ST), tibialis anterior (TA), and extensor digitorum longus (EDL)].

In anaesthetized cats, a 10% HRP solution or a 2% WGA-HRP solution in 5% DMSO was injected intramuscularly or applied as a nerve soak unilaterally or bilaterally to one of the three muscles (or muscle nerves). Other hindlimb nerves were ligated and cauterized. Animals either remained anaesthetized or were allowed to recover and were perfused after 4 days. Transverse sections of the lumbosacral cord were cut and processed for HRP demonstration using Mesulam's TMB procedure (modified).

The TA and EDL motor nuclei were located on the lateral border of the ventral horn, while ST occupied a more ventro-medial position. The rostro-caudal distributions of the 3 motoneuron pools exhibited considerable interanimal variation and in many cases were more extensive than those found by Romanes (J. Comp. Neurol. 94:313, 1951). For example, the TA motor nucleus extended as far rostrally as caudal L5 in one animal and as far caudally as rostral S2 in another animal. The number of cells in the motoneuron pools was also significantly greater in some cases than was found by Romanes (1951).

Spinal segments L4-S2 were examined for afferent labelling and for all 3 muscles, label extended from L4-S1, and in some cases, S2. Afferent labelling was found ipsilaterally throughout the medial-lateral extent of laminae I-II in most cases and was also frequently found in the medial and central parts of laminae VI-VII. On occasion, afferent label was found in laminae III-V and laminae IX-X.

The superficial dorsal horn labelling may be ascribed to the terminal regions of small diameter afferent fibers, while labelling in laminae VI, VII and IX is more likely due to large diameter afferent fibers. In some nerve soak experiments, the results indicated a preferential uptake/transport of HRP by small diameter afferent and efferent fibers. Supported by NSF Grant No. BNS 8415917.

384.5 ANATOMICAL INVESTIGATION OF THE MEDIAL AND LATERAL PLANTAR MOTOR COLUMNS IN THE RAT. D.P. Crockett*, S.L. Harris* and M.D. Egger. Dept of Anatomy, UMDNJ-Rutgers Med. Sch., Piscataway, NJ 08854.

The intrinsic plantar muscles of the hind foot are innervated by the medial and lateral plantar nerves, which are distal branches of the tibial nerve. The main action of these muscles is the downward (plantar) flexion of the toes. As part of a continuing study of cutaneous reflexes involving the plantar musculature, we conducted an anatomical study of the plantar motor columns in the spinal cords of Sprague-Dawley rats (213-485 g; N = 12). Initially, six medial and lateral plantar nerve pairs were labelled by direct application of a 20% solution of HRP (w/v in 2% DMSO) to the proximal cut nerves (2-4 hr), sectioned in the ankle. After survival times of approximately 48 to 72 hr, the animals were transcardially perfused and the relevant portions of the spinal cord were serially sectioned on a freezing microtome (60 μm-thick sections). Two cords were sectioned in each of the three major planes. HRP was detected using TMB as a chromagen. We found the plantar motor pool located in the extreme dorsolateral portion of the ventral horn, typically extending from either the rostal portions of the sixth lumbar spinal segment (L6) or the caudal aspects of L5 into L4. Measurements of cross-sectional areas were made by camera lucida tracing of HRP-labelled cell bodies onto a digitizing graphics pad from material sectioned in the transverse ($\bar{X}$ = 610 μm^2, S.D. = 151, N = 293), horizontal ($\bar{X}$ = 605 μm^2, S.D. = 148, N = 405) and sagittal ($\bar{X}$ = 617 μm^2, S.D. = 151, N = 273) planes. The three distributions were basically unimodal and were not statistically different. The size distribution of motoneuron perikarya innervating the intrinsic muscles of the foot (at least in the rodent) is unlike that of the larger, more proximal hind limb muscles, where bimodal distributions are observed, presumably representing alpha- and gamma-motoneurons. We examined the possibility of topographical organization within the plantar motor columns by individually labelling the medial (N = 3) or lateral (N = 3) plantar nerves (30% HRP). We observed much overlapping in the distribution of HRP-labelled motoneurons, examined in horizontal sections. Preliminary analysis suggests, however, that the medial plantar motor column is somewhat more caudally positioned. Thus, though there may be some topographical differences between the two motor pools serving the intrinsic plantar flexors, the anatomical distributions show considerable overlap. (Supported in part by NSF grant BNS-8518437)

384.6 **DENDRITIC ARCHITECTURE OF TWO MOTOR NEURON POPULATIONS SUPPLYING NECK MUSCLES IN A CRYPTODYRAN TURTLE, PSEUDEMYS SCRIPTA.** R.J. Callister and E.H. Peterson. Dept. Zool. and Biomed. Sciences, and Dept. Basic Sci. Coll. Osteopathic. Med., Ohio Univ. Athens, OH 45701.

Vertebrate neck muscles, unlike limb muscles, are sometimes supplied by two populations of motor neurons. In P. scripta, e.g., muscles which retract and protract the head are innervated by the ventral motor nucleus a musculotopically organized motor neuron population in the tip of the grey matter, and by the medial motor nucleus which lies on the dorsomedial grey/white border and is not musculotopically organized (Yeow & Peterson, '86, J. Comp. Neurol. 243:145-165, 166-181). To better understand the functional role of these two motor neuron populations we have examined their dendritic architecture by infiltrating the C4 nerve to m. RCCQ (chief head and neck retractor in cryptodyran turtles) with free HRP or Cholera Toxin HRP conjugates, either unilaterally or bilaterally. Sections were cut in transverse or horizontal planes and reacted with TMB or cobalt intensified DAB. Our results confirm that m.RCCQ is innervated unilaterally by ventral and medial nuclei. These two motor neuron populations differ in size and dendritic architecture. Ventral motor neurons resemble cells innervating limb muscles in turtles (Ruigrok et al., '84 J. Comp. Neurol. 230:413-425), with dendrites radiating preferentially in the ventrolateral funiculus. These processes rarely invade the medial funiculus and never cross into contralateral spinal cord. Medial motor neurons are larger on average than ventral cells and they have two major dendrite systems. Medial processes arch through the ipsilateral and contralateral medial funiculi and extend into the contralateral medial motor nucleus. These dendrites form bundles with medially directed dendrites of other ipsilateral and contralateral medial cells. A second dendritic system runs longitudinally along the ipsilateral dorsomedial grey/white border at the level of the medial motor nucleus. Thus the two dendritic systems of medial cells provide a potential substrate for interactions with other medial motor neurons both ipsi- and contralaterally. These data suggest that single neck muscles are supplied by two morphologically distinct populations of motor neurons, and that these two populations may play different functional roles in the control of head movements.

384.7 MORPHOLOGY OF MEDIAL LONGITUDINAL FASCICULUS AXONS IN THE UPPER CERVICAL SPINAL CORD OF JUVENILE TURTLES, PSEUDEMYS SCRIPTA. A.M. Brichta and E.H. Peterson. Dept. Zool. and Biomed. Sci. and Dept. Basic Sci. College Osteop. Med. Ohio Univ. Athens, OH 45701.

As part of a study aimed at understanding the control of cervical musculature we are using anterograde transport of HRP to characterize morphological populations of medial longitudinal fasciculus (MLF) axons which terminate in upper cervical cord. We used juvenile turtles to minimize transport distances and the number of sections over which single axons must be reconstructed. Experiments were conducted in vitro or in vivo. In some cases we backfilled motor neurons by infiltrating C2 or C3 muscle nerves with HRP.

MLF axons are 0.3-3.0um in diameter and appear to cluster in 3-4 size classes. Collaterals of these parent axons segregate into two broad size groups which can be further subdivided on the basis of axonal branching patterns and terminal arbor morphology. Serial reconstructions show that large axon collaterals (0.6-2.0um) ramify in upper ventral horn (laminae VII-VIII). They divide into 2-4 smaller daughter branches which traverse the ventral horn and terminate laterally in fine varicose processes. In contrast the more numerous, smaller (<.6um diam) collaterals have less extensive arborizations, and may terminate as single, transversely oriented branches. Two subgroups could be distinguished on the basis of terminal arbor morphology. One derives from thick parent axons and ramifies in medial ventral horn as a linear array of numerous, large (>2um diam) varicosities. The other subgroup may branch off either thick or thin parent axons, has fewer, smaller (.6-1.8um) varicosities, and extends closer to the lateral border of the grey matter. Occasionally transversely oriented processes of both types appear to wind together in bundles. Typically the arbors described above are collateral branches of MLF axons which continue caudally in the medial funiculus, but in some cases they appear to be terminals branches of individual axons, suggesting that some MLF axons may have a restricted target in the upper cervical cord. These data suggest that there may be several morphological classes of MLF axons which terminate in the upper cervical cord, and raise the question of whether they may play different functional roles in the control of cervical motor neurons.

384.8 SERIAL RECONSTRUCTION OF SYNAPTIC CONTACTS ON CERVICAL MOTOR NEURONS IN A CRYPTODYRAN TURTLE, PSEUDEMYS SCRIPTA. M.B.L. Yeow and E.H. Peterson. Dept. Zool. and Biomed. Sciences, and Dept. Basic Sciences, College Osteop. Medicine. Ohio Univ. Athens, OH 45701.

As part of a study aimed at understanding the control of neck musculature we are attempting to characterize synaptic contacts on cervical motor neurons. Our quantitative observations to date are based on complete reconstructions of 14 boutons and 44 boutons estimated to be at least 80% complete. We have also examined shorter series or single sections through >500 additional boutons. All material was taken from the C3 segment; motor neurons were identified by their distinctive location and morphology (Yeow & Peterson, J. Comp. Neurol., '86, 243:145-165, 166-181).

Cervical motor neurons receive at least 7 morphological types of synaptic contact: S, F, Sp, G, T, Cs, Cf. These generally resemble boutons contacting limb motor neurons in other vertebrates (Yeow & Peterson, '85, Soc. Neurosci. Abs. 11:883), however we observed no M-type contacts (putative Type II afferents, Conradi et al., '83, Br. Res. 265:31-39). Boutons vary widely in size, from small en passant or terminal swellings to large globular bodies which make multiple synaptic contacts. Bouton volume (X=1.65 $\pm$1.8um^3; range: .05-10.1um^3) is significantly correlated with the area of apposed motor neuron membrane (X=1.47 $\pm$.99um^2; range .11-4.45um^2). Individual boutons bear 1-4 active zones (AZs).

To assess the potential role of individual boutons in recruiting cervical motor neurons we examined the relation between bouton size and the number and area of its AZs. There is a significant correlation between bouton size (volume or total appos. area) and (1) the number of AZs, (2) individual AZ area, and (3) total AZ area. Areas of individual AZs are generally smaller and more homogeneous (X=.18um $\pm$.14um^2) than total AZ areas (X=.24 $\pm$.21um^2), suggesting a tendency to restrict the size of individual AZs. Thus large boutons increase their total AZ area by increasing both the number and size of individual AZs. If individual AZs act as indivisible, independent units of transmitter release (Walmsley et al., '85, J. Neurosci. 5:2095-2106; Tremblay et al., '83, Br. Res. Rev. 6:299-314) then large boutons may generate larger and more variable synaptic potentials in cervical motor neurons than smaller contacts.

384.9 INNERVATION OF TONGUE MUSCULATURE IN THE MACAQUE. A. Sokoloff and T. Deacon. (SPONS: H. Eichenbaum). Dept. Anthropology, Harvard University, Cambridge, MA 02138.

Although the mammalian tongue is necessary for normal respiration, mastication, deglutition and vocalization, little is known of the neuroanatomical system controlling tongue movements. This is in part due to the complexity of muscle fiber organization within the tongue body. To determine the somatotopic innervation of intrinsic tongue musculature, 1-3 microliters of wheatgerm agglutinin-HRP were injected into specific regions of the tongue body and genioglossus and geniohyoid muscles in anaesthetized macaques (M. fascicularis). Tongue and brainstem tissue was processed with tetramethyl benzidine.

The hypoglossal nucleus of the macaque can be divided into medial and ventral (V) columns at caudal levels and medial (M), intermediate (I), ventrolateral (VL) and dorsolateral (DL) columns at its middle and rostral extent. There is also a caudal ventrolateral cell group (CV) which is separate from the main body of the hypoglossal nucleus. Injections in the genioglossus labeled motoneurons in I between M and DL. Cells in V were labeled with geniohyoid injection. Injections involving superior longitudinal fibers labeled cells in rostral DL while inferior longitudinal injections labeled cells in caudal DL. Injections within transverse and vertical muscle groups labeled cells in M, VL and CV. The interdigitation of transverse and vertical fibers within the tongue make it difficult to distinguish their independent sources of innervation. However, injections confined to the septal midline (which exhibits relatively few vertical fibers) predominantly labeled cells in M and CV but not VL, while more lateral intrinsic injections (into areas containing mostly vertical fibers) predominantly labeled cells in VL. This suggests that projections from the medial column terminate in transverse fibers and those from the ventrolateral column terminate in vertical fibers. Injections into anterior as opposed to posterior intrinsic musculature (transverse and vertical fibers) do not result in topographically differentiated labeling of motor neurons in M and VL. CV cells were not labeled with geniohyoid injections. Lateral tongue injections labeled motoneurons on one side of the hypoglossal nucleus only. Retrograde transport of HRP to the facial nucleus was not seen.

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384.10 PHYSIOLOGICAL STUDIES OF PELVIC FLOOR MUSCLES. B. Dubrovsky, Depts. Psychiat. and Physiol., McGill Univ., Montreal, Que.H3A 1A1

Most clinical evaluations on the functional state of the anorectal region are basically decided from manometric studies. However, analysis of pressure in terms of forces acting within the anorectal region is difficult. Pressure and force measurements do not always correspond. Activity of pelvic floor muscles is an important source of the forces acting in the region. Information on this issue comes mainly from recording of pressure changes concomitantly with EMG's from both, the internal (IAS) and external (EAS) sphincters of the anus. Due to the concentric arrangement of these muscles, the attribution of a recorded pressure or pressure change to activity in one or other of the sphincters can be particularly hazardous. The activity and response characteristics of the levator ani (LA) muscle complex, an important component of the pelvic floor apparatus, is not well known. Previous work from this laboratory (Dubrovsky et al., Exp. Neurol. 88:277 (1985)) has shown that the levator ani and EAS correspond with different spinal neuronal circuits; that both muscles can be independently activated, and further that bilateral pudendal section does not abolish levator ani activity as does the EAS. We explored the response characteristics of the LA and EAS to rectal distention. In chloralose (80 mg/kg) anesthetized cats, both LA muscles, and the EAS were exposed and threaded for bifocal recording of EMG activity. It is well known that rectal distention elicits a relaxation of the IAS and a contraction of the EAS. The latter response cannot be maintained for more than a minute. In contrast the LA responds with a sustained discharge of up to 3-4 minutes to rectal distention of 5-10 cc of air. The response is highly sensitive to the rate of infusion; a sudden inflation produces a larger phasic discharge, and a slow inflation (ca 20 sec) produces only a weak discharge. Threshold for the response with rectal inflation is lower in the LA than in the EAS. A fast 1 cc air inflation elicits a LA discharge with negligible effect on the EAS. Under Flaxedil, muscle responses to the same stimuli cannot be seen, indicating a muscle origin for the recorded responses. Cortical projections for these muscles were studied by recording field and single neuronal activity around pericruciate cortex. Two loci, one in the pre and other in the postcruciate region, both 2 mm from the medial wall, were found to be places of clear reversal of the field potential together with maximal neuronal discharge. Latencies of 12-15 msec were observed in the intact preparation. After section of the dorsal columns there was a significant decrease (2/3) of the amplitude of the field potential, and neuronal discharges appear much later (ca 25-35 msec). As both LA and EAS have contrasting segmental behavior, and a fast path for cortical projection, both should be considered in discussions of pelvic floor.

(Supported by NIH).

384.11 SPINAL CORD INJURY CAUSES LOSS OF DISTANT MOTONEURONES. E. Eidelberg. Veterans Administration Hospital, and Division of Neurosurgery, The University of Texas Health Sciences Center at San Antonio, TX, 78284.

Spinal cord transection causes "paralysis", loss of sensation, "spinal shock", partially reversible muscular atrophy and supersensitivity to acetylcholine, and - as a delayed effect - signs of reinnervation of motor units. We asked whether some of these effects might be due to death of lumbar motoneurones. We counted the total number of probable alpha motoneurones (by size, morphology and position) in L_4 and L_5 in adult rats. The controls (N=6) had 5710 +810 cells. Rats whose thoracic spinal cord had been transected at T_{6-8} showed loss of cell counts of the order of 30%, beginning on the 4th day post-transection. This loss was attenuated by local anesthetization of the cord, prior to and in the intended site of, the transection. Preliminary data suggest that the loss of motoneurones was attenuated also by a Ca^{2+} channel blocker injected intravenously. This effect of cord transection may be mediated by excessive axonal discharge in descending pathways, and the entry of an excess of Ca^{2+} into their target cells.

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384.12 LATERAL AND VENTRAL FUNICULI IN MOTOR RECOVERY FOLLOWING SUBTOTAL SPINAL CORD LESIONS IN ADULT RATS. J. W. Little, R. M. Harris, R. C. Sohlberg* Seattle V.A.M.C. and Univ. of Washington, Seattle, WA, 98108

Significant recovery of motor function is observed following subtotal spinal cord lesions in experimental animals and human patients. The descending pathways responsible for this recovery are not yet understood. This study examines the role of the lateral and ventral funiculi in this recovery process.

Adult female rats underwent subtotal spinal cord section in the mid-thoracic region, sparing the left lateral and/or ventral funiculi. Motor behavior was examined at least three times per week through four weeks. Four individual motor functions were graded on a 4-point scale for each hindleg: swing phase of gait, stance phase of gait, one-legged hopping, and hindleg withdrawal to light grasp of the foot. These four scores were summed to yield a composite variable for quantifying motor recovery. Initial and final motor deficits were consistently greater in the right than the left hindleg, and rates of recovery were slower. Rats with sparing of the left lateral and ventral funiculi showed fewer initial deficits in the left hindleg, fewer final deficits in the right hindleg, and faster motor recovery than those with sparing of only one funiculus. There is a suggestion of two temporal phases of motor recovery. Animals with mild deficits recovered during the first 3 to 5 days. Those with severe deficits did not recover during this early period, but did recover slowly during the second and third weeks. Some animals with intermediate lesions showed partial recovery during the early period and a second phase of recovery during the late period. Luxol Fast Blue staining of the lesion sites revealed absence of myelinated fibers in the right half of the cord in most animals and variable sparing in the left half.

These studies demonstrate that motor recovery regularly follows section of 1/2 to 3/4 of spinal long tracts. The rate and extent of that recovery depends upon the quantity of long tracts spared, though either lateral or ventral funiculus fibers are able to mediate comparable locomotor recovery. The two time-periods of locomotor recovery suggest that two neuronal mechanisms may underlie the behavioral substitution by the spared long tracts.

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384.13 EXAGGERATED REFLEXES IN CATS FOLLOWING SPINAL CORD HEMISECTION. R.L. Carter*, L.A. Ritz, E.W. Scott*, C.F. Shank*, and G.W. Sypert. Depts. of Neurosurgery and Neuroscience, University of Florida Health Center, Gainesville, FL., 32610.

Previous studies in mammalian central nervous systems suggest that partially denervated neurons display an enhanced synaptic transmission from remaining afferent systems, e.g., Decima and Morales, 1983. We are interested in whether the clinical manifestations of spinal cord lesions, e.g., spasticity, are correlated with electrophysiological measurements of flexor and extensor monosynaptic reflex arcs.

Adult cats, between 2.0 and 4.7 kg, were randomized to control or experimental groups. Left hemisections at L5-L6 cord junction were performed aseptically on experimental animals, who underwent a terminal procedure 1-6 months postoperatively. Each animal was decerebrated and CO_2 and vital signs were monitored. Dopamine was given as a pressor agent if needed; hemodynamically stable animals received equivalent doses and data were collected pre- and post-administration. Laminectomies from L4-L7 were performed and bilateral leg dissections exposed flexor (to semitendinosus, semimembranosus, and posterior biceps muscles) and extensor muscle nerves (to medial and lateral gastrocnemius and soleus muscles). L6L7 or L7S1 ventral and dorsal roots were stimulated sequentially and the responses measured in the muscle nerves, for each side.

Fourteen control and ten experimental animals' responses have been compared. Each control animal had normal and symmetric reflexes, tone and gait. There appears to be an inherent asymmetry of the electrophysiological monosynaptic reflex (MSR) in controls, with the right side being slightly larger. In each experimental case, the animals had normal preoperative gait, tone, and reflexes. Postoperatively, the lesioned animals went through a stage of areflexia, with each animal eventually demonstrating hyperactive cutaneous and deep tendon reflexes. Most animals also exhibited hypertonia. In all but two experimental animals, the MSR measurement from either extensor or flexor nerves, was enhanced on the lesioned side (1.6 - 7 times larger than the intact side). Dopamine did not appear to affect the size of the MSR in this paradigm. The evoked potentials will be evaluated with regards to amplitude, latencies, rise times, and half-widths. Histological evaluation of lesion sites in six animals to date have confirmed the accuracy of the hemisections.

These preliminary observations indicate that there is a correlation between the clinical findings of hyperreflexia and the enhanced electrophysiological records of MSRs on the lesioned side of an animal. Experiments with thoracic hemisections and with lumbar dorsal column lesions are to follow.

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384.14 BEHAVIORAL AND MORPHOMETRIC STUDIES USING A COMPUTERIZED SPINAL INJURY DEVICE. S.K. SOMERSON* AND B.T. STOKES, Depts. Bio-Med. Eng. and Physiol., Ohio State University, Columbus, OH 43210

A behavioral and histological analysis was performed on three previously defined groups of spinal cord injured rats. Displacement (time and magnitude) of the spinal cord was controlled using biomechanical feedback during the injury process; the displacement amplitude was kept constant (1.54 +/- .02) while the time of compression was varied between the groups. The three levels of injury were designated as light, intermediate, and heavy and impact durations were 14, 19, and 24 milliseconds, respectively. Laminectomies were performed at midthoracic cord (T-9 or T-10) and the vertebral column was rigidly fixed during injury by using vertebral clamps rostral and caudal to the exposed cord.

Motor performance was based on scores from three tests. Animals were handled for 2 weeks prior to surgery. The tests were administered twice before surgery and over a three week post-injury time period (on days 1, 3, 8, 14, and 21). Tests included: open field walking (scored on a modified Tarlov scale), inclined plane analysis, and grid walking (an agility test). Light group animals recovered to pre-operative levels in both the open field walking and inclined plane assessments, but grid walking scores indicated a mild deficit in the coordination between fore and hind limbs. The intermediate and heavy groups rats' showed statistically identical results in the open field walking and inclined plane tests. Scores did not recover to normal values indicating lasting deficits in general mobility and postural compensation for bodily displacement in space.

Morphological results reinforced the behavioral data. There was exceptional consistency in the amount of tissue damage. Fractional lesion area (% total area at site of maximum lesion) was nearly identical in the intermediate and heavy groups: 1.52% and 1.18% uninjured fibers remained in these respective groups. The light injury animals had 66.2% of their neuropil intact. Three dimensional reconstruction and determinations of lesion volumes are currently in progress.

The peak force generated during the displacement controlled injuries varied between animals in each group according to the mechanical properties of their spinal cords. Overlap in the range of peak force occurred only between the intermediate and heavy groups. This overlap of the force envelopes could explain the similar behavioral and histological measurements in the two groups.

Specific injury protocols therefore resulted in reproducible responses in both neurological and pathological measures as they have in previous functional studies (Somerson and Stokes, 1985). In addition a predictable group of marginally injured animals can be created by this protocol. (Supported by USPHS-10165)

384.15 COMPARISON OF ANTERIOR AND POSTERIOR EXPERIMENTAL SPINAL CORD CONTUSIVE INJURY. E. Fujii* and J. R. Wrathall, Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, D.C. 20007

Clinically, the most common traumatic spinal cord lesion occurs at lower cervical levels due to anterior contusion caused by fracture of the vertebral body. Most experimental studies have examined lesions produced by posterior injury. We have therefore produced and compared anterior and posterior contusive injuries in the rat.

Injuries were produced by a weight-drop technique (EXP.NEUROL. 88: 108, 1985) in which a 10 g weight was dropped from 5 cm height. Two groups of rats were injured at C7 using an impounder with a round tip (2.4 mm diam.), with the weight dropped on the anterior (ACSW) or posterior (PCSW) dura. A third group (ACLW) was injured by anterior contusion at C7 using an impounder with a tip of the same area but elliptical in shape (3.6 X 1.6 mm). A fourth group (PTSW) was subjected to posterior contusion at T8 using the impounder with the round tip. The rats were tested for neurological function at 24 hr, 1, 2, 3, and 4 wks after injury. Use of limbs in locomotion was assessed as well as performance in other tests of reflex and complex behavior (EXP.NEUROL. 88: 123,1985). At 4 wks, the rats were perfused and spinal cord tissues were examined for histopathology.

All groups exhibited maximal neurological deficit at 24 hr with a less severe chronic deficit by 4 wks. Injury at C7 produced forelimb deficit in locomotion, grasping and reflex tests, but less hindlimb deficit than with the same impounder at T8. Anterior contusion produced less overall deficit than posterior using the same impounder. The elliptical impounder resulted in greater functional deficit than the round impounder. The extent of the histopathological lesions was consistent with the extent of functional deficit in the different groups. The most severe cervical lesions were observed in the PCSW group, where cavities typically replaced most of the gray as well as the more central white matter. The least severe lesions were present in the ACSW group, where white matter microcysts and partial demyelination typically characterized the lesion epicenter (area of maximal damage). Comparison of anterior vs posterior injury groups indicated greater involvement of ventral-medial white matter after anterior injury. However, even with anterior injury, when the epicenters exhibited central cavities, the rostral and caudal "tails" of the lesions were in the dorsal funiculus.

The results indicate that experimental cervical anterior spinal cord contusive injury can be produced in rats. The resulting functional deficit and histopathological lesion after injury depends upon both the direction of injury (anterior vs posterior) and the shape of the impounder used (round vs elliptical).

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384.16 IMPLANTATION OF NEURONS INTO THE CONTUSED SPINAL CORD: A BIOASSAY OF THE MICROENVIRONMENT AT THE INJURY SITE. D.W. Hoovler, C.A. Fischofer* and J.R. Wrathall. Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, D.C. 20007.

Contusive injury results in mechanical disruption of spinal cord tissue as well as biochemical changes that may lead to additional tissue destruction ("secondary injury") via the production of a toxic environment at the injury site. In order to examine the postulated development of a neurotoxic microenvironment, we have implanted exogenous neurons into the injury site of contused spinal cord at different times following injury and analyzed survival of these neurons at various times after implantation.

Dorsal root ganglia (DRG) of adult Sprague-Dawley rats were dissociated in 0.25% collagenase and viable neurons labeled with the fluorescent dye acridine orange. Five ul of neuron suspension (6000 viable neurons) was injected via a 33 gauge steel needle inserted 0.8 mm below the dura at the dorsal midline of each spinal cord. Neurons were implanted into uninjured spinal cord (controls) as well as the injury sites of rats subjected to contusion produced by a 10 g weight dropped 2.5 cm onto the exposed dura (Wrathall, et al., Exp. Neurol. 88:108, 1985) either immediately (within 10 min), 1 wk or 4 wks before implantation. Animals were perfused at 10 min, 2 or 18 hrs after implantation, and longitudinal frozen sections (14 um) of the injury/implantation site examined to calculate the total number of DRG neurons and the percentage with normal-appearing nuclei at the implantation sites.

In all groups, the average number of neurons per site was somewhat reduced at 18 hr as compared to 10 min or 2 hrs after implantation. However, no significant difference was found in the total number of DRG neurons per implantation site when control and contused groups were compared at the same time after implantation. In all groups, the average percentage of neurons exhibiting a normal-appearing nucleus (vesicular, with a prominent nucleolus) showed a slight downward trend with time after implantation. The percentage of neurons with normal-appearing nuclei was not significantly different in comparisons of control and contused groups at the same times after implantation. Thus, although there was an indication of some reduction in total neuron number and viability over time after implantation, no differences in neuron survival were detected after implantation into contused as compared to uninjured spinal cord.

No evidence of an injury-induced neurotoxic microenvironment at the site of contusion was obtained in these experiments. The results suggest that diffusable neurotoxic factors are not involved in "secondary injury" after contusion, or if so, that endogenous spinal cord neurons are differentially sensitive to their effects. (Supported by NIH-NINCDS, NO1-NS-2-2310).

384.17 SOMATOSENSORY EVOKED POTENTIALS AS AN INDEX OF SPINAL CORD INJURY IN THE RAT. A. Raines, K.L. Dretchen, K. Marx* and J. Wrathall. Departments of Pharmacology and Anatomy, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

The treatment of spinal cord injury with new modalities in man initially requires the demonstration of an improvement in functional capacity in some model system capable of detecting changes in functional state. We have recently described a graded spinal cord injury in the rat employing a weight-drop technique (Exp. Neurol. 88: 108-149, 1985) in which behavioral deficit and tissue damage (analyzed morphometrically) were highly correlated with the height from which a 10 gram weight was dropped to cause injury.

Four weeks after no (0), mild (2.5 cm), moderate (5.0 cm) or severe (17.5 cm) weight drop and consequent graded injury, rats were assessed behaviorally and then anesthetized with chloral hydrate and placed on a respirator. Somatosensory evoked potentials (SEP's) were elicited by contralateral supramaximal sciatic nerve stimulation with bipolar platinum iridium electrodes and recorded with bipolar silver ball electrodes, one placed over the somatosensory cortex. Stimuli were applied at a frequency of 0.2 Hz amplified 10K with filters set at 0.1 and 3.0 K Hz. Evoked potentials were stored on magnetic tape and 512 responses were averaged with a signal averager. SEP peak to peak amplitude, integrated area and latency were measured. These 3 parameters were correlated with height of weight drop, behavioral deficit, inclined plane test and Tarlov score. SEP area correlated (correl. coef. = .714) with amplitude. Both SEP area and amplitude correlated with weight drop height, behavioral deficit, Tarlov score and inclined plane, with the amplitude providing a better correlation in each comparison. No attempt was made to resolve the polyphasic potential, as inter-individual differences were appreciable. Latency did not correlate with weight drop height, behavioral score, inclined plane or Tarlov test. Mild injury usually resulted in a relatively uniform modest reduction of SEP amplitude and area; severe injuries usually resulted in failure of response. Rats in the moderate injury group tended to respond bimodally, with some showing little SEP depression and others exhibiting little or no recordable SEP.

The present experiments demonstrate that SEP amplitude and area can be used in the rat to monitor the functional integrity of neural pathways; this should make it possible to assess the influence of pharmacologic and other interventions on the course of recovery from a spinal cord injury and thus identify novel interventions for the treatment of spinal cord injury.

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385.1 EMG AND CYCLIC HAND MOTION: A FREQUENCY RESPONSE STUDY.
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Agarwal and Gottlieb noted (CRC Crit. Rev. 12, 1985) "In non-isometric conditions, the EMG-force relationship is a significant problem which needs to be resolved. . . ." While a major part of this problem (for intact systems) is detection of generated muscle force, this force may be inferred given: 1) an estimate of muscle activation (e.g., the surface EMG envelope), 2) accurate records of limb kinematics, and 3) a model for the muscle-load system. For our purposes, it is important that such a model be: 1) homeomorphic, 2) lumped-parameter, and 3) minimally complex (whether linear or nonlinear, with possibly time-varying parameters) for the ranges of frequency and amplitude studied.

Normal human subjects, seated with forearm pronated and supported to the wrist, were instructed to make self-paced, extension-flexion cyclic hand movements in the vertical plane for 16 s at a single frequency. With multiple trials, this frequency ranged from 1 Hz to the maximum voluntary frequency (7-12 Hz). Hand motion was detected by a phase- and amplitude-calibrated accelerometer. Bipolar surface EMGs were detected from extensor digitorum and extensor carpi radialis. After digitization, the EMGs were high-pass filtered, rectified, and smoothed (amplitude demodulated). Auto- and cross-spectral analysis was performed.

Peak demodulated extensor EMG led peak extension by an angle that increased from about 90° at 1 Hz to about 230° at 8-12 Hz. The log(peak displacement/peak EMG)- and phase-frequency values are consistent with a linear, third-order homeomorphic model, with transfer function:

$$\frac{\text{Position}}{\text{force}} = \frac{3.726 \times 10^6 (s + 848.2) e^{-0.010s}}{(s + 111.3)(s^2 + 13.35s + 70.99)}$$

Model linearity was supported since harmonic bands (1st, 3rd, 5th) in the motion were present in the EMGs. Added mass affected frequency response data and model response similarly, provided that model stiffness increased proportionally with mass. For certain voluntary frequencies (1-3 Hz), a band considered to be hand tremor occurred at 7-9 Hz. Broadband parametric variation of the model's short-range stiffness element generated similar resonance oscillations, whereas direct forcing of this element could not. A proportional, but delayed (about 10 ms), relation between demodulated EMG and nonisometric muscle force was indicated.

385.2 RHYTHMIC PRECISION: VARIABILITY IN TIMING OF PIANO SCALES AS A FUNCTION OF TEMPO.
D.L. VanEerd*, C.L. MacKenzie, L. Wilcox*, E.D. Graham*, D.B. Huron* & B.L. Wills* (SPON: E. Roy). Music Performance Laboratory, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

In piano performance, an important "technical ability" is to play scales quickly with rhythmic precision. For scales, arpeggios and thirds, the pianist's goal is to play evenly, with equal intervals between notes. As speed increases (internote intervals decrease), one might predict that the variability is proportional to the timed intervals (after Schmidt et al., 1978) in a manner analagous to Weber's Law for discriminability in sensory systems. The purpose of this research was to examine rhythmic precision as a function of playing speed.

Five skilled pianists (with Grade 10 or ARCT with the Royal Conservatory of Music in Toronto) played 2 octave ascending scales in C major with standard fingering (123123412312345). Each trial was preceded by 8 metronome beats; metronome settings corresponded to 2.0, 3.1, 4.0, 6.1, 8.0, 10.0, 12.3 and 13.9 keystrokes/sec. Performance on a grand piano was monitored by a microcomputer system measuring positions in time of the 88 keys (Wills, MacKenzie, Harrison, Topper & Walker, 1985). Internote intervals (INIs) were defined as the time between consecutive key downs, and rhythmic precision was operationalized as the standard deviation (SD) and coefficient of variation (CV=SD/M) of INIs within a trial.

Results indicated significant variation in INIs as a function of tempo, both in an absolute sense (SD) and a relative one (CV). SD of INI was a U-shaped function of tempo, with minimum variability between 4-6 notes/sec. This is consistent with Wagner (1971). CV was a monotonically increasing function of playing speed, indicating that relative variability increases with tempo. Typical long-short patterns were seen in consecutive INIs (see Wing, 1977), and this effect was additive to playing speed. Implications for models of underlying sources of timing and motor delay are discussed.

385.3 A DIMENSIONLESS MUSCLE-TENDON MODEL FOR USE IN COMPUTER STUDIES OF POSTURE AND MOVEMENT: ITS STATIC, DYNAMIC AND STIFFNESS PROPERTIES.
E.L. Topp*, F.E. Zajac, and P.J. Stevenson* (SPON: P. Dev) Rehab. R & D Center, Palo Alto VA Medical Center, Palo Alto, CA 94304, and Mechanical Engineering Dept., Stanford University.

Computer simulation of the neuromusculoskeletal system can be used to study and evaluate coordination strategies. Meaningful simulations of neural control of the body require musculotendon actuator models that incorporate the static and dynamic character of skeletal muscle and tendon. We have developed a low (2nd)-order dynamical model that captures the salient features of muscle and tendon, can itself be studied to understand musculotendon properties, and is computationally efficient enabling many actuators to be simulated simultaneously.

We assume that (a) fibers in each muscle act in parallel, insert at the same pinnation angle (α) on tendon, have the same length and specific tension, and develop length-dependent passive forces that sum with their active forces; (b) active muscle has a short-range stiffness that increases linearly with its force; (c) active force is velocity- and length-dependent according to fiber force-velocity and isometric force-length (f-ℓ) relationships, with the latter scaled by activation; and (d) maximum shortening velocity (V_o) is independent of both length and activation. These properties, when combined, are called the musculotendon contraction dynamics (1st-order). We also assume that (a) as an approximation to E-C dynamics, activation has 1st-order dynamics with different excitation and relaxation time constants; (b) activation is driven by one neural control, which is somewhat related to the level of recruitment and average firing rate of the muscle (the EMG); and (c) tendon obeys a non-linear stress-strain relationship that is actuator independent. We chose nominal parameters and functions for the actuator-independent constitutive properties of muscle fibers and tendon. By using maximum isometric force, optimal fiber length (ℓ_o) and V_o as dimensional quantities to form the overall dimensionless dynamics, we found that musculotendon contraction dynamics depends only on the ratio of tendon slack length to ℓ_o (and α). For high ratios (~10) as found for some shank muscles (Gordon et al., RESNA Proc., 1986), tendon then has the significant effect of broadening the musculotendon range of motion associated with the ascending region of the isometric f-ℓ curve, slowing force build-up, and dominating musculotendon elastic energy storage and stiffness. (Supported by NIH grant NS 17662 and the Veterans Administration.)

385.4 PHYSIOLOGICAL CLASSIFICATION OF MUSCLES INTO AGONIST-ANTAGONIST MUSCLE ACTION GROUPS: A THEORY AND METHODOLOGY BASED ON MECHANICS
F.E. Zajac, M.E. Gordon*, and M.G. Hoy. Mechanical Engineering Dept., Design Division, Stanford University, Stanford, CA 94305, and Rehab. Res. & Dev. Ctr., Palo Alto VA Medical Center.

Measurement of EMG activity from muscles has become common in motor control studies. Interpretation of these EMG patterns is often based on two notions: a) muscles can be classified into agonist-antagonist groups; and b) a group has a specific action on the body (e.g., extend-flex the knee), where the body is regarded as an articulated chain of rigid-body segments. We assume a planar body (for now) and that each physiological agonist-antagonist group (PHYS AG-ANTAG GR) should be associated with a distinct action-reverse action on the body. We define a muscle's action on the body to be the direction in which the muscle acts to accelerate the body. This direction will be a vector (muscle action vector) where the ith element is either +1, -1, or 0, depending on whether the action is to positively, negatively or zero accelerate the ith body segment. We show that all elements of a muscle action vector are, generally, non-zero; i.e., a muscle force affects the acceleration of all body segments, even those to which the muscle neither attaches nor spans, even though its torque acts only on the attached and spanned segments. This fact follows by recognizing that it is the corollary to the better known fact that acceleration of all body segments must be taken into account in determining muscle torques. All muscles with the same muscle action vector are collected into one set, and muscles with vectors negative to this action vector into another. These two muscle sets form, by definition, one PHYS AG-ANTAG GR. In contrast, one set of an anatomical agonist-antagonist group (ANAT AG-ANTAG GR) is defined as those muscles developing the same torque directions on the segments attached and spanned; the other set produces opposite torque directions.

We show that any given ANAT AG-ANTAG GR is a subset of some PHYS AG-ANTAG GR. Though an ANAT AG-ANTAG GR does not depend on body segment orientation, the remaining subset of the PHYS AG-ANTAG GR does. Thus, two muscles not belonging to any ANAT AG-ANTAG GR can at one time be agonists, at another time antagonists, and at still other times, neither. The number (z) and composition of the PHYS AG-ANTAG GRs also depend on mechanical constraints external and internal to the body since the constraints reduce the number of degrees-of-freedom, say, to n, and $z < 2^{n-1}$. Thus, for a highly constrained body with z less than the number of ANAT AG-ANTAG GRs, different ANAT AG-ANTAG GRs will belong to the same PHYS AG-ANTAG GR. We are now defining the PHYS AG-ANTAG GRs in such tasks as human posture, jumping, walking and pedaling. (Supported by NIH grant NS17662 and the Veterans Administration.)

385.5 POSTURAL SYNERGIES DICTATED BY SEGMENTAL ACCELERATIONS FROM MUSCLES AND PHYSICAL CONSTRAINTS M.E. Gordon*, F.E. Zajac, M.G. Hoy (SPON: M.C. Carter). Mechanical Engineering Dept., Design Division, Stanford University, Stanford, CA 94305, and Rehab. Res. & Dev. Ctr., Palo Alto VA Medical Center.

It has been proposed (Nashner and McCollum, Behav. & Brain Sci., 8:135, 1985) that postural regulation is simplified by the organization of muscles into synergies. Our approach is to organize muscles into physiological agonist-antagonist (PHYS AG-ANTAG) groups based on muscle contributions to accelerations of all the body segments (see accompanying abstract). We define a muscular synergy as a group of muscles which work together to accomplish a purposeful motor task. Objectives for this study were to see how synergies are determined from muscle contributions to segmental accelerations and from physical constraints associated with posture.

The body was modeled as four rigid-body segments: foot, shank, thigh, and torso. The musculoskeletal system was modeled in detail, with separation of single and double joint muscles. Static force-length properties were included, based on a dimensionless musculotendon actuator model scaled to each actuator. Posture was studied as an optimization problem with the objective of maximizing the acceleration of the body from a disturbed position towards a desired one. The disturbed positions corresponded to leaning forward and backward, with the knees extended either by the use of muscles to cause equal accelerations of the shank and thigh segments, or by the use of a knee brace. To keep the feet flat on the ground, the net segmental accelerations were always constrained.

We found synergies consistent with those of Nashner and McCollum. Also consistent with their observations, our optimization predicted "clockwise curved" postural trajectories, with muscles activated in distal to proximal sequence. Analysis of the muscle contributions to segmental accelerations showed that in many positions of the body, synergies are composed of muscles in one PHYS AG-ANTAG group. However, at times soleus and gastrocnemius act to accelerate the body segments in *opposite* directions (i.e., they are *physiological antagonists*). Even so, because the relative magnitude of their contribution to acceleration of each segment is different, they function *synergistically*, together with the other muscles, in restoring posture. With the knee constrained in extension by a brace rather than by activation of muscles, different synergies were found. In conclusion, postural synergies are greatly influenced by physical constraints and by both the direction and magnitude of muscle contributions to segmental accelerations. (Supported by NIH grant NS17662 and the Veterans Administration.)

385.6 SYNERGISTIC CONTROL OF UNIARTICULAR AND BIARTICULAR MUSCLES IN HUMAN JUMPING: A COMPUTER SIMULATION STUDY M.G. Hoy, F.E. Zajac, E.L. Topp*, C.T. Cady*, and W.S. Levine* Rehab. Res. & Dev. Ctr. (153), VA Medical Center, Palo Alto, CA 94304, and Mechanical Engineering Dept., Stanford University.

Biarticular muscles that are anatomical extensors at one joint and anatomical flexors at the other joint (e.g., rectus femoris) share anatomical function with both uniarticular flexors and extensors. Accordingly, these muscles may be activated synergistically with uniarticular flexors or extensors, e.g., during the swing and stance phase of locomotion, respectively. Biarticular muscles, however, can influence segmental accelerations differently than uniarticular muscles by exerting torque on more body segments, and thus, may function differently in coordination of movement. To determine whether jumps can be controlled by synergistic activation of uni- and biarticular muscles, we used computer simulation to examine the actions of uni- and biarticular muscles in coordination of human countermovement jumps.

The human musculoskeletal model comprised planar, rigid-body segments (trunk, thigh, shank, foot), uniarticular extensors of hip, knee, and ankle, and biarticular muscles (gastrocnemius (GAS), rectus femoris (RF), hamstrings (HAM)). A dimensionless model was used for musculotendon dynamics (Topp et al., this volume); specific parameters for the model are described in a previous report (Gordon et al., RESNA Proc., 1986). Countermovement jumps were simulated by allowing the body to fall freely from a standing position and then activating muscles to brake the downward motion and propel the body upwards.

Although jumps could be produced with the uniarticular muscles alone, generally jump height was lower and horizontal kinetic energy was higher than in jumps produced by coactivation of uni- and biarticular muscles. However, some variations in muscle strength made control of high jumps using few synergies impossible. The highest jumps were produced when RF and GAS were activated after the uniarticular muscles. Coactivation of HAM with either RF or GAS decreased jump height. Coactivation of uni- and biarticular muscles made control of jumps more robust.

Uniarticular extensor muscles thus function differently than biarticular muscles in human jumps. Biarticular muscles are not a single functional group, but should be activated separately to produce the highest jumps. Results indicate that synergistic activation of uni- and biarticular muscles is an inappropriate control strategy for coordination of jumps; rather, both timing and magnitude of muscle activation are important in control of jumping. (Supported by NIH grant NS 17662 and the Veterans Administration.)

385.7 LEARNING-RELATED DIFFERENCES IN HUMAN SMU RECRUITMENT DURING POSITION TRACING. L.D. Abraham and N. Benjuya. Neuromuscular Lab, Dept. of Physical Education, Univ. of Texas, Austin, TX 78712

Motor-learning-related EMG changes have been reported, based on surface EMG, usually describing decreased antagonist coactivation as a significant feature. This study investigated the activity of single motor units (SMUs) during the learning of a novel tracing task, which required precise control of the position of both ankles. (See Benjuya & Abraham, Neurosci. Abstr., 1986, for report of a parallel isometric task.) Twenty, right-leg dominant, normal humans were tested in single learning sessions. The subject was seated with each foot secured to an instrumented pedal which allowed only ankle plantarflexion (PF) and dorsiflexion (DF). The subject was instructed to trace a triangular shape on an oscilloscope screen, controlling the vertical and horizontal motion of the cursor with movement of the right and left feet respectively (50 deg. arc = 5 cm). The task was repeated until it was completed in fewer than 10 sec three consecutive times with less than 2 deg. of instantaneous spatial error. The number of trials to criteria ranged from 50 to 140. Throughout each session, EMG recordings of SMU activity were obtained from bipolar intramuscular fine wire electrodes in the soleus (SOL) and tibialis anterior (TA) muscles of the right leg. In all, thrity individual SMUs from each muscle were identified off-line by amplitude and waveform. Triangle tracing required a combination of increasing (I), static (S), and decreasing (D) PF or DF. The right foot demands for the task were, in order: I-PF, D-PF, I-DF, S-DF, D-DF. Skill performance was quantified off-line by comparing the actual tracing with the criterion triangle. The triangle was partitioned into segments, and average tracing error (TE) and time per segment (TPS) were calculated. ANOVA was used to assess differences in TPS, TE, and SMU frequency among segments and between pre- and post-learning trials (first 20 trials vs last 20 trials). Comparison of pre- and post-learning performance revealed improved TE scores in the I-PF and D-DF segments, as well as at the corners. TPS scores became faster in the I-PF and D-DF segments, and also in the S-DF segments. Thus the major improvements were during the (bipedal) hypothenuse and the (sustained) extreme DF. TA SMU frequency was highest in the D-PF, I-DF, and S-DF segments, and decreased in all segments. SOL SMU frequency showed little change with respect to learning. Antagonist coactivation, defined as parallel changes in TA and SOL frequency, was generally higher in the pre-learning trials. Some segments, however, showed no change in coactivation, while SOL increases in three segments led to increased coactivation. These increases were apparently related to difficulty in controlling the cursor at segment transitions.

385.8 LEARNING-RELATED DIFFERENCES IN HUMAN SMU RECRUITMENT DURING ISOMETRIC TRACING. N. Benjuya and L.D. Abraham. Neuromuscular Lab, Dept. of Physical Education, Univ. of Texas, Austin, TX 78712

Characteristics of single motor unit (SMU) firing during isometric contractions have been described (e.g. De Luca et al., J. Physiol., 329: 113-128, 1982). This study investigated the activity of SMUs during the learning of a novel tracing task, which required precise isometric control of torque at both ankles. (See Abraham & Benjuya, Neurosci. Abstr., 1986, for report of a parallel ramp movement task.) Twenty right-leg dominant, normal humans were tested in single learning sessions. The subject was seated with each foot secured to an instrumented pedal which detected ankle plantarflexion (PF) and dorsiflexion (DF) torque. The subject was instructed to trace a triangular shape on an oscilloscope screen, controlling the vertical and horizontal motion of the cursor with torque at the right and left ankle respectively (25 N-m range = 5 cm). The task was repeated until it was completed in fewer than 10 sec three consecutive times with less than 2 deg. of instantaneous spatial error. The number of trials to criteria ranged from 40 to 150. Throughout each session, EMG recordings of SMU activity were obtained from bipolar intramuscular fine wire electrodes in the soleus (SOL) and tibialis anterior (TA) muscles of the right leg. In all, thirty individual SMUs from each muscle were identified off-line by amplitude and waveform. Triangle tracing required a combination of increasing (I), static (S), and decreasing (D) PF or DF. The right foot demands for the task were, in order: I-PF, D-PF, I-DF, S-DF, D-DF. Skill performance was quantified off-line by comparing the actual path with the criterion triangle. The triangle was partitioned into segments, and average tracing error (TE) and time per segment (TPS) were calculated. ANOVA was used to assess differences in TPS, TE, and SMU frequency among segments and between pre- and post-learning trials (first 20 trials vs last 20 trials). Comparison of pre- and post-learning performance revealed greatly improved TE scores for all segments, reflecting initial difficulty throughout. Post-learning TPS scores were faster in all segments, especially in the D-PF, I-DF, and S-DF segments (those requiring control of only one foot). TA SMU frequency was highest in the D-PF, I-DF, S-DF, and D-DF segments, and decreased in all post-learning segments. SOL SMU frequency showed small decreases in I-PF and small increases in D-DF. Antagonist coactivation, defined as parallel changes in TA and SOL frequency, was completely absent in pre-learning performance but appeared in post-learning at a very low level in the D-DF (last) segment. This unusual shift was apparently related to the difficulty along the terminal bipedal segment (the hypotenuse of the triangle).

385.9 H-REFLEXES IN A MOTOR SKILL ACQUISITION TASK. A.M. Baylor and N. Benjuya. Neuromuscular Lab, Univ. of Texas, Austin, Tx 78712.

H-reflexes were studied in the acquisition of a foot task involving tracing a triangular shape on an oscilloscope screen (vertical cursor = right foot and horizontal cursor = left foot). The beam was controlled by isometric plantar and dorsi flexion torque around the ankle joint. Seven subjects repeated the task in blocks of 20 trials until criterion performance was achieved. Electromyographic (EMG) recordings were made from the soleus (initial agonist) and tibialis anterior muscles of the right leg. The command, "trial number, ready, go", was given in regular intervals, and subjects were instructed to begin each trial on "go". H-reflex stimulations were presented near the beginning of each trial and timed with respect to the "go" signal at 0, 500, 1000, 1500, or 2000 ms. Control blocks of 10 H-reflexes were given initially, finally and between each block of 20 trials. H-reflexes were expressed as percent of control value with the control block selected on the basis of similar M responses.

Total number of trails to reach criterion ranged from 35 to 200 (M=122). Data from the first 20 trials were compared to the last 20 trials. The average time to complete 1 trial dropped from 9 sec (first block) to 4 sec (last block) with the greatest reductions in the first 5 trials. H-reflexes were grouped into 7 time intervals with respect to onset of agonist EMG and ranged from 600 ms after EMG onset to 1500+ ms prior to EMG.

In the final block, subjects tended to be close to control H-reflex values; however subjects differed in how the control values were achieved. Four of the subjects showed higher pre learning values with post learning reductions toward control especially in the early intervals; two showed pre learning depression with post learning increases especially in later intervals, and one showed little change from control values. When the pre to post learning differences are expressed as a percent of pre trials, pre to post differences within individual subjects ranged from a 58% reduction in early intervals to a 157% increase in a later interval. In the final block, subjects tended to respond later to the "go" signal; thus creating more responses in later H-reflex intervals.

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385.10 **IDENTIFICATION OF THE TIME-COURSE OF CHANGES IN HUMAN ANKLE DYNAMIC STIFFNESS DURING VOLUNTARY CONTRACTIONS.** R.E. Kearney and I.W. Hunter, Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montréal, Québec, Canada, H3A 2B4.

It is now well established that joint stiffness dynamics change dramatically with steady-state changes in operating point parameters such as mean torque, mean position, and perturbation amplitude. However, little is known about the time-course of changes in stiffness dynamics after the operating point changes. Last year we reported on the use of a novel, time-varying system identification technique to track the time-course of changes in dynamic ankle stiffness during voluntary contractions of triceps surae (Soc. Neurosci. Abst. (1988) 11:214). Reliable estimates of the dynamic stiffness were obtained before and after the start of the contraction but not during the transient phase. The objective of the present work was to determine the reason for this and to modify the analysis procedure to obtain reliable estimates of stiffness dynamics during the transient phase of the contraction.

The basic technique requires an ensemble of responses in which: (1) The pattern of perturbation across the ensemble is the same as along each realization; and (2) The nonstationarity is the same in each realization. Identification may then be performed across the ensemble, where conditions are stationary, rather than along a realization where conditions are time-varying.

Examination of our experimental data revealed two potential problems. First, there was some input variability; the cross-ensemble input pattern was not identical to that along each realization probably because of varying load characteristics. Secondly, the nonstationarity was not identical in each realization because of tracking jitter; the delay between the command signal and the onset of the voluntary contraction varied somewhat from trial to trial. Simulations indicated that tracking jitter was the likely cause of the unreliable estimates of stiffness dynamics during the transient phase of the contraction.

The analysis procedure was therefore modified to compensate for tracking jitter. This involved: (1) Identifying the onset of the contraction in each trial; (2) Time shifting the torque and position records to align the torque onset in all records; (3) Shuffling realizations within the ensemble to restore the required cross-ensemble perturbation pattern; (4) Interpolating the resulting irregularly sampled cross-ensemble data; and (5) Computing the stiffness impulse response functions as before.

Simulations demonstrated that this procedure could indeed successfully compensate for the effects of tracking jitter. Analysis of experimental data yielded robust estimates of dynamic stiffness throughout all phases of the contraction. The dynamic stiffness was found to follow a time-course very similar to that of the mean torque. However, in the data analysed to date, there were strong indications that stiffness changes preceded torque changes.

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385.11 **EVOKED POTENTIAL AND LOAD PERTURBATION CHARACTERIZATION OF THE SHORT AND MEDIUM LATENCY SYNERGY OF PRE-TIBIAL FLEXORS AND VASTUS MEDIALIS IN HUMANS.** J.D. Brooke, W.E. McIlroy*, N.J. Mallory*, D. Wright* and P. Yoon*. Neurophysiology Lab. School of Human Biology, Univ. of Guelph, Guelph, ONT Canada N1G 2W1.

Heteronymous paths for muscle synergies are well reported, some appearing human specific (Brooke & McIlroy Electroenceph. clin. Neurophysiol. 1985, 60, 39-45). We are currently characterizing such a projection between human pre-tibial flexors and vastus medialis. Six experiments involved twenty one healthy adult subjects. Surface electrodes transduced tibialis anterior (TA) and vastus medialis (VM) EMGs which were amplified (Grass P511 at 3 Hz - 3 kHz band) and computer sampled at 1 kHz. Common peroneal nerve stimulation (CPNS) was transcutaneous for 1 ms square wave at caput fibula, anode distal. VM EMG responses, as peak to peak waveform amplitude or area, were identified against the 95% band of EMG from background contraction. In Experiment 1 (E1) means of 20 samples per subject revealed significant VM responses from CPNS at group I latency, with VM prior contracted (ca. 20% of max) isometrically or isotonically (both when pedalling or episodic), but with no response relaxed. E2 showed graded responses in VM when stimulation ranged from 0.7 times threshold for motor nerve activation (MT) of TA, through 1.0, 1.4, 1.8, 2.1 to 2.4 MT, with peak occurring 1.4 to 2.1 MT. In E3, VM responses from 6 subjects at 1.4 MT were unaffected by vibration of TA which reduced the average homonymous reflex to 30% of control. Such VM respqnses still appeared in E4, with TA lightly contracted and then directly contracted by electrodes on the belly of the muscle. Homonymous responses in TA were rare. One concludes that Ib fibres likely constitute the afferent limb of this short latency pathway. As possible independent indicators of this range of stimulation intensities, in the fifth experiment first negative and positive potentials concurrently evoked over the sensori-motor cortex were evaluated in three subjects. However no significant positive relationship was found, even with presentation order controlled. TA and VM also acted synergistically in E6, when subjects on a stationary ergometer suddenly experienced increased resistance to pedalling (leading to 50% of initial velocity by ca. 20 ms post onset of disturbance). The coordinated muscular activity was initiated at a medium latency following perturbation onset ($\bar{X}$ = 104 ms), followed by synergies to restore the pedalling at 'voluntary' latencies ($\bar{X}$ = 168 ms). Complementary firing of tibialis anterior and vastus medialis appears to be a significant synergy of the human lower limb, a conclusion strengthened by the analogy between the short and medium latency responses. There is a need to evaluate its functional role and additional convergence. Supported by NSERC (Canada) Grant #A0025 and the Banting Research and Bickell Foundations.

385.12 THE BEHAVIOR OF SPINDLE RECEPTORS DURING MUSCLE CONTRACTION DEPENDS ON THE OPPOSING FORCE. R. López Torres* and E.J. Muñoz-Martínez. Depto. Fisiología. CINVESTAV. Apdo. Postal 14-740. 07000 México, D.F. MEXICO

Spindle afferents may fire during muscle contraction even though the firing could not be attributed to activation of intrafusal muscle fibers but it must rather be sought in the contractile events outside the spindle (Hunt & Kuffler, 1951). The ceasing of spindle discharges results from receptor unloading. Thus, a different receptor behavior should be expected if both the force opposing the contraction as well as the initial load are changed.

Our experiments were performed in cats under barbiturate anaesthesia. The spinal roots of the L7 and S1 segments were sectioned near the spinal cord; recordings were taken from small dorsal root filaments and single motor axons were stimulated. The medial gastrocnemious or the soleus muscles were dissected and attached to strain gage transducers which permited maximal displacements of 1 mm. Two transducers were used alternatively. The maximal working ranges were 10 Kg for one transducer and 100 g for the other.

It was found that the response of Ia afferents to single twitches or to tetanic contractions of single motor unit varied depending on the initial tension and the force opposing the contraction. Background discharges ceased in most fibers during isometric twitch contraction of single motor units, but stimulation of other motor axons induced Ia firing in coincidence with the twitch peak. On the other hand, at least 50% of the Ia fibers increased their firing frequency during tetanic contractions of single motor units provided that the initial tension was greater than 25 g but, interestingly, the background firing ceased if the opposing force was changed from 1 Kg to 100 g. We propose that, on the average, spindle receptors provides information on the degree of muscle shortening during contraction. In addition, our results show that the spindle discharge during single twitches and tetanic contractions depends on the location of both the muscle spindle and the motor unit.

J. Physiol. 113: 298-315, 1951.

385.13 COMPUTER SIMULATION OF THE ROLE OF THE CEREBELLUM IN ADAPTIVE REFLEX CONTROL. M.G. Paulin* (SPON: C. Norris). Dept. of Otolaryngology - Head & Neck Surgery, School of Medicine, University of Southern California, Los Angeles, CA 90033.

The cerebellum is involved in regulating movements so that they are smooth and accurate. Damage to the cerebellum can prevent reflexes from adapting to changes, suggesting that reflex modification is due to plasticity on side arcs through the cerebellum. But my computer simulations show that the regulatory function alone can explain the cerebellum's apparent learning ability.

I have assumed that the cerebellum controls reflexes by modulating signal flow in extracerebellar pathways, as described by MacKay and Murphy (1). My hypothesis is that the effect of cerebellar dysfunction on the ability of reflexes to adaptively modify their dynamics is a consequence of this role.

The simulated reflex is a linear system which initially relies on a feedback loop to hold its output near the intended path during a maneuver. While the system 'practices' maneuvers, parameters on the forward path are automatically adjusted so that less feedback is required. The system evolves from a mainly closed-loop into a mainly open-loop system. In the final adapted state it responds accurately without feedback.

Feedback modulation, simulating the assumed role of the cerebellum, affects the system's sensitivity to noise and the ability to correctly modify parameters on the forward path. Adaptation slows and may stop if the feedback signal is poorly modulated.

The simulation shows that it is not necessary to assume that cerebellar plasticity plays a special role in reflex adaptation.

(1) MacKay W.A. and Murphy J.T. (1979). Cerebellar modulation of reflex gain. Progress in Neurobiology 13, 361-417.

385.14 TRANSECTION-INDUCED CHANGES IN THE DISTRIBUTION OF SURAL POST-SYNAPTIC POTENTIALS IN TRICEPS SURAE MOTONEURONS OF THE ADULT CAT. J. Kehler*, L. LaBella* and D.A. McCrea (SPON: A. Storey). Dept. of Physiology, U. of Manitoba, Winnipeg, Canada R3E 0W3.

Postsynaptic potentials (PSPs) produced by low threshold sural nerve stimulation were examined in triceps surae (TS) motoneurons in both unlesioned (UL) and chronic spinal (CS) cats (2-5 months post-transection). Only the first PSPs occurring at twice threshold (2T) stimulation and at latencies $\leq$ 5ms are reported. First effects were predominantly excitatory in TS motoneurons of both preparations. A clear increase in the incidence of these EPSPs occurred in CS animals, with a decrease in the earliest latency. Using chloralose anaesthesia in the acute experiment, 59% of the PSPs in UL animals were excitatory (n=27) with 25% of them occurring at latencies between 2.0-2.5ms, while in CS animals 70% of PSPs were excitatory (n=33) with 52% of them occurring between 1.5-2.5ms. In other experiments, the sural nerve was separated into medial (MS; the branch running through the popliteal fossa) and lateral branches (LS; the branch running along the inner aspect of biceps femoris). In UL barbiturate-anaesthetized animals MS stimulation caused EPSPs 19% of the time (n=21; earliest latency 2.5ms) while in CS, the incidence of EPSPs was 60% (n=20; earliest latency 1.8ms). LS stimulation only produced IPSPs in both UL and CS animals. It appears that the excitatory component of sural effects in TS motoneurons is due to MS alone, and that even with moderately deep barbiturate-anaesthesia this excitation is enhanced after chronic spinalization.

Preliminary studies focusing on sural effects in soleus (SOL) motoneurons have revealed only IPSPs (latency range 3.0-5.0ms) in UL animals upon stimulation of either the MS or LS branch of sural at 2T. However, in CS animals early latency EPSPs (earliest 1.8ms) have been found upon stimulation of the MS but not LS branch and while the incidence and amplitude of LS-IPSPs are virtually unchanged after transection, MS-IPSPs are either reduced or absent. The appearance of MS-EPSPs after transection and the differential changes among MS and LS inhibitory effects suggests that these 2 divisions of the sural nerve may access separate populations of interneurons; at least in pathways to the SOL motoneuron pool. Given indications in the literature (eg. Pinter et al, Exp. Brain Res. 45:133, 1982) that rubrospinal and pyramidal tract stimulation preferentially facilitate sural EPSPs in fast extensor motoneurons and sural IPSPs in slow extensor cells, we would like to speculate that certain descending systems utilize interneurons in the pathway from the MS branch to alter the balance of excitation to fast and slow motoneurons. This speculation assumes that the distribution of sural PSPs among SOL cells is representative of the distribution to all slow ankle extensor motoneurons.

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385.15 MOTOR LEARNING MODIFIES ONLY THE LONG LATENCY COMPONENT OF THE BLINK REFLEX. C. Evinger and K. A. Manning. Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Reflex blinks arise from a two-step neural process. First, using stimulus magnitude information, the blink generator computes an initial downward eyelid movement. This early component is preprogrammed and insensitive to sensory feedback. Second, using information obtained continuously from sensory feedback, the blink generator produces the late component of the blink. Two component models also apply to other reflexes and may be a fundamental organizational principle of mammalian reflexes.

While it is well known that simple motor learning paradigms can modify reflexes, it is unknown whether these changes occur throughout the reflex or in only one component. The present study analyses the two components of the blink reflex after (1) motor learning induced by adaptive plasticity or habituation, and (2) a reflex modification paradigm.

In humans, rabbits, and rodents, airpuffs, light flashes, or electrical stimulation of the supraorbital branch (SO) of the trigeminal nerve evoked reflex blinks. Blink amplitude and orbicularis oculis muscle activity were monitored. Adaptive plasticity paradigms increased the amplitude of the blink by requiring the animal to blink against a constant upwardly directed force applied to the upper eyelid or decreased the amplitude of the blink by making the subject blink with a constant downwardly directed force on the upper eyelid. Stimulus presentation rates greater than 0.17 Hz caused habituation of the blink reflex. Reflex modification produced by preceeding (100-200ms) the SO stimulation by a 20 ms tone decreased the amplitude of the evoked blink.

The adaptive plasticity paradigm using the upwardly directed force produced a 16-20% increase in blink amplitude and using the downward force effected a long-term 30-40% decrease in blink amplitude. In habituation, rapid presentation of 7 stimuli caused a 20-60% reduction of blink amplitude which returned to normal following 5 minutes without stimulation. Reflex modification produced a 20-60% reduction in blink amplitude on tone-SO trials but, unlike habituation or adaptive plasticity, did not influence subsequent trials or SO only trials.

Despite the variety of species and paradigms, all conditions gave one identical result. The early component of the blink reflex always remained constant. Every modification of the reflex arose exclusively from the late component. Demonstrating that the longer latency pathways of the blink reflex are the site of motor learning provides a focus for cellular analysis.

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385.16 A NEW METHOD FOR HUMAN LIMB PARAMETER ESTIMATION. M.R. Zomlefer and D. Khalili*. Electrical Engineering and Computer Science Department, Santa Clara University, Santa Clara, California 95053.

Determination of human body segmental parameters is essential to the accurate design of prosthetic and orthotic devices, reconstructive surgery and rehabilitation, sports medicine and physical therapy (Dempster, 1955, Drillis et al. 1966). Body segmental parameters are necessary for the modelling and quantization of the biomechanical properties of limbs, joints and muscles. These parameters are also critical to the understanding of the coordination of movement.

A new non-invasive method for the determination of certain body segment parameters is proposed and formulated. The limb masses, center of masses and inertial properties are all obtained using biomechanical measurements and modern estimation theory. In addition, through a series of "Experiments" it is possible to derive individual joint parameters and dynamic properties (e.g., stiffness and viscosity). This same approach can be used to estimate segmental torque-joint angle properties IN VIVO.

An interconnected robotic system consisting of two double link planar robot arms is attached across the human body segments. Sets of measurements are made for distinct joint angles. Then, by applying the measurement data in the equations of the static state of equilibrium of the system (robot arms + human limb), the mass, and the center of mass for the limb segments are computed. The accuracy of the results by using this procedure principally depends on the accuracy of the force sensors.

385.17 PREDICTIVE CONTROL OF HUMAN MOVEMENT. B.J. McFadyen and J.S. Frank* Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

The measurement and modelling of human sensori-motor control has concentrated on feedback, adaptive and, so-called, feedforward processes. The term feedforward control has been subject to great controversy arising from the varied perspectives of psychology, physics, physiology and engineering. The classical definitions are rooted in engineering science. One definition relates to shunting an input forward in the control process to avoid delay by bi-passing certain system components, while the other relates to gaining appropriate information prior to an inputs occurrence in order to anticipate it. What these classical definitions lack for the description of human sensori-motor control is the inclusion of cognitive processes such as memory. Although human movement literature has used the term feedforward control, it often has been applied erroneously with respect to its classical definitions.

This theroetical paper explores this issue and suggests the replacement of "feedforward control" with the more general term, "predictive control". It is hoped that this will allow more constructive communication and better grounds on which to base measurement and modelling of the motor control system.

385.18 NONEQUILIBRIUM PHASE TRANSITIONS IN MULTI-DEGREE OF FREEDOM MOVEMENTS. J.A.S. Kelso*, G. Schöner*, J.P. Scholz*, and H. Haken* (SPON: A. Nash). Center for Complex Systems and Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431; Haskins Laboratories, New Haven, CT 06511; Institute for Theor. Phys., Stuttgart Univ., FRG.

The movements of animals and people are ordered spatiotemporal structures even though they arise from microscopic sources (e.g. neuronal, skeletomuscular, vascular) of huge dimensionality. How are such ordered states to be understood? The interdisciplinary field of synergetics (e.g. Haken, H., Synergetics: An introduction, Springer, Berlin, 3rd Ed. 1983) studies the spontaneous formation of patterns in nonequilibrium systems and offers a means by which the behavior of very high dimensional systems may be described in terms of a few macroscopic quantities called order parameters. In coordinated biological motion the phase relation (ϕ) among interacting components may be a relevant order parameter characterizing different modes of coordination. In experiments on oscillatory human hand movements (Kelso, J.A.S., Bull. Psychon. Soc. 18:63, 1981; Am. J. Physiol. 246, R1000, 1984), abrupt transitions between one mode of coordination ($\phi \simeq 180$ deg) and another ($\phi \simeq 0$ deg) were observed as a control parameter (pacing frequency) was scaled beyond a critical value. This phenomenon (analogous, perhaps, to locomotory gait changes, e.g. from trotting to galloping) is reminiscent of the nonequilibrium phase transitions studied in synergetics. Indeed, Haken, Kelso and Bunz (Biol. Cybern., 51, 347, 1985) have modeled such transition behavior explicitly. Order parameter dynamics for relative phase were derived from oscillator equations for the two hands with a nonlinear coupling between them. Here we elaborate after Schöner, Haken and Kelso (Biol. Cybern. 53, 247, 1986) a detailed stochastic analysis of the transition behavior that serves to (1) test the validity of the phase transition paradigm (2) establish time scales relations (e.g. among local relaxation times, equilibration time etc.), which are prerequisites for dynamic modeling of the transition and (3) illustrate how the switching from one coordinative state to the other is effected by order parameter fluctuations. Recent observations (Kelso, J.A.S. & Scholz, J.P. In H. Haken (Ed.) Complex Systems: Operational Approaches in Neurobiology, Physics and Computers, Springer, Berlin, 1985) and new experiments are consistent with the stochastic theory in revealing enhanced fluctuations and critical slowing down of the order parameter (ϕ) near the transition. Data and theory thus converge on the interpretation that the emergence of coordinated movement patterns may be understood--in a quite detailed fashion--in terms of the physics of nonequilibrium processes.

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385.19 THE SPACE-TIME BEHAVIOR OF SINGLE AND BIMANUAL RHYTHMICAL MOVEMENTS: DATA AND A LIMIT CYCLE MODEL. B.A. Kay*, J.A.S. Kelso*, E.L. Saltzman*, G. Schöner* (SPON: I.B. Johanson). Dept. of Psychology, Univ. of Connecticut, Storrs, CT.; Center for Complex Systems and Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33432; Haskins Laboratories, New Haven, CT 06511.

How do space and time relate in rhythmical tasks that require the limbs to move singly or together in various modes of coordination? And what kind of minimal theoretical model could account for the observed data? Earlier findings from human cyclical movements were consistent with a nonlinear, limit cycle oscillator model (Kelso, J.A.S., Holt, K.G., Rubin, P. & Kugler, P.N. J. Mot. Behav. 13, 226, 1981) although no detailed modeling was performed at that time. In the present study, kinematic data were sampled at 200 samples/second and a detailed analysis of movement amplitude, frequency, peak velocity and relative phase (for the bimanual modes, in-phase and anti-phase) performed. As frequency was scaled from 1 to 6 Hz (in steps of 1 Hz) using a pacing metronome, amplitude dropped inversely and peak velocity increased. Within a frequency condition, the movement's amplitude scaled directly with its peak velocity. These diverse kinematic behaviors were modeled explicitly in terms of low-dimensional (nonlinear) dissipative dynamics with linear stiffness as the only control parameter. Data and model are shown to compare favorably. The same model offers a unified treatment of a number of fundamental aspects of movement, including 1) the postural steady state (when the linear damping coefficient, α, is positive, 2) the onset of movement (when the sign of α becomes negative); 3) the persistence and stability of rhythmic oscillation [guaranteed by a balance between excitation (via $\alpha\dot{x}$, $\alpha < 0$) and dissipation (as indexed by the nonlinear dissipative terms, $\beta\dot{x}^3$ and $\gamma x^2\dot{x}$). This balance determines the limit cycle, a periodic attractor to which all paths in the phase plane ($x,\dot{x}$) converge]; 4) frequency and phase-locking between the hands; and 5) switching among coordinative modes (the latter properties due to a nonlinear coupling structure, see Haken, H., Kelso, J.A.S., Bunz, H. Biol. Cybern. 51, 347, 1985). In short, we show how a rather simple dynamical control structure requiring variations in only one system parameter can describe the spatio-temporal behavior of the limbs moving singly and together. The model is open to further empirical tests which are underway.

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385.20 **EFFECTS OF PRE-CUED DIRECTION AND FORCE INFORMATION ON CNV.** W.A. MacKay and M. Bonnet*. Dept. of Physiology, University of Toronto, Toronto M5S 1A8, CANADA

Although efferent motor signals must ultimately code both movement direction and muscle force information, at processing stages within the sensorimotor continuum one may be represented independently of the other. For example, directional information is precisely coded in many cortical area 5 neurons, independently of force parameters. In this study, the scalp potentials associated with performance of a pre-cued reaction time (RT) task were analyzed to distinguish differences linked to the parameters being prepared. The warning stimulus (WS, on a panel of 4 LEDs) provided FULL, DIRection, FORCE or NO advance information about the ensuing reaction. The response stimulus, 1.5s later, specified both the direction (right forearm flexion or extension of about 10°) and force level (weak or strong). Mean RTs varied both with the amount and type of advance information given. For practised subjects, mean RTs measured to agonist EMG onset were approximately, FULL: 152 ms, DIR: 177, FORCE: 197, NO: 225.

Using source derivation methods (see Crammond et al., EEG & clin. Neurophysiol. 61:396-410), the current sink responsible for most of the CNV was localized to the parietal lobe. Changes in voltage gradients (vectors) between the 8 recording leads divided the CNV temporally into 4 intervals, measured from WS onset: 1; 300-500ms, 2; 500-700, 3; 700-1100, 4; 1100-1500. Phase 1 was characterized by a frontal negative trend and parietal positivity. In all other intervals the maximum development of negativity was in the parietal lobe. The 4 advance information conditions were distinguished mainly by the scalp vectors during intervals 1 and 3. Increasing negativity in phase 1 focused on the arm premotor area for FULL information, but migrated progressively caudally for DIR and FORCE. For NO, a positive-going focus in the arm somatosensory region dominated. In phase 3, NO information produced a maximum negative trend centered over the central sulcus, whereas it was situated postcentrally for all other conditions. Laplacian derivations indicated a modest constant negativity precentrally for FULL and FORCE, which was reduced both by NO and DIR. The postcentral area developed a continuously augmenting negativity which was maximal for FULL and DIR and markedly reduced by FORCE and NO. The small vertex negativity showed very little difference under the different conditions. During choice RT preparation, scalp potentials indicate that somatosensory cortex may be primarily concerned with directional information, whereas the precentral area may give relatively more emphasis to force information. M.B. was a NSERC France-Canada Exchange scientist.

386.1 TOPOGRAPHY OF PRIMARY SOMATIC SENSORY CORTEX (SI) IN OLD WORLD PRIMATES, CERCOPITHECUS AND MIOPITHECUS Susan Warren & Mary Carlson, Depts.Psychiatry, Anatomy & Neurobiology and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110

With the application of micromapping techniques to the study of topography of sensory cortex came the provocative finding by Paul and colleagues in 1972, that SI in Macaca mulatta was not characterized by a single somatotopic projection of cutaneous input. Rather they showed two separate projection patterns for the hand corresponding to separate SI architectonic fields, areas 3b and 1. A later study by Kaas et. al.('79) also reported multiple topographies in SI of Macaca but proposed a mirror-image pattern in 3b-1 in contrast to the serial pattern proposed in the first study. We mapped SI in several species of Macaca (Carlson et.al.'82) finding evidence for multiple hands areas, but as Iwamura and colleagues ('83,'85), we concluded that area 1 was not somatotopically organized.

These studies of Cercopithecus aethiops and Miopithecus talapoin, were intended to look for common features in SI organization that might clarify the complex topographic details still contested in Macaca. General features of SI organization in all these species are: 1) receptive fields (rfs) on the ulnar palm and dorsal hairy hand are located medial, and rfs on the radial palm lateral, to the digit area; 2) digits are organized from D5 to D1 in the mediolateral plane; 3) in area 1 the glabrous and dorsal hairy digit rfs from the same digit segment are found in close proximity but intermingled thoughout the digit area; 4) the predominant input in the SI hand area was from digit tips; and 5) shifts in the distal-proximal progressions of rfs on the digits occur without respect to SI architectonic borders.

Additional findings from these current studies in C. aethiops and M. talapoin are: 1) rfs on dorsal hairy digits are found in area 3b as well as area 1, but with half the frequency as in area 1; 2) rf progressions in SI are best characterized as a continuous spiral from the ventral to dorsal surfaces, from proximal to distal, and back again; and 3) rfs on the distal hairy digit often serve as a landmark for a reversal of such spirals to more proximial digit segments hairy rfs but do not always correspond to the 3b-1 border. This mixing of glabrous and hairy inputs and complex variations in distal-proximal sequences contrasts with the segregation of inputs and somatotopic patterns found in prosimian primates (Carlson,'85).

From these recent studies we conclude that the difficulty in finding a generally acceptable "pattern" in the SI of Old World anthropoids relates to the complexity and variability of rf sequences on the digits and the difficulties of relating rf sequences precisely to architectonic subdivisions within SI.

386.2 SIGNIFICANCE OF TOPOGRAPHY IN PRIMARY SOMATIC SENSORY CORTEX (SI) FOR TACTILE DISCRIMINATION CAPACITY IN OLD AND NEW WORLD PRIMATES Mary Carlson and Pia Nystrom* Dept. of Psychiatry and McDonnell Center for Study of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110

Over the last 15 years micromapping studies of SI have suggested general trends in evolution of complex cortical areas in primates. SI in prosimian primates is characterized by a single somatotopically-organized hand area in contrast to the multiple cutaneous hand areas in Old World (OW) anthropoids (Carlson'85). Kaas and colleagues ('83) have described multiple cutaneous area SI areas in New World (NW) anthropoids. The separate projection of the glabrous surface of the hand to SI cytoarchitectural area 1 appears as a recent acquisition of most anthropoid primates. As surgical removal of area 1 in Macaca produces deficits on texture discrimination tasks (Carlson'81) we reasoned that anthropoid primates should have superior texture capacity to the prosimian with only a single hand area.

Tactile capacity of the prosimian, Galago, was compared to the OW anthropoid, Macaca, and NW anthropoids Saimiri and Cebus, on a series of easy, moderate and difficult level size and texture discrimination tasks. Initial acquisition scores may indicate learning as well as sensory abilties while threshold performance is more indicative of sensory abilties alone. During size acquisition Galago required significantly more trials and made more errors than Macaca, but when tested on size threshold both species were similar in performance. Galago also was retarded relative to Macaca during acquisition of texture tasks, and continued to perform significantly below Macaca on texture threshold tasks. These results suggest a selective "deficit" for texture in Galago. On size acquisition tasks Saimiri performed better than Galago and similar to Macaca. On texture acquisition and threshold tasks Saimiri performed at the level of Galago and not Macaca, although in contrast to Galago, Saimiri has multiple SI hand areas. Cebus, another New World species with multiple SI hand areas, but with a more expanded digit representation than Saimiri (similar to that of Macaca) was tested next. On size acquisition and thresholds Cebus performance was indistinguishable from Macaca and Saimiri but was inferior to Macaca on all texture tasks, performing at the level of Galago and Saimiri.

Our failure to find superior texture discrimination capacity in NW species with multiple SI hand areas leads to the conclusions: 1) the greater differentiation of hand areas in SI may have evolved independently in OW and NW primate families and may serve different behavioral functions; and 2) the size and complexity of SI areas may not be as predictive of tactile capacity as are the response properties of SI neurons. (Supported by PHS grant MH40157).

386.3 LOCALIZATION OF HUMAN SECOND SOMATIC SENSORY CORTEX (SII) USING VIBRATION STIMULATION AND PET. H. Burton, P.T. Fox & M.E. Raichle (SPON: S. Eliasson). Depts. Anatomy & Neurobiology and Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110

Regional cerebral blood flow (rCBF) was recorded in 43 paid, normal volunteers utilizing positron emission tomography (PET). Here we report on a region of interest (ROI) within the parietal operculum that is likely to be the second somatic sensory area (SII). Previously (Burton et al., Soc. Neurosci. Abst., 1985) we reported on the somatotopic organization in primary somatic sensory cortex (SI) by mapping rCBF changes (ΔrCBF) produced by cutaneous vibrations (130hz, 2mm) of fingers, feet and lips.

CBF scans were acquired during rest and stimulation following intravenous boluses of ^{15}O-labeled water. Images of ΔrCBF were created by a pixel-for-pixel subtraction of control state CBF from stimulated state. The subtraction format images showed large focal ΔrCBF in the rolandic cortex and lesser foci in the vicinity of parietal operculum, medial frontal cortex (supplementary motor area?) and cerebellum. Foci of ΔrCBF were identified by a computerized maximum detection algorithm using a cubic (10 cc) ROI and inter-slice interpolation. A stereotactic method of anatomical localization (Fox et al., J. Comput. Assist. Tomogr. 9:141, 1985) was used to determine the coordinates of these maxima. The stereotactic coordinates were plotted in mm from the center of the AC-PC line after proportionate corrections for brain size.

In 25/69 scans involving finger stimulation, two distinct ROIs were identified in the contralateral parietal cortex. The maximum ΔrCBF was centered in SI in all cases (Horizontal:48, Frontal:-6 to -12, Sagittal:38-42) and a second focus was located on or near the outer bank of the parietal operculum (H:10-22, F:0 to -6, S:42-58). In this probable SII, ΔrCBF were 1/3-1/2 the values seen in SI but exceeded background levels of 1-2 ml/min. Only subjects with a focal ΔrCBF of >5 ml/min were used to obtain the average coordinates for SII. The active zone was a small oval that appeared on 1 or, rarely, 2 adjacent slices. Minimal scatter from these coordinates was noted in the localization of peak ΔrCBF from individuals. Coordinates for the hallux in SII were similar to those from the hand but the scatter from individuals was greater. Only a single SI-SII ROI was seen following lip stimulation.

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386.4 REPRESENTATION OF THE BODY SURFACE IN THE SOMATOSENSORY CORTEX OF THE WESTERN POCKET GOPHER (Thomomys).
P.X. Joris* and R.J. Nudo (SPON: R.B. Masterton)
Coleman Mem. Lab., Univ. of Cal. at San Francisco, San Francisco CA94143.

Differentially enlarged representations of specific body parts in the primary somatosensory cortex (SI) have been correlated with specialized tactile behaviours and tactile surfaces, in a number of earlier-studied mammals. No detailed somatosensory map of a true burrowing mammal has been derived.

Pocket gophers are highly specialized rodents living in a predominantly tactile environment, succesful in evading above- and in-ground predators. The hairy skin extends behind the teeth so that the large curved incisors are exposed when the mouth is closed. The external fur-lined cheek pouches, used to transport food, extend from the face to the shoulders, and are easily everted. The claws of the forepaws are large and curved. Limbs are jointed so that pocket gophers can run backwards almost as fast as forward.

The somatotopic organization of the SI region was mapped in the Western pocket gopher using microelectrode multiunit mapping techniques, under Ketamine-Acepromazine or Nembutal anesthesia. A single, systematic, cutaneous representation on the lateral convexity was found, with a general orientation similar to that in SI in the rat (Chapin, J.K. and Lin, C.-S., J.Comp.Neurol., 229, 199-213,1984). Greatly magnified representations of the face and forepaw were found lateral to the hindpaw and trunk representation which showed the belly rostral to the back. The representation of the forepaw was large in comparison with the rat, with the representation of hairy dorsal skin surfaces of the digits surrounding that of the glabrous skin representation at its caudal edge. Caudolateral to the forepaw representation, a vibrissal field was found, with an internal topography similar but proportionally smaller to the rat. Interspersed between forepaw and mystacial vibrissae, the representation of the external skin of the cheek pouch was found; a smaller representation of the internal pouch fur was located more rostrally. Cheek and chin representations were situated rostrolateral to the forepaw representation. A large representation of the upper and lower lip were lateral to the chin representation. Still more lateral, bordering a very large incisor representation, a second, far smaller cutaneous representation, presumptively SII, was found.

The overall representation of the body surface in proportion to the entire neocortex appears to be substantially larger in the pocket gopher than in any other mammal studied to date.
(Supported by the Coleman Fund and NIH grant 1 F05 TW03675-01.)

386.5 LAMINAR ORGANIZATION OF AREA 3b OF CAT SOMATOSENSORY CORTEX. H.D. Schwark, T. Hirai* and E.G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Anatomical and physiological characteristics of single neurons in the forepaw region of area 3b in cat somatosensory cortex were examined using intracellular and extracellular techniques. Particular attention was paid to the laminar position of the neurons. The experiments were performed on paralyzed, barbiturate-anesthetized adult cats. Peripheral receptive fields were stimulated using hand-held probes, and stimulating electrodes were positioned in the medial lemniscus (ML), the ventroposterolateral nucleus of the thalamus (VPL), and cortical area SII.

Glass-coated tungsten microelectrodes were used for extracellular recordings, and small microlesions were made during the experiments to permit localization of the recorded neurons. Layers III, IV and V contained the highest percentage of neurons with cutaneous receptive fields (54%), and layer VI contained the lowest percentage (29%). Orthodromic latencies following VPL stimulation were similar for neurons in all layers except layer II, in which neurons were activated at significantly longer latencies ($p<.001$). The latency of activation of layer II neurons following stimulation of SII was also significantly longer than for neurons of the other layers ($p<.05$).

Intracellular recordings were made with glass micropipettes filled with HRP. After determining the receptive field characteristics and responses to electrical stimulation by extracellular recording, the neurons were penetrated and stained by iontophoresis of HRP. The results of these experiments were supplemented by others in which isolated neurons were labeled by extracellular iontoporetic injections of HRP into areas 3a, 3b or 1. These injections were processed by the cobalt-intensified DAB method, and resulted in complete filling of a number of neurons adjacent to the injection site. The results of both intracellular and extracellular labeling experiments revealed a laminar-specific organization of axonal arborization within area 3b. In addition to this laminar-specific organization, a number of stained neurons sent axons to other areas of SI or to motor cortex. In some cases these projecting axon collaterals traveled exclusively within the gray matter for distances as long as 4 mm. Two examples were seen in which axons arising from area 1 neurons gave rise to collateral projections to area 3a as they traveled to area 4.

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386.6 THALAMIC PROJECTIONS TO THE POSTERIOR PARIETAL CORTEX IN RHESUS MONKEY. Jeremy D. Schmahmann * and Deepak N. Pandya. Departments of Anatomy and Neurology, Boston University School of Medicine, Boston, MA 02118 and Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA 01730.

Thalamocortical connections of rhesus monkey superior (SPL) and inferior (IPL) parietal lobule were investigated in ten hemispheres using WGA-HRP.

Rostral IPL receives afferents from pulvinar oralis (PO), caudal IPL from pulvinar medialis (PM), and caudalmost IPL, area Opt, from PM as well as lateral dorsal nucleus (LD). In addition, rostral and caudal IPL receive projections from the dorso-medial (DM) and intralaminar nuclei medially, and the lateral posterior (LP) and ventrolateral (VL) nuclei laterally.

The lateral posterior nucleus provides most of the projection to SPL, including the lateral and medial cortices and the intraparietal sulcus. Additionally, the area of SPL on the convexity of the hemisphere is connected to PO, DM and intralaminar nuclei medially, and to VL laterally. The medial SPL, area PGm however, receives only occasional projections from these other nuclei.

These results indicate that the main line of projection from thalamus to IPL is derived from the PO, PM and LD nuclei, and to SPL from the LP nucleus. These main projections are flanked medially by connections from DM and intralaminar nuclei, and laterally by the VL and LP (to IPL) and VL nuclei (to SPL).

Supported in part by NIH training grant T32 NS07152 (JDS) and NIH 16841 (DNP).

386.7 POSTNATAL REGRESSIVE CHANGES IN THALAMIC PROJECTIONS TO SOMATIC SENSORIMOTOR CORTEX OF THE MACAQUE: A QUADRUPLE FLUORESCENT DYE TRACER STUDY. S.S. Cheema, I. Darian-Smith, C. Darian-Smith*, and A.W. Goodwin. Anatomy Dept, Univ. of Melbourne, Australia, 3052.

Pre- and post-natal regressive processes have a determinant role in the development of the neuronal circuitry of the mature brain. Retrograde labeling of neurons, using different tracers injected into adjacent zones of cortex, allows the identification of the extent of the collateral branching of these cells. We have used this procedure to follow postnatal changes in the thalamocortical neuron populations projecting to the somatic sensorimotor cortex of the macaque.

The hand representation in the sensorimotor cortex of 7 macaques, aged 2 days to 5 years, were each injected with 4 fluorescent dyes (fast blue, diamidino yellow, rhodamine latex microspheres, and Evans blue). The cortical sites injected were at the rostral and caudal lips of the postcentral gyrus, in precentral motor cortex, and in the post-arcuate area. After 12 to 16 days the thalamic sites of fluorescent, retrogradely labeled cells were plotted using a computer-linked microscope digitizer.

In the adult macaque those neurons projecting to areas 3b and 1 were mainly within VPL, but some were in the anterior pulvinar. Cells projecting to areas 2 and 5 were in the anterior pulvinar and in LP. Cells projecting to area 4 were mainly within VLp (for terminology see Jones: The Thalamus, Plenum, 1985), and those projecting to area 6 were more anterior in VLa. Although there was considerable intermingling of neurons with different cortical projections, double-labeled cells were rare, confirming (a) that the injections of the different dyes did not overlap, and (b) that the cortical terminations of these cells were quite restricted.

In the newborn monkey many thalamocortical neurons had localized cortical terminations as in the adult. However, up to 25% of cells in some regions were double-labeled, indicative of widely distributed cortical endings. The commonest collateral projections were (a) to areas 4 and 6, (b) 3b/1 and 2. A few neurons with widely separated terminations in pre- and post-central gyri were also seen. At 12 weeks after birth neurons with extensive collateral branching were still present but reduced in number, but by 6 months had largely disappeared.

In summary, in the newborn macaque substantial numbers of thalamocortical neurons project widely to different parts of the sensorimotor cortex, but by 6 months this distribution is quite restricted. It follows that a selective elimination of thalamocortical connections must occur postnatally, a process that may relate to the coincident development of manual skills.

Supported by NH&MRC Australia, Ian Potter, Jack Brockhoff, and Australian Brain Foundations.

386.8 CAN SI CUTANEOUS NEURONS PROVIDE A KINESTHETIC SIGNAL? Dan A.D. Cohen & J.F. Kalaska, Centre de Recherche en Sciences Neurologiques, Universite de Montreal, Montreal, Que. H3C 3J7.

Experiments suggest that kinesthetic sensations of position and movement arise primarily from proprioceptive input. While recordings show that certain cutaneous receptors can signal joint angle changes, cutaneous input is usually attributed a minor role at best. Since gating and other central modulatory mechanisms could alter this ascending cutaneous input, peripheral studies cannot provide definitive answers to the kinesthetic potential of cutaneous afferent signals. We studied area 3b & 1 cells with cutaneous RFs, to determine whether their activity during normal limb movements contained potential kinesthetic information. We recorded several hundred single cells in 2 monkeys while they made reaching movements of the arm in 8 directions away from a central starting position. Cells had RFs on all surfaces of the arm proximal to the wrist. Many cutaneous cells were not active in the task but responded intensely when we stimulated mechanically their RF during the movements. Other cells emitted graded changes in activity with movements in different directions. In most cases, these responses were much weaker than the activity resulting from standard tactile stimulation of their RF. RF location affected the probability and intensity of response during movement. We found differences between cells receiving hair vs. "skin" input. "Hair" cell responses during movement tended to be more intense and were usually excitatory. This activity was caused by brushing of hairs against other parts of the body or by bending of hairs in the RF as the arm changed posture during movement. Cells receiving slowly-adapting "skin" input were generally less active although some SA skin cells sensitive to lateral skin stretch were very active during certain movements. Rapidly-adapting skin cells were relatively inactive. Inhibitory responses were more common than for hair cells, so that skin cells frequently had a reciprocal relation to movements in opposite directions. These movements were such that skin surfaces from different parts of the limb and body rarely came into direct contact with each other. Thus, the activity of skin cells most likely parallels the response of peripheral non-hair cutaneous receptors to the mechanical deformation of the skin caused by changes in the posture of the limb during movement. Overall, skin cell activity, although of lower intensity, provides a more unbiased signal about direction of movement and limb position than does hair cell activity. Thus, there exists in areas 3b & 1 during limb movement a pattern of mainly low-intensity cutaneous activity which varies in a meaningful fashion with limb movements. Nevertheless, SI proprioceptive neurons were much more active overall than cutaneous cells during the same movements, supporting their predominant role in kinesthesia. This work supported by MRC Grant MT-7693.

386.9 RESPONSES IN PRIMARY SOMATOSENSORY CORTEX (SI) DURING ACTIVE TOUCH IN PRIMATES. R. Sinclair* and H. Burton. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110

The responses of SI cortical neurons were examined during an active touch task involving textured surfaces. A Macaca Fascicularis was trained to stroke his hand, from an extended to retracted position, over a textured Nyloprint surface within a specified range of velocity and force. Eight tactile blocks, covered with 2 of 5 possible textured (ridged) surfaces (smooth, 10% Ridge/Groove ratios for 1500 and 3050 μm, and 50% R/G for 3050), were presented in pseudo-random sequence. During the strokes, each tactile block rested on a digital scale to measure force. To begin a trial, the animal put his hand in a stirrup that restricted finger placement on the surfaces. The stirrup swung at the end of a rod connected to a potentiometer used to measure position. Neuronal activity was recorded through a chronic stainless steel chamber implanted over the left SI area; transdural recordings were made with glass-coated Pt-Ir electrodes. Receptive field and minimum punctate force threshold were determined using Stoelting Von Frey hairs. Impulse timing was stored with analog samples of stroke velocity and force on a PDP 11/34 for subsequent analysis.

Certain response patterns emerged from our current sample of 38 single units (s), and 24 clusters (c). Most cells studied (31s/22c) showed an increased firing rate during the stroke as opposed to the time between strokes. The sample is strongly biased toward cells that respond during the stroke. Unless the receptive field was cutaneous and in contact with the surfaces, there was no evidence of responses to stroking. Two cells (2s) responded only to the bump formed by a middle contact strip. Other cells (10s/9c) showed this bump response as well as responding during contact with the surfaces. Another subset of cells (11s,13c) responded to surfaces with ridges of any spatial period, but did not respond to smooth surfaces. Other cells (7s,1c) responded only when smooth surfaces were contacted. These last 2 response patterns were often recorded in the same vicinity. Response magnitudes for another group of cells (6s,5c) were proportional to spatial period (roughness). A differential (contrast) response was seen in 3 cells (2s,1c) that depended on the spatial period of the preceding or following surface. Responses to a smooth surface, for example, were greater if preceded by a rough surface than if by a smooth surface.

These results from SI cortex during active touch revealed neuron types not previously noted with passive stimulation. These include cells with contrast effects, cells with off responses to textured stimuli, and cells with responses correlated with the spatial period of the surface. NINCDS Grant #NS0989

386.10 THE RESPONSIVITY OF S-I CORTICAL NEURONS CHANGES SYSTEMATICALLY WITH REPETITIVE TACTILE STIMULATION. M. Diamond*, O. Favorov*, M. Tommerdahl*, D. Kelly* & B. Whitsel. Departments of Physiology & Mathematics, University of North Carolina, Chapel Hill, NC 27514.

Extracellular recordings of the response of single S-I neurons to repetitive controlled tactile stimuli were obtained from monkeys and cats. Two modes of tactile stimuli were used: i) unidirectional, surface-parallel, constant velocity brushing stimuli--- each stroke across the skin regarded as a single stimulus repetition; and ii) trains of intermittent vertical displacement stimuli--- each train of sinusoidal displacements regarded as a single stimulus repetition. Ten to 200 stimulus repetitions were delivered in each run. For the brushing stimuli, the stimulus parameters that were varied in different runs included velocity, traverse length, direction, and interstimulus interval (ISI). For the vertical displacement stimuli the parameters varied were indentation amplitude, train duration, and ISI (the interval between trains).

The data indicate that single somatosensory cortical neurons frequently show a change in responsivity (exhibit a "trend") in the course of repetitive natural stimulation. The trends exhibited by a given S-I neuron are: i) highly reproducible--- determined by performing series of identical stimulus runs extending over several hours, ii) stimulus-specific--- revealed by the fact that a neuron can exhibit radically different trends when different stimulus parameters are used, and iii) exquisitely sensitive to systemic administration of subanesthetic doses of a variety of general anesthetics as well as clinically effective doses of central depressants.

When the same stimulus conditions are used for all the neurons sampled in the course of a single microelectrode penetration, neurons which exhibit similar trend behavior occur in groups. Penetrations parallel to the pial surface reveal that such "trend groups" correspond to aggregates of cortical columns approximately 350 to 750 microns in diameter. We believe that an analysis of trend behavior may reveal the formation of an extensive pattern of columns, some which have their responsivity enhanced as the stimulus is repeated, while others experience a progressive reduction in responsivity to the same stimulus. We are interested in i) defining how neurons belonging to the same or different trend groups interact with each other, ii) determining if such interactions lead to the formation of functional cortical columns, iii) determining if the trend group pattern is related to the pattern of metabolic activity which is evoked in S-I by repetitive tactile stimulation, and iv) assessing whether the formation of these hypothesized functional columns is sensitive to agents and manipulations known to influence the somatosensory capacities of man.

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386.11 ROLE OF KINETIC AND STATIC CUES IN HUMAN SUBJECTS' EVALUATION OF DIRECTION OF CUTANEOUS STIMULUS MOTION. G.K.Essick* and M.H.McGuire* (SPON: W. Maixner). Dent. Res. Cntr., Univ. of N.C., Chapel Hill, N.C. 27514

Psychophysical experiments were conducted to determine whether human subjects' capacity to evaluate direction of tactile motion is based on kinetic (i.e., velocity) and/or static (i.e., positional) cues. A servomotor was used to apply constant velocity brushing stimuli to the skin overlying the mandible. The stimuli were applied through an aperture in a teflon plate which lightly contacted the skin. A complete factorial design was used to determine the effects on directional sensitivity (d') of three parameters: (i) stimulus velocity, (ii) the width of skin contacted, and (iii) the length of skin traversed. To reduce the availability of kinetic cues a "slotted" aperture (consisting of two slit-like openings each .25 cm wide, separated by a distance of 1 cm) was employed in some experiments.

Data obtained from 4 subjects who were studied using a continuously open aperture indicate that d' is optimal whenever stimulus velocity is 6 cm/sec. Moreover, for continuous motion at velocities between 2-16 cm/sec, d' increases as the width of skin contacted is increased, reflecting spatial summation of kinetic cues. In those experiments using the slotted aperture, the d' values were found to approximate those obtained with the 1 cm long continuously open aperture, except when the velocity of stimulation was between 4-16 cm/sec. Within this velocity band the d' values obtained with the slotted aperture were less than those obtained with the 1 cm long continuously open aperture. This difference in directional sensitivity is interpreted to reflect the loss of kinetic cues whenever the slotted plate is employed.

These preliminary results suggest that human subjects usually base their report of stimulus direction on positional cues whenever the skin overlying the mandible is tested. However, kinetic cues appear to contribute to the capacity whenever the stimuli move at velocities between 4-16 cm/sec. In conclusion, it appears that the neural mechanism underlying subjects' analysis of direction of cutaneous motion involves both static and kinetic channels.

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386.12 A MODEL FOR TACTILE LETTER RECOGNITION. K.H. Pasman, J.R. Phillips and K.O. Johnson. Bard Laboratories of Neurophysiology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

A model of spatial pattern recognition in the somatosensory system was investigated. This model relates the neural representation of letters of the alphabet presented to the skin to human letter recognition performance (eg. percent correct identification, pattern of confusion between letters). The core of the model is a decision procedure which compares a representation of the stimulus with a collection of 26 templates stored in memory and determines the appropriate response. This process was explored from both the neurophysiological input and psychophysical output ends. Psychophysical results, summarized in confusion matrices, were used to infer the relative similarity of the internal templates. Letter representations based on neurophysiological data were also used to infer the relative location of the templates, and thereby predict psychophysical performance.

The neural letter representations are distributed patterns of activity in the three mechanoreceptor populations of primate glabrous skin reconstructed from single fiber recordings. Letter representations were also obtained from extracellular single unit recordings in areas 3b and 1 of macaque somatosensory cortex. Each of these neural letter representations was then converted to a vector in a multidimensional space to serve as input for the decision process. Inner products between all pairs of letter vectors were calculated and used to project these vectors into 26 dimensions. The internal templates for the 26 letters were also represented as points in this space. These templates were derived directly from the stimulus letter forms. Alternatively, relative template positions were obtained from neurophysiological data or psychophysical letter recognition studies.

The goal of this analysis is to extend present decision theoretic models for two-alternative forced-choice experiments to the analysis of multidimensional data. Decision rules based on proximity measures (such as a nearest-neighbor rule) or parcellation of the decision space (as signal detection theory does for the one-dimensional case) were investigated.

The peripheral neural data for this study were collected by S.S. Hsiao. Supported by NIH Grants GM07057 and NS18787.

386.13 THE NEURAL TRANSFORMATION OF SCANNED SPATIAL PATTERNS IN THE SOMATOSENSORY SYSTEM. S.S. Hsiao*, J.R. Phillips, and K.O. Johnson. Bard Laboratories of Neurophysiology, Johns Hopkins University School of Medicine, Baltimore Md. 21205

Raised letters of the alphabet ranging in height from 2.0mm to 8.5mm and tetragonally spaced dot arrays (dot spacings from 0.87mm to 6.5 mm) were used as spatial stimuli in an investigation of the transformation of spatial patterns in the ascending somatosensory pathways. The patterns were repeatedly scanned across the monkeys finger pad such that the entire surface scanned the receptive field of a neuron in successive sweeps. Single unit recordings were made in the peripheral afferents in the anesthetized monkey and in areas 1 and 3b in S1 cortex of the awake monkey. The responses from each neuron were reconstructed to form plots representing the spatio-temporal interaction of the neuron with the embossed patterns. These spatial event plots were then used to infer the neural transformations occurring in the pathways leading toward spatial pattern recognition.

Two stages in the transformation process are being investigated; the transformation from the embossed patterns to the representation in the primary afferents and the transformation from the primary afferents to the cortical representations. The peripheral and cortical spatial event plots are considered as two dimensional time varying neural images that are coded in populations of identical neurons. These images are input to the transformation model which predicts the subsequent level of neural representation. Initially the transformation is modeled as a linear and position invariant transfer function. This model is then tested by comparing actual neural responses from the alphabets to predicted patterns generated using the model. Differences between the actual and predicted responses are then used to modify the transformation until a close fit occurs between the predicted patterns and the higher neural representations. The basic model used for the peripheral transformation consists of summing responses from spatially distributed, weighted point spread functions. This summed response describes the sensitivity profile for each of the primary afferent types.

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386.14 SOMATOSENSORY EVOKED POTENTIALS FOLLOWING SURGICAL EXCISION OF HUMAN SOMATOSENSORY OR MOTOR CORTEX. C.C. Wood, T. Allison, G. McCarthy, D.D. Spencer*, and P.D. Williamson*, VA Medical Center, West Haven, CT and Yale University School of Medicine, New Haven, CT 06516.

Stimulation of the median nerve at the wrist elicits a series of somatosensory evoked potentials (SEPs) that can be recorded noninvasively from the human scalp and body surface. Although considerable progress has been made toward identifying the generators of these potentials, the specific cytoarchitectonic areas responsible for cortical SEPs N20-P30, P20-N30, and P25-N35 remain in dispute. In scalp recordings, N20-P30 is largest at parietal sites contralateral to the median nerve stimulated, P20-N30 is largest frontally, and P25-N35 is largest in the peri-Rolandic region. SEPs with similar morphologies and larger amplitudes are seen in cortical surface recordings during surgery, with N20-P30 largest in the somatosensory hand area on the postcentral gyrus, P20-N30 largest in the motor hand area on the precentral gyrus, and P25-N35 largest on the postcentral gyrus in close proximity to the central sulcus. Some investigators (e.g., Desmedt, Papakostopoulos) have proposed that P20-N30 and N20-P30 are generated by separate sources, with P20-N30 originating in motor or supplementary motor cortex and N20-P30 originating in somatosensory cortex. Others (e.g., Broughton, Allison) have proposed that P20-N30 and N20-P30 reflect opposite sides of the same equivalent dipole source located in somatosensory area 3b which, in humans, is located on the posterior bank of the central sulcus.

To test these alternative hypotheses, we recorded scalp and cortical surface SEPs following surgical excision of somatosensory or motor cortex in two patients undergoing surgery for relief of medically intractable seizures of the hand and arm. Removal of the somatosensory hand area abolished both P20-N30 and N20-P30, as well as P25-N35. These results hold both for cortical surface recordings immediately following the excisions and for subsequent scalp recordings following a period of recovery. In contrast, all three sets of potentials were present following removal of motor cortex.

Because P20-N30 was present following removal of the motor hand area, it cannot be generated in motor cortex even though its cortical surface amplitude is largest there. Instead, the data support the Broughton-Allison hypothesis that P20-N30 and N20-P30 are generated by a tangentially oriented source in somatosensory area 3b. The results indicate that P25-N35 is also generated in somatosensory cortex (probably by a radially oriented source in area 1), although they do not rule out a small contribution from motor cortex.

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386.15 SOMATOSENSORY EVOKED POTENTIALS FOLLOWING SURGICAL EXCISION OF SOMATOSENSORY OR MOTOR CORTEX IN THE MONKEY. T. Allison, C.C. Wood, and G. McCarthy, VA Medical Center, West Haven, CT and Yale University School of Medicine, New Haven, CT 06516.

In Old World monkeys, median nerve stimulation elicits cortical somatosensory evoked potentials (SEPs) similar in many respects to those of humans (Arezzo et al., EEG Journal, 1979, 46: 155-172), although with shorter latencies and somewhat different relative amplitudes. Monkey P10-N20 and N10-P20 are largest over motor and somatosensory cortex, probably analogous to human P20-N30 and N20-P30, respectively. Like human P25-N35, monkey P12-N25 is recorded in close proximity to the central sulcus; however, it is much larger relative to P10 and N10 than are analogous potentials in humans. Thus, cortical SEPs in the monkey are dominated by initially positive-going potentials (P10 and P12) both anterior and posterior to the central sulcus, whereas N20-P30 in humans is seen over a much broader region of the postcentral gyrus.

To evaluate alternative hypotheses for the sources of these potentials, we recorded epidural and cortical surface SEPs before and after excisions of the somatosensory or motor hand representation areas in the monkey (Macaca fascicularis). SEP recordings were made in two ways: (1) from an array of 24 implanted epidural electrodes spanning the sensorimotor region; and (2) from multiple arrays of 16 cortical surface electrodes following a craniotomy which exposed sensorimotor cortex while keeping intact the skull region containing the epidural array. In different monkeys, somatosensory and motor hand representation areas were removed by aspiration. Following the excisions, cortical surface SEPs were again recorded and the dura and bone flap were replaced to approximate their original positions. Epidural recordings were repeated following a recovery period.

The effects of somatosensory and motor cortex excisions were similar in both cortical surface and epidural recordings. Removal of the somatosensory hand area abolished P10-N20, N10-P20, and P12-N25. In contrast, all three sets of potentials were present following removal of the motor hand area. These results exactly parallel those in humans (see Wood et al., this meeting). Together, the human and monkey data strongly support the hypothesis that human P20-N30 and N20-P30 (and analogous potentials in the monkey) originate in area 3b of somatosensory cortex and are recorded over frontal and parietal cortex because of the tangential orientation of the equivalent source. The data also support the hypothesis that human P25-N35 and monkey P12-N25 are generated predominantly by a radially oriented source in somatosensory area 1, although they do not rule out a small contribution from motor cortex.

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386.16 THE ASYMMETRY OF THE CORTICOTHALAMIC CROSS-TALK. E. Ramon-Moliner. Department of Anatomy, School of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.

The connections between cortex and thalamus are known to be reciprocal. However, it is an asymmetrical reciprocity. Whereas the corticothalamic fibers originate and terminate in a precise somatotopic manner, with little overlapping of their terminal arborizations, the thalamocortical system is made out of fibers which ramify very early within the white matter of the hemispheres to originate widely diverging preterminal and terminal branches distributed over extended cortical areas. In Golgi material of the rat each axon leaving the thalamus may originate terminal branches distributed over a span of at least 9 mm^2 on the surface of the cortex, perhaps more. This is compatible with a terminal columnar distribution. In cat, microinjections of radioisotopes in the thalamus lead to labeling of cortical areas no smaller than 800 mm^2. This is puzzling in view of the well known fact that the various sensory systems are very precisely mapped on the cortex. There must be a physiological "focusing mechanism" (possibly the large number of cortical local circuit neurons) whereby only the terminal fibers in the center of the cone of divergence must suceed in eliciting evoked potentials. The study of Golgi material has long led to accept that the termination of the specific afferent plexus in the cortex occurs mainly in layers IIIb and IV but autoradiographical material points to layer I as another important target. The presence of the horizontally oriented neurons in this layer must contribute an additional enhancement of input divergence. By contrast, the corticothalamic projections revealed by radioautography after microinjections in the cortex are always confined to small tissue volumes within the thalamus, in the order of 2.5 to 8.0 mm^3, which is about the same size of the volume of cortex labelled at the injection site, indicating very little tendency to ramifying divergence. Unless highly effective dampening mechanisms exist, the reciprocal connections between cortex (C) and thalamus (T) must generate reverberating sequences. If connections with little divergence are indicated by $\rightarrow$, and those with high divergence by $\twoheadrightarrow$, the corticothalamic crosstalk can be symbolized by: ....T $\twoheadrightarrow$ C $\rightarrow$ T $\twoheadrightarrow$ C $\rightarrow$ T $\twoheadrightarrow$ C .... In this reverberating spread the thalamus constitutes (anatomically) a collection of precise targets and a source of diffuse projections while the cortex constitutes a source of accurate projections and a large target which is poorly aimed at.

387.1 INTRINSIC CIRCUITRY OF THE CEREBRAL CORTEX: THE LOCAL AXONAL PROJECTIONS OF CORTICOTHALAMIC PROJECTION CELLS. E. L. White and A. Keller*. Unit of Morphology, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva, Israel.

Horseradish peroxidase (HRP) was injected into the vibrissal area of the ventrobasal thalamus in young adult, male CD/1 mice. The next day, the injected region of thalamus was lesioned. Four days later, the mice were perfused with aldehydes, their brains were removed, tissue chopped at 100 μm and reacted with diaminobenzidine to demonstrate the HRP. The result of this procedure was to label, within the barrel region of SmI cortex, both the local axon collaterals of corticothalamic (CT) projection cells (by the retrograde transport of HRP) and thalamocortical (TC) axon terminals (by lesion-induced degeneration). Examination with the light microscope of thick sections through barrel cortex showed layer IV to contain clusters of HRP-filled processes; the clusters resembled barrels in their size, shape and distribution. Examination with the electron microscope confirmed that many of the HRP-filled processes in layer IV were CT axon collaterals, however most were small diameter dendrites. The highest concentration of CT axon terminals occured in lower layer V and in upper layer VI, regions in which the somata of CT projection cells occur. CT axon terminals invariably were presynaptic at asymmetrical synaptic junctions; postsynaptic elements were nearly all irregularly shaped dendrites which were non-spiny or which possessed very few stubby or sessile spines. We conclude that these dendrites belong to non-spiny stellate cells. Rarely, a CT axon collateral synapsed with a spine belonging to an HRP-labeled dendrite of a CT cell. The numbers of CT and TC synapses varied greatly among dendrites of different cells. Supported by N.I.H. # NS 20149-03, BSF # 3201/83.

387.2 THE BARRELS OF RODENT SOMATOSENSORY CORTEX ARE DIRICHLET DOMAINS. S. Senft* (SPON: Robt. Grubb). Depts. of Neurology and Neurosurgery and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Rodent somatosensory cortex contains columnar cell groups, named "barrels", which respond principally to movements of single facial vibrissae. At least two major questions remain unanswered: What are the forces which create barrels, and what functional advantages proceed from this type of neural organization?

We have identified a geometrical property of barrels which may clarify research into both their ontogeny and operation. Namely, we have been able to characterize them as Dirichlet domains. A Dirichlet domain is a bounded region having the property that all points within it are nearer to a specific ("center") point inside that region than to the corresponding center point of any other region. Domain boundaries, therefore, are defined mutually by the perpendicular bisectors between neighboring centers, and in two dimensions form networks of convex polygons. Dirichlet domains occur widely in nature - from crystal packing to animal nesting behavior - and often are created through a process of nucleation followed by isotropic competition for territory.

Cortices from adult and neonatal mice were removed, flattened, fixed, and frozen-sectioned at 75 microns parallel to the pial surface. Barrels were stained for cytochrome oxidase. Images of these sections were obtained by video microscopy, then digitized and contrast-enhanced with a Grinnell image processor. Dirichlet domains were fitted to the images by in-house programs based on a numerical method (Honda, Int. Rev. Cytol. 81, 1981).

Barrels were found, both by visual inspection and by numerical criteria, to conform closely to Dirichlet domains. This finding suggests that nucleating events (thus far unobserved) may initiate a competitive formation of barrels. Furthermore, this result may provide clues to barrel function. A problem faced by the nervous system, considered from the point of view of decision theory, is to match arbitrary values of input to a fixed set of responses. An efficient theoretical solution to this problem is to impose Dirichlet domains on the input space - with a "center" for each possible response. Then every input value will automatically be associated with its closest, most appropriate, reaction. In neural terms this strategy will result in regions having uniform response characteristics and can relax requirements for precise labelling of afferent projections.

Similar patterns seen in other cortical and sub-cortical areas of the brain suggest that Dirichlet construction could be a widespread means of neuronal organization.

(Supported by NIH grant 17763).

387.3 RECEPTIVE FIELD ARCHITECTURE IN THE WHISKER REPRESENTATION OF RAT SOMATOSENSORY CORTEX. T.J. Pincince* and J.P. Donoghue. (SPON: A. Megela-Simmons). Center for Neural Science, Brown University, Providence RI 02912.

The receptive field (RF) properties of neurons in the primary somatosensory cortex (SI) were characterized as part of a larger study to identify the network properties of assemblies of cortical neurons. The RF properties of over 200 single cells in the SI whisker representation were recorded extracellularly in urethane anesthetized rats.

Three different response patterns characterize SI neurons: (a) "unidirectional-phasic" neurons (60% of total population) exhibit a transient burst of activity at either onset or offset of whisker displacement, (b) "bidirectional-phasic" cells (32%) exhibit transient responses to both the onset and offset of stimulation, (c) "pseudo-tonic" cells (8%) respond continuously to sustained, low amplitude whisker vibration which other cell types could not follow. Stable displacements of the whiskers evoked only a phasic response in "pseudo-tonic" cells. Cells with tonic activity related to whisker displacement could not be found.

Cells were classified on a 1 to 4 scale according to response amplitude and the ability to follow a 1 Hz natural whisker stimulus. Scaling was corroborated by post stimulus time histograms. Class 1 cells responded consistently to small, low velocity manually or mechanically controlled whisker displacements; peak response to whisker displacement exceeded 50 Hz. Class 4 cells were unresponsive to peripheral stimulation. Layer IV cells were the most responsive in SI: 72% of these cells were Class 1 or 2. Class 4 cells accounted for 28% of the total population of SI cells but only 10% of layer IV cells. Only 40% of superficial and deep layer cells were Class 1 or 2 and over 35% of the cells were Class 4. Thus neurons in these layers are less responsive than in layer IV.

RF size, orientation and row preference were characterized for individual cells. Average RF size was between 2 and 3 whiskers. Single-whisker units did not constitute the majority of cells recorded in SI. Single-whisker RFs comprised 34% of all cortical cells, but 52% of layer IV cells. Most cells in the superficial (57%) and deep layers (88%) had multiple-whisker RFs. Within the total population, RF size and response amplitude were uncorrelated.

A RF is said to have a row preference if all the whiskers in the receptive field are found in the same row. Eighty-five percent of the cells with two-whisker RFs had a row preference but only 45% of the cells activated by 3 or more whiskers exhibit a row preference. Orientation is defined as the location of the primary whisker, the whisker that drives the cell best, relative to the other whiskers that lie within the cell's RF. Seventy-one percent of the cells had oriented RFs and 73% are oriented with the primary whisker most caudal.

Thus all SI cells appear to be phasically activated by whisker stimulation and the majority of cells in SI have multiple-whisker RFs that are characterized by slight row preference and caudal orientation. These results suggest that precise perception of the environment by the animal would require integrative network properties, perhaps mediated by the size, shape and orientation of RFs in SI. (Supported by NIH grant NS22517 and ONR #N0014-81-K-0136)

387.4 INFLUENCE OF LOCAL CORTICO-CORTICAL CIRCUITS ON LAYER V RECEPTIVE FIELDS IN RAT SI BARRELFIELD CORTEX. J.L.U. Guise and J.K. Chapin. Dept. of Cell Biology and Anatomy, Univ. of Texas Health Science Center, Dallas, TX 75235.

The aim of this study was to determine whether the large receptive fields (RF's) of layer V neurons in the barrelfield region of the primary somatosensory (SI) cortex of the rat are dependent on local cortico-cortical circuits. In neuroanatomical studies we have shown that granular aggregates (barrels) in layer IV of this region receive the strongest ventrobasal thalamo- cortical inputs, whereas layer V receives the strongest cortico-cortical inputs. Furthermore, RF's of single neurons recorded in layer IV include only one or a few whiskers, while RF's in layer V are much larger, often encompassing all of the mystacial whiskers.

The experimental protocol involved: 1) microelectrode penetration of barrelfield cortex and determination of a layer IV neuronal RF in order to precisely localize the position in the barrelfield, 2) driving the electrode down to layer V to find a neuron with a large RF, 3) generation of post-stimulus histograms of the response to standardized vibratory stimulation of each whisker in the RF, 4) driving a bipolar stimulating electrode (100μm diameter;20μm tip) into another barrel, usually 3-7 barrels away (about 2mm), 5) stimulating through the electrode (0.1ms pulses;1Hz;10-60μA) to check for direct orthodromic responses, 6) passing current through the electrode (100μA;5s) to lesion the area, 7) testing the response of the layer V neuron again to vibration of the whisker associated with the lesioned barrel, and 8) histological reconstruction of the lesioned area at the site of the stimulating electrode.

Both layer IV and V neurons typically responded to vibration of the center whisker in their RF's with 6-10ms latencies. However, response latencies of layer V units increased as whiskers progressively more distant from RF centers were stimulated. Microstimulation of another barrel produced orthodromic responses of 1-3ms latency in layer V neurons, whereas most layer IV neurons did not respond to microstimulation. After lesioning the other barrel, the recorded layer V neuron no longer exhibited the clear, short-latency response previously elicited by vibration of the whisker corresponding to the lesioned barrel. However, the absence of the normal post-excitatory inhibition unmasked a weak, longer-latency response. The recorded unit still responded normally to vibration of other off-center whiskers, and of the center whisker.

These results provide evidence suggesting that the large size of layer V RF's is based on local cortico-cortical projections, an hypothesis which is also supported by our neuroanatomical demonstration that supra- and especially infra-granular layers receive the vast majority of local cortico-cortical input. Local SI cortical circuits may therefore function to produce a convergence of inputs from adjacent cortical regions.

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387.5 2DG LABELED POPULATIONS OF NEURONS AND THEIR RELATIONSHIP TO WHISKER COLUMNS IN MOUSE SMI (BARREL) CORTEX. J.S. McCasland and T.A. Woolsey. Depts. of Neurology and Neurosurgery and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

We previously reported (Soc. Neurosci. Abstr. 11, 751, 1985) a modified high-resolution 2DG technique for convenient histological processing, improved label retention compared with the original protocol, and compatibility with other histochemical procedures. This method was used to label functional columns in barrel cortex following single-whisker (C3-only) stimulation in the behaving animal, and demonstrate the presence, particularly in layers II and III, of larger numbers of labeled cells in the stimulated column than are found in the corresponding column in normals (all whiskers intact). We attribute this increase in labeling after a decrease in overall whisker stimulation to the absence of inhibitory influences, normally produced in the C3 column by the activation of neighboring whiskers.

These findings have led us to focus on the relative numbers of activated neurons in neighboring columns, and the spatial patterning of these neurons within each column. Our analysis points to two main conclusions:

First, by constructing polar plots analogous to those used to measure orientation selectivity in visual cortical neurons, we find in the C3-only case that each column shows a substantial mean vector (substantially skewed polar distribution relative to barrel centers) which is quite similar from one tangential section to the next, suggesting that small portions of whisker columns, or polar "zones", show tight functional coupling throughout the vertical extent of the column in different cortical layers. There are indications that these zones extend beyond the barrel-defined column boundaries.

Second, by comparing total labeled cell counts for the barrel-defined columns we confirm an asymmetry observed by Durham et al. in which more activated cells are found in the anterior barrel columns (D row) corresponding to ventral whiskers. In the C3-only case, however, this asymmetry is reversed, so that more labeled cells are found in B row columns than in D. This reversal of functional asymmetries is consistent with the idea that functional connections in this system are preferentially maintained by those neurons which are normally more heavily stimulated, so that the C3-derived terminals would be found preferentially in the less-stimulated columns of the B row whiskers. This model leads to testable predictions.

(Supported by N.I.H. Grants NS17763 and NS07057).

387.6 Cortical Projection Patterns of the Medial Division of the Nucleus Posterior Thalami in the Rat. S. M. Lu* and C.-S. Lin (SPON:B.R.Schofield). Dept of Anatomy, Duke Univ. Med. Ctr., Durham, N.C. 27710.

The medial division of the nucleus posterior thalami (POm) in the rat may have several parts. Thus, the cortical projections have been demonstrated to terminate in several somatosensory and motor areas. Similarly, the ascending afferent projections from the spinal cord and cerebellum only terminate in certain portions of the POm. The purpose of the present study is to examine whether the POm can be subdivided on the basis of differences in cortical projection patterns.

Either a mixture of HRP and WGA-HRP or PHA-L was injected iontophoretically into either the POm or the somatosensory cortex. The transported HRP were visualized by reacting the sections with diaminobenzidine methods and heavy metal intensification. Immunocytochemical methods were used to localize the transported PHA-L.

Retrogradely labeled neurons are observed in both POm and the ventrobasal complex (VB) after injection of HRP into either layer I or the middle layers of the primary somatosensory cortex (SI). The labeled neurons in POm are resticted to its lateral region next to the medial border of the VB. The number of labeled neurons is greater in POm than in VB after layer I injections. More labeled neurons are observed in both POm and VB after injections in the middle layers, but the number of labeled cells is greater in VB than in POm.

Following PHA-L injections into the lateral portion of POm, labeled axons and terminals can be found in SI, secondary somatosensory cortex (SII) and the premotor area (area 6). The terminals are primarily in layers I and IV in SI and SII, and in layers I and III in the premotor area. In SI, the labeled terminals are distrbuted unevenly: they are concentrated in the septal areas of the barrel field, but are evenly distributed in the non-barrel portions of SI. By comparing the laminar projection patterns of VB and POm, we found that POm projects more heavily to layer I and less heavily to layer IV. In the barrel field, the axon arborizations from the two nuclei are interdigited with each other, that is VB axons arborize in the center of the individual barrel, while the POm axons arborize in the surrounding septal regions.

Since more neurons are labeled in POm following injections in the middle layers than after layer I injections, two populations of neurons may exist in POm with different laminar projections. Furthermore, it is likely that a more medial and rostral part of POm may project to motor area. We are currently investigating this possibility.

(Supported by NIH grant 06233 to C.S.L.)

387.7 AXONAL TRAJECTORIES BETWEEN THE VENTROBASAL COMPLEX AND SOMATOSENSORY CORTEX IN THE MOUSE. K.L. Bernardo* and T.A. Woolsey. Dept. of Neurological Surgery, Division of Exp. Neurology and Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Point to point whisker-related topographic maps have been found at all three central stations in the somatosensory system of the mouse. Although topography is retained at these endpoints, little is known about the organization of the fibers connecting these points. Nearest neighbor relationships might or might not be retained by afferent projection fibers. We have recently been able to demonstrate thalamocortical axon tracjectories directly.

An in vitro brain slice preparation (Bernardo et al., J. Neurosci. Meth., in press) has been used to label third order afferent fibers in the somatosensory system of the mouse with horseradish peroxidase. In 400-800 micron brain slices the entire pathway between the ventrobasal complex (VB) and somatosensory cortex was labeled. The thalamocortical fibers are organized into a plexiform system of bundles which appears to be fairly constant from animal to animal. The principal finding is that nearest neighbor relationships of individual fibers are not retained at many points along the pathway.

Bundles of fibers projecting from VB course between regularly spaced groups of VB neurons. Third order afferent fibers often leave VB by way of the fiber bundle closest to their perikarya, but they do not invariably do so. This abolishes nearest neighbor relationships for these axons before they have even left VB. Once lateral to the complex, the axon bundles converge upon and traverse the reticular nucleus of the thalamus. Axon bundles then rotate, analogous to the coiling of fibers in a strand of rope about a central axis. This accounts for the well known 180^{0} rotation between thalamic and cortical maps. Laterally, fiber bundles converge and diverge, in a fairly complex fashion, within the internal capsule (IC). Individual axons have not been found to bifurcate proximal to the subcortical white matter (SCWM), so that nearest neighbor relationships are once again abolished within the IC. Individual fibers diverge widely after single bundles of fibers reach a point just proximal to the SCWM.

At the level of the SCWM afferent fibers have been found to make a small dorsally concave loop prior to taking one of two possible courses: some of the fibers will ascend directly into the overlying cortex, usually with a transverse ascent, angled towards the dorsal surface; other fibers will run in the SCWM for a variable distance prior to ascending into the cortex. We conclude that fibers running in the SCWM, and through the cortex, "seek out" appropriate cortical regions to reestablish the nearest neighbor relationships that exist in VB.

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387.8 SPECIFICITY OF THALAMOCORTICAL CONNECTIONS IN THE RAT SOMATOSENSORY SYSTEM. P.W. Land, D.J. Simons and S.A. Buffer, Jr. Dept. of Neurobiology, Anatomy and Cell Science, Dept. of Physiology and Center for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Physiological and anatomical studies in rodents have found a somatotopic representation of the mystacial vibrissae within the ventrobasal complex (VB) of the thalamus. Recently, we identified metabolic and structural correlates of this somatotopy in VB of adult rats using a histochemical stain for the enzyme cytochrome oxidase (CO). Like the barrels in layer IV of the somatosensory cortex (Sml), thalamic barreloids (Van der Loos, 1976) revealed by CO histochemistry are arranged in a pattern of rows and arcs similar to the distribution of vibrissae on the face. In order to assess the specificity of connections between individual barrels in Sml and their corresponding barreloid in VB, adult Long-Evans rats received iontophoretic depositions of HRP in layer IV of Sml under tribromoethanol anesthesia. Twenty-four hours later, animals were deeply anesthetized with ether and perfused transcardially with 2% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Sections through the cortical injection site and VB were reacted with cobalt-intensified DAB after which they were rinsed in 0.1 M buffer and reacted for CO. An adjacent series of thalamic sections were prepared according to a TMB procedure.

When HRP depositions were confined to the CO-rich hollow of a single barrel, 80 to 100% of retrogradely labeled thalamic neurons were located in the barreloid isomorphic to the injected barrel. Injections nearer the side of the barrel but still within the CO-reactive hollow tended to yield a greater number of labeled cells in non-corresponding barreloids. Injections that spanned the septa between barrels labeled cells distributed among adjoining barreloids. Interestingly, none of the injections produced significant retrograde labeling of cells in adjacent layer IV barrels.

These results are in accord with previous studies of the rat Sml cortex. For example, approximately 90% of neurons recorded within the barrel hollows have receptive fields restricted to the one appropriate facial whisker. The data also suggest that a certain proportion of the cells with multiwhisker receptive fields in layer IV could result from direct convergence of inputs from 2 (or more) barreloids onto single cortical barrels. In layer IV, inhibitory interactions among different whiskers may be mediated indirectly by cells in non-granular laminae.

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387.9 SENSORY DEPRIVATION BY NEONATAL WHISKER TRIMMING ALTERS FUNCTIONAL ORGANIZATION IN SmI "BARREL" CORTEX. D.J. Simons and P.W. Land. Center for Neuroscience, and Dept. of Physiology and Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Sch. of Medicine, Pittsburgh, PA 15261.

The functional effects of neonatal tactile sensory deprivation were examined in adult rats whose mystacial vibrissae were removed by simple whisker trimming. Rats had either row-C vibrissae or all whiskers except those in row-C trimmed to within 1mm of the skin surface from birth to 45-60 days of age, after which the clipped hairs were allowed to regrow for 3-8 weeks. Glass micropipettes were used to examine the extracellularly-recorded activity of individual neurons in the cytochrome-oxidase rich centers of the layer IV barrels in response to controlled deflections of the vibrissae produced by electromechanical stimulators. Data were obtained from deprived and non-deprived barrels (i.e. corresponding to trimmed and intact whiskers respectively) and from normal animals. Results showed that cells in non-deprived barrels had response properties similar to those in control, i.e. normal, barrels. By contrast unusual properties were observed for most, but not all, cells in deprived barrels. These abnormalities included a) increased rates of spontaneous activity, b) exceptionally vigorous (in terms of spikes/stimulus) discharges to whisker deflections, and c) decreased selectivity to whisker displacements at different deflection angles. Many cells in deprived barrels could be strongly driven from whiskers associated with adjacent deprived or non-deprived barrels, whereas neurons in non-deprived barrels were driven weakly or not at all by whiskers associated with adjacent deprived barrels. Interestingly, response profiles from deprived barrels lacked the pronounced 100 msec period of post-excitatory suppression that characterizes the profiles observed in normal and in non-deprived barrels.

These results may reflect alterations in intracortical inhibitory mechanisms and/or abnormal patterns of afferent input to the barrels from thalamic relay nuclei. The findings underscore the importance of early sensory experience in establishing the functional organization of the somatosensory cortex.

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387.10 SPATIAL DISTRIBUTION OF INTRINSIC CORTICAL NEURONS THAT EXCITE OR INHIBIT LAYER 2/3 PYRAMIDAL CELLS: A PHYSIOLOGICAL STUDY OF NEOCORTEX IN VITRO. L.R. Silva* and B.W. Connors (SPON: M.F. Davies). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Although anatomical studies have revealed some aspects of intracortical connections, little is known about their functional properties. We have approached this problem by recording from single neurons while chemically exciting small groups of presynaptic cells. Coronal slices of rat somatosensory cortex (400 μm thick) were prepared using standard techniques. While recording intracellularly from physiologically identified pyramidal cells in layer 2/3, L-glutamate (2-3 mM; 20-70 pl) was applied to the slice by pressure ejection from a micropipette (10 μm tip). Intracellular responses were recorded during Glu application in layers 2-6 at a series of lateral distances from the recording electrode. At the end of the experiment, Glu ejection sites were marked and the slice resectioned and stained with cresyl violet, to identify cortical layers.

Glu applied near the recording site directly excited the impaled cell. More laterally, Glu application in layer 2/3 resulted in a prominent set of IPSPs (up to 10 mV hyperpolarizing responses when the membrane potential was held just below firing threshold). IPSPs were sometimes preceded by a much briefer depolarizing EPSP. IPSPs could be evoked by Glu applications up to 650 μM lateral to the recording site. Application of Glu in layer 4 predominantly generated EPSPs. This was observed with Glu stimuli at lateral distances up to 500 μM. Glu-evoked inhibitory responses were also observed from layer 4, although much less frequently. Glu application in layer 5 resulted in IPSPs or in a brief EPSP followed by a more prominent IPSP. Layer 6 application also typically resulted in IPSPs, although excitatory responses were observed. Glu-evoked inhibition arose from at least two different types of IPSP: 1) a fast response that reversed around -69 mV (n=5); 2) a longer latency response that reversed around -84 mV (n=5). When bicuculline methiodide (500 μm) was pressure ejected near the recording site, Glu application that had previously caused a prominent IPSP, or a mix of EPSP and IPSP, resulted in a large excitatory response. This occurred when Glu was applied in layers 2-6.

These data suggest that glutamate directly excites both excitatory and inhibitory cortical neurons in layers 2-6, and that this may be useful in revealing aspects of intracortical circuitry. In particular, 1) layer 2/3 pyramidal cells receive strong excitation from layer 4, and strong inhibition from layers 2/3, 5 and 6; 2) the inhibition is made up of at least two kinds of IPSP and is at least partially blocked by bicuculline.

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387.11 PYRAMIDAL TRACT NEURONS WITH OTHER SUBCORTICAL COLLATERALS IN THE SOMATOSENSORY CORTEX OF THE ADULT RAT. (SPON: S.E. Fish) R.W. Rhoades, K.A. Koralek and H.P. Killackey. Dept. of Psychobiology, University of California, Irvine, CA 92717.

During the past several years attention has been focused on cortical neurons which initially send processes to multiple targets and later eliminate one process. However, the presence and distribution of cortical neurons which project to multiple targets in the adult animal has received less attention.

We addressed this question with the following double label paradigm. Groups of rats were injected with two different fluorescent retrograde tracers (fast blue and either diamidino yellow or fluoro-gold) into two distinct targets. In one group of rats one tracer was injected into the pyramidal tract below the decussation at high cervical levels and a second tracer was injected into the brainstem trigeminal complex. In a second group of rats one tracer was injected into the superior colliculus and the second tracer was injected into either the pyramidal tract (below the decussation) or the brainstem trigeminal complex. After an appropriate survival time, the rats were sacrificed and their brains prepared for histological analysis. The resultant sections were examined with a light microscope under epifluorescence illumination.

All combinations of injections resulted in some double labeled cells in somatosensory cortex and adjacent cortical regions. In cases involving superior colliculus injections, double labeled neurons were scattered throughout the region in which both populations of singularly labeled cells were intermixed. Thus, they did not appear to be differentially distributed. However, neurons which were double labeled from the spinal cord and the brainstem trigeminal complex were differentially distributed. The majority of these double labeled neurons were located in the cortical region where the limb and face representation of primary somatosensory cortex abut. Other double labeled neurons were scattered among single labeled neurons in the cortical areas lateral and posterior to primary somatosensory cortex.

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387.12 CORTICOCORTICAL CONNECTIONS OF THE RAT SOMATOSENSORY CORTEX. J. Olavarria, R.C. Van Sluyters and H.P. Killackey School of Optometry and Neurobiology Group, Univ. of Calif., Berkeley, CA 94720 and Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

We have recently reported that the overall pattern of callosal projections in the rat somatosensory cortex are both more extensive and detailed than had been previously reported (Olavarria et al., Br. Res. 291:364, 1984). In the present study, we have examined the contribution of discrete parts of primary somatosensory cortex (SI) to the overall pattern of corticocortical connnectivity.

Adult pigmented rats received single injections of about 0.1 ul of horseradish peroxidase (HRP) in SI. After 24 hours the rats were sacrificed and the brains removed. Both cortices were separated from the rest of the brain, flattened, sectioned parallel to their surface and reacted for HRP histochemistry using tetramethylbenezidine for the chromogen.

A single injection of HRP into SI results in multiple foci of label in both the ipsilateral and contralateral hemispheres. Ipsilaterally, there are at least four foci of label in other neocortical areas: (1) rostrally in motor cortex, (2) caudally in the cortex between SI and visual cortex, and posterior and lateral to SI (3) in SII and (4) in a separate area rostral to SII which in the squirrel has been designated the parietal ventral area (PV) (Krubitzer et al., J.C.N., in press, 1986). The focus of label in both SII and PV clearly shifts in relationship to the injection site in SI. For example, an injection into the face region of SI produces a band of label that is continuous across dorsal portions of SII and PV, while an injection into the hind limb representation in SI results in separate foci of label at opposing extremes of the ventral portions of SII and PV. This suggests that these two areas are topographically organized with respect to both SI and the body surface.

In the contralateral hemisphere, the densest focus of label was located in the homotopic portion of SI. In addition, less dense label could be detected in SII, PV and motor cortex indicating that the rat corpus callosum interconnects heterologous, as well as homologous cortical areas.

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387.13 NEONATAL INFRAORBITAL NERVE SECTION ALTERS THE PATTERN OF CALLOSAL CONNECTIONS IN SOMATOSENSORY CORTEX OF THE RAT. K.A.Koralek and H.P.Killackey. Dept. of Psychobiology, University of California, Irvine, CA 92717.

In the parietal cortex of the rat, callosal projection neurons and their terminations are distributed in a pattern which both surrounds and is embedded within primary somatosensory cortex. The present study demonstrates that this distribution can be modified by a peripheral manipulation at birth.

The infraorbital nerve of neonatal Sprague-Dawley rats was severed unilaterally and the pups were allowed to grow up to 30-35 days of age. Under ketamine anesthesia, multiple (40 to 50) injections of HRP (Sigma type VI) were placed in the cortex ipsilateral or contralateral to the nerve cut, so as to be evenly distributed throughout parietal cortex. After a 24 hour survival period, the animals were perfused and the non-injected hemisphere was flattened, sectioned tangentially and processed with tetramethylbenzidine.

The pattern of distribution of callosal projections is not detectably altered in the hemisphere ipsilateral to the lesion. However, in the hemisphere contralateral to the nerve cut, the pattern differs considerably from normal. First, within primary somatosensory cortex, labeled cells and fibers which normally form a lattice-like pattern around the representations of the vibrissae are missing. Instead, there are aberrant clusters of labeled cells and terminations within portions of the affected region. Second, the dense continuous band of retrograde and anterograde labeling normally present along the postero-lateral border of primary somatosensory cortex is disrupted.

The different patterns of distribution of callosal projections in normal animals and in animals whose infraorbital nerves have been severed suggest that peripheral input plays a role in shaping the distribution of callosal projections in both primary and non-primary sensory cortical regions.

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387.14 MORPHOLOGICAL CHANGES IN RAT SOMATOSENSORY CORTEX FOLLOWING PRENATAL LIMB REMOVAL. D.R.Dawson and H.P. Killackey, Dept. of Psychobiology, University of California, Irvine, CA 92717

Recently we reported the existence of detailed morphological patterns outside of the cortical vibrissae field, in regions of rat somatosensory cortex coextensive with the physiological representations of the forelimb and hindlimb. These regions were found to possess consistent internal organization when reacted for the enzyme succinic dehydrogenase (SDH), and their configuration could be disrupted by depriving the cortex of normal peripheral input (nerve section or limb removal) on the day of birth. The present study examines changes in these same cortical patterns in response to prenatal peripheral insult.

Rats were visualized in utero on embryonic day 16 of gestation (sperm plug = day 1) and a small incision was made in the uterus. The forelimb was exposed and cauterized, and the incision sutured. Natural birth occurred on E 22 or 23. On postnatal day 14, pups were sacrificed under ether anesthesia and perfused with 10% glycerol. Cortex was separated from underlying white matter and flattened between glass slides. The tissue was quick frozen in isopentane, cut in a cryostat, and reacted for SDH.

Prenatal removal of the limb produces profound disruptions of the normal pattern within the cortical forepaw region, ranging from random clusters to homogeneous blurs. The total percent of area devoted to the forepaw does not change (relative to the normal contralateral pattern). The adjacent cortical hindpaw region, however, does increase in size in response to complete forelimb removal, in some cases by as much as 100% (relative to the contralateral hindpaw region). The hindpaw region of cortex seems to increase by invading the region of low SDH activity which lies between the forepaw and hindpaw representations. Other regions adjacent to the forepaw region, such as the representation of the lower lip, show no increase in size following the same manipulation. We tentatively interpret these findings to be a reflection of the close proximity of the two limb representations at the level of their first synaptic stations in the brain, and are currently investigating this in greater detail.

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387.15 SOMATOSENSORY CORTEX TOPOGRAPHY AFTER NERVE REPAIR IN NEONATAL MARMOSET MONKEYS. J.T. Wall, M.F. Huerta, & J.H. Kaas. Vanderbilt University, Nashville, TN 37240.

The primate hand is innervated by the median, ulnar, and radial nerves. If neonatal marmosets are reared with sectioned and ligated median nerves, a normal topographical representation of the hand does not develop in the primary somatosensory (area 3b) cortex (Huerta et al., this volume). The present study asked two questions. (1) Following median nerve section in neonatal marmosets, is cortical topography different if the sectioned nerve is repaired, instead of ligated? (2) Does a normal hand representation develop after neonatal nerve repair, or are abnormal topographical patterns, like those seen after nerve repair in adult monkeys (Wall et al., J. Neurosci., 6, 1986, 218), developed instead?

The median nerves of 1-week old marmosets were sectioned and repaired. One year later, the topographical organization in the cortical area 3b hand representation was assessed with multiunit mapping methods in ketamine anesthetized animals.

There were two major findings. (1) The topography in marmosets with repaired nerves differed from the topography in marmosets with ligated nerves. After ligation, the representation of the median nerve skin on the radial hand was missing, and the cortical zone normally representing this skin was taken over by representations of hand regions that remained innervated by other nerves. In contrast, after repair a representation of the radial hand reappeared in the cortical zone that was taken over by other nerves after ligation. (2) The topography in marmosets with repaired nerves was abnormal. After repair, neurons at some cortical sites did not have normal receptive fields: some sites had abnormally large receptive fields or fields on discontinuous zones of skin, whereas other sites were unresponsive to cutaneous stimuli. In addition, normal topographical gradients for digit and palm inputs in the rostrocaudal cortical direction, and for radial and ulnar hand inputs in the lateromedial direction, were not always clear. Similar cortical abnormalities have been observed after nerve repair in adult monkeys.

These findings suggest that: (1) denervation-related cortical changes are reversible in neonates, (2) normal cortical representations are not always reestablished after neonatal nerve repair, and (3) neonatal and adult circuits may have similar limitations in their capacity to normalize input disorders after nerve repair.

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387.16 ELECTRICAL STIMULATION OF A PERIPHERAL CUTANEOUS NERVE RESULTS IN WIDESPREAD CHANGES IN CORTICAL RECEPTIVE FIELDS G.H. Recanzone*, T.T. Allard*, W.M. Jenkins, and M.M. Merzenich. Coleman Mem. Lab., UCSF, San Francisco CA 94143.

The effects of electrical stimulation of a peripheral cutaneous nerve on primary somatosensory cortex (SI), within and far removed from the zone of cortical representation of the stimulated nerve, was investigated in a series of normal adult cats. Each cat was anesthetized with sodium pentobarbitol and the ulnar nerve in the distal forelimb was electrically stimulated at levels that massively excited large myelinated afferents at 20 PPS for 6-8 hours. Tungsten microelectrodes (~1MΩ@ 1kHz) were used to define multi-unit cutaneous receptive fields (RF) in SI before and after electrical nerve stimulation. Iridium floating microelectrodes were used to define cutaneous RFs at a given cortical site prior to and every hour during the stimulation period.

Receptive field sizes increased progressively in all cats. RFs monitored with floating electrodes showed clear increases within 2 hours. A comparison of RF size before stimulation and after 6-8 hours of stimulation showed that RFs increased in area from 1.3X to 7.8X (mean = 3.2X). Similar changes of RF size were also observed outside of the cortical representation of the stimulated nerve, for example, in the representation of the hindpaw.

Receptive field locations commonly changed following electrical stimulation. In some cases, RFs at a given cortical site moved from one digit to another following electrical stimulation. These changes were seen in the cortical representations of both the forepaw and the hindpaw.

Preliminary results indicate that these progressive, nerve-stimulation induced changes in cortical receptive fields are reduced or reversed by the systemic administration of naloxone.

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387.17 THALAMO-CORTICAL SYNAPTIC EXCITATION ANTAGONIZED BY EXCITATORY AMINO ACID BLOCKERS IN SOMATOSENSORY PATHWAYS. J.-I. Oka, T.P. Hicks and R.S. Metherate. Depts. of Med. Physiol., The University of Calgary, Calgary, Alberta, T2N 4N1 and Microsurgical Res. Labs., Roy. Vic. Hosp. McGill U., Montreal, Quebec H3A 1A1.

Whereas the relay of somatic sensory information is entirely routed through the thalamus of mammals, very little information is available to date concerning the pharmacological identity of the excitatory synaptic transmitters of the functionally distinct classes of thalamic neurones subserving this modality. The recent introduction of new classes of receptor-specific antagonists for the excitatory amino acids has enabled the direct testing of the hypothesis that these substances act as thalamo-cortical transmitters. Recently Tsumoto et al. (In press) has suggested that in the primary visual relay system to cortex from the lateral geniculate nucleus, glutamate and/or aspartate probably are active in such excitatory processes. We have tested this idea in the thalamo-cortical relay system of the cat's somatic sensory pathway, joining the ventroposterior lateralis nucleus (VPL) and the primary somatosensory cortex.

Of 95 cells recorded extracellularly, 40 cells were examined with at least one amino acid antagonist through one or more complete series of tests, using: 1) electrical stimulation of the VPL to activate cells *synaptically*, as well as using 2) light, tactile stimulation of the cutaneous surface to activate cells *naturally* and finally, using 3) the amino acid agonists, quisqualate and N-methyl-D-aspartate (NMDA) or d,l-homocysteate (DLH) to activate the cells *microiontophoretically*. Twenty-three tests on these 40 cells demonstrated concomitant antagonism of quisqualate- and NMDA/DLH-induced excitation with the blockers, CIS-PDA, kynurenic acid, high doses of 2APV, or GDEE, at times when airpuff induced excitation or electrical activation of the cells was incompletely eliminated. With another 18 tests, a selective reduction by 2APV of NMDA/DLH-induced excitations was demonstrated concomitant with a lack of reduction, or only a weak interference with the airpuff or tactile-evoked response, or of the synaptic activation following electrical stimulation of VPL.

One possible interpretation of these data is that it is likely that an excitatory amino acid is the endogenous synaptic transmitter of some, or many, thalamo-cortical neurones, and that the exact nature of the post-synaptic receptors is likely to be of the non-NMDA type. Furthermore, it must be considered that some other non-amino acid transmitter mediates some additional component of the excitatory response. An additional hypothesis is that more than one population of amino acid receptors may jointly be involved in the synaptic process.

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387.18 IONTOPHORETIC APPLICATION OF BETA-ALANINE, GLYCINE, TAURINE AND THEIR ANTAGONIST, STRYCHNINE, IN CAT SOMATOSENSORY CORTEX. N.Tremblay*, R.Warren* and R.W.Dykes. Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

Iontophoretic techniques are a useful way to test hypotheses about mechanisms underlying the functional properties of cortical neurons. In primary somatosensory cortex, neurons are divisible into functionally distinct classes based upon their adequate stimulus and adaptation rate. In area 3b cells may be classified as either rapidly adapting (RA) or slowly adapting (SA) to sustained stimuli and may be differentiated further by the presence or absence of a receptive field, their threshold, and less often by their directional selectivity and response lability. By blocking GABA-mediated inhibition with bicuculline methiodide, Dykes et al (1984) showed an increase in receptive field size in RA regions but not in SA regions. The present study was designed to look for another inhibitory transmitter which might control receptive field size in SA regions. Strychnine was employed as an antagonist because it interferes with three putative inhibitory neurotransmitters, B-alanine, glycine and taurine.

Cats were anesthetized with sodium pentobarbital (35mg/kg) and a craniotomy performed to expose the somatosensory cortex. Carbon fibers were inserted into the central barrel of 5-barrel micropipette assemblies. These were pulled, and the barrels filled with the following solutions :strychnine hydrochloride (.01M,pH3.5; in NaCl 165mM), L-glutamic acid (0.5M,pH8.0), B-alanine (0.5M,pH3.5), and NaCl 3M as a control for current artifacts.

During data collection, sets of ten somatic stimuli were delivered at two second intervals once every minute. Two hundred and twenty-four cells were studied. Strychnine was tested in both RA and SA regions. Three effects were documented:(i)In RA regions, the receptive field increased in 11 out of 27 cases whereas none of the 11 receptive tested in SA regions enlarged. (ii) In 11 of 24 cases a receptive field was revealed in RA regions and only 2 of 28 cells tested in SA regions developed a receptive field. (iii)In 15 of 51 cells tested in RA zones strychnine reduced the threshold for somatic stimuli but only 1 of 39 cells isolated in SA zones showed this effect. In a second series of experiments, we tested B-alanine, glycine and taurine in order to examine their effects on somatosensory cortical neurons . We obtained no evidence that one of the three hypothesized inhibitory neurotransmitters controlled receptive field size in SA regions. Nevertheless, neurons were most effectively inhibited by B-alanine. In 16 of 16 cases B-alanine (5-25nA) decreased the responses to peripheral stimuli. Strychnine antagonized the effect Produced by B-alanine in 9 of 11 cells. More equivocal results were obtained with glycine and taurine which were less effectively antagonized by strychnine.

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387.19 GLUTAMIC ACID DECARBOXYLASE, CHOLECYSTOKININ AND SOMATOSTATIN IMMUNOREACTIVITIES OF SOMATOSENSORY CORTEX AND THALAMUS OF THE RACCOON. C.-S. Lin, S.M. Lu, G.P. Standage and G.S. Doetsch. Dept. of Anatomy, Duke University Medical Center, Durham, NC 27710 and Depts. of Anatomy, Surgery (Neurosurgery) and Physiology, Medical College of Georgia, Augusta, GA 30912.

The comparatively-enlarged and highly-differentiated somatosensory cortex of the raccoon has been used recently as a model to study basic aspects of functional cerebral plasticity and its possible anatomical substrates. The aim of the present experiments was to extend these studies by examining some neurochemical features of the raccoon somatosensory cortex and thalamus. Specifically, we determined the distribution and morphology of neurons that are immunoreactive to glutamic acid decarboxylase (GAD), cholecystokinin (CCK) and somatostatin (SS).

GAD-immunoreactive neurons were found in all layers of the cortex and also in the white matter. Most remarkable were the many GAD-positive cells that were located in layer I and the white matter compared to other species, such as the rat, cat and monkey. All GAD-immunoreactive neurons had the characteristics of non-pyramidal cells; most displayed either a multipolar or bitufted morphology.

In the thalamus, the distribution of GAD-immunoreactive neurons varied greatly from one nuclear region to another. VPL and VPM were found to be very sparsely labeled, containing only scattered GAD-positive neurons that were typically small in size. In contrast, VPI was densely packed with GAD-immunoreactive cells.

Cortical neurons that were immunoreactive to CCK were located primarily in layers II/III, V and VI; labeled cells occasionally were found in layer I. Very few, if any, CCK-positive neurons were present in the white matter. The morphology of most of the CCK-positive cells was multipolar, bipolar or bitufted.

SS-immunoreactive neurons were found in layers II/III, V and VI of the cortex and also in the white matter. A few SS-positive cells were located in layer I. The majority of the SS-immunoreactive neurons were either bitufted or multipolar in morphology.

The results show that the distribution of neurons that are immunoreactive to GAD, CCK and SS in the somatosensory cortex of the raccoon is similar to that in other species. However, a considerable number of immunoreactive neurons are located in layer I and the white matter of the raccoon, but not in other animals. The distribution of GAD-positive neurons in the thalamus of the raccoon is also similar to that in other species, except for the dramatic difference between the sparsely-labeled VPL/VPM complex and the densely-labeled VPI nucleus.

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387.20 GABAERGIC MODIFICATION OF TEMPORAL CODING TO HIGH FREQUENCY STIMULI IN SII. K. Alloway and H. Burton. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

GABAergic inhibitory circuits restrict the receptive field size of neurons in the primary somatic sensory cortex (Dykes et al., J. Neurophysiol., 1984). We have extended those findings to neurons in the second somatic sensory cortical area (SII) (Alloway and Burton, Somatosens. Res., 1986). Our analysis suggested that GABAergic circuits may limit the temporal fidelity of SII neurons that respond to low frequency (10-50 Hz) vibrations. Here we report the results from neurons that respond to high frequency (>200 Hz) vibrotactile stimulation.

Cats, implanted with a chamber over SII, were placed in a sling apparatus and non-traumatically immobilized. The animals were respired with nitrous oxide and 1% halothane. During isolation of neuronal activity, the animals were paralyzed and halothane was briefly discontinued. Single units were recorded with a Pt-Ir wire in the central barrel of a 9 barrel glass pipette. Side barrels contained 0.3 M glutamate, 0.25 M GABA, 2.0 M NaCl, and 5-6 mM bicuculline methiodide (BMI). Electrode penetrations were made in an area of SII that receives inputs from Pacinian receptors in the distal forelimb. Vibrotactile stimulation was provided by a modified acoustic speaker controlled by a microcomputer; a PDP 11/34 computer collected and stored spike discharges for on- and off-line data analysis.

Neuronal responses (n = 13) to high frequency mechanical sinusoids have been analyzed for changes in entrainment (%E) and frequency following probability (FFP). %E (peak half-cycle spike count divided by total spike count) varied across neurons but usually increased with increasing stimulus amplitudes (10-110 microns). In neurons responding best to 300 Hz, average %E was 74.8%. BMI (25-75 nA) and glutamate (25-150 nA) failed to improve the mean %E at preferred (300 Hz) or nonpreferred (<300 Hz) frequencies. In 7-8 neurons, however, BMI reduced the threshold amplitude needed to elicit periodic discharges.

All neurons normally fired on intermittent cycles, a result that was reflected by values of FFP ([peak half count minus remaining half count] divided by number of cycles) below 1. Thus, FFPs ranged from .005 to .131 for 300 Hz stimuli at amplitudes of 50-60 microns. BMI and glutamate significantly increased the average FFP values by 54% (n = 11) and 40% (n = 10), respectively. In two neurons that responded only to the initial onset of vibration, both drugs caused the cells to discharge intermittently during stimulation.

These results suggest that GABA circuits do not act to segregate submodality inputs to SII, but may limit temporal fidelity and, thereby, transform the temporal code representing activity in peripheral channels. - Supported by NINCDS grant NS22012.

387.21 CHOLINERGIC MODULATION OF NEURONAL RESPONSES TO PERIPHERAL STIMULATION IN RAT SOMATIC SENSORY CORTEX. J.P. Donoghue and K.L. Carroll*. Center for Neural Science, Brown University, Providence RI 02912.

Diffuse afferents to cerebral neocortex, such as the basal forebrain cholinergic pathway, have been postulated to modulate the cortical processing of specific sensory inputs. To address this possibilty, the effect of iontophoretically applied acetylcholine (ACh) on sensory response properties in the primary somatic sensory (SI) cortex was investigated in urethane anesthetized albino rats. Single neurons in the SI whisker representation were recorded during natural stimulation of the mystacial vibrissa under three conditions: (a) with no applied drug, (b) during local ACh application and (c) after a five minute recovery period. Laminar distribution of cells was identified by correlating electrode depth and normal discharge characteristics with histological recontructions of electrode tracts.

ACh application increased the response elicited by whisker stimulation (termed a Type I effect) in most SI neurons. Peak discharge under these conditions increased an average of 81% over control. Of cells with Type I responses, 75% showed an increase in both peak response and background discharge; the remainder exhibited an increase in peak response with little or no change in background activity. Frequently, these cells could not be activated by peripheral stimulation in the control state, but in the presence of ACh, whisker deflection evoked a clear response without increasing background activity. Glutamate application to the same cells however, increased both sensory response and background discharge.

Type II effects, found in 20% of recorded SI cells, were characterized by a depression of the sensory evoked discharge during ACh application. Usually the Type II effect was a accompanied by a decrease in and background activity. In others, the decrease in peak response was coupled with a paradoxical increase in background activity. Type I and Type II effects were similar in that both were evoked at comparable current intensities (Type I-40±3 vs. Type II-34±4 nA) and antagonized by atropine.

Type I and Type II effects were largely segregated into different cortical lamina. Of the cells tested in supra and infragranular layers 88% had Type I responses. Only 18% of layer II and III cells affected by ACh were classified Type II; layer V cells showed only Type I effects. In contrast, 70% of layer IV cells showed decreased sensory responses during ACh application.

These studies show that muscarinic cholinergic actions can markedly enhance the effectiveness of sensory inputs to certain SI neurons, while suppressing sensory activation in others. The laminar segregation of this differential action of ACh may increase the contribution of supra and infragranular layers and diminish the contribution of the granular layer in processing sensory input from the whiskers. These effects may provide a mechanism for selective attention and gating of sensory information in cerebral neocortex. Supported by NIMH grant #MH40972.

CORTICAL AND SUBCORTICAL ORGANIZATION III

388.1 THE ROLE OF THE FRONTAL CORTEX IN COGNITIVE-MOTIVATIONAL INTERACTIONS: A NETWORK THEORY. D.S. Levine, Dept. of Mathematics, Univ. of Texas at Arlington, Arlington, TX 76019.

Neural network models are suggested to explain a variety of clinical and lesion data on the mammalian prefrontal cortex. The orbital frontal cortex, via its connections with the limbic system, hypothalamus, and reticular formation, is suggested to be involved in the mediation of incentive motivation toward or away from specific stimuli. This suggestion is based on the perservation and enhanced reaction to novelty that occurs with frontal damage. Hypotheses are proposed abouth the excitatory or inhibitory nature of some connections to and from the orbital area.

The dorsal frontal cortex, via its connections with secondary sensory areas of the cortex, is suggested to be involved in the expectation of one stimulus given the occurrence of another. Relationships are discussed with the contingent negative variation, corollary discharge, and the coding of higher-order temporal chunks of stimuli. It is shown how attention can be biased toward longer time sequences of stimuli in preference to shorter sequences. Finally, the incorporation of motivational data into behavioral plans is analyzed using diffuse connections between the orbital and dorsal areas of the frontal cortex.

388.2 TOPOGRAPHIC ORGANIZATION OF CORTICO-CORTICAL CONNECTIONS OF THE PREFRONTAL COMPONENT OF MEDIAL PRECENTRAL CORTEX IN RATS. R.L. Reep and J.V. Corwin* SPON:(Don W. Walker). Depts. of Physiological Sciences and Neuroscience, Univ. of Florida, Gainesville, FL 32610; and Dept. of Psychology, Univ. of New Orleans-Lakefront, New Orleans, LA 70148.

Medial precentral cortex lies between the cingulate and somatic sensorimotor areas, and extends from the frontal pole to retrosplenial cortex. Its rostral portion (rPCm) belongs to prefrontal cortex by virtue of its connections with the mediodorsal thalamic nucleus. We have found, on the basis of HRP and autoradiographic studies, that rPCm engages in uniquely widspread cortico-cortical connections. Like other rodent prefrontal fields,rPCm has connections with limbic and paralimbic regions. However, it alone also has reciprocal connections with visual, somatic sensory, and auditory areas. In the visual and somatic sensory areas, efferents from rPCm terminate in well defined columns 300-500um in diameter. Labeling is heaviest in layers I, III and V. Occasionally the spacing between adjacent columns is also about 500um, suggesting that efferents from other sources interdigitate with those from rPCm. Efferents to the agranular subfield of retrosplenial cortex are focused in layer I, diffuse in deeper layers. Labeling is continuous in the mediolateral and rostrocaudal dimensions throughout this terminal field. Other ipsilateral cortical regions to which rPCm projects are the lateral agranular, anterior cingulate, and presubiculuar fields. In caudal PCm and contralateral rPCm, labeling appears diffuse throughout all cortical layers and no columns are seen. rPCm projects bilaterally and diffusely to the insular and entorhinal cortices. Ventrolateral orbital cortex receives a bilateral projection terminating primarily in layers I, III and V. Quantitative grain counts will be presented for many of these terminal fields.

In view of the above findings, and the role of rPCm in behavioral neglect, we consider rPCm to be a unique multimodal association region of rodent prefrontal cortex, distinguished largely by its diversity of isocortical connections in addition to more typical connections with limbic and paralimbic regions.

Supported by the University of Florida Division of Sponsored Research and by the Maxwell Fund.

388.3 LAMINAR ORIGIN OF CORTICAL PROJECTIONS TO THE FRONTAL CORTEX IN THE RHESUS MONKEY. H. Barbas. Depts. of Health Sciences and Anatomy, Boston Univ. and Sch. of Med., Boston, MA 02215.

The laminar origin of cortical projections to the frontal cortex was studied in 17 adult rhesus monkeys with the use of the retrograde transport of horseradish peroxidase (HRP). The frontal regions injected with HRP included the premotor cortex, anterior periarcuate, periprincipalis, ventrolateral area 12, and orbital area 11. Counts of retrogradely labeled neurons were obtained from every 20th section throughout the entire cerebral cortex. The boundaries of architectonic zones containing HRP labeled neurons were determined from matched sections stained for the visualization of cell bodies, myelin, or acetylcholinesterase. Labeled neurons were grouped into systems of projections reflecting their origin in visual, somatosensory, auditory, or motor cortices. Within each system, in turn, labeled neurons were classified into one of five categories representing gradations of architectonic differentiation.

Most labeled neurons were located in layers III and V, with a few in VI. In all systems studied, projections which originated from the least architectonically differentiated areas (category 1) arose mainly from layers V and VI. A gradual increase in supragranular labeling was observed in regions which showed an increasing laminar differentiation (categories 2-5).

The results suggest that the laminar origin of frontally directed projections depends on the laminar organization of the regions which give rise to the projecting afferent fibers. Frontally directed projections from limbic cortices, which show a rudimentary laminar organization, emanate mainly from the deep layers. Projections from increasingly more differentiated cortices arise progressively from the upper, or supragranular, layers. Thus, as the cortical architecture gradually changes from limbic areas toward the primary cortices, the origin of frontally directed projections gradually shifts from predominantly infragranular to predominantly supragranular layers.

Supported by NSF grant BNS 83-15411.

388.4 DEMONSTRATION OF INDIVIDUAL FIBERS FROM THE SUBSTANTIA INNOMINATA TO THE FRONTAL CORTEX OF THE RAT, USING PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). K.M. Carnes & J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Kidney bean lectin (PHA-L) was used as an anterograde axonal tracer to label fibers from the magnocellular areas of the basal forebrain (especially the ventral pallidum and other parts of the substantia innominata). Cells in these regions provide inputs to all non-striatal parts of the telencephalon, including the cerebral cortex, amygdaloid complex and olfactory bulb; a high proportion of the fibers to the neocortex have been reported to be cholinergic (Mesulam et al., Neuroscience 10:1185-1201, 1983).

Iontophoretic or pressure injections of PHA-L were made through micropipettes; after survival periods of 7-14 days the animals were killed and their brains reacted immunohistochemically with antibodies against PHA-L (Gerfen and Sawchenko, Brain Res. 290:219-238, 1984). Complete series of sections were taken through the rostral part of the hemisphere, in order to allow reconstruction of individual labeled fibers in the frontal cortex; a 1-in-5 series of sections was taken through the remainder of the brain.

Small injections (ca. 500 μm in diameter) in the substantia innominata labeled fibers in many parts of the cerebral cortex, from the frontal cortex to the entorhinal cortex, as well as in the amygdala and olfactory bulb. In the frontal cortex labeled fiber segments with varicosities were found in all layers, but were concentrated in the superficial (I and II) and deep (V and VI) layers.

Reconstruction of single fibers from serial sections suggests that individual fibers have different morphologies, with some ramifying in the deeper layers of the cortex, and others concentrated in the superficial layers. The branches of individual fibers appear to be concentrated within an area about 1 mm in diameter, although distal fiber segments may extend 0.5 mm beyond this limit. In addition, separate fibers labeled from a restricted injection (and therefore arising from nearby cell somata) may be concentrated within the same 1 to 2 mm area of the cortex. These results are in agreement with previous data from double retrograde tracer experiments, which indicated that individual cells in the substantia innominata project to restricted areas (ca. 1-1.5 mm) of the cerebral cortex (Price and Stern, Brain Res. 269:352-356, 1983).

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388.5 AFFERENT CONNECTIONS OF THE PREFRONTAL CORTEX IN CAT: A RETROGRADE ANALYSIS UTILIZING HRP AND DIAMIDINO YELLOW. G.S. Ruffa*, D.D. Spencer, C.A. Greer, M.L. Schwartz and S.S. Spencer, Sections of Neurosurgery, Neuroanatomy and Department of Neurology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The afferent input to the prefrontal cortex in cat was studied utilizing the retrograde transport of horseradish peroxidase (HRP) or diamidino yellow (DY). Prefrontal cortex (PFC) in both humans and monkeys has been found to be essential for the performance of certain cognitive behaviors (Goldman- Rakic et al., Hndbk. Child Psych. Biol. Inf. Develop., 1983) and in humans is frequently noted for the development of medically intractable epileptic foci. We wished to examine the possibility that topographical variation in the afferent connections of the PFC may be related to the susceptibility of different regions of the PFC to kindling (Spencer et al., Soc. Neurosci. Abst., 1986).

Adult cats were anesthetized and the region of the presylvian sulcus pressure injected with either 0.6ul of 2% DY or 2% WGA-HRP. Following a 2 - 7 day survival the animals were perfused and routinely processed for either fluorescence microscopy (DY) or with TMB for HRP localization. Alternate sections were stained with cresyl violet (DY) or counterstained with neutral red (HRP) for anatomical orientation. The location and relative density of retrogradely labelled cell bodies was determined with light microscopy and plotted on drawings of corresponding transverse sections.

The pattern of labelling was comparable for DY and HRP injected cats and was generally consistent with prior studies of PFC, particularly that of Cavada & Reinoso-Suarez (JCN, 242:293, 1985). The densest pattern of label was found within the different cytoarchitectonic regions of the ipsilateral PFC as well as in the homotopic PFC of the contralateral hemisphere. Moderate label was found in the ipsilateral cingulate gyrus, anterior ectosylvian area and within the cortex of the entorhinal and the suprasylvian sulci. Moderate labelling of cell bodies was also found within the cortex of the contralateral entorhinal sulcus. Finally, labelling was observed in several thalamic nuclei, with the densest concentration in mediodorsal nucleus.

The results will be discussed relative to subdivisions of the PFC that may differ in the extent and topography of their afferent connections and their susceptibility to kindling.

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388.6 REGIONAL DEVELOPMENT OF THE HUMAN FRONTAL NEOCORTEX: CHANGING PATTERNS OF ACETYLCHOLINESTERASE ACTIVITY I. Kostović, Dept. of Anatomy, Medical Faculty, Zagreb, Yugoslavia

Previous histochemical evidence from this laboratory has demonstrated the existence of acetylcholinesterase (AChE) reactive basal forebrain and thalamic inputs to the developing human frontal cortex. Now we report data on the early regional development and changing chemoarchitectonic patterns of AChE reactivity in the human frontal cortex. We performed a correlated histochemical and Nissl study on postmortem brain tissue of fetuses, infants, children and adults, ranging from 8 weeks of gestation (w.g.) to 26 years. Although local differences in AChE reactivity exist in the subplate and marginal zone as early as 18 w.g., first regional differences in chemoarchitectonics appear between 22. and 26. w.g., when basolateral cortex can be distinguished from dorsolateral prepolar cortex by presence of strong AChE reactivity of subplate zone and discrete band within the cortical plate. The precentral cortex displays weaker activity restricted mainly to the subplate and marginal zone. AChE reactive nonpyramidal cells of subplate and marginal zone are distributed throughout different cortical regions. Between 26. and 28. w.g. (several weeks prior to the first regional cytoarchitectonic differentiation of the frontal cortex into precentral motor, "premotor" and prefrontal cortex) there is a dramatic increase in staining density within the prospective layer IV of the cortical plate. In the basolateral prefrontal cortex AChE staining is extremely dense within the layer IV and lower part of layer III with the appearance of columnar pattern. The premotor and the precentral cortex show only the modest laminar staining. In the early postnatal cortex one can observe the dramatic decrease in staining density within the layer IV, disappearance of columnar pattern and of dense band in the marginal zone. Concomitantly, there is gradual (deep to superficial) appearance of AChE reactive neurons in cortical layers and by third postnatal month layers VI and V contain numerous AChE reactive neurons. In two specimens (11 and 14 years) AChE reactivity has been also found in pyramidal cells of layer III of frontal cortex (no specimens from 8 postnatal month to 11 years have been analyzed). In young adults (23 and 26 years) frontal cortex shows an increase in the intracortical fibre staining and strong pericellular and cellular staining of pyramidal cells in layers III and V (Kostović, Neurosci Abstr 8/2:933, 1982). In conclusion, AChE input from "noncholinergic" (thalamus) and "cholinergic" (basal forebrain) structures densely innervates cortical neuropil during the early cytoarchitectonic differentiation of the frontal lobe. The dramatic changes of chemoarchitectonics during postnatal areal development indicate prolonged involvement of cholinergic and "cholinesterasic" systems in the development of cortical innervation and suggest "plasticity" of the human cortex during neonatal period, childhood and puberty.

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388.7 KINDLED FOCI IN THE PREFRONTAL CORTEX OF THE CAT: METABOLIC ANALYSES WITH 2-DEOXYGLUCOSE. D.D. Spencer, S.S. Spencer, G. Ruffa*, J. Partington* and C.A. Greer, Sections of Neurosurgery, Neuroanatomy and Department of Neurology, Yale Univ. Sch. of Med., New Haven, CT 06510.

In humans with frontal lobe epileptic foci metabolic studies have shown a decrease in activity proximal to the focus during interictal periods. Most animal models of epiltogenic events have focussed on metabolic studies during epiltiform activity in regions other than the frontal lobes. We wished to establish (1) the susceptibility of prefrontal cortex (PFC) in the cat to electrical kindling and (2) the characteristics of metabolic activity within PFC during interictal periods.

Four adult female cats were kindled daily via bipolar stimulation of the PFC until they reached criterion, either 5 consecutive generalized seizures or a maximum of 120 stimulations. At 72 hrs. following the final kindling session the cats were administered 100uCi/kg of 14C-2-deoxyglucose (2DG) and sacrificed 45 min. later. The brains were routinely processed for 2DG autoradiography and the brain sections subsequently stained with cresyl violet for histological correlation.

Two principal stimulation sites were histologically identified, dorsolateral PFC and dorsomedial PFC. Each of these areas produced seizures which were clinically distinct. The cats stimulated in the dorsolateral region required more stimuli to reach criterion than did cats stimulated in the dorsomedial region of the PFC. 2DG analyses revealed that cats stimulated in the dorsolateral PFC had decreased 2DG uptake adjacent to the stimulation site and increased 2DG uptake in ipsilateral entorhinal and prepyriform cortex. In contrast, cats stimulated in the dorsomedial region exhibited increased 2DG uptake adjacent to the stimulus site as well as in the ipsilateral insular cortex and areas 2, 4 and 6.

The results demonstrate a differing susceptibility to kindling in subdivisions of the PFC correlated with different clinical characteristics of the induced seizures and different interictal metabolic patterns. These observations will be discussed relative to parallel studies on the afferent connections of the PFC (Ruffa et al., 1986).

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388.8 CORTICAL AREAS IN THE MEDIAL FRONTAL LOBE OF THE CAT. S.Y. Musil* and C.R. Olson (SPON: R.W. Cholewiak). Dept. of Psychology, Princeton, N.J. 08544.

The medial frontal lobe of the cat is thought to contain several cortical areas. However, regional variations of frontal lobe cytoarchitecture are gradual and have given rise to conflicting systems of areal parcellation. As an independent means for characterizing and delimiting frontal lobe areas, we have assessed regional variations in afferent connectivity. We report here on patterns of retrograde labeling observed following 25 deposits of distinguishable retrograde tracers in 16 cats.

Anterior cingulate cortex (AC), defined as cortex with thalamic input from the anteromedial thalamic nucleus (AM) but not from the mediodorsal nucleus (MD), was confined to a zone above and immediately rostral to the corpus callosum. Tracers deposited in this region were transported to association cortex throughout the frontal lobe and to posterior limbic, insular and entorhinal territories.

Medial frontal cortex, defined as cortex with stronger input from MD than from AM, occupies the remainder of the medial frontal lobe. Projections from the mediodorsal thalamic nucleus, the ventral complex of the thalamus, the insula and posterior limbic cortex terminate throughout medial frontal cortex and exhibit topography with respect to the dorsoventral cortical axis. In contrast, projections from other brain regions are spatially restricted and provide a basis for demarcating three discrete cortical divisions, as described below.

Infralimbic cortex (IL), which occupies the ventroposterior rim of the medial frontal lobe, is unique in receiving intense projections from primary olfactory (prepiriform) cortex and from the hippocampal subiculum.

The medial frontal eye field (MFEF), a dorsal strip adjacent to the cruciate sulcus, is distinguished by input from visual association cortex, including area 7.

Intermediate prefrontal cortex (IPF), located between IL and MFEF, resembles these areas in receiving afferents from frontal, insular and posterior limbic association areas. It differs from them in lacking subicular, olfactory and visual association connections. IPF, as defined here, includes cortex commonly regarded as "prelimbic", but our results do not support the interpretation of prelimbic cortex as a discrete entity.

In conclusion, we have analyzed patterns of afferent connectivity thropoughout the cat's medial frontal lobe and have used the results as a basis for delimiting four cortical areas.

388.9 THE ORGANIZATION OF THE CONNECTION OF THE PREFRONTAL CORTEX WITH THE PREMOTOR AREA IN MACAQUE MONKEYS. T.Arikuni, K.Watanabe* and K.Kubota, Dept.of Neurophysiology, Primate Research Institute, Kyoto Univ., Inuyama,Aichi,484,Japan.

The efferent and afferent connections of the prefrontal cortex (areas 8A and 45 of Walker) with the premotor area (area 6 of Brodmann) were investigated in macaque monkeys by means of anterograde and retrograde labeling techniques. Under a deep pentobarital anesthesia a single dose of wheat germ aggulutinin conjugated horseradish peroxidase (WGA-HRP) (0.1 ul, 10%, Sigma VI) was injected into various parts of the arcuate cortex in six Macaca fuscata and one Macaca rhesus . After a survival time of 3 days, monkeys were deeply anesthetized. And the brains were perfused through the heart by a mixture of glutaraldehyde (1.25%), paraformaldehyde (1.0%) and phosphate buffer (pH 7.2), and removed immediately. They were cut at 50 um on a freezing microtome. Every fourth section was collected and treated with TMB, some of which were counterstained lightly by 1% neutral red solution. The sections were examined by light microscopy under bright- and dark-field illumination.

Following a single injection of WGA-HRP into area 8A, labeled terminals and cells appeared dorsomedially in one or two circumscribed sites of area 6, and dorsolaterally also in one or two circumscribed sites of area 6, consisting of 1-6 vertical bands, being the width ranging from 250 to 350 um. In each band, labeled terminals were found throughout the entire cortical depth of the premotor area. Labeled cells were located mainly in layers III and V, and sparsely in layers II, IV, and VI of the premotor area. A closer examination revealed that one band consisted of a densely packed core and a lesser packed peripheral zone. When bands appeared in groups of two to six, there were spaces devoid of both terminal and cellular labelings or of sparsely labeled terminal and cellular labelings between bands at intervals of 100 to 500 um. Reconstruction of serial sections showed that these bands extended in a longitudinal or oblique direction, but not in a transverse direction.

Following a single injection of WGA-HRP into area 45, similar findings were obtained. Labeled terminals and cells always appeared in two circumscribed sites of the premotor area: one locus in the dorsolateral premotor area and the other locus in the dorsomedial premotor area. They also consisted of several bands.

It was concluded that in the monkey cells of small areas of the prefrontal cortex (areas 8A and 45) project to multiple sites of the premotor area and projected sites of the premotor area reciprocally project to the same original areas of the prefrontal cortex.

388.10 POSTERIOR GRANULAR FRONTAL CORTEX IN THE PROSIMIAN PRIMATE GALAGO: ANATOMICAL EVIDENCE OF HOMOLOGY WITH THE FRONTAL EYE-FIELD OF HIGHER PRIMATES. T.M. Preuss and P.S. Goldman-Rakic, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The frontal cortex of higher primates contains several areas marked by a granular layer IV. By contrast, the frontal cortex of non-primate mammals is agranular. In order to reconstruct the evolution of higher primate granular frontal cortex, we have examined frontal cortical anatomy in a prosimian primate species, Galago crassicaudatus (thick-tailed bushbaby). Prosimians are particularly useful for evolutionary studies as they possess a combination of ancestral mammalian and derived primate traits.

Examination of the cytoarchitecture of Galago frontal cortex reveals a region with a granular layer IV, although Galago appears to possess fewer architectonic subdivisions of granular frontal cortex than do higher primates. We have so far investigated one of these subdivisions, the posterior granular area (GrP), in detail, using injections of WGA-HRP, which involved GrP in 3 cases, and an injection of tritiated amino acids in lateral posterior parietal cortex, which resulted in dense anterograde labeling of GrP. GrP possesses charcteristics which strongly suggest that it is homologous to the lateral frontal eye-field (FEF; Walker's areas 8a and 45) of higher primates. These include: (1) large layer V pyramidal cells in addition to a distinct granular layer IV; (2) dense reciprocal connections with a large-celled, lateral (multiform) subdivision of the thalamic mediodorsal nucleus (MD) distinguishable from an adjacent, small-celled (parvicellular) subdivision; (3) strong reciprocal connections with parietal area 7 as well as connections with visual association areas; (4) a very dense projection to the intermediate grey layer of the superior colliculus and a lighter projection to the stratum opticum.

The complex of anatomical features which characterizes higher primate FEF, and its likely homologue in Galago, is not found in non-primates. For instance, non-primate frontal oculomotor areas are agranular, the lateral part of MD (with which they are connected) cannot be subdivided into distinct multiform and parvicellular subnuclei, and their projection to the tectum is light and essentially restricted to the intermediate grey layer (Segal et al., Exp. Brain Res., 51: 423-432, 1983). The detailed similarities between Galago GrP and higher primate FEF, and the differences between primates and non-primates, suggest that primate frontal cortex possesses modifications or specializations of oculomotor circuitry, and that these modifications evolved very early in primate history, prior to the divergence of prosimians and higher primates.

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388.11 FOREBRAIN AFFERENTS TO AGRANULAR PREFRONTAL CORTEX DEMONSTRATED BY FAST BLUE AND HORSERADISH PEROXIDASE RETROGRADE LABELING IN THE RABBIT. Sheryl R. Ginn, Charles M. Gibbs*, Shirley L. Buchanan and D. A. Powell. Neuroscience Lab., WJB Dorn VA Hospital and Univ. of South Carolina, Columbia SC.

The frontal cortex has long been implicated in mediating learning and memory processes. Previous research from our laboratory (J Comp Physiol Psychol 96:755-774, 1982) indicates that the midline frontal cortex is involved in the initial processing of information; i.e., lesions in this area attenuate autonomic, but have no effect on somatomotor, conditioning. In an attempt to explore the anatomical substrates involved in autonomic conditioned responses, New Zealand albino rabbits (Oryctolagus cuniculus) of both sexes received unilateral pressure injections of the fluorescent dye Fast Blue (4%, 0.2 µl) or horseradish peroxidase (HRP; 25-50%, 0.05-0.2 µl) in agranular prefrontal cortex. Injections were made beginning 2.0 mm anterior to bregma and continuing rostrally to 10 mm anterior to bregma in different animals. All injections were made 1 mm lateral to the midline and were placed 1.0-3.0 mm below the dural surface. Following a 2-6 day survival period, animals were sacrificed and the tissue processed for either fluorescent dye analysis with a Leitz Dialux 20 microscope equipped with Leitz Fluorescence filters A (excitation-360 NM) and D (excitation-390 NM) or reacted for HRP analysis according to the tetramethyl benzidine procedure of Mesulam (J Histochem Cytochem 26:106-117, 1978).

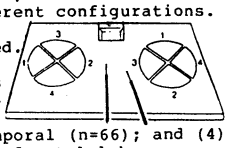
Labeled cells were observed in both ipsilateral and contralateral prefrontal cortex. A moderate number of labeled cells were observed in the dorsal portion of the anterior claustrum. Scattered cells were also observed in the diagonal band of Broca and the basal forebrain, particularly the nucleus basalis of Meynert. Some labeled cells were found in the ipsilateral ventral tegmental area and lateral hypothalamus. However, the heaviest label was found in the ipsilateral thalamus; the mediodorsal N., the ventrolateral N. and ventromedial N. showing the heaviest labeling. Label was also observed in the anteromedial nucleus as well as midline thalamic nuclei, including the periventricular N. and N. reuniens. These results are similar to those previously reported in rat and monkey and provide an anatomical basis for further studies of the agranular prefrontal cortex during associative learning.

Supported by VA Institutional Research Funds

HUMAN BEHAVIORAL NEUROBIOLOGY: DISORDERS

389.1 DEFICITS IN UNIMANUAL AND BIMANUAL TAPPING IN PATIENTS WITH FRONTAL-LOBE LESIONS. G. Leonard*, B. Milner and L. Jones. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Lesions involving the frontal cortex in both human and non-human primates impair the execution of complex motor acts, even though the precentral gyrus is spared (Deuel, 1977; Kolb and Milner, 1981). In the monkey, unilateral lesions of the supplementary motor cortex give rise to deficits in bimanual tasks where the two hands must perform different actions simultaneously (Brinkman, 1984). The present study examines the performance of patients with unilateral excisions from either the frontal or temporal cortex on three motor tasks, using the apparatus (Thurstone, 1944) shown below. Subjects completed three tasks of increasing difficulty: (1) repetitive unimanual tapping (15 s) on one sector; (2) sequential unimanual tapping (30 s) on the four sectors in numerical sequence; and (3) bimanual tapping (30 s), in which the subject had to tap, in the same numerical sequence, the sectors of two plates that were numbered in different configurations.



In all, 151 patients and 60 normal control subjects were tested. The patients were assigned to the following four groups on the basis of side and site of cortical excision: (1) left frontal (n=13); (2) right frontal (n=22); (3) left temporal (n=66); and (4) right temporal(n=50). Within the frontal-lobe groups, the excisions varied with respect to size and locus; all removals involved the lateral cortex (sparing the precentral gyrus), and many also invaded the medial frontal region. All subjects were right-handed.

For all groups the right hand was consistently faster than the left on the unimanual tasks. On all three tasks the left and right temporal-lobe groups performed normally. In contrast, both the left and right frontal-lobe groups were impaired with either hand on the sequential tapping task, and they also had a deficit on bimanual tapping. For the repetitive task, only women with frontal-lobe lesions were impaired. The deficits on these tasks bore no obvious relation to the locus or extent of frontal-lobe removal. Our findings demonstrate that the frontal cortex plays a critical role in the execution of these motor tasks, and that the effects of unilateral frontal-lobe lesions are manifested bilaterally.

389.2 EFFECTS OF NEOCORTICAL LESIONS ON FINGER-FORCE COORDINATION IN HUMAN SUBJECTS. L. A. JONES, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Recent studies of motor function in non-human primates have demonstrated various kinds of manual impairment following lesions involving the frontal cortex. Smith et al. (1981) reported that unilateral lesions of the supplementary motor area in monkeys result in changes in the forces generated during prehensile grasping; there is a significant increase in the rate of force change during the dynamic phase of grasping and the mean static force is also higher.

This study examined the performance of patients with unilateral excisions from either the right frontal (n=13), right temporal (n=18) or left temporal (n=20) lobe, and of normal control subjects (n=14), on a force-tracking task. Subjects were required to generate a target force, which ranged from 10% to 40% of the maximum finger force, by isometrically flexing either one, two or three fingers. The time taken to produce the correct force or combination of forces was recorded. The subject's hand was positioned so that the distal interphalangeal joint of each of the four fingers rested on a strain-gage force transducer. An offset was added to the output of the force amplifier, and, using a digital meter as feedback, the subject's task was to generate, as quickly as possible, sufficient force with a designated finger or fingers to reduce the offset to zero. The meter provided an error signal of the summed absolute difference between the actual forces exerted and the target forces.

The time taken to produce the target forces increased monotonically with the number of fingers involved in the task. There were no differences between the subject groups in the time taken to reach the target when using one or two fingers. However, only patients with excisions from the right frontal lobe were significantly slower than normal control subjects when generating a set of forces with three fingers, and this deficit was bilateral. The impairment in force coordination seen in the right frontal-lobe group could reflect: (1) a deficiency in using external feedback to modulate the forces generated, and/or (2) an inefficiency in monitoring the motor output to the fingers, including knowing what force was being produced by which finger. These hypotheses are currently being investigated.

389.3 THE EFFECTS OF HUMAN AGING ON P3 POTENTIALS IN STIMULUS-RESPONSE INCOMPATIBILITY TASKS. C. Christensen, A. Pfefferbaum*, J. Ford*, and B. Kopell* Dept. Psychology, Vassar College and Veterans Administration Medical Center, Palo Alto, CA 94304.

We have recently shown that effects of stimulus–response incompatibility on reaction time (RT) and P3 latency depend on the nature of the task in young adults (Pfefferbaum et al., EEG Clin. Neurophysiol., in press). In this study we compare the behavioral and electrophysiological responses of young and older adults on these same tasks.

Ten young (20±2 yr) and ten older (70±3 yr) women were tested with an ERP paradigm that used the words "left," "right," "LEFT," and "RIGHT" as stimuli. The stimulus sequence was presented as several separate runs with varying response instructions. Ss were instructed to make left or right button presses according to 1) the meaning of the stimulus (WORD task), 2) the case in which the stimulus was written (CASE task), or 3) both the case and meaning of the stimulus (CASE/WORD task). In each task, half of the trials called for a response that was incompatible with the stimulus. For all three tasks an additional stimulus sequence was presented in which stimulus evaluation was made more difficult by blurring of the words.

Reaction times and P3 latencies were shortest for both groups in the WORD, followed by the CASE, then the CASE/WORD tasks. Across tasks older Ss responded about 120 msec. more slowly than did young Ss and their P3 latencies were prolonged by approximately 55 msec. RTs of older Ss were especially long in CASE/WORD, though there was not an associated prolongation of P3 latency. The effects of degrading the stimulus lengthened RT in all tasks; incompatibility had the same effect in all but the CASE task. P3 latency increased with degrading but it increased on incompatible trials only in the WORD task. These effects were observed in both young and older Ss.

The results for young Ss suggest that P3 latency is increased by incompatibility in the WORD task because they adopt a speeded, automatic response strategy that may predispose to parallel stimulus and response processing. More cautious responding leading to serial processing in the CASE/WORD task would limit the extent to which a P3 effect of incompatibility is observed. The pattern of results for older Ss is similar to that of younger Ss. The bias toward more cautious responding by older Ss reported by others did not change their strategies in the WORD task in ways that removed the incompatibility effect on P3 latency.

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389.4 OLFACTORY FUNCTION AND HEMODIALYSIS: TREATMENT EFFECTS. P. Conrad*, J. Corwin*, L. Katz*, G. LeFavour*, M. Serby*, J. Rotrosen*. (SPON: H. Ryer) Psychiatry Service (116A), NY VA Medical Center, NY, NY 10010.

There is evidence that hemodialysis may have an effect on neurological functioning. For example, demyelinization, peripheral neuropathy and destruction of the axon cylinder have been associated with hemodialysis (Thomas, 1976). As well, plasma choline levels have been shown to be inversely correlated with nerve conduction velocity in people receiving chronic hemodialysis (dialysands). Basal plasma choline levels asymptote at approximately twice normal and are not appreciably lower after a dialysis session (Rennick et al., 1975). This observed change in choline levels is apparantly unique in comparison to commonly measured uremic toxins. Behaviorally, Schiffman et al. (1978) demonstrated reduced ability to judge qualitative odor differences and reduced hedonic responses in dialysands.

We investigated the relationship among olfactory identification, memory, learning and dialysis as follows. We administered a 20-item, 40-trial yes/no olfactory identification task and a Buschke Selective Reminding list learning task 1/2 hour before and again 1/2 hour after a dialysis session to 10 subjects. Standard lab values were also recorded before and after a treatment. Eleven age-matched controls received the olfactory test once.

Results were that olfactory performance was significantly worse at baseline for dialysands as compared to normals; olfactory performance worsened significantly after a dialysis treatment as compared to baseline. There were, however, no parallel changes in memory scores from baseline to postdialysis.

Because there was no decrease in performance on the cognitively harder memory task and there was a worsening on the easier olfactory task, we hypothesized that the acute deficit was not due to anemia, fatigue, disequilibrium related to the actual treatment or uremia. The physiological mechanisms underlying the acute and chronic olfactory deficits are unknown. It is possible that a neurochemical or other substance necessary for olfactory integrity is being removed acutely by the dialysis procedure or is chronically unavailable in metabolic or structural pools. Given the unique behavior of plasma choline levels during the dialysis treatment, and the suggestive findings associating choline dysregulation and neural dysfunction in dialysands, choline-related systems are possible candidates for the mechanism.

389.5 THE RELATIONSHIP OF SOME VISUAL AND PERCEPTUAL VARIABLES TO INDIVIDUAL DIFFERENCES IN MOTION SICKNESS SUSCEPTIBILITY. R.L. Ladowsky and K.-P. Ossenkopp. Department of Psychology, University of Western Ontario, London, Canada N6A 5C2.

Although the induction of motion sickness depends on the presence of an intact vestibular apparatus in man and other species (Phys. Rev., 1970, 50, 1), individual differences in motion sickness susceptibility (MSS) do not appear to be related to vestibular sensitivity (Aviat. Space Environ. Med., 1976, 47, 931). The present study examined whether individual differences in some visual and perceptual variables might account for individual differences in susceptibility to motion sickness.

Thirty males and 30 females were given two motion sickness questionnaires to complete. One questionnaire required subjects to make overall self ratings of MSS while the other questionnaire examined developmental aspects of MSS. Subjects were also given several paper and pencil tests which are considered to be visual-perceptual in nature. An eyesight examination measured near and far acuity, both monocularly and binocularly, near and far vertical and lateral phoria, and stereopsis. The duration of the spiral movement aftereffect was also measured in each subject. Finally, MSS while executing head movements in a rotating chair was measured using the Well-Being Scale of Reason and Brand (1975, Academic Press).

In males and females the correlations between MSS in the rotating chair and overall self-ratings of MSS were $r = .49$, $p < .05$, and $r = .51$, $p < .05$, respectively. In males, the correlations of childhood and adulthood MSS with chair rotation susceptibility were $r = .61$, $p < .05$ and $r = .30$, $p < .05$, respectively, and in females, the correlations of childhood and adulthood MSS with chair susceptibility were $r = .70$, $p < .05$ and $r = .36$, $p < .05$, respectively.

Spiral movement aftereffect durations were found to be significantly longer in susceptible than in resistant individuals ($p < .05$), as has previously been reported (Br. J. Psychol., 1968, 59, 385; Aviat. Space Environ. Med., 1976, 47, 931), but this measure appeared to predict chair rotation susceptibility scores more strongly than it predicted questionnaire MSS scores. Other visual-perceptual tests appeared to correlate more highly with questionnaire scores than with chair scores. In a previous study those males and females who indicated relatively poor vision on a questionnaire also rated themselves as significantly more susceptible to motion sickness. In this study, no single measure of eyesight was highly correlated with any of the MSS measures. The results of this study suggest that a variety of factors are related to MSS and that the relative importance of each factor may depend on the way in which susceptibility to motion sickness is measured.

389.6 DEFICITS IN LEFT-HEMISPHERE LINGUISTIC FUNCTION IN TURNER'S SYNDROME. W. Heller* and J. Levy* (SPON: P. Tueting). Depts. of Psychiatry and Behavioral Sciences, Univ. of Chicago, Chicago, IL 60637

Dichotic-listening studies consistently report that women with Turner's syndrome (TS), who typically have impaired visuo-spatial ability but normal verbal IQ (VIQ), show smaller right-ear advantages (REA's) than normal women. To account for this, various authors (e.g. Gordon and Galatzer, Psychoneuroendocrinology, 1980, 5, 225-234) have proposed that language is represented in both hemispheres, resulting in normal left-hemisphere (LH) specialization for verbal function and impoverished right-hemisphere (RH) specialization for visuo-spatial function. If so, the reduction in REA's in TS women should be due to enhanced left-ear (LE) relative to normal right-ear (RE) performance. However, inspection of the data reported in Gordon and Galatzer (1980) reveals that smaller REA's were due to reduced RE performance in TS women. To investigate the possibility that TS women have a deficit in LH function, as suggested by these data and by reports that TS women are inferior to normal women in verbal fluency (Waber, Dev. Med. Child Neurol., 1979, 21, 58-70) and in digit span (Silbert et al., Behav. Gen., 1977, 7(1), 11-12), unilateral vertically-oriented nonsense syllables were tachistoscopically presented to the right and left visual-field (RVF and LVF) of 16 TS women and 35 age-, SES-, and VIQ-matched controls. Prior research (Levy, Heller, Banich and Burton, J. Exp. Psych.: Hum. Percept. Perf., 1983, 9, 329-359) has shown that top-letter (TL) errors are equally common in the two fields, whereas bottom-letter (BL) errors are more frequent in the LVF. Therefore, analysis of error patterns yields a more sensitive measure of hemispheric specialization for linguistic processing than total correct. For both TS and normal women, BL errors were more frequent than TL errors ($p<.01$), and more so for the LVF ($p<.0001$), reflecting the linguistic superiority of the LH. TS women made more RVF errors than controls ($p<.025$), but LVF errors did not differ between groups, results that are similar to those of Gordon and Galatzer (1980). The reduced RVF performance of TS women was due to a selective increase in BL errors for the RVF ($p<.01$), indicating that linguistic encoding for the LH was significantly reduced for TS women. For LVF performance, error patterns were identical for TS and normal women, disconfirming the hypothesis that language representation is greater in the RH of TS compared to normal women.

389.7 ABNORMALITIES IN EEG AND CEREBRAL BLOOD FLOW IN PARANOID SCHIZOPHRENICS DURING ACTIVATION. J. Herman, M. Devous*, H. Roffwarg, J. Raese, L. Jennings*, F. Bonte*, R. Paulman*, R. Gregory*, and P. Rampy*. Departments of Psychiatry, Nuclear Medicine and Medical Computing Resources Center, Univ. of Texas Health Science Ctr. and Dallas Veterans Administration Medical Center, Dallas, Texas 75235.

The EEG of schizophrenics differs from normals, especially in frontal (Morihisa et al., Arch Gen Psychiatr, 719, 1983) and left hemispheric regions (Guenther et al., Biol Psychiatr, 249, 1986). Abnormalities in the above studies are made more evident by procedures requiring specific mental activities. Differences between schizophrenics and normals in EEG correlate regionally with decreased glucose uptake (Buchsbaum et al., Drug Res, 193, 1982). Weinberger et al., (Arch Gen Psychiatr, 114, 1986) found patients and controls to be indistinguishable at rest by rCBF. However, the blood flow of schizophrenics decreased in dorsolateral pre-frontal cortex during the Wisconsin Card Sort (WCS), a task requiring bilateral frontal cortical involvement.

We report a study of twenty male inpatients, including 12 paranoid schizophrenics (PS) and eight non-paranoid schizophrenics (NPS) who were compared to ten normal male controls (NC), matched for age and handedness. EEG was collected from 6 left and 6 right 10-20 sites on each hemisphere. RCBF was measured by the three dimensional technique of dynamic single-photon emission computerized tomography (DSPECT). Resting measurements and the activation procedure (WCS) were performed with simultaneous measurement of rCBF and EEG. EEG segments free of eye movement artifact were transformed to the frequency domain. Mean values of power in delta, theta, alpha and beta frequencies were calculated for each recording site. Values of rCBF for comparison to EEG sites were extracted from the 3D pseudo-color images.

A conditions by groups effect in EEG was evident in delta and significant in alpha frequencies in pre-frontal, frontal and mid-temporal sites (P=.058). NCs and NPSs evidenced activation (lower amplitude) in these frequencies at these sites. The PS group, however, showed an opposite effect in delta and alpha at the frontal-temporal sites: their amplitude was consistently increased (deactivation) by the WCS. Blood flow, when computed as a ratio of region of interest to whole hemisphere value, decreased nonsignificantly in PSs but not NCs or NPSs during the WCS. The decreases were observed in bilateral superior frontal, right middle and inferior frontal, and right superior, middle and inferior temporal regions. The reduced rCBF in PSs was also suggested by visual inspection of activated images. Thus, the WCS in PSs induced bilateral-frontal deactivation in EEG, but confirmatory evidence from rCBF requires further analysis.

389.8 EFFECTS OF CORTICOTROPIN-RELEASING FACTOR ON PLASMA ACTH AND CORTISOL CONCENTRATIONS IN SCHIZOPHRENIA. P.B. Chappell,* M.A. Smith, J.C. Ritchie, K.R.R. Krishnan,* D. Reed,* J. Rivier,* W.W. Vale and C.B. Nemeroff. Dept. Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710, Salk Institute, La Jolla, CA 92138.

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide widely distributed in the mammalian CNS. It functions as a hypothalamic hypophysiotropic hormone, stimulating pituitary ACTH and β-endorphin secretion in laboratory animals and humans. It has been postulated that CRF may play a central role in regulating the behavioral, autonomic and endocrine responses to stress. Clinical studies have demonstrated that patients with endogenous depression, anorexia nervosa and panic disorder have a blunted ACTH response to exogenous CRF when compared to the responses of normal control subjects. Moreover, previous work from our laboratory and that of Gold and his colleagues has shown that CSF concentrations of CRF-like immunoreactivity are elevated in patients with endogenous depression and anorexia, supporting a hypothesis that hypersecretion of CRF may underlie the hypercortisolemia of these disorders. In contrast, CSF CRF concentrations are not elevated in schizophrenic patients. The purpose of the present study was to assess the ACTH and cortisol responses to CRF in schizophrenia.

Eight patients (seven male and one female, mean age 34 ± 3) that fulfilled Research Diagnostic Criteria (RDC) for schizophrenia were studied. Three met criteria for undifferentiated, two for paranoid, one for residual and two for schizoaffective subtype. Four were medication-free and four were maintained on low-dose neuroleptics. Eight normal volunteers (seven male and one female, mean age 34 ± 2) served as controls.

Ovine CRF (1μg/kg) was administered as an IV bolus at 2000 hours and blood samples were obtained over the following two hours for measurement of ACTH and cortisol. Plasma ACTH concentrations were measured by a sensitive and specific radioimmunoassay, and cortisol by a modification of Murphy's competitive protein binding radioassay.

No significant differences in the ACTH and cortisol responses to CRF were detected, confirming a previous study by Gold and his colleagues. There was no difference in response to CRF in one patient that was studied both drug-free and during antipsychotic drug therapy. The greatest ACTH response occurred in two undifferentiated schizophrenics who were actively hallucinating during the study. It would appear that the hypothalamic-pituitary-adrenal axis in patients with chronic schizophrenia is not severely dysregulated as it is in depression, anxiety and anorexia.

389.9 DISTRIBUTION OF ERPS FROM SCHIZOPHRENICS AND NORMALS: MODALITY, TASK, AND STIMULUS SIGNIFICANCE. Althea M.I.Wagman, A. Summerfelt, William T. Carpenter, and Douglas Heinrichs. Maryland Psychiatric Research Center, Baltimore, MD 21228.

A number of ERP studies comparing schizophrenics (SZ) to normals (N) have found diminished P3 in tasks requiring detection of a low probability stimulus in the odd-ball paradigm. These studies differ somewhat in findings regarding responsiveness of the SZ P3 to stimulus and task conditions. This study approached the resolution of this problem by assessing several components of the ERP in both the visual and auditory modality to determine whether: 1) SZ P3 was unusually low in both modalities 2) the SZ amplitude reduction was due to the inability of stimulus parameters to engage the P3 3) the difference between SZ and N was confined to the P3 4) the topology of the ERP was different in the two groups.

Eighteen sequentially admitted, drug-free, DSMIII SZ outpatients consented to participate. Their performance was compared to that of 18 SADS-L Ns. Schizophrenics were tested during the 4th week of a drug-free period. Symptoms of psychosis were rated on the test day with BPRS. In the auditory procedure, Ss identified low probability high frequency tones under "Attend" instructions or read an article while the same high and low frequency tones were presented in the "Ignore" condition. The visual task required response to one of 3 spatial patterns which occurred infrequently. The order of presentation of these procedures was randomized across Ss. ERPs were recorded from bilateral frontal and central electrodes and Pz. Another electrode was placed over Cz for the auditory and Oz for the visual task. For all target identification procedures Ss were instructed to withhold response of the right forefinger until the end of the ERP sampling period.

Principal component analysis was used to develop 4 orthogonal components to explain variance in each modality for the groups independently. These solutions produced components which could be identified as Slow Wave, P3, P2, and N1 for each group. A second analysis which combined the data from both groups was used to evaluate between group differences for each component within each modality. There was similarity between the groups on the P3 component when stimuli were to be ignored, and when probability alone was the relevant variable. Both groups produced large readiness potentials while waiting to respond to targets. Schizophrenics were impaired relative to normals primarily in the activation of the P3 component by low probability stimuli only when they were task relevant.

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389.10 POST-STROKE MOOD DISORDERS: IN-HOSPITAL PROGNOSTIC FACTORS AT ONE AND TWO YEAR FOLLOW-UP. A. W. Forrester*, R. G. Robinson, (SPONS Barry Gordon) The Johns Hopkins Univ Sch Med., Balt. MD.

In a prospective study of 103 stroke patients, psychiatric examinations consisting of the Present State Examination (PSE), Hamilton Depression Rating Scale (HDRS), Zung Depression Scale (ZDS) to quantify depression; Mini-Mental Status Examination (MMSE) to measure cognitive impairment; the Johns Hopkins Functioning Inventory (JHFI), to measure activities of daily living (ADL); and the Social Functioning Exam (SFE) were made during the acute post-stroke period and at one and two years follow-up.

In previous studies, we have reported that lesion location is the most important variable in determining the severity of post-stroke depression (Robinson et al, Brain 107, 81, 1984). Over the first 6 months of follow-up, however, there was an important interaction between depression and impairment variables with depression in-hospital predicting ADL 6 months later and vice versa. In the present study, we examined the relationship between in-hospital measures and outcome at one and two years follow-up. In hospital measures of depression were signficantly correlated with these measures at 1 year follow-up (N=35) (PSE, r=.64; HDRS r=.56; ZDS r=.24). In addition, there was a significant relationship between depression as measured in-hospital and the JHFI (ADL) scores at 1 year follow-up (r = .52, .32 and .26 for ZDS, PSE and HDRS respectively). No significant correlations were found between JHFI scores in-hospital and depression scores at 1 year follow-up. In contrast, among patients interviewed at 2 years post-stroke (n = 41), there was a significant, but weaker relationship between in-hospital depression and JHFI scores at 2 years follow-up (r = .33, p < .05, .22 NS, .18 NS for ZDS, PSE and HDRS).

These data suggest that, over the first year post-stroke, the existence and severity of depression may have an important impact on the course of recovery in activities of daily living. Other work (not presented here) has also demonstrated that between one and two years post-stroke there was a significant improvement of depression scores in patients with major depression in-hospital. Thus, the weakened relationship between depression and ADL during the period between one and two years post-stroke may reflect improving depression scores without concomitant changes in ADL's. This is consistent with our previous finding that impairment does not cause depression or lead to its recovery but that once depression exists it can significantly impact on daily activities.

389.11 ACUTE EFFECTS OF ETHANOL ON EVENT-RELATED POTENTIALS IN A SUSTAINED ATTENTION TASK. J.M. Stapleton, J.R. Rohrbaugh, R. Parasuraman,* H.W. Frowein,* E.A. Zubovic,* M. Linnoila* and M.J. Eckardt. Section of Clinical Brain Research, Laboratory of Clinical Studies, NIAAA, Bethesda, MD 20892.

Event-related potentials (ERPs) and behavioral performance measures were recorded from 12 normal volunteers following acute ethanol administration (0, 0.45, 0.80 or 1.05 g/kg initial dose + 0.24 g/kg/h maintenance dosing) during a task which has previously been shown to be sensitive to decrements related to time on task (Nuechterlein et al., 1983, Science 220:327). ERPs were recorded from standard 10-20 system sites (Fpz, Fz, Cz, Pz, Oz, C3, C4), using a Grass polygraph with a time constant of 12 sec and a half-amplitude high-frequency cutoff at 70 Hz, and digitized online at a rate of 200 Hz. Trials containing eyeblink or other artifacts were deleted during averaging. Ethanol was administered orally, with orange juice added to make a constant volume, and the initial dose was consumed over a 30-min period. Testing began at 90 and 180 min after the beginning of drinking. Visually degraded stimuli (single digits) were presented at a 1 sec ISI with a target probability of 25% and a keypress response required.

The three ethanol doses produced peak blood ethanol levels of 42, 74, and 92 mg% with significant and linear differences in subjective level of perceived intoxication. Behaviorally, ethanol impaired performance overall as indicated by number of hits, or by the signal detection measures of sensitivity d prime or a prime, with a linear dose-response curve. In addition to this overall effect, the higher ethanol doses enhanced the decrement in performance over time.

The ERP measures showed a diferent pattern of results. P3 showed a dose-related decrease in amplitude and increase in latency, but no effect of time on task. N2 was less sensitive to the effects of ethanol, and showed a small but reliable decrease in amplitude and increase in latency across time. There were no statistically significant effects on N1 of ethanol or time on task. None of the ERP measures showed an interaction of ethanol dose with time on task, as was evident in the behavioral performance measures.

These data indicate that ethanol does impair performance in sustained attention tasks, a finding that may have practical relevance in situations, such as driving, that require continuous performance. They also suggest that the mechanism of the decrement in performance over time in these tasks is independent of the mechanism of ERP changes at least in terms of ethanol sensitivity.

389.12 NEUROPHYSIOLOGICAL AND COGNITVE CHANGES IN RECENTLY DETOXIFIED ALCOHOLICS R.F. Kaplan*, K.A. Yurko, and J.T. Becker. University of Connecticut Health Center, Farmington, CT 06032

The alcoholism literature suggests that EEG abnormalities and cognitive dysfunction associated with chronic alcohol misuse are at least partially reversible following the cessation of drinking. From a clinical perspective the time course of cognitive recovery is important because treatment intervention typically involves psychotherapy and educational programs. The data presented here are preliminary findings from a larger investigation designed to examine neurophysiological and cognitive changes following cessation of drinking.

The subjects were five male alcoholics (X age=42 years, X education=13 years) with at least nine years of problem drinking who were recruited from the Alcohol Treatment Center, University of Connecticut. Each subject was studied repeatedly at day 6, 21, and 45, respectively. Eight channels of bipolar EEG were recorded from the left and right frontal, temporal, centro-parietal and occipital regions of the skull and quantified using power spectral density methods. Each subject was also administered three comparable forms of a neuropsychological test battery including the Digit Symbol Substitution Test, the Verbal Paired-Associate Learning Test, the Symbol Digit Substitution Test, the Stroop Color Test, the Symbol-Digit Paired Associate Learning Test, the Witkin's Embedded Figures Test, the Four Word Short-term Memory Test and the FAS.

A repeated measures multivariate analysis of variance (MANOVA) was used to examine the EEG and behavioral scores. Pearson correlation coefficients were calculated to examine relationships between the quantified EEG scores and scores on the neuropsychological tests. The results showed increased absolute power in the alpha (9-12 Hz) frequency from the temporal and occipital regions and increased absolute power in the theta (5-8 Hz) frequency from both the frontal and occipital leads between days 6 and 45. There were also significant improvements over time in performance on the Witkin's Embedded Figures Test, a test of visuoperceptual skills, the Stroop Test, a test of perceptual interference and on the FAS, a test of verbal fluency. Of particular interest were the high correlations between EEG theta and alpha power and test performance for these three tests at each test point. In conclusion it appears that there is both normalization of the EEG and specific types of cognitive recovery occurring during the first 45 days of the alcoholics' abstinence and a relationship between these changes.

This research was supported by NIAAA Alcohol Research Center Grant #5 P50 AA03510.

389.13 MEMORY FOR SEMANTIC ORGANIZATION IN PICTURES FOLLOWING DAMAGE TO LANGUAGE REGIONS IN THE LEFT HEMISPHERE. D. W. Zaidel. Dept. of Psyhology, UCLA, Los Angeles, CA 90024.

As in language, pictorial organization conveys semantic information which can be studied systematically after brain damage. Pictures are closer to a veridical representation of reality than language and provide an alternative method of probing the conceptual/semantic system in the brain. Here, the relationship between memory for pictorial semantics and language was studied in patients who have suffered from specific type of language breakdown due to stroke. The damage was localized in frontal versus posterior regions in the left hemisphere and resulted in nonfluent (N=8) or in fluent aphasia (N=7), respectively.

Memory was measured in two separate series of stimulus scenes with 10 stimuli per series. There were 3 types of scenes: organized (real-life arrangement), unorganized (random arrangement), or incongruous (seeming real-life arrangement). The task was to recognize single figures and entire scenes in separate 5-choice arrays. Responses were indicated through pointing. For each stimulus series there were 20 responses, 10 for single figures and 10 for whole scenes.

The results showed a dissociation between the two groups of patients on all 3 types of semantic organization. Performance of the nonfluent patients was similar to that of normal control subjects and to nonaphasic patients with frontal lobe damage (Zaidel, D. W., INS Proceedings, 1985) whereas the fluent group was similar to nonaphasic patients with posterior left hemisphere damage (Zaidel, D. W., Brain, 106, 1986). A dissociation between memory for single figures and whole scenes was present only in the nonfluent group.

Taken together, the results suggest (1) that pictorial semantics as linguistic semantics are dissociable and subserved by different cortical regions in the left (and right) hemisphere, and (2) that the structure of the pictorial semantic system is independent of the integrity of the linguistic system itself.

389.PO SPATIAL APHASIA IN BRAIN DAMAGED SIGNERS U. Bellugi, H. Poizner*. Neurolinguistics Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037.

American Sign Language (ASL) displays the complex linguistic structure found in spoken languages, but conveys much of its structure by manipulating spatial relations, thus exhibiting properties for which each of the hemispheres of hearing people shows a different predominant functioning. The study of brain-damaged deaf signers offers a particularly revealing vantage-point for understanding the organization of higher cognitive functions in the brain, and how modifiable that organization may be. We have had the unique opportunity to analyze the breakdown of sign language following localized lesions to the left hemisphere. These studies are part of a systematic program which examines the effects of brain lesions on sign language processing and on spatial cognition in deaf signers with either left or right hemisphere lesions.

We present new analyses of grammatical breakdown for a visual gestural language. We found one patient who shows a complete agrammatism; that is, she omits all grammatical markers, even when they occur on single signs, producing extremely short ungrammatical sign utterances. Her pattern of deficits parallels those found in hearing patients with similar lesions. Another patient shows a different pattern of grammatical impairment involving inappropriate additions and substitutions of grammatical markers, which in ASL are superimposed movements co-occurring with the sign. Futhermore, in this patient, there is breakdown of syntactic mechanisms, which in ASL are conveyed by manipulating spatial relations. Unlike the previous patient, his aphasia is modality specific, unique to signed rather than spoken language. In contrast to the sign language deficits of these left lesioned patients, nonlanguage spatial cognitive processing was largely intact.

These results, seen in the perspective of other converging evidence from right hemisphere lesioned signers (Bellugi, Poizner & Klima, MIT Press, in press), show that the underlying specialization of the left hemisphere for language does not rest upon speech or sound.

389.PO PATTERNS OF VISUAL PERCEPTUAL IMPAIRMENT IN MAN SUGGEST MULTIPLE CORTICAL VISUAL SYSTEMS. P. J. Eslinger. Department of Neurology, Univ. of Iowa College of Med., Iowa City, IA 52242.

Impairments of visual perception often occur after damage to posterior cerebral regions. The impairments can be quite different in nature, compromising only "spatial" aspects of perception or only "pattern recognition" aspects of perception. These behavioral differences are hypothesized to be dependent upon the location of cerebral damage. We studied 2 groups of patients who showed vastly different patterns of visual perceptual impairment following cerebral infarction, localized with a standard method of CT scan analysis. The four patients of Group 1 were severely impaired on standardized tests of spatial perception while pattern recognition was normal. The lesions in this group were localized to the parietal lobe. The 5 patients of Group 2 showed clear impairment in pattern recognition tests (e.g., facial discrimination), but normal spatial perception. The lesions in this group were localized to the infracalcarine region and occipital-temporal junction.

The results indicate that dissociations in visual perceptual performance may be associatied with different loci of cerebral damage. The spatial aspects of visual perception appear to be mediated through superior visual system structures (supracalcarine and parietal cortices), while pattern analysis and recognition is more closely associated with inferior visual system structures (infracalcarine and temporal cortices). Together with other neuropsychological findings from human and non-human primate studies, the results support the existence of superior and inferior visual cortical systems devoted, respectively, to spatial and pattern recognition aspects of visual perception. Supported by NIH Grant PO1 NS 19632-02.

HUMAN BEHAVIORAL NEUROBIOLOGY: METHODS

390.1 DOSE RELATED PHYSIOLOGIC, BEHAVIORAL, AND COGNITIVE EFFECTS OF INTRAVENOUS NICOTINE IN NAIVE HUMAN SUBJECTS. P.A. Newhouse, T. Sunderland*, K. Thompson*, P.N. Tariot*, H. Weingartner*, and D.L. Murphy*, LCS-CNS, NIMH, Bethesda, MD, 20892

Nicotine, a centrally acting cholinergic agonist, has been reported to have a range of physiologic, behavioral, and cognitive effects in the human including autonomic stimulation, hormonal release, and enhancement of memory. However, most prior investigations using cigarettes have been hampered by failure to control the dose and rate of administration, or to account for other effects of smoking, and using chronic smokers, who are presumably tolerant, as subjects. In an attempt to control these factors and as part of a study examining the feasibility of using nicotine in Alzheimer's Disease where there appears to be a loss of central nicotinic receptors, we evaluated the effects of a range of intravenous doses of nicotine in naive subjects.

11 normal nonsmoking volunteers (ages 21 to 57, mean 32) received single blind 60 minute continuous infusions of nicotine bitartrate or placebo by peripheral vein. Doses were 0.125, 0.25, and 0.5 µg/kg/min of nicotine base. Blood pressure, pulse, and EKG were monitored by telemetry. Cognitive testing and behavioral ratings (both subject and observer) were performed before, during, and after drug administration. Blood was drawn for plasma nicotine and levels of ACTH, prolactin, and cortisol.

Results showed that there were significant dose dependent rises in systolic and diastolic blood pressure and pulse with a linear shaped dose response curve. Temperature and respiratory rate showed no dose relationship. Peak plasma nicotine values were in the 10-20ng/ml range similar to that seen after smoking. ACTH and cortisol showed small but consistent increases during or immediately after drug infusions at the 0.25 (ΔMAX ACTH=9.0pg/ml, ΔMAX cortisol=5.74mg/dl) and the 0.5 (ΔMAX ACTH=15.15pg/ml, ΔMAX cortisol=7.85mg/dl) doses. Prolactin showed no dose related changes. Preliminary results of cognitive testing showed increases in short term free recall on drug versus baseline at the 0.25 and 0.5 dose accompanied by decrease in intrusion errors at the 0.5 dose. There was also evidence for better delayed recall of previously remembered items on drug as compared to baseline at the 0.25 dose. Behavioral effects included dose related increases in subject rated anxiety, irritability, and fear accompanied by a sense of decreased alertness. These effects did not appear to be due to physical side effects as these were not marked at any dose, although there were marked interindividual variations in behavioral response. These studies provide evidence of specific dose related effects of nicotine on a variety of systems and provide a basis for further investigation of the central and peripheral effects of nicotine and nicotine cholinergic agonists.

390.2 QUANTAL RATE OF PSYCHOBIOLOGIC CLOCK. M.Clynes, M.Rynn*, Research Center, N.S.W. Conservatorium of Music, Sydney 2000, Australia.

Recent studies (1) appeared to show a quantumization in the rate of psychobiologic clocks involved in thinking and performing music, of 0.48 ± .02 percent, about one tenth of a metronome step. This was observed in 170 performances of a top string quartet (Sydney String Quartet) over a number of years. They also showed a degree of stability of overall timing, independent of environment, of the order of 0.4% in performances years apart.

We now confirm the quantal findings with a study of the histographic distribution of 90,000 tap intervals (30 runs) carried out by a non-musician, the taps being in accordance with repeatedly thought (not played, or heard) 11 bars of Mozart's Concerto K467. The subject produced 3000 taps at each experimental run corresponding to 69 repetitions of the 11 bars, pausing between successive repetitions for a few seconds. Each mentally thought performance had 44 taps. Intervals between taps were measured to a resolution of 10^{-5} sec and stored on a computer.

It had been found previously with tapping to thought music by musicians (2) that tapping times become more stable as larger groups of taps are included in a sample, s.d. 3.9% for single taps, 1.2% for groups of 10 taps, 0.8% for groups of 100 taps, 0.4% for groups of 1000 taps. This indicates that the stability of musical concepts exceeds the ability to execute the corresponding pattern in any one instance. Thus, means for each music excerpt (44 taps) were considered for quantumization rather than individual taps, which also include the effects of musical microstructure (3, 4), and noise of the neuromuscular effector system. 2070 means were plotted in a histogram, relative to the median value for each run, and showed a striking quantum size of 0.46 percent. (This corresponds to 2.3 milliseconds per tap at the observed tap rate).

The non-musician, while showing more than three times the spread of timing fluctuations from one mental performance to the next than musicians, was yet subject to the same quantal psychobiologic clock rate - thus, unlike the stability that relates to musical concepts, the quantumization is not related to musical ability or proficiency, but is rather a basic phenomenon of the psychobiologic clock that undoubtedly is also involved in activities and serial behavior other than musical thought. The quantum in the rate seems to reflect discreteness within the psychobiologic clock.

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390.3 RECENT ADVANCES IN NEUROCOGNITIVE PATTERN ANALYSIS. Alan S. Gevins. EEG Systems Laboratory, 1855 Folsom St., San Francisco, CA 94103

We use the generic term "Neurocognitive Pattern (NCP) Analysis" to refer to procedures being developed to extract spatiotemporal neurocognitive patterns from the unrelated "noise" of the brain. Recordings with up to 64 scalp channels during highly controlled tasks are now routine in our laboratory, as is the extended signal processing sequence required to extract minute neurocognitive signals from gigabyte sets of single trial data. More robust measures of the degree of "functional interdependency" between electrodes have been developed and applied to a bimanual visuomotor task recorded with 26 channels from 7 people. The results were clear-cut and consistent with prior neuropsychological models of the rapidly shifting cortical network accompanying expectancy, stimulus registration and feature extraction, response preparation and execution, and "updating" to feedback about response accuracy. Predictive patterns have been identified which distinguish trials that subsequently had accurate or inaccurate performance. Along with pilot analyses of intracerebral recordings in a primate model, these results suggest that it is possible to characterize "functional interdependencies" of event-related processing between local neural areas by measuring the wave congruence and lag time of appropriately preprocessed low-frequency macropotentials. Many of these results cannot be explained by single equivalent current dipole source models, but could, in principle, be understood in the context of a distributed network of specialized processing nodes. However, unique determination of this network is a formidable problem, since event-related signals emitted by each node are obscured by unrelated brain activity and are overlapping both in time and in space when recorded at the scalp. We are attacking this problem with a number of technical developments: use of magnetic resonance images to relate the EEG electrode positions to underlying cortical structures; use of spatial deconvolution to remove the "blurring" effect of conduction through skull and scalp; and incorporation of multichannel MEG data.

This research is supported by the National Institutes of Neurological and Communicative Diseases and Strokes, the National Science Foundation, and the Air Force Office of Scientific Research.

390.4 BRAIN STATE AND ATTENTIONAL MECHANISMS IN THE TRANSITION TO A DE-EXCITED STATE OF CONSCIOUSNESS: A NEUROPSYCHOLOGICAL MODEL OF TRANSCENDING: A.T. Arenander. CNRG, Dept. of Biol. and Physiol. Sci., Maharishi International Univ., Fairfield, IA 52556.

Research on the Transcendental Meditation (TM) technique has found changes in electroencephalographic, respiratory, cardiovascular, and other physiological parameters suggestive of a state of de-excitation of the nervous system. During the process of de-excitation or transcending, sustained periods of marked coherence in brain activity and spontaneous respiratory suspension are closely correlated with subjective reports of awareness without thought. A model is proposed to explain the process of transcending that is based upon cognitive and neural mechanisms similar to the well-known orienting reflex (OR).

According to the model, orienting and habituating processes, coordinated by the prefrontal cortex and the basal forebrain (PFBF), interact to produce a sequence of progressive de-excitations of neural activity, while conscious awareness is maintained. The brainstem (BS) core, in particular the mesencephalic reticular formation and the locus coeruleus, are suggested to be involved in maintaining awareness by producing ORs to the stimulus at each stage in the sequence of de-excitation. The regulation of attentional processes during transcending may be based upon an integrated hierarchy of control systems, including PFBF, BS, hippocampal, and amgydaloid elements converging on the thalamocortical system. An important aspect of the orienting mechanisms in the model are neural reference systems, coordinated by a hippocampal-based comparator, that evaluate the changing configuration of both stimulus and brain state. Consistent with OR theories, the process of transcending would markedly enhance the signal-to-noise ratio of the neural system and facilitate the conscious analysis and perception of modes of cognitive activity not ordinarily available to conscious awareness. The model of transcending is supported by a large body of cognitive neuroscience research on brain state and attentional mechanisms.

390.5 Latency of Late Positive Components and Memory for Prototypes in Immediate and Delayed Testing Conditions. J.G. Banghart , R.M. Vardaris , J. Lindsey, J. Maticic and D. Schafer, Dept. of Psychology, Kent State University, Kent OH 44242.

It has recently been suggested that LPC latency reflects a qualitative difference at encoding between remembered and forgotten items (Neville et al., 1986; John son et al., 1985). Posner and Keele (1970) found that prototypes were remembered better than instances in a behavioral task. The purpose of this study was to investigate the differences between prototypes and their instances behaviorally and electrophysiologically.

Right-handed male students volunteered for the study. Subjects were assigned to the immediate testing group(I) or the delayed group (D), and learned to categorize a series of dot patterns. After 90 trials subjects in I were asked to categorize another set of stimuli which consisted of prototypes, noninstances and old instances. Subjects in D were tested on the same stimuli a week later.

ERPs were collected during both phases of the experiment from electrodes attached to Fz, Cz and Pz. EOG was monitored and trials containing eye-movement were excluded from analysis. Raw data trials were averaged in groups of fifteen. The components P2, P3, P4 and P5 were the maximal positive peaks from 200 to 600 msec.Results indicated that behaviorally prototypes were forgotten at a faster rate than instances. Prototypes had shorter latencies than instances in I and D at frontal and central locations (except for P5 at Cz in D). Latency at Pz was longer for prototypes than for instances for P3(D), P4 (D and I) and P5(D). Latency for noninstances was either intermediate between prototypes and instances or equivalent to instances. Latency for P3 for noninstances was shorter in I than D at all electrode locations.

The results appear to reflect encoding differences between prototypes and instances with more elaboration associated with prototypes at the frontal and central locations. The finding that for P3, P4 and P5 latency for prototypes at Pz was longer than for instances and noninstances suggests that late positive components recorded from the parietal locations reflect a factor other than the qualitative memorial factor recently proposed.

390.6 REPETITION EFFECTS ON INTRACRANIAL EVENT RELATED POTENTIALS RECORDED DURING LEXICAL DECISION. M.E.Smith, G.Heit, E.Halgren. Brain Research Institute, UCLA, and, VA Southwest Regional Epilepsy Center, Los Angeles, CA.

We have described the component structure of human medial temporal lobe event-related potentials (MTL-ERPs) during recognition memory, and the manner in which those components differ between repeated and nonrepeated words (Smith et al, SNS 9:647; SNS 10:846; Electroenceph. Clin. Neurophysiol. 63:145-159). Components with similar intracranial distribution are also observed during lexical decision tasks, where subjects are required to discriminate words from nonsense letter strings. Repetition improves behavioral performance in this task (e.g. Forbach et al, Mem. & Cognit 2:337), although this effect was not obtained in the amnesic patient H.M. (Gabrieli et al, SNS 10:383). In the present study we compare the effects of stimulus repetition on MTL-ERPs recorded during lexical decision with those obtained during recognition memory. Recordings were obtained from multiple electrodes implanted bilaterally in the amygdala, hippocampus, and para hippocampal gyrus, of 8 patients being evaluated for surgical treatment of epilepsy. In the lexical decision task, half of the words were very common, and half were less common, in terms of frequency of occurence in the language. Repetitions were seperated by an average lag of 2.5 minutes, and 60 intervening items. Subjects were required to discriminate words from nonwords. In the recognition test, subjects were required to detect repetitions of previously presented words. As in our earlier studies, both tasks elicited a characteristic sequence of long-latency MTL-ERPs. Item repetition had a similar effect in both tasks, with a usually negative component occuring at 400-500 ms ("MTL-N4") being larger to nonrepeated words, and a later typically positive component occuring 550-650 ms ("MTL-P3") that was largest to repeated words. In lexical decision this difference was larger for uncommon words than for common words. However only small effects of repetition were seen for nonwords. These effects parallel those found in behavioral studies, suggesting that limbic structures play an important role in the repetition priming effects seen in lexical decision, in addition to contributing to overt judgments of recent memory familiarity.

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390.7 PRECORTICAL GATING IN THE HUMAN VISUAL SYSTEM DURING SELECTIVE ATTENTION: A TOPOGRAPHICAL ANALYSIS. M. Oakley*, R.G. Eason, and T. McCandies*. Dept. of Psychology, UNCG, Greensboro, N.C. 27412.

In 1983 Eason, Oakley, & Flowers reported that internal canthus recordings of averaged VER-components corresponding in latency and polarity to the b-wave and afterpotential of the human ERG can be modulated by selectively attending to peripheral locations in the visual half-field in which task-relevant stimuli appear (Physiol. Psych., 1983). These results were replicated in two followup experiments (Eason, Bull. Psychon. Soc., 1984). Even though neuroanatomical evidence for the existence of centrifugal fibers in the optic nerve of mammals, including humans, continues to mount (Itaya-Br. Res., 1985; Terubayashi, et al, Neurosci. Let., 1983; Larsen & Moler, Acta Ophthal., 1985; Wolter, Graefes Arch. Ophthal., 1979), considerable skepticism remains as to their existence. Partly for this reason, and partly because the 1983 findings have not been replicated by others (Mangun, et al., Psychophysiol., 1986), the question of precortical gating at a retinal level remains highly controversial. Indeed many cognitive scientists remain skeptical as to whether precortical gating occurs at any level as a function of attention and other behavioral states.

Using stimuli too weak to elicit measurable ERGs, and with electrodes placed at frontal scalp regions, Oakley, et al (Soc. Neurosci. Abstr., 1985) recently reported preliminary evidence for precortical gating during selective attention at sites central to the retina. They found a negative potential with a peak latency of approximately 50 msec (recorded at Fp2 and Fp4) to be significantly greater when subjects attended to the peripheral spatial location of a given half-field in which task-relevant stimuli appeared than when they attempted to ignore that same location.

The present work replicates and extends these findings. Two experiments have been conducted. In Experiment I the same paradigm and procedures were used as in the 1985 study. In addition to frontal scalp locations, data have been obtained from central, parietal, and occipital areas over both hemispheres. An early component (40-60 msec) recorded at all electrode sites was affected by the attention manipulation except at C3 and C4. Also, the attention effect tended to be more pronounced in right hemisphere recordings.

In Experiment II the angular distance from the point of fixation to the site of retinal stimulation was varied to assess whether the observed attention effect could be an artifact produced by subjects inadvertently shifting their eyes toward the relevant visual field. The results negate this possibility.

The results of these studies provide further support for precortical gating during spatial selective attention in humans.

390.8 SACCADES AND SWITCHING OF SPATIAL ATTENTION: EVOKED POTENTIALS. L. Anllo-Vento* and M.R. Harter (SPON: W.L. Salinger). Psych. Dept. Univ. of North Carolina at Greensboro, Greensboro, N.C. 27412.

Selective attention to a certain point in visual space does not require a concomitant shift in gaze. However, there are units in the brain that respond with increased firing in anticipation of a saccade towards a relevant visual location. This study elucidates the effect that an imminent saccade has on ERP measures of visual selective attention in humans.

Eight adult females were subjects in the study. Three successive stimuli constituted one trial. A small red arrow, pointing to left or right, was flashed in the center of the visual field and served as a cue to indicate which field was relevant. Three-hundred milliseconds later, a red square or circle (S1) appeared 8.5 degrees in the left or right field. A third smaller red square or circle (S2) was foveally presented 300 msec after S1. In the Saccade condition, subjects were required to move their eyes in the direction of S1 when it appeared in the relevant visual field. When S2 matched S1 in shape, they had to respond by lifting their index finger from a reaction-time microswitch. In the No-saccade condition, subjects fixated on the center of the screen at all times, and only responded to the match between S1 and S2 when S1 appeared in the direction signaled by the arrow. ERPs were recorded from occipital (O1-O2), parietal (P3-P4), and frontal (F3-F4) electrodes. Saccades were recorded on-line (EOGs), and those trials in which the subject failed to perform the eye movement task adequately were excluded.

Selective attention effects were manifested in a negative deflection peaking at about 150 msec after S1 (N150 or N1), and preceding the saccade by approximately 50 msec. Increased N150 amplitude was found over the hemisphere contralateral to the relevant visual field. This effect was greatest over parietal cortex, although it was also evident over the occipital and frontal sites. The latency of this attention effect indicates that subjects switched their attention within 450 msec after the presentation of the arrow.

Pre-saccadic effects were evidenced in an overall reduction of N150 amplitude over occipital and parietal, but not frontal cortical areas. At the parietal electrodes an interaction was also found between attention and pre-saccadic effects, showing that the anticipation of a saccade reduced the amplitude of N150 following relevant, but not irrelevant S1s.

These results corroborate in part previous findings in single unit studies (Wurtz, R.H. et al., Prog. Psychobiol. Physiol. Psych., 9:43, 1980) in that they show changes in neural activity resulting from the anticipation of a saccade to a relevant location. They may indicate that such activity exerts an inhibitory effect upon ongoing attentional processes.

390.9 P1-N1-P2 AMPLITUDE LATERALIZATION DURING CPT PERFORMANCE R. J. Strandburg, J. T. Marsh, W. S. Brown, R. F. Asarnow*, D. Guthrie*, Department of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024

Previously, we demonstrated significant right lateralization of the N1 ERP component at parieto-temporal leads during performance of the span of apprehension task (Span) which requires detection of a briefly presented (50 msec) target letter within a 12 letter array. This might represent the selection of an initial parallel processing strategy for locating the target within the array (in contrast to a serial scan) thus preferentially engaging the right hemisphere.

In this study we employ a version of the continuous performance task (CPT) which does not involve visual search but does require discrimination of a fixed target among spatially varying distractors. Single digits were presented at a fixation point every 1.25 sec for 50 msec and were surrounded by up to nine distractor digits. Subjects were required to respond to the repetition of the same digit on successive trials which occurred on 18% of the stimulus presentations. Right handed college students (7 male, 8 female) were studied.

ERPs were recorded from the 19 International 10-20 System loci referenced to linked earlobes. Lateral and vertical eye movements were monitored separately with bipolar leads. A 2.5 sec epoch including both the target and preceding stimulus was digitized online. Peak-to-peak measurements were made for the P1-N1-P2 complex. In addition, baseline to peak amplitude was measured for the P3 component.

We observed a significant right lateralization for the P1-N1 and N1-P2 measures at the T5/T6 leads. While there was some suggestion of right laterality at P3/P4, this was not statistically significant, nor was lateralization observed at O1/O2 where these components were largest or at any other lateral suggesting a high degree of localization. Replicating previous findings, the third positive component (P450) was significantly greater to targets than non-targets. We did not, however, observe lateral asymmetry for this component.

Tentatively, we suggest that the right lateralization over visual association areas for both the Span and CPT is not simply the outcome of spatial localization but the initial establishment of figure/ground relationships between target and distractors. Further on in processing (P3) this asymmetry disappears.

390.10 SHORT-TERM MEMORY FOR SPATIAL LOCATION: A TARGET DIRECTED LOCOMOTION TASK IN NORMAL HUMAN SUBJECTS. R.E. Steenhuis and M.A. Goodale. Dept. of Psychology, University of Western Ontario, London, Ontario, N6A 5C2.

A recent demonstration that human subjects are able to walk very accurately without visual feedback to a target placed up to 21 m away has been interpreted as evidence for an accurate short-term memory for spatial locations (Thomson, J. Exp. Psychol. 9:427-443, 1983). Thomson found that performance accuracy depended on time taken to reach the target and not on target distance. Subjects walking with eyes closed could be as accurate as they were with vision, providing they reached the target within 8 s of last viewing. Performance accuracy was found to decrease dramatically if the subject took more than 8 s to reach the target. The present study was undertaken to investigate further the proposed short-term memory for spatial location.

In these experiments, subjects walked with eyes open or closed to a target placed at different distances (3 m intervals up to 21 m away) in a corridor or in an open field. In some experiments, delays were introduced between viewing the target and walking so that a distance previously reached within 8 s would now take more than 8 s to reach. In all the experiments, when subjects walked with eyes closed, a linear relation between target distance and performance accuracy was found. As the distance to the target increased accuracy decreased. The distance to targets more than 3 m away was significantly underestimated. Not surprisingly, with vision, performance was highly accurate at all distances. Delays of 4 s or less, which increased the time from viewing the target to completion of a walk to more than 8 s at some distances, did not produce a significant effect on performance for walks with eyes closed. Performance accuracy was the same at a given distance whether the walk took more than 8 s or less than 8 s. However, 30 s delays produced a significant decrease in accuracy at all distances. Thus, the relation between accuracy and distance remained linear. No evidence of an abrupt decrease in accuracy of the type previously reported was found under any condition. Moreover, subjects walking with eyes closed did not perform at distances beyond 3 m as if they had vision, even when they were given practice.

No support was found for a highly accurate and transient short-term memory of spatial location which allows subjects walking with eyes closed to perform as if vision is available. Rather performance accuracy was found to depend on distance and on a more slowly decaying short-term memory. Supported by Grant No. A6313 from the Natural Sciences and Engineering Research Council of Canada to M.A. Goodale.

390.11 SELECTIVE ATTENTION ALTERS HUMAN AUDITORY SENSORY PROCESSING AT 20-50 MSEC. M. Woldorff*, J.C. Hansen, and S.A. Hillyard, Department of Neurosciences, UCSD, La Jolla, California 92093.

Recordings of Event-Related Potentials (ERP's) were used to address the controversy as to whether selective auditory attention in humans involves "gating" or filtering of irrelevant sensory input at lower levels of the auditory pathway. The earliest ERP change that has been consistently elicited by attended stimuli is the negative "Nd wave", which onsets as early as 60 msec. Hillyard et al. (Science, 182:177-180, 1973) suggested that the Nd wave may reflect an early "stimulus set" selection of an attended channel of input. The possibility exists, however, that gating of irrelevant channel inputs can start at even earlier levels of the auditory pathways.

To investigate further the possibility of short latency attention effects, 10 subjects were presented with dichotic tone sequences consisting of very brief tone pips of 5000 Hz (left ear) and 3400 Hz (right ear) at a high rate (ca. 5/sec). The task was to listen selectively to the tones in one ear at a time and to press a button upon detecting occasional "target" tones of slightly lesser intensity than the 60 dB SL "standard" tones. Two parallel recording systems were employed: one appropriate for Brainstem Evoked Responses (BER's) and the other for the longer latency waves. To verify the experimental effects, a complete replication with 16 new subjects was carried out using different tone pip parameters.

Switching attention from one ear to the other had no effect on the BER's. Thus these data provided no evidence for pre-thalamic gating or modulation as a mechanism for early selective attention. The auditory ERP's did, however, show a significant modulation in the mid-latency range in both experiments. The mid-latency attention effect appeared as a greater positivity to attended ear stimuli in the latency range 20-50 msec over central and frontal scalp areas. This "P20-50 wave" peaked at a latency similar to that of the mid-latency component Pa, a wave that may reflect activity from primary auditory cortex (Celesia, Brain, 99:403-414, 1976; Kraus et al., Electroenceph. clin. Neurophysiol., 54:275-287, 1982). Because of the short onset latency of this ERP effect, these data strongly support the hypothesis that selective auditory attention can bias processing of inputs at an early stage.

390.12 THE SPATIAL ALLOCATION OF VISUAL ATTENTION AS INDEXED BY EVENT-RELATED BRAIN POTENTIALS. G.R. Mangun* & S.A. Hillyard. Department of Neurosciences, University of California, San Diego, La Jolla, California 92093

Numerous behavioral studies have investigated how processing resources are allocated across the visual field during focused attention. One concept that has emerged is that of an attentional "spotlight" consisting of a small (≤1 degree) discrete zone of facilitation at an attended location. In contrast, other behavioral results suggest that the effects of attention fall off continuously as a function of distance from an attended locus (i.e., a spatial gradient). We have presented electrophysiological data which is in line with either a diffuse spotlight or gradient model of attention (Mangun, G.R. & Hillyard, S.A., Soc. Neurosci. Abstr. 11: 879, 1985). To further map the spatial distribution of attention the present study used event-related brain potentials (ERPs) to index visual processing as the locus of attention was shifted across the visual fields.

Stimuli consisting of vertically oriented bars were flashed at three locations: one in each of the lateral visual fields (5.3 degrees eccentricity) and one on the vertical meridian. With eye position maintained on a central point, subjects were instructed to attend to each position in turn during separate experimental runs in order to detect infrequent shorter bars (i.e., targets). ERPs to lateral stimuli were compared when attention was directed to them, as opposed to when it was directed to the midline or opposite field stimuli.

Selective visual-spatial attention was evident in the ERPs as an amplitude modulation of the evoked fronto-central N150 and parietal N175 components and of an evoked P135/N185/P230/N280 sequence over occipital scalp when the evoking stimulus was attended as compared to when it was not attended. Attended stimuli also elicited broader "endogenous" central N270 and parietal N280 components that could be dissociated from the amplitude enhancement of the earlier ERP components. Attentional gradients appeared as systematic decreases in amplitude of the sensory components of the ERP for the lateral stimuli as attention was focused at increasing distances from the locus of the evoking stimuli.

In summary, the effects of attention to points in space are first seen in the ERP as enhancements of the early exogenous waves which exhibit graded decreases in amplitude as attention is shifted across the visual field. This is followed by longer latency attention-related waves which are specific for the attended stimuli and probably endogenous in nature.

390.13 THE EFFECTS OF SELECTIVE ATTENTION ON SCALP-RECORDED SOMATOSENSORY EVOKED POTENTIALS IN HUMANS. G. McCarthy and C.C. Wood, VA Medical Center, West Haven, CT and Yale University School of Medicine, New Haven, CT 06516

We report a study of the effects of selective attention on somatosensory evoked potentials (SEPs) elicited by left and right median nerve shocks delivered percutaneously to the wrists. Fourteen subjects received constant-current shocks delivered randomly to the two wrists with intervals of 300-500 msec. On 10% of the trials a lower intensity target shock was presented. In Attend-Left conditions, subjects were required to respond with a foot press to targets on the left side and to ignore all stimuli on the right. In Attend-Right conditions, responses were made to right side targets only. Behavioral data indicated that focusing attention to a single side significantly improved performance over a divided attended condition.

SEPs evoked by non-targets on the attended side were compared to the SEPs evoked by the same stimuli when the other side was attended. The presynaptic nerve volley recorded over the brachial plexus did not change as a function of attention condition. The figure below presents SEPs from frontal (F), central (C), and parietal (P) scalp on the side ipsilateral (left) and contralateral (right) to the stimulus. Solid and dashed lines indicate stimuli delivered to the attended and unattended sides, respectively. The initial cortical responses over the contralateral side were unaffected by the attentional manipulation. The most consistent finding was a ramp-shaped positive offset for attended stimuli over the ipsilateral scalp beginning at about 22 msec and continuing to about 125 msec. This was followed by an enhanced positive peak at 190 msec. Over the contralateral scalp, a much smaller negative offset between 50-100 msec was obtained, followed by a negative offset at 140 msec.

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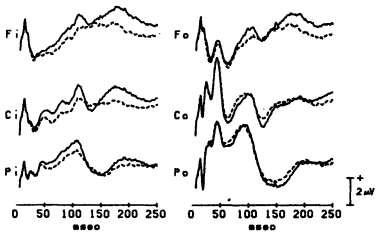

390.14 Chemical Anatomy of the Human Pineal Gland
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It has been established in animals that norepinephrine (NE) stimulates the circadian production of melatonin in the pineal gland by induction of the rate limiting enzyme for melatonin synthesis, N-acetyl Transferase. Evidence also indicates that the superior cervical ganglion (SCG) is critical in this peripherial noradrenergic innervation of the pineal gland. In humans, the pineal gland is innervated anteriorly via the habenular complex (habenula and fibers of the posterior commisure), and posteriorly via axons which course with the Great vein of Galen, although the anatomic path by which the SCG innervates the pineal is unclear.

We report a HPLC method to simultaneously determine catechol and indole content in human pineal gland; including NE, 5-hydroxytryptophan, normetaphrine, homovanillic acid, serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), N-acetyl serotonin and melatonin. The levels of NE, 5-HT and 5-HIAA are higher in the pineal gland than in its surrounding peri-vascular structures. There is an anterior to posterior decrease in NE content within the pineal from subjects known to be awake just prior to death. This gradient of NE roughly coincides with the distribution of Bodian positive fibers, apparently entering the pineal via the habenular complex.

In the pineal gland from subjects dying in their sleep, there is a highly significant (3 to 5 fold) increase in the NE content in the posterior portion of the pineal gland. In addition, uniform levels of N-acetyl-serotonin, the direct precursor to melatonin, are found in the pineal from these subjects, but no melatonin is detectable. The chemical anatomy of the human pineal gland will be discussed in relation to its afferent innervation, as will the possible forensic utility of these preliminary observations.

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390.15 THE INFLUENCE OF GENDER AND HANDEDNESS ON MANUAL PERFORMANCE IN THE SIMULTANEOUS BLOCK DESIGN TASK. J. DeLuca*, D.A. Rasile*, M.E. Kovaleski*, R.G. Burright* and P.J. Donovick. Dept. Psychology, SUNY Binghamton, Binghamton, NY 13901.

The present investigation was designed to address whether hand use preference in a visuo-constructive task can be modulated by activation of specialized cognitive systems which appear to be most dependent on right cerebral hemisphere functioning.

Male and female college students were administered a common laterality examination and based on their results, placed into one of our handedness categories (clearly right; leaning right; leaning left; clearly left). Each subject was then presented with a printed picture of a design and asked to simultaneously construct two replicas of the design, one with each hand. It was hypothesized that if hemispheric arousal has an asymmetric influence on manual activity, then the spatial nature of the block design task should result in activation of the right hemisphere, leading to a relative increase in left hand activity when compared with hand usage on a "neutral" (presumably nonlateralized) task.

Analysis of hand preference in the simultaneous block design task did reveal a significant overall preference for the left hand in construction of the designs for both males and females. This strong left hand preference was observed among both right handed and left handed subjects. However, the magnitude of this left hand preference was greater in right handers. Furthermore, sex interacted with handedness. That is, among right handers, males showed a greater preference for the left hand than females in simultaneous block design construction; but among left handers, females showed a greater left hand preference than males.

These results present the first evidence that a simultaneous, bilateral block design presentation, analogous to that observed in dichotic listening and dichhaptic stimulation, yields lateral preferences which are consistent with current models of perceptual asymmetry in the cerebral hemispheres of humans. While further work is clearly required, the simultaneous block design task ultimately may serve as a valuable research and clinical tool for the Neuropsychologist interested in cerebral lateralization.

390.PO SEQUENTIAL RECALL WITHOUT DIRECT CONNECTIONS: A POSSIBLE BASIS FOR SINGLE-INTERVAL SENTENCE CONSTRUCTION IN HEBBIAN NETWORKS. R. Martin. Chemistry Dept., Brooklyn College, Brooklyn, NY 11210.

Models of sequential recall typically rely on trails of strengthened connections that transmit discharge activity directly from each set of cells to the next within each sequence. This approach appears inadequate, however, when connection trails are highly intertwined and connections that guide recall correctly in some situations increase the likelihood of "wrong turns" at other times. While it is possible to envision superordinate command cells that correctly guide recall across small numbers of such crisscrossed connection trails (much as switchmen manage railyard traffic (1,2)), current network models appear unable to cope with the much larger connection tangles that form when hundreds or thousands of pathways cross repeatedly in more complex nets. An attractive method by which to navigate through such apparently intractable tangles is to eliminate or severely weaken links between neurons within each sequence, and to rely instead on superordinate cells that radiate stimulation, in one step or time interval, directly to separate groups of subordinate cells. Sequential recall will occur if subordinate units that receive the greatest amount of stimulation discharge first, if cells that are somewhat less effectively stimulated fire next, ... , and if the least stimulated cells, at the end of the sequence, discharge last. This method of "stimulation gradient sequencing" requires that lateral inhibition prevent cells at different positions along the gradient from firing concurrently, and that links be strengthened and weakened according to approx. six standard learning rules, which elsewhere have been shown to enable use of concept hierarchies and manipulation of concepts within one step or time interval (3). Stimulation gradient sequencing appears to be a computational method of sufficient power -- in combination with other mechanisms -- to produce grammatically and logically correct sentences because direct links between cells that generate each kind of sentence are absent or ineffective. As a result there is virtually no interference between connection paths responsible for sentence generation and "entanglement errors" are avoided.

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(2) Martin, R. (1986). A preliminary model of neural mechanisms for sentence production. In: Simulation at the Frontiers of Science, J. Young, D.W. Ingalls & R. Hawkins, eds., SCS: San Diego, 30-35.

(3) Martin, R. (1986). Use of explicit constraints to evaluate a potentially unique, neural model for single-interval concept manipulation. In: Modelling and Simulation Methodology in the Artificial Intelligence Era, M.B. Elzas et al., eds., forthcoming.

390.PO RIGHT HEMISPHERE SUPERIORITIES: MORE APPARENT THAN REAL? M.S. Gazzaniga and C.S. Smylie*. Dept. of Neurology, Cornell Univ. Med. Col., New York, NY 10021.

Right hemisphere damaged patients have repeatedly exhibited deficits in tasks using stimuli that are difficult to verbally identify such as the recognition of unfamiliar, similar-looking faces as well as other hard-to-name stimuli. In like manner, split-brain patients have exhibited superior performance on similar tests from the right as opposed to the left half brain. Results of this kind are commonly interpreted to suggest the right brain is specialized for certain perceptual functions. At issue is whether or not the right brain is structurally different from the left or whether the superior performance reflects processing strategies that result from the left brain being unequivocally dominant for language encoding. In short, do the left brain's automatic attempts to verbally encode hard-to-name stimuli, interfere with a simple perceptual task? In the present study, a callosum sectioned split-brain human was serially presented two hard-to-name color stimuli, with an interstimulus interval (isi) of either 1000 or 180 msec. The stimulus pair was randomly presented to the left or right brain and a simple yes/no judgment on whether the two stimuli were the same was made. In an experimental run the first block of 40 trials utilized a slow isi, followed by 40 trials with a fast isi, followed by a block of slow isi trials. The results revealed no significant difference in error rate between the two hemispheres, but a highly significant difference in reaction times (RTs) when the two colored stimuli were separated in presentation by 1000 msec. In this condition, the left brain was up to 250 msec slower than the right in carrying out the judgment ($p < .005$). However, there was no RT difference with a 180 msec isi. In addition, follow up tests also showed a left brain superiority for colored stimuli that were easy-to-name, such as red, green and blue. The results suggest that when the left hemisphere's automatic verbal encoding processes are activated in an effort to name and encode hard-to-name stimuli, extra resources are drawn upon that slow down and potentially interfere with the left brain's capacity to make a simple perceptual judgment. Such reported deficits are always relative to the right brain, which does not automatically name and verbally encode new information and thereby is free to respond directly to the perceptual task. (Aided by USPHS Grant 1 RO1 NS22626-01.)

391.1 BEHAVIORAL AND BIOLOGICAL EXPLANATIONS FOR EXCESSIVE PREGNANCY-ASSOCIATED WEIGHT GAINS D.J. BOWEN* AND J.W. DRYDEN* (SPON:M. Lee). Dept. of Psychology, Texas Tech Univ., Lubbock, TX 79409.

Some women gain excessive weight during and/or after their pregnancies. Possible explanations for this phenomenon include increases in sweet food consumption and biological changes that occur during and after pregnancy. Two studies using rats were conducted to examine these explanations. In Study One pregnant and non-pregnant naive female Albino rats were provided with either bland chow or bland chow plus a 30% w/v glucose solution. At Day 20 of pregnancy and at Day 20 of the postpartum period one-half of the rats from each pregnancy-by-food group were killed, adipose tissue pads were weighed, and adipose cell size and number analyses were performed. In Study Two multiparous and primiparous female Albino rats were provided with either bland food or bland food plus glucose solution before, during, and after pregnancy. During and after pregnancy adipose tissue was removed from each rat, and several biochemical analyses were performed. Body weight, consumption of both foods, and water consumption were measured daily before, during and after pregnancy in both studies. Consumption of sweet food, but not of bland food, increased during and after pregnancy. Both adipose tissue weights and cell size increased during pregnancy. After pregnancy rats provided with glucose solutions and not allowed to lactate gained the most adipose tissue of any group. Results of both studies indicate that both behavioral and biological variables are involved in excessive weight gains associated with pregnancy.

391.2 N^6(L-2-PHENYLISOPROPYL) ADENOSINE, AN ADENOSINE A_1 AGONIST, STIMULATES FOOD INTAKE IN SATED RATS. A.S. Levine, M. Grace*, C.J. Billington*, D.D. Krahn*. Neuroendocrine Research Laboratory, VA Medical Center, Minneapolis, MN 55417.

Some of the central actions of opioids are thought to be mediated by adenosine. Opioids stimulate feeding in non-deprived rats, but decrease food intake in hungry animals (perhaps due to their sedating effects). We previously reported that central administration of adenosine results in a decrease in food consumption in food deprived rats (Pharmacol. Biochem. Behav. 19:23-26, 1983). In the present study we evaluated the effect of the A_1 adenosine agonist, N^6(L-2-phenylisopropyl) adenosine (L-PIA),[1] on food intake in non-deprived rats. Intracerebroventricular administration of L-PIA to rats at 0900 hours (lights on) increased food intake at a variety of doses over a 4 hour period (Table).

	Food Intake (g)		
	0-1 hr	0-2 hr	0-4 hr
Saline	0	0.1 ± 0.1	0.1 ± 0.1
L-PIA 10 µg	1.4 ± 0.4	1.8 ± 0.4†	1.8 ± 0.4†
1 µg	0.9 ± 0.4	1.8 ± 0.7†	2.0 ± 0.7†
0.1 µg	0.8 ± 0.8	0.8 ± 0.8	1.0 ± 0.8

We next administered L-PIA to rats for 4 days at 0900 hours. As has been demonstrated for opioid ligands, R-PIA enhanced food intake more on the 4th day of administration compared to the 1st day of injection (Table).

	Food Intake (g/2 hr)	
	DAY 1	DAY 4
Saline	0.1 ± 0.03	0
L-PIA 10 µg	0.4 ± 0.3	2.5 ± 0.8†
1 µg	0.7 ± 0.2	2.0 ± 0.9†
0.1 µg	0.5 ± 0.2	0.9 ± 0.5

The adenosine antagonist, caffeine, failed to suppress L-PIA (10 µg) induced feeding following peripheral administration (Table). High doses of peripherally administered naloxone (10 mg/kg) decreased L-PIA induced feeding.

	Food Intake (g/hr)
Saline	0.4 ± 0.4
L-PIA + Sal	1.8 ± 0.1
L-PIA + NLX (10 mg/kg)	0.3 ± 0.2
L-PIA + Caff (5 mg/kg)	1.8 ± 0.7

These data suggest that the adenosine ligand L-PIA affects feeding behavior in a similar manner to opioids.

391.3 NALTREXONE SUPPRESSES FOOD INTAKE IN OBESE MEN. T. A. Spiegel*, E. E. Shrager, M. Morrison*, A. J. Stunkard*, C. P. O'Brien and E. Stellar. Obesity Research Group, Department of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

Food intake of 13 obese male volunteers was measured in laboratory lunches Monday to Friday for 3 consecutive weeks. In a double-blind procedure, subjects were given increasing doses of naltrexone (25 to 200 mg/day) the first 4 days of the second week. There was a significant reduction in food intake averaged across the 4 drug days compared to the first placebo week. (Mean reduction = 30%; $F = 11.0$; $df = 2,24$; $p = .0003$). However, the suppression of food intake did not occur until the second day on the drug and food intake remained suppressed on Friday of the drug week (when placebo was given). This pattern of suppression in food intake was seen in 11 of the 13 subjects, whose mean reduction in food intake across days 2 to 5 of the drug week was 45%. Food intake was not reduced during the drug week in the other 2 subjects. Food intake remained suppressed in 9 of the subjects in the placebo week following the drug week. Water intake was not affected.

Rates of ingestion during meals and subjective ratings of palatability, hunger, and emptiness suggested that the drug reduced appetite rather than promoting an early onset of satiety.

Six subjects reported nausea or other adverse reactions on one or two days during the drug week. Food intake of these subjects was suppressed. However, food intake was also suppressed in 5 of the 7 subjects who did not report adverse reactions. Transaminase levels were also elevated at the end of the experiment in 3 subjects.

Weight changes during the drug week were minimal in 9 of the subjects (mean change = +.02 lbs). The other 4 subjects lost 3 to 7.2 lbs. during the drug week.

These results suggest that an endogenous opioid system may be involved in the regulation of appetite and food intake in man. However, the continued suppression of food intake in subjects after the drug week suggests that a conditioned taste aversion may have caused the reduction in food intake, especially in subjects reporting nausea.

391.4 DELAY OF CANCER ANOREXIA FOLLOWING INJECTION OF 6-HYDROXYDOPAMINE INTO THE CORPUS STRIATUM. T. Foley-Nelson, W.T. Chance, J.L. Nelson, L. Cao* and J.E. Fischer*. Dept. Surgery, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

The development of anorexia is a major complicating factor in the aggressive therapy of cancer. Our previous results employing insulin treatments to correct cancer anorexia suggested complex alterations in dopamine (DA) metabolism in the corpus striatum (CS) and nucleus accumbens (NA) of tumor-bearing rats. To permit a more direct assessment of the role of brain DA in mediating cancer anorexia, endogenous stores of DA were reduced in these brain areas using 6-hydroxydopamine (6-OHDA) and the effects of methylcholanthrene (MCA)-induced sarcomas on food intake were then examined. Adult (282±3 g), male, Fischer 344 rats were treated with desmethylimipramine (30 mg/kg, ip) and anesthetized with ether. Sixteen of these rats received bilateral injections (3 ul) of 6-OHDA (4 ug/ul) into either the corpus striatum (A = +1.5 mm from bregma, L = ±3.0 mm, V = -5.5 mm) or nucleus accumbens (A = +3.2 from bregma, L = ±2.0 mm, V = -8.0 mm), while an additional 12 rats were subjected to control operations. To aid the recovery of feeding following surgery, wet mash was offered to the rats for the next 7 days. One week following surgery, MCA sarcomas were transplanted (sc) in all the 6-OHDA-treated and half of the control rats, and the rats were returned to a diet of rat chow pellets. Significantly reduced food intake was first observed in the tumor-bearing control (TB-C) rats 22 days after transplant, while significant anorexia was delayed in the 6-OHDA-CS group until day 27. Analysis of variance across days 21 to 28 revealed significant group differences in food intake between nontumor-bearing and TB-C rats as well as between nontumor-bearing and 6-OHDA-NA rats. No significant differences were observed for this period between nontumor-bearing and 6-OHDA-CS or between TB-C and 6-OHDA-NA groups. Therefore, only the TB rats treated with 6-OHDA in the CS demonstrated a significant reduction in anorexia. Examination of neurotransmitter changes in the CS and NA revealed significant decreases of DA in the CS for 6-OHDA-CS (35%) and 6-OHDA-NA (24%) groups, with no alterations in DA metabolites for these groups. Greater and more specific reductions were observed in the NA with significant reductions in DA (58%), 3-methoxytyramine (50%), 3,4-dihydroxyphenylacetic acid (28%) and homovanillic acid (36%), occurring only in the 6-OHDA-NA group. Therefore, these results suggest that alterations in striatal DA metabolism may be important in the etiology of cancer anorexia, and that the anorexia may be subject to manipulation with the proper dopaminergic pharmacological agent.

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391.5 RAPID DEVELOPMENT OF TOLERANCE TO CONTINUOUS, PERIPHERAL ADMINISTRATION OF SATIETIN. V. E. MENDEL, Depts. An. Physiol., An. Sci. and Food Intake Lab., Univ. of Calif., Davis, CA 95616.

Satietin, a 50kd glycoprotein isolated from human plasma (hSAT) produces anorexia in rats which have been fasted from 0 to 96 hrs. All previous studies of hSAT but one have used single bolus injections. That study by Mendel et al., 1986, reported a continuous decrease of food intake by rats receiving four consecutive daily bolus ICV injections (25µg $rat^{-1}day^{-1}$). The objective of the present study was to determine whether continuous, intraperitoneal infusion of hSAT would hold food consumption below normal.

Thirty-seven rats were divided into five groups. Gr. I (n=7), saline; Gr. II (n=8), 1.0 mg/kg; Gr. III (n=7), 0.5 mg/kg; Gr. IV (n=8), 0.25 mg/kg and Gr. V (n=5) untreated controls. All rats were fed Purina Lab Chow for 7 days before surgery during which food intake (FI), water intake (WI) and body weight (BW) were measured daily. Surgery was performed under chloral hydrate anesthesia during the last two hr. of light (900-1100H). Alzet pumps (Model 2001) were implanted and rats returned to their home cage. (Two rats were lost to infection, Gr. I and Gr. III). Food and water intake decreased in all groups following surgery. Food intake/BW decreased signficantly more in Gr. II than in the other groups ($P<0.01$) on the first day following surgery. On the 2nd day post-surgery, FI/BW was significantly less in Gr. II and IV when compared to Gr. I. After day 2 no differences between Gr. I and hSAT groups were observed for the next 5 days. Body weight was significantly reduced in all groups following surgery when compared to the intact animals. Gr. I and II continued to lose weight for the next 2 days then began to gain weight during the remaining 4 days; Gr. III also lost weight on Day 2 postsurgery. Water intake/food intake rato (WI/FI) was significantly higher ($P<0.01$) than Gr. I on Day 2 in groups II and IV. A trend to consume more water per unit FI continued until Day 6. One mg/kg intraperitoneal bolus injection of hSAT has been reported to be the lowest effective peripheral dose but when given continuously it appears that tolerance rapidly develops after which food intake is not reduced. These results question whether satietin is a physiological controller of food intake.

391.6 ESTROGEN BASED CONDITIONED TASTE AVERSION: A NEUROETHOLOGICAL MODEL OF ANOREXIA NERVOSA. C.R.Gustavson and J.C.Gustavson*.Psychiatric Ethology Laboratory,Univ.of Texas Med.Branch,Galveston,TX 77550.

Our proposed model contains 3 components: (1)Estrogens produced at sexual maturation cause malaise in females that (2)have experienced disruption of normal neurological organization during fetal development due to mild CNS masculinization. The mild masculinization may have resulted from a variety of intrauterine events (e.g., maternal pathology, pollutants, or medical treatments such as diethylstilbestrol). The net result is an altered response (illness) to the presence of estrogens, which do not become abundant until maturation. The estrogen produced illness establishes a series of conditioned taste aversions, making available foods unpalatable and precipitating drastic weight loss. The dramatic weight loss is accompanied by reduced production of the nauseating estrogen, and weight gains are accompanied by a return of malaise. (3)In coping with this feedback loop, an evolved behavioral starvation system is inappropriately evoked, possibly via basic metabolic changes such as ketosis. The behavioral details of the starvation system appear bizarre in a nutritionally abundant environment. Concomittant changes in social interactions are especially reflected within the family setting

Experimental results from 3 studies have verified the illness producing potential of estrogen. Adult male rats injected with estradiol following consumption of a flavorful fluid readily acquired aversions to this fluid, whereas identically treated females failed to acquire the aversion. Follow-up exlperiments controlling for potential estrogen induced anorexia have indicated that postnatally androgenized female rats would upon maturation acquire aversions with this proceedure.

391.7 EFFECTS OF ANOREXIGENIC DRUGS ON APPETITIVE AND CONSUMMATORY BEHAVIOR. D.L. Wolgin, Florida Atlantic Univ., Boca Raton, FL 33431

In order to dissociate the effects of anorexigenic drugs on appetitive and consummatory behavior, rats were given sweetened milk for 30 min either in bottles or by infusion through an intraoral cannula. In the first experiment, amphetamine (AMP; 0, .25, .5, & 1 mg/kg) had no effect on the intake of cannula-fed rats, but suppressed the intake of bottle-fed rats at the two highest doses. Although increased activity was observed at the highest dose, bottle-fed rats drank less than cannula-fed rats at each dose of the drug.

Fenfluramine (FEN; 0, 2.5, 5, & 10 mg/kg) produced a dose-dependent decrease in intake with both methods of feeding, but the effect was greater in bottle-fed rats. Although FEN had marked sedative effects at the two highest doses, bottle-fed rats drank less than cannula-fed rats at each dose of the drug.

In a final experiment, the effect of AMP (1 mg/kg) was assessed in a conditioned aversion paradigm. Rats were given four conditioning trials in which a .1% sodium saccharin solution was provided for 15 min followed by an injection of AMP. Again, bottle-fed rats showed greater suppression of intake than cannula-fed rats. This difference was not due to differences in conditioning because when switched to bottles, the cannula group's intake fell to the level of the bottle group's. Taken together, these results demonstrate that anorexigenic drugs affect the appetitive phase of feeding more than the consummatory phase.

391.8 TOLERANCE TO AMPHETAMINE ANOREXIA: RESISTANCE TO EXTINCTION AND RAPID REACQUISITION. A. Streather and R. E. Hinson. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, N6A 5C2 Canada.

The present experiment investigated conditions which lead to a loss of tolerance to amphetamine anorexia. Thirty six rats were injected with 3 mg/kg AMP ip followed 20 minutes later by 30 minutes access to milk. After 22 sessions milk consumption had increased from a mean of 3.75 ml to 17.00 ml. Next, these tolerant animals were split into four groups: One group continued to received milk contingent on AMP (CONT-CONT), a second group received milk 24 hr after AMP (CONT-NONCON), and two other groups were not given AMP, of which one received saline followed by milk (CONT-SAL), while the other received neither injection nor milk (CONT-O). All animals were maintained at 85% ad lib weight during this phase. Tests for tolerance to anorexia were given 12, 24, 48 and 72 days into this phase. As expected, the CONT-CONT group acquired more tolerance. Unexpectedly tolerance was not lost in any other group. In order to determine if other conditions might lead to a loss of tolerance, all animals were next left undisturbed in their home cages for 21 days with ad lib food and water. At the end of this time all animals were reduced to 85% ad lib and given an AMP anorexia test. All animals had become significantly less tolerant. Finally, all animals were once again given milk contingent on amphetamine as during the original acquisition phase. Reacquisition of tolerance occurred within six administrations of AMP compared to 22 injections required during original training.

The above findings demonstrate that once tolerance to AMP anorexia has developed, it is very resistant to extinction in rats maintained at 85% ad lib. It appears that ad lib access to food is necessary if tolerance to AMP-anorexia is to be extinguished. Furthermore, even when tolerance is apparently totally extinguished it is acquired more rapidly during a reacquisition phase than during original training.

Just as procedures other than simply the administration of the drug are needed for tolerance to develop to AMP-induced anorexia, the withdrawal of drug is not by itself sufficient to bring about a loss of tolerance.

391.9 BRAIN UPTAKE INDEX OF L-CARNITINE AND PALMITIC ACID IN BRAIN SITES ASSOCIATED WITH FEEDING IN LEAN AND OBESE ZUCKER RATS. R.B.S. Harris and R. J. Martin. Department Foods and Nutrition, University of Georgia, Athens, GA 30602

Although fatty acids are a minor energy substrate in the brain, specific regions oxidize fatty acids at high rates. It has been shown that fatty acid metabolism in the lateral (LH) and ventromedial hypothalamus (VMH) of rats change in response to a shift in peripheral energy balance status (Kasser et al., Am. J. Physiol., 1985). In addition, in vivo brain uptake of metabolites was found to be altered in fasted rats (Kasser et al., Physiol. Behav. 36:1161, 1986). Hypothalamic uptake of palmitate was increased 300%. This pattern was also observed in the brain stem, where palmitate uptake was 75 to 185% higher in fasted than in fed rats.

In this study we examined the brain uptake pattern of palmitate in the Zucker fatty rat. This animal model of obesity is hyperphagic and may also display this unique pattern of fatty acid uptake in regions of the brain associated with feeding. We also measured brain uptake of carnitine since this is a cofactor for fatty acid uptake into mitochondria. The brain uptake index of L-carnitine and palmitic acid in the LH, MH, cortex (CTX) and area postrema (AP) of lean and obese Zucker rats was determined. Eight week old female Zucker rats were anesthetized. A 0.2 ml volume of Krebs Ringer bicarbonate buffer, containing 1.0 mM palmitate, 0.25 mM carnitine, 2% BSA, 5 µCi/ml $^{3}H_2O$, 1 µCi/ml ^{14}C-1-L-carnitine and 4 µCi/ml ^{14}C-1-palmitic acid was injected into the carotid artery. Fifteen seconds later the rat was decapitated and the brain removed. Brain regions were digested overnight in Doles solution. $^{3}H_2O$ and carnitine were isolated from the palmitate by separating and washing the aqueous and heptane layers of the solution. Carnitine uptake appeared to be fourfold higher than that of palmitate in all areas except CTX. Uptake was highest in AP and lowest in CTX. There were no phenotype differences in uptake. Palmitate uptake was similar in the LH, VMH and CTX and increased in the AP. AP palmitate uptake was higher for obese than lean rats. These data parallel those made of carnitine palmityl transferase (CPT) in similar brain areas from Sprague-Dawley rats (Martin et al., in press, 1986). This study suggests that there is significant uptake of both carnitine and palmitate in the brain. The phenotypic differences in palmitate uptake in the brain stem area containing the AP may be of importance in the defective 2-deoxy-D-glucose response of the Zucker fatty rat (Ikeda et al., Am. J. Physiol. 239:E379, 1980) since it has been shown to be the major site of 2DG stimulation of feeding (Ritter et al., Science 213:451, 1981).

391.10 HYPOTHALAMIC OBESITY IS REVERSED BY DEXAMETHASONE BUT NOT BY CORTICOSTERONE. S.A. Assimon* and R.M. Gold (SPON: N.R. Carlson). Department of Psychology, Division of Neuroscience and Behavior, University of Massachusetts, Amherst, MA 01003.

Parasagittal knife cuts alongside the paraventricular nucleus of the hypothalamus (PVN) produce overeating and obesity in rats. The PVN is also the hypothalamic site of origin of corticotropin-releasing factors. Since PVN knife cuts disrupt the hypothalamic-pituitary-adrenal axis, perhaps this altered functioning mediates the obesity.

PVN knife cuts (KC) or sham surgery were performed on thirty female rats. They then received either corticosterone (CORT) (4, 8, 16, 32 mg/kg), dexamethasone (DEX) (0.05, 0.1, 0.2 mg/kg), or vehicle.

At 4 and 8 mg/kg, CORT did not influence the knife-cut induced weight gain. At the higher doses, CORT enhanced the weight gain. In contrast, DEX attenuated the knife-cut induced weight gain. Also, DEX sometimes produced glycosuria in PVN-KC rats. Insulin (5 or 8 u/kg) mitigated the weight loss of PVN-KC rats exposed to DEX but did not promote weight gain.

The differential effects of these 2 glucocorticoids may lie in the fact that CORT and DEX have different negative feedback effects on the hypothalamic-pituitary axis as suggested by differential binding affinities at hypothalamic-pituitary axis (DeKloet et al., 1977) and their differetnial ability to inhibit CRF and ACTH at these sites (Sakakura et al., 1981).

391.11 REVERSAL OF ADRENAL DEFICITS BY ACTH DOES NOT REVERSE HYPOTHALAMIC KNIFE-CUT OBESITY. A. Sylvan*, J.M. Watt*, J.S. Meyer, R.M. Gold, S. Gath* & L. Crevier*. Neuroscience and Behavior, Psychology Department, University of Massachusetts, Amherst, MA 01003.

Hypothalamic knife cuts alongside the paraventricular nucleus (PVN) reliably produce obesity and hyperphagia. We have previously demonstrated that these cuts also produce adrenal deficits. They diminish the corticosterone response to ether stress and reduce adrenal size. If these deficits are the result of damage to CRF axons as they exit the PVN, then the ACTH response to stress should also be diminished. We therefore measured stress elicited ACTH 15 days after bilateral knife cuts and found that ACTH levels were in fact reduced.

Knife cuts produce obesity and lower ACTH levels. Adrenalectomy blocks obesity and raises ACTH levels. Therefore we examined the effect of exogenous ACTH on knife cut obesity. Subjects were 40 Sprague-Dawley (Charles River) female rats maintained on Purina Mouse Chow. Subcutaneous injections of ACTH gel (8 units) or vehicle were given twice daily for fifteen days after knife cut or sham surgery.

As expected, the knife cuts produced rapid weight gain and hyperphagia. Adrenal weights and corticosterone response to ether were diminished by the knife cuts. ACTH administration resulted in reversal of the adrenal deficits produced by the knife cuts. However, ACTH had no effect on body weight gain or food intake. These results support our previous findings that knife cuts compromise the hypothalamic pituitary adrenal axis. However, they suggest that the obesity is not mediated by changes in ACTH or corticosterone levels. These findings do not rule out a role for CRF in the control of food intake.

391.12 EFFECTS OF ADRENALECTOMY AND HYPOTHALAMIC KNIFE-CUTS ON WHITE BLOOD CELLS. E.J. Weeks Jr.*, M. Yanik* and R.M. Gold. Psychology Department, University of Massachusetts, Amherst, MA 01003.

We have previously shown that obesifying parasagittal hypothalamic knife-cuts placed alongside the paraventricular nucleus of the hypothalamus (PVN) lower both ACTH and corticosterone levels in serum, and arrest adrenal growth. The cuts accomplish this presumably by severing CRF positive axons. Adrenalectomy (ADX) also lowers corticosterone (CORT) levels, but elevates ACTH.

In female rats, adrenalectomy increased the total white blood cell (WBC) count from 12,140 to 23,900 cells/ul, all of the increase being attributable to lymphocytes. ADX reduced the number of monocytes. PVN cuts alone had no effect on WBC (13,860) or monocyte counts. When both ADX and PVN cuts were combined, the WBC's increase was mitigated (15,983), and the monocytes were no longer suppressed.

These data suggest that ACTH and CORT have opposing actions on WBC's. ACTH appears to increase the number of lymphocytes and the total WBC count, while suppressing monocytes. CORT, in contrast, appears to decrease lymphocytes while stimulating monocytes. Implications for appetite regulation via monocyte hormones will be discussed.

(SPON A. Trehub)

391.13 PLASMA CORTICOSTERONE, GLUCOSE, AND BODY WEIGHT AS A FUNCTION OF TIME OF DAY AFTER A 19 HOUR DEPRIVATION. S.L. Sommerville*, V.J. Perez, and C.J. Smith*. Dept. of Psychology, Texas Tech University, Lubbock, TX 79409.

Studies employing food deprivation often fail to recognize basic changes due to time of day in their interpretation of physiological or behavioral data. This study examined the effects of food deprivation held constant for all animals, but initiated and terminated at different times of day. Sixty-four adult male CD-1 mice were weighed and started on a 19 hour food deprivation schedule designed so the periods ended at one of eight times: 0700h (hours), 0800h, 0900h, 1000h, 1900h, 2000h, 2100h, and 2200h. Animals were re-weighed and decapitated at those times and trunk blood was collected for fluorometric determinations of plasma corticosterone (PC) and plasma glucose (PG). Data obtained from the PC, PG, and body weight changes was analyzed by univariate ANOVA's. Pairwise comparisons between means were determined using non-directional t-tests.

During morning hours, there was a declining pattern in the percentage of body weight lost, with the greatest loss (10%) occurring at 0700h, while in evening hours, there was an increasing pattern of weight lost, with the greatest amount (7%) occurring at 2200h. Each of these patterns coincide with cycles of nocturnal lipogenesis and diurnal lipolysis. The maximum levels for PC (23.2 ug%) were reported at 0700h and at 1900h (16.6 ug%). The levels of PC form a pattern indicative of the neuroregulatory cycle of glucocorticoid release, with the greatest reactivity to stress (deprivation) occurring during morning hours. Similar patterns were also noted in PG levels, with the maximum (138.0 ug% and 125.8 ug%) occurring at 0700h and 1900h, respectively. These findings may be explained by regulatory mechanisms controlling metabolic processes and ultimately glucose utilization. Whenever deprivation paradigms are employed, these findings indicate that the time of day food deprivation is initiated and terminated must be considered. Constant deprivation lengths are not sufficient to ensure that all animals are responding in the same manner. The effects of neuroregulatory cycles on activity levels, patterns of lipolysis and lipogenesis, and consummatory behaviors are supported by these results.

391.14 ALDOSTERONE'S INFLUENCE ON SELECTION OF DIETARY MACRONUTRIENTS. T.L. Thomas, A.W. Knehans, and L.D. Devenport. Department of Psychology, University of Oklahoma, Norman, OK 73019.

Aldosterone (Aldo) has potent effects on weight gain and fat metabolism. Free fed animals exhibit dose-related weight gains relative to controls, and impaired weight loss and decreased survival rates during restriction, despite having larger fat pads (1,3). This weight gain is independent of Aldo's effects on sodium and potassium retention and does not depend upon or interact with gonadal hormones or ovariectomy (2). Blood analyses reveal depressed Free Fatty Acid and Blood Urea Nitrogen levels, indicating impaired fuel mobilization. To determine whether this altered metabolism would influence dietary preferences, animals were allowed to self-select among these sources of calories.

To accomplish this, 40-day-old male Sprague-Dawley rats (Sasco, Omaha) were adrenalectomized (ADX) and given Aldo replacement of 0, 25, or 125 ug/kg/24 hr. delivered by mini-pump (Alzet). Sham operates were also included as controls. Folowing a 48 hour recovery period, animals were given free access to food cups containing each of the macronutrients (4). Intakes and weight gains were recorded daily. Animals were sacrificed by decapitation and blood was collected for analysis. Inspection for possible adrenal regeneration and mini-pump performance took place at this time.

Aldo-infused animals exhibited a dose-related increase in preference for fat relative to ADX controls. Protein intakes also tended to increase in response to Aldo, but this effect was dose-dependent. These findings demonstrate mineralocorticoid potentiation of fat preference and caloric intake. In addition, this profile of nutrient selection resulted in larger weight gains by Aldo groups than have previously been observed in single diet experiments.

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391.15 HYPOTHALAMIC REGULATION OF THERMOGENESIS: STIMULATION OF THE PARAVENTRICULAR NUCLEUS ELICITS THERMAL RESPONSES IN BROWN ADIPOSE TISSUE M.H. Brown Department of Psychology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

Brown adipose tissue (BAT) is important both for nonshivering and for diet-induced thermogenesis. While metabolic mechanisms of thermogenesis have been well characterized (Physiol Rev, 64: 314-386, 1984), relatively little is known about central neural mechanisms that might regulate thermogenesis. Interscapular BAT (IBAT) is innervated by sympathetic nerves that originate from cervical and upper thoracic segments of the spinal cord. Injections of retrogradely transported neural tracers into this region of the spinal cord result in labelled cells in several hypothalamic structures including the ventromedial nucleus (VMN), the lateral hypothalamic area (LHA) and the paraventricular nucleus (PVN [e.g., Neurosci Lett, 17: 307-312, 1980]). Thus, anatomical pathways for possible neural regulation of thermogenesis have been described.

Recent experiments have provided evidence that the hypothalamus may indeed influence metabolic function in BAT. Electrical stimulation of the VMN but not the LHA causes increased lipogenesis in IBAT (Nature, 284: 62-63, 1980) and an initial 0.2 °C decrease followed by a 0.8 °C rise in the temperature of IBAT (Nature, 289: 401-402, 1981). The thermal response is similar to that elicited by electrical stimulation of the nerves that innervate IBAT as well as to the thermal response of the tissue to intraventricular injection of norepinephrine (NE [Perkins et al. Amer J Physiol, 232: R101-R109, 1977]). The possibility exists, however, that an additional hypothalamic nucleus, the PVN, may be involved in the regulation of BAT and thermogenesis. Noradrenergic fibers arising from the medulla ascend through the hypothalamus and some of these terminate in the PVN (Br Res Rev, 4: 275-325, 1982). Among these ascending fibers are axons that pass ventrally and medially to the VMN. Thus, the thermal and lipogenic responses in BAT following stimulation of the VMN may be due to indirect stimulation of noradrenergic inputs to the PVN. While the possibility that the PVN is involved in regulation of thermogenesis apparently has not been directly tested, Zeisberger et al. (In: Jansky, L. [ed] Depressed Metabolism and Cold Thermogenesis, Prague, Czechoslovokia, Charles University, 1975, p. 182-187) reported that injection of norepinephrine (1 µg in 1 µl) into the brains of guinea pigs caused increased body temperatures, particularly in interscapular adipose tissue, and a 50% increase in resting oxygen consumption. The authors did not specify which neural structures were included in the injection site, however, it appears that the PVN was among the hypothalamic sites effected by the injection. The present investigation was carried out to test whether thermal responses in BAT following stimulation of the VMN are due to indirect stimulation of the PVN through stimulation of fibers of passage. Electrical stimulation (a 30 s train of square-wave pulses 0.5 ms in duration at a frequency of 50 Hz and a constant current of 60 or 100 µA) at sites between the VMN and the PVN or just lateral to the PVN resulted in thermal responses in IBAT that were similar to those reported by Perkins et al. after stimulation of the VMN. An injection of NE bitartrate (1 µg in 0.33 µl of saline) aimed at a site just lateral to the PVN also produced a thermal response in BAT. These results suggest that the PVN is involved in the neural regulation of thermogenesis in BAT. (Supported by NIMH Grant MH 37877 to A. A. Nunez.)

391.16 COLD EXPOSURE DURING LONG DAYS IN SYRIAN HAMSTERS: EFFECT OF PINEALECTOMY ON ENERGY BALANCE. F.A.Caputo* S.Hoffman*, G.Smith* and N.E.Rowland (SPON: G.M. Hope). Dept Psychol, Univ Florida, Gainesville FL32611

Syrian hamsters (M. auratus) with free access to running wheels undergo testicular regression in the cold with a stimulatory photoperiod. We now examine whether this effect is reversible by pinealectomy.

Adult male hamsters were housed in a L14:10D cycle at 23°C. Half of the animals were housed in running wheels (exercise EX condition) and the other half were in standard cages (sedentary SED condition). After adaptation, half of the EX and SED groups were pinealectomized (PX); the remaining hamsters received a sham craniotomy (SH). Two wks later, one half of the hamsters in each of the 4 groups were placed in cold (4°C) with the L14:D10 cycle still imposed. The remainder stayed in the warm (23°C). Body weight, food intake and activity (EX groups) were recorded for 7 wk. Hamsters were then sacrificed for measures which included interscapular brown fat (IBAT) and testes weights, and plasma glucose concentration.

Cold exposure increased food intake in all groups. IBAT weight was increased in SH groups, and cold decreased weight gain except in EX/SH condition. Cold exposure also caused testicular regression, and this was exacerbated by EX in the SH but not PX groups. Thus, the cold + EX effect was slightly attenuated by PX. These data indicate that cold is a sufficient stimulus for gonadal changes, and this is not entirely dependent upon the pineal. Instead, considerations of energy homeostasis seem important. [NSF BNS- 8216528].

Group (Ns 6-8)	Food (g/24h)	ΔBWT (g/7wk)	Testes (g/pr)	IBAT (g)	PGlu (g/l)	Wheel (krev)
SH/23/SED	11.2	25.4	3.44	.24	.92	---
SH/23/EX	12.6	16.9	3.46	.18	.96	11.4
SH/4/SED	17.0*	6.2*	2.26*	.34*	.99	---
SH/4/EX	23.2*	21.0	1.15*	.28*	1.13	1.1*
PX/23/SED	11.0	24.9	3.48	.27	.96	---
PX/23/EX	13.3	16.3	3.82	.22	.93	11.9
PX/4/SED	20.4*	5.0*	2.41*	.33	1.06	---
PX/4/EX	23.5*	4.7*#	2.09*#	.23	1.13	1.6*

Cold differs from corresponding warm condition *P<.05; PX differs from corresponding SH group #P<.05, #P<.07.

391.17 MONOSODIUM GLUTAMATE-INDUCED ALTERATIONS IN FEEDING BEHAVIOR, BLOOD PRESSURE AND NEUROPEPTIDE Y LEVELS. D.R. Wallace, S.M. Gabriel and R. Dawson. Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610.

Monosodium glutamate (MSG) administered neonatally to rodents results in significant neuronal degeneration in the arcuate nucleus of the hypothalamus (ANH) and other circumventricular organs. As a consequence of the neurotoxic action of MSG, a complex constellation of neuroendocrine, neurochemical and behavioral disturbances is manifested. The present study examined normal and pharmacologically stimulated feeding, basal blood pressure and neuropeptide levels in MSG-treated and control rats.

Male rats were injected with either MSG (4 mg/g) or 18.7% NaCl (w/v) on postnatal days 2 and 4 (MSG-Lo) or postnatal days 2, 4, 6 and 8 (MSG-Hi). One group of adult MSG-treated and control rats were examined for deficits in pharmacologically elicited feeding, deprivation induced feeding and baseline feeding measures. A second group of adult MSG-treated and control rats had basal blood pressure measured using the tail cuff method. All rats were checked for pituitary, testicular and optic nerve atrophy. Neuropeptide Y (NPY) content was measured by radioimmunoassay in brain regions taken from MSG-Hi, MSG-Lo and control rats.

Twenty-four hour food intake was not significantly reduced in MSG-Hi or MSG-Lo compared to controls, however, the MSG-Hi consumed significantly ($p<0.05$) less food in the dark part of the light cycle than controls. MSG-Hi consumed significantly ($p<0.05$) less food than controls after a 48 h fast and both MSG-Hi and MSG-Lo lost significantly ($p<0.005$) more body weight in response to the 48 h fast than controls. Log dose-response curves using diazepam, guanfacine and morphine were performed and food intake responses to the antagonist yohimbine, idazoxan and naloxone were also evaluated in MSG-Hi, MSG-Lo and control rats. MSG-Hi ate significantly ($p<0.05$) less than controls during the dark phase and significantly ($p<0.05$) more than controls during the light phase of the light cycle in response to naloxone (1 mg/kg). Both MSG-Hi and MSG-Lo demonstrated an attenuation in morphine (1 mg/kg) stimulated food intake when compared to controls. MSG-Lo consumed significantly ($p<0.05$) more food than controls in response to guanfacine (0.01 mg/kg). MSG-Hi and MSG-Lo showed a significant ($p<0.05$) attenuation of diazepam stimulated feeding when compared to controls. Blood pressure (mmHg $\pm$ SE) was significantly ($p<0.001$) reduced in MSG-Hi (113 $\pm$ 3) and MSG-Lo (112 $\pm$ 2) versus controls (127 $\pm$ 2). NPY levels (pg/mg wet wt. $\pm$ SE) were significantly ($p<0.001$) reduced in the hypothalamus of MSG-Hi (508 $\pm$ 38) and MSG-Lo (504 $\pm$ 22) compared to controls (763 $\pm$ 53), but differences were not present in other brain regions.

In summary, MSG treatment (Hi or Lo) resulted in significant alterations in feeding behavior, blood pressure and hypothalamic NPY content.

391.18 POSSIBLE SENSORY INNERVATION OF RAT INGUINAL-SUBCUTANEOUS FAT. D.S. Hall, J. Dark and R.B. Fishman, Dept. of Psychology, Univ. of California, Berkeley, CA 94720

Early anatomical data suggests that afferent innervation does not arise from white adipose tissue (Havel, R.J., Handbook of Physiol., Sect. 5: 575-582, 1965). Because several observations suggested to us that this may not necessarily be the case, we undertook to re-evaluate this question using current anatomical procedures.

Under chloropent anesthesia, a single crystal of 'True Blue' (TB) fluorescent tracer was inplanted into inguinal-subcutaneous fat of male rats. The incision in the fat pad was closed immediately with an application of methyl-acrylate (Krazy glue; T.C. Bour & A.O. Humbertson, Soc. Neurosci. Abst., 1985) to prevent spread of the tracer. Animals were autopsied 5-7 days later and dorsal root ganglia (DRG) and corresponding spinal cord from T-10 to L-5 were dissected and examined histologically. The inguinal fat pad and underlying muscle and overlying skin also were observed for presence of fluorescence.

Fluorescent cell bodies were consistently observed in DRG's T-13 to L-2 and often in L-3 as well. The densest labelling of cells was always in L-1 and L-2. The number of labelled cells per DRG ranged from 2- 20; however, these numbers may represent underestimates because TB crystal size varied between animals and in no case did fluorescence completely or evenly fill the fat pad. No evidence of tracer was seen in motorneurons within the spinal cord, indicating that the TB did not spread to adjacent muscle. Likewise, neither skin nor muscle evidenced fluorescence.

These data clearly indicate that neural processes presumably mediating sensory information arise from inguinal white adipose tissue. The nature of this information, its specific site of origin, and its central destination remain unknown.

Supported by NIH Grants HD-14595 and NS-19790.

MOLECULAR BIOLOGY OF GENE EXPRESSION AND NUCLEIC ACIDS VII

392.1 MOLECULAR CLONE OF THE cDNA FOR DARPP-32, A DOPAMINE- AND CYCLIC AMP-REGULATED INHIBITOR OF PROTEIN PHOSPHATASE-1. R.M. Lewis, T. Kurihara*, J. Eisler* and P. Greengard, Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, N.Y. 10021.

DARPP-32 (dopamine- and cyclic AMP-regulated phosphoprotein, Mr-32,000) is a cytosolic protein which is phosphorylated in response to dopamine or 8-bromo-cyclic AMP in intact neurons. When phosphorylated, DARPP-32 is an effective inhibitor of protein phosphatase-1. DARPP-32 and phosphatase inhibitor-1 contain regions of peptide sequence homology. Phosphorylated DARPP-32 is dephosphorylated most efficiently by phosphatase-2A or -2B (calcineurin). DARPP-32 is enriched in regions of the nervous system containing dopaminergic nerve terminals. Within these regions, DARPP-32 appears to be localized in cells possessing D-1 dopamine receptors (dopamine receptors coupled to adenylate cyclase). As one step towards studying the control of expression of DARPP-32, we have cloned the cDNA for bovine DARPP-32.

Poly A+ RNA isolated from bovine caudate nucleus was used to prime cDNA synthesis in the Okayama-Berg plasmid primer. A new linker DNA was developed from a modified portion of the lac i and lac z gene of pUC18, which allowed this plasmid to act as an expression vector. The library was screened with two synthetic oligonucleotide probes derived from a glutamate-rich region of DARPP-32. One probe contained all possible codons for the peptide sequence of this region; the second probe contained two sequences based on codon usage and potential thermal stability. Partial nucleotide sequences of four clones which hybridized with both probes were determined, and the derived peptide sequences were compared with the known peptide sequence of DARPP-32 to confirm the identity of the clones. Colonies from three of these clones could also bind monoclonal antibodies to DARPP-32. The insert from the longest clone (~1700 bp) was used to probe Northern blots of bovine caudate poly A+ RNA. Two bands (~1700 and ~1900 bp) were detected. The potential significance of the two poly A+ RNAs is under investigation, and the entire sequence of the cDNA for DARPP-32 is being derived.

392.2 **Isolation and Sequence of a cDNA Encoding Bovine Chromogranin A.** A.L. Iacangelo*, H.-U. Affolter* and L.E. Eiden*; E. Herbert*; and M.Grimes* (SPON: J.A. Ruth). Lab. of Cell Biology, NIMH, Bethesda, MD 20892; Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201; and Dept. of Chemistry, Univ. of Oregon, Eugene, OR 97403.

Chromogranins are a family of acidic glycoproteins located within the secretory vesicles of the adrenal medulla. Chromogranin A is the major protein of this family, comprising 40% of the soluble protein of the secretory vesicle. Chromogranins are released with catecholamines and enkephalins from chromaffin cells upon cholinergic stimulation. The function of chromogranin A is unknown, but recently chromogranin A was shown to bind calcium, indicating a possible role in neuroendocrine secretion. A cDNA encoding prechromogranin A was isolated from a bovine adrenomedullary clone bank, and sequenced. Using this cDNA, we have determined the primary structure of chromogranin A and studied the distribution of its messenger RNA in neuroendocrine tissues.

A cDNA library, constructed from adrenal chromaffin cell mRNA by the method of Okayama and Berg, was screened for the presence of a cDNA encoding chromogranin A by hybridization with a 30-residue oligonucleotide probe. Four clones were isolated: the longest clone, pCHRG4A, was subcloned into M13 and sequenced.

Chromogranin A, as determined by the sequence of pCHRG4A, is a 48.3 kD protein, with an apparent molecular weight of 75 kD by polyacrylamide gel electrophoresis. Chromogranin A contains eight pairs of basic amino acid residues; these may represent cleavage sites for the generation of smaller polypeptides from the parent chromogranin molecule. This protein is rich in glutamic acid which occurs frequently as oligoglutamic acid. The N-terminal amino acid sequence of chromogranin A, as determined from the cDNA, is identical to the partial sequences reported for human and bovine chromogranin A and parathyroid secretory protein I. Chromogranin A is similar to rat ß-granin, a 21 kD protein isolated from beta cells of the pancreatic islets, differing by a single amino acid from the sequence of the first 14 amino acids of ß-granin.

Chromogranin A is encoded by a 2100 base mRNA which is present in the parathyroid and adrenal glands, pituitary, and caudate nucleus of the brain by Northern blot analysis and *in situ* hybridization histochemistry (Siegel et al. this volume). Chromogranin A mRNA is most abundant in the parathyroid, where its presence and sequence similarity to secretory protein I suggest that these two proteins are identical. The chromogranin A mRNA was also detected in rat adrenal gland and in tissue from a human pheochromocytoma.

392.3 TRANSLATION OF TYROSINE HYDROXYLASE mRNA IN XENOPUS OOCYTES. A. Weiner* & K.L. O'Malley* (SPON: J. Schwob). Washington Univ. Sch. Med., St. Louis, MO 63110.

We are interested in defining the structural characteristics of tyrosine hydroxylase that contribute to its overall enzymatic activity. A cloned cDNA for this catecholamine biosynthetic enzyme allows for site specific modifications that can subsequently be translated in vitro to assess activity levels. Xenopus oocytes provide a convenient expression system in which a large variety of foreign mRNAs have been translated. We have used this system to test the efficiency and stability in translation of TH mRNA derived from a rat pheochromocytoma cell line, PC12. Poly A^+-mRNA was prepared from PC12 cells that had been treated with 1 μm dexamethasone and 1 mM dibutyryl cAMP, agents known to induce TH mRNA levels. Poly A^+-RNA was then microinjected into mature oocytes by established techniques. Control oocytes were injected with sterile water. Both groups were then incubated for various time periods in Barth's modified medium at 19°C. In oocytes injected with RNA, TH activity increased over a 13-day time period reaching levels 12-fold higher than water-injected controls.

Because TH is an iron-containing protein, these results imply oocytes contain sufficient amounts of iron for incorporation into newly synthesized TH molecules. Additionally, TH mRNA appears to be stable, enzymatic activity accumulated in a linear fashion over the 2-week period assayed. We now plan to inject oocytes with RNA transcribed from a TH cDNA in which specific mutations can be made. Interestingly, in water-injected oocytes an endogenous low level of tyrosinase activity was detected.

392.4 IDENTIFICATION & INTERRELATIONSHIP OF GENES ENCODING CATECHOLAMINES BIOSYNTHETIC ENZYMES. E. Brown*, D. Kursar*, G. Coker* & K.L. O'Malley* (SPON: T.J. Cicero). Washington Univ. Sch. Med., St. Louis, MO 63110

As part of our ongoing investigation of the structure and regulation of the genes encoding catecholamine biosynthetic enzymes, we have isolated rat genomic clones corresponding to messages transcribed by tyrosine hydroxylase (TH) and a closely related gene (O'Malley, J. Neurosci. Res., 1986, in press). The rat TH codes for a mature messenger RNA of approximately 1.9 kb. This message is derived from a gene of approximately 7 kb isolated on a single phage clone spanning 12 kb. We have tentatively identified the closely related rat adrenal cDNA as coding for dopamine β-hydroxylase (DβH). Genomic blotting data suggests that the TH gene and the gene putatively encoding DBH may be contiguous in the chromosome. To investigate this possibility, we have isolated genomic DNA corresponding to the rat adrenal clone. This clone encodes a mature mRNA of 5.5 kb derived from a single copy gene that is at least 20 kb in length. Under moderate stringency conditions, this gene and the TH gene cross-hybridize, suggesting a high degree of homology between them. We are rescreening a rat genomic library in order to obtain the complete adrenal gene, and possibly the link between it and the TH gene.

The TH mRNA and phenylalanine hydroxylase (PH) mRNA are 50% homologous (Ledley et al., Biochemistry 24:3389, 1985), suggesting that these two proteins evolved from a common ancestor. These authors suggested that there are two major domains in PH and TH, a conserved region responsible for hydroxylase activity and a non-conserved region contributing to substrate specificity and regulation. Ledley et al. report that this boundary is marked by an intron/exon junction. We find such a junction in the TH gene at the position marked by the homologous and non-homologous domains. These results suggest a similar organization between the two genes. Further characterization will provide insight into the evolution and structural relationship between the TH and PH genes.

392.5 NUCLEOTIDE SEQUENCE OF FELINE GLUTAMIC ACID DECARBOXYLASE. Y. Kobayashi*, D.L. Kaufman*, and A.J. Tobin (SPON: N. Wexler). Department of Biology, Molecular Biology Institute, and Brain Research Institute, University of California, Los Angeles, CA 90024.

In our continuing studies of developmental regulation in GABAergic neurons, we have analyzed the nucleotide sequence of a cDNA containing most of the coding region of glutamic acid decarboxylase (GAD) from feline brain. This clone, obtained from a lambda gt-11 feline occipital cortex library, contains 2.3 kb of feline cDNA adjacent to codon 1006 of E. coli beta-galactosidase. The fusion protein resulting from the bacterial transcription and translation of this recombinant is enzymatically active, converting glutamate to GABA and CO_2 (Kaufman, et al., Science, in press). We have subcloned this insert into M13 mp10 and determined its complete nucleotide sequence.

The sequence shows an open reading frame of at least 521 amino acids, consistent with our estimates of the molecular size of GAD (>60kD). Comparison of the feline GAD sequence with sequences in two large databases reveals no significant homologies. When compared with complete and partial sequences of other pyridoxal enzymes, the GAD sequence was homologous only with the pyridoxal binding site of DOPA decarboxylase from porcine kidney (Bossa, et. al., Biochem. Biophys. Res. Comm. 78, 177-184, 1977). GAD also shows extensive homology with the DOPA decarboxylase of Drosophila (J. Hirsh, personal communication). The similarities among these enzymes suggests that neurotransmitter-producing enzymes might have a closer evolutionary relationship than do pyridoxal phosphate enzymes in general.

We are currently extending our analysis to human GAD cDNAs.

This work was supported by a grant to AJT from NIH (#NS 22256) and a program project grant to Dr. A.V. Delgado-Escueta (#PO1 NS 21908). DLK is supported by a USPHS Training Grant in Cell and Molecular Biology (#GM 07185).

392.6 DISTRIBUTION AND LOCALIZATION OF NOVEL NEURAL POLYPEPTIDES STUDIED WITH IMMUNOLOGICAL AND MOLECULAR PROBES. J. Hempstead*, R. Ziai*, L. Sangameswaran*, G. Goodall* and J. I. Morgan* (SPON: M. J. Sedivec). Roche Inst. Molec. Biol., Nutley, NJ 07110.

The developing cerebellum expresses three polypeptides; a hexadecapeptide termed cerebellin, a putative calcium binding protein named PEP-19 and an immature neuronal marker, NSP. We have followed the developmental expression of these molecules in cerebellum and a neuroblastoma line using immunocytochemistry, RIA, HPLC and in situ hybridization confirmed by northern transfer analysis. The results show that both cerebellin and PEP-19 are restricted to cerebellar Purkinje cells and their level of expression increases dramatically after birth. Cerebellin was found by HPLC and RIA to be present exclusively in cerebella from chicken, mouse, rat, rabbit, dog and man, and is thus relatively well conserved. The phylogenetic distribution of PEP-19 is in hand. While cerebellin is restricted to cerebellum, PEP-19 is also found by HPLC and immunocytochemistry to be present in GABAergic neurons in other brain regions such as olfactory bulb. However, cerebellum is the richest source of the polypeptide.

NSP was found by in situ hybridization to be present in all fetal and neonatal neurons. HPLC showed no significant variations in the concentration of NSP from one brain region to another. NSP is not found in juvenile or adult rat brain. It is detectable in high concentrations in a neuroblastoma cell line. Upon quasi differentiation of these cells the level of NSP drops precipitously to 5% of its value in log phase cultures. The mechanism of transcriptional control will be discussed.

392.7 MOLECULAR CLONING OF A NOVEL BRAIN-SPECIFIC POLYPEPTIDE, PEP-19. L. Sangameswaran*, R. Ziai*, J. Hempstead* and J. Morgan* (SPON: D. Marshall). Dept. of Neurosciences, Roche Inst. of Mol. Biol., Nutley, NJ 07110.

We have previously shown that by rapid isolation procedures and HPLC mapping techniques peptide markers could be identified from specific regions of the brain. One such peptide, designated PEP-19, was purified to homogeneity from adult rat cerebellum. It had a molecular weight of 7.6 kDa and contained 61 amino acids with a blocked N-terminus. The peptide was sequenced following proteolytic and chemical fragmentation. Although PEP-19 has a unique sequence it bears a limited sequence homology with S-100 and other calcium binding proteins. It is a developmentally regulated polypeptide in that the concentration increased from 0.1 nmol/g of cerebellum at birth to 2 nmol/g at 20 days *post partum*. Adult rat cerebellum is the predominant source of PEP-19. By HPLC it was also detected in olfactory bulb but not in other regions of the brain. Immunocytochemical studies showed PEP-19 to be localized in Purkinje cells and stellate neurons in rat cerebellum.

In vitro translation studies with poly A^+ mRNA isolated from adult rat cerebellum indicated the 7.6 kDa polypeptide to be the primary translation product. We have recently isolated clones from a rat brain λgt11 library screened with the antibody against PEP-19. Sequencing of these clones are currently in progress.

392.8 ISOLATION AND ANALYSIS OF GENES INVOLVED IN SPECIFIC NEURAL PROCESSES IN THE BRAIN OF THE SONGBIRD, S. CANARIA. D. F. Clayton, M. E. Huecas*, and F. Nottebohm. The Rockefeller University, New York, NY 10021

The molecular analysis of genes influencing the structure and function of higher nervous systems requires an appropriate experimental subject. The songbird offers unique advantages for such a study: large and discrete brain nuclei are dedicated to the learning and production of a complex but analyzable behavior, song. A number of fundamental neural processes are displayed in the song control system, including neurogenesis (even in adults), learning, sensory-motor integration, lateralization of function and hormone-dependency.

We are now engaged in the production and screening of cDNA libraries from specific regions of the canary brain. Libraries are constructed in vectors utilizing SP6/T7 transcription promoters to facilitate secondary screening and analysis by in situ hybridization. Our goal is to isolate clones which meet one or more of the following criteria: they are a) expressed uniquely in cells participating in the song control system; b) expressed in ventricular zone cells which contribute to adult neurogenesis; or c) differentially expressed during specific functional or seasonal states of the song control nuclei. Our strategies for accomplishing this goal will be presented, as well as our initial results.

392.9 IDENTIFICATION OF cDNA CLONES FOR RAT CATECHOL-O-METHYL-TRANSFERASE (COMT). MH Grossman[1], XQ Breakefield[2], A Foote[2], CR Creveling[3], R Weinstein*[1] and B Zhao[2]. Dept. Peds., Temple Univ. Sch. of Med., Phila.,[3]PA 19133; [2]Molec. Neurogenetics. Shriver Ctr., Waltham, MA 02254; [3]Lab. Bioorganic Chem., NIH-NIAMDD, Bethesda, MD 20205.

Catechol-O-methyltransferase (COMT: EC 2.1.1.6) metabolizes, by O-methylation, the catecholamine neurotransmitters dopamine, epinephrine and norepinephrine, catechol steriods, and the catechol drugs L-DOPA, alpha-methyl DOPA and isoproterenol. It has been established that multiple molecular weight and isoelectric point forms of the enzyme exist in a variety of rodent tissues. The exact nature of the relationship of the forms of COMT to each other has not been determined, however, the use of a cDNA for the COMT enzyme may help resolve this question.

To achieve this goal, we have made use of a well characterized rabbit anti-serum to highly purified rat liver COMT enzyme. Initially an mRNA was isolated by the procedure of polysome immunoadsorption. When the immune-specific mRNA was translated **in vitro**, it coded for a single polypeptide of MW 23,000 and pI 5.2, that of the major soluble COMT enzyme.

In addition, the antiserum was used to screen a rat liver cDNA library (donated by Richard Hynes, MIT) which was cloned into the expression vector lambda gt 11. With this vector, hybrid proteins specified by the inserted cDNAs are made. Screening of antigen-producing clones from this library is achieved through immobilization of lambda phage plaques on nitrocellulose, induction of expression with isopropyl thio-β-D-galactopyranoside (IPTG) and incubation with COMT antiserum. By this procedure we have identified two groups of clones from separate screenings. Seven putative positive (pp) clones were detected from a first screening of about 70,000 plaques and six from another screening of about 14,000 plaques. This gives a combined frequency of about 0.1%, which is slightly below that of our estimate of the abundance of the COMT mRNA in rat liver, based on immune-precipitation of COMT protein from translation products of total poly A^+ mRNA **in vitro**. Blot analysis of plaque-purified clones has revealed that clones RL 24, RL 28, and RL 13, as well as clones RL 30 and RL 26, cross-hybridize on Southerns and also hybridize to the same size message, from rat liver, on Northerns (about 2 kb and 1.8 kb respectively). All of the clones have been sized and range from 0.22 kb to 0.7 kb.

Experiments are underway to confirm one or more of these clones. These include positive hybrid selection, blocking **in vitro** translation of rat COMT mRNA, Northern blot analysis, **in situ** hybridization analysis, nucleotide and amino acid sequence analysis, and other cross-hybridization procedures. Several of these strategies incorporate the aid of the purified COMT mRNA. This work was supported in part by a grant from the Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A.

392.10 PHENYLETHANOLAMINE N-METHYLTRANSFERASE: STUDIES ON mRNA AND GENE STRUCTURE. D.K.Batter, S.R.D'Mello*, L.M.Boyle*, A.E.Gioio* and B.B.Kaplan. Molecular Neurobiology Program, Western Psychiatric Institute & Clinic, Univ. of Pittsburgh Sch. Med., Pittsburgh, PA 15213

Phenylethanolamine N-methyltransferase (PNMT) catalyzes the conversion of norepinephrine to epinephrine, thereby contributing to the expression of the adrenergic phenotype. To understand the molecular mechanisms regulating the synthesis of this enzyme, we have begun studies on the PNMT gene and its mRNA (Batter et al., Trans. Amer. Soc. Neurochem. 17: 294,1986). We report here a further characterization of PNMT cDNA clones, and a PNMT genomic clone.

Beginning with a cDNA library constructed from bovine adrenomedullary RNA, a clone containing a 650 bp insert complementary to PNMT mRNA was identified (Kaplan et al., 1985 In: "Gene Expression in Brain," C. Zomzely-Neurath and W.A. Walker (Eds.), Wiley & Sons, N.Y. pp. 1-22). This insert was used as a probe in northern blot analysis identifying PNMT mRNA as a single band approximately 1100 nucleotides in length. Restriction fragments of PNMT cDNA were subcloned into M13 mp18 and mp19 vectors and sequenced using the Sanger dideoxy chain termination method. The absence of a 3'-terminal stretch of poly(A) residues suggested that our cDNA corresponded to an internal region of the mRNA. Rescreening of the cDNA library has tentatively identified a clone overlapping the 650 bp clone which contains a poly(A) 'tail'. Sequencing of this clone will permit the characterization of the 3'-end of PNMT mRNA.

PNMT gene structure was initially investigated using Southern analysis. The 650 bp cDNA was used to probe restriction enzyme digests of bovine brain DNA. Autoradiography revealed a small number of hybridization signals per digest, consistent with the presence of a single copy of the PNMT gene per haploid genome. The 650 bp cDNA was then employed to screen a bovine genomic library for clones containing coding portions of the PNMT gene. After screening 1.5×10^6 pfu, one recombinant phage (λP1) was isolated. A restriction map for λP1 was constructed and exon-containing fragments identified by Southern blotting. These exon-containing fragments were sub-cloned into M13 vectors and sequenced by the Sanger dideoxy technique. To date, a comparison of this data with the cDNA sequence has allowed identification of two exons (and, therefore, one intron). In addition, the orientation of PNMT gene transcription has been determined. Blotting experiments with the 3'-end cDNA probe and oligonucleotide-extended transcripts should enable us to identify the remaining exonic regions, complete the characterization of the gene, and ultimately elucidate the primary structure of PNMT. (Work supported by USPHS Grants MH00518, MH29670 and NS19608).

393.11 COMPLEMENTARY DNA CLONING OF HETEROGENEOUSLY DISTRIBUTED MONKEY CORTEX RNAs. G.H. Travis, C.C.G. Naus, J.H. Morrison, J.G. Sutcliffe* (SPON: W.G. Young). Research Institute of Scripps Clinic, La Jolla, Calif. 92037.

The cerebral cortex possesses striking morphologic and laminar heterogeneity of neurons. One explanation for this is that cells from different areas or of different morphologic class express unique sets of proteins. This suggested to us that an analysis of individual cortical proteins with a heterogeneous distribution pattern would be useful for delineating new neural pathways and possibly shedding light on the function of specific cell groups. In this study we used subtractive hybridization to identify complementary DNA (cDNA) clones representing messenger RNAs (mRNAs) present in cerebral cortex but absent from cerebellum. This strategy was designed to eliminate mRNAs encoding proteins required by all neurons, in addition to proteins present specifically in glial cells.

Cytoplasmic poly(A)+ RNA was prepared from primary visual, motor, dorsal prefrontal cortex and cerebellum of young adult monkeys (Macaca fascicularis). A cDNA library was constructed in pUC18 using RNA from the three cortical areas. Radio-labelled cDNA from cortex was hybridized to an excess of cerebellar RNA, and the single-stranded DNA was separated from DNA-RNA hybrids on hydroxyapatite. The unhybridized cDNA fraction was used to probe 25,000 clones from the cortex library. Approximately 1,200 clones were selected and probed pair-wise with unsubtracted cDNA from cortex and cerebellum. Forty-eight clones giving a differential signal were used to probe Northern blots containing RNA from the three cortical areas, cerebellum and monkey liver. Eight clones gave hybridization signals in cortex but not in cerebellum. Four others gave signals in cerebellum at lower abundance than cortex. All of these subtractive-selected clones were "brain specific" (absent in liver RNA). Clones were analyzed by Northern blots with RNA from 6 different monkey brain regions. A heterogeneous distribution pattern was observed with each clone. Selected cDNAs were subcloned into an SP6 in vitro transcription vector, and used to make probes for in situ hybridization analysis on monkey brain sections. On a cellular level, novel regional and laminar distribution patterns were observed (see Naus et al., this issue).

These results are in agreement with the hypothesis that neuronal heterogeneity is related to the expression of unique sets of mRNAs by different cells. However, only a small portion of RNA species displayed regional specificity between cortex and cerebellum. Thus it appears unlikely that all neuronal heterogeneity can be accounted for by region exclusive expression of moderately abundant RNAs. We are continuing our characterization of these clones both at the level of gene expression and by the distributions of their encoded proteins through sequence analysis.

392.12 SINGLE COPY AND REPETITIVE TRANSCRIPTS IN THE MURINE CEREBELLUM U.A.O.Heinlein[*1], H.Schaal[*1], D.Goldowitz[*], and W.Wille[1]. [1]Inst. for Genetics, Univ. of Cologne, D-5000 Köln 41, F.R.G. and [*]Dept. of Anatomy, Thomas Jefferson Univ., Philadelphia, PA 19107

An attempt was made to identify cerebellum-specific cDNA sequences by screening a cDNA-library in the pcD-system derived from adult cerebellar poly(A)$^+$RNAs (Wille et al., Soc. Neuroci. Abstr. 11:798, 1985; Schaal et al., submitted). In principle, we applied a three step differential screening procedure: colony screening, Southern and and Northern blot analysis. In the two former cases ss-cDNAs from cerebellum (Cb) and total brain minus cerebellum (TB$^-$), respectively, have been used as probes. As controls served ss-cDNAs from liver and kidney.

After the second screening approx. 40% of the selected clones contained repetitive elements. Two clones, both containing L1 sequences (Voliva et al., Nucleic Acids Res., 11:8847, 1983), identify an abundant large transcript (RNase sensitive !) predominantly transcribed in the Cb of mouse and rat. This transcript has neither been found in poly(A)$^+$RNA of TB$^-$, kidney and liver, nor in neonatal Cb. In Northern blots of total RNA the signal in mouse and rat Cb is at least 20-30fold more intense than in TB$^-$, thymus, muscle, spleen, liver, lung and heart. We confirmed these data by localizing the L1-like transcript in cerebellar granule cells exclusively.

One of the cDNA-clones, CebA847, contains a single copy portion and a repetitive element. The single copy part hybridizes with two transcripts (approx. 2.2 and 2.5 kb) of Cb poly(A)$^+$RNA exclusively, the repetitive element recognizes an additional transcript of about 0.5 kb in different tissues. The cDNA has been sequenced, and genomic and cDNA clones from both portions have been isolated. While the analysis of longer single copy cDNA clones (derived from a gt11 library; D.Barthels from our lab[1]) is in progress, the analysis of the repetitive element revealed the following properties: NO member of the known repetitive families; interspersed in approx. 10^4 copies per genome; about 0.3-0.4 kb long (genomically with different flanking sequences (Heinlein & Wille, in preparation).

This work has been supported by grants Wi563/3-2 and /3-3 from the Deutsche Forschungsgemeinschaft SPP "Biochemie des Nervensystems" to W.W.

392.13 MOLECULAR CLONING AND SEQUENCING OF THE cDNA FOR AN OLFACTORY NEURON SPECIFIC PROTEIN: OMP. K. E. Rogers*, P. Dasgupta*, M. Grillo*, U. Gubler*+, F. L. Margolis. *Roche Inst. Molec. Biol. and +Dept. Molec. Genetics, Roche Res. Ctr., Nutley, NJ 07110

Olfactory marker protein (OMP) is a developmentally regulated protein of unknown function located exclusively in chemoreceptor neurons of the nasal epithelium. In order to investigate the regulation of OMP gene expression, a cDNA library enriched for OMP-mRNA was constructed in the plasmid pMG-5. The library was screened for OMP-cDNAs using a synthetic oligodeoxynucleotide probe whose sequence was predicted from established amino acid sequence data. Upon screening 8000 colonies, 4 clones were identified containing approximately full length inserts of 2.3 kilobases. The clones were shown to contain OMP-specific sequences by their ability to hybrid select OMP-mRNA out of olfactory mucosal poly A+mRNA as demonstrated by *in vitro* translation of the selected mRNA and subsequent immunoprecipitation of the products. The complete nucleotide sequence of one clone was determined. This demonstrated that the cDNA indeed contained the complete OMP coding region of 486 nucleotides, followed by 1630 nucleotides 3' untranslated region. This latter included the polyadenylation signal 16 nucleotides upstream of the polyA tail. No other open reading frame larger than 7 codons was observed in register. A portion 5' to the coding region is absent from this clone. The changes in OMP mRNA following olfactory bulbectomy were examined by RNA blot hybridization using the labelled cDNA. These studies indicated that the levels of OMP mRNA declined significantly within a few days following bulbectomy and began to increase approximately 4 weeks post-bulbectomy. The size of the OMP mRNA remained unchanged. OMP mRNA was not detected in 14 non-olfactory tissues, which argues that no other mRNA arises from the OMP gene by differential splicing of the primary transcript and that cryptic unexpressed OMP mRNA is not present in any other tissue. However, OMP-mRNA was present not only in olfactory epithelium but also at low levels in olfactory bulb where OMP positive cells have not been detected. The unexpected presence of OMP-mRNA in olfactory bulb was confirmed by *in vitro* translation and immunoprecipitation of the products. This evidence suggests that OMP-mRNA is either axonally transported or exists in a cell population which has not been immunohistochemically detected. Recently, we have isolated 3 Charon 4A phages which contain OMP gene sequences from a Hae III rat genomic library. These clones are currently being characterized to facilitate study of the regulation of OMP gene expression.

392.14 CLONING AND EXPRESSION OF MESSENGER RNA FOR VITAMIN D-DEPENDENT CALCIUM BINDING PROTEIN. G.D. Frantz*[1], T.L. Wood[1*], S. Christakos*[4], and A.J. Tobin[1,2,3] (SPON: R.P. Saneto). [1]Department of Biology, [2]Molecular Biology Institute, and [3]Brain Research Institute, University of California, Los Angeles, CA 90024; and [4]Department of Biochemistry, New Jersey Medical School, Newark, NJ 01703.

Mammalian 28 kD vitamin D-dependent calcium binding protein (CaBP) is found primarily in brain and kidney. The highest concentration of CaBP is in the cerebellum, where, in the rat, it comprises 1-2% of the total soluble protein. Immunocytochemistry reveals that CaBP is present in specific neuron types throught the brain. In the cerebellum, CaBP is limited to the Purkinje cells.

In order to study the regulation of CaBP expression, we have obtained a putative CaBP cDNA from a lambda gt-11 mouse cerebellar cDNA library. Out of 40,000 independent recombinants screened, one reacted strongly with a polyclonal antibody to CaBP. This recombinant contained a 1.2 kb insert.

We have used this putative CaBP cDNA to study the appearance of CaBP RNA in rat cerebellum, kidney, and liver. Both cerebellum and kidney contain three RNA species (1.9, 2.8, and 3.2 kb) that hybridize to our probe at relatively high stringencies (0.1 X SSC, 55°). The cerebellum contains significantly more CaBP RNA than kidney; liver contains no detectable CaBP RNA.

We have subcloned the putative CaBP cDNA into M13 mp18 and into pIBI76. We are now determining its nucleotide sequence by the dideoxy method. We have used the pIBI subclone to produce single stranded RNA probes for in situ hybridization. This will allow us to determine the cellular distributions of CaBP RNA in the brain and to study its regulation during development and in response to vitamin D deprivation.

This work was supported by grants to AJT from NIH (#NS 20356 and NS 22256) and to SC from NIH (#NS 20270) and NSF (#PCM 84-02840). TLW has been supported in part by USPHS Training Grant #GM-07191 in Integrative Biology.

392.15 **REGIONAL VARIATIONS IN CHOLECYSTOKININ GENE EXPRESSION IN THE RAT BRAIN.** M.Voigt and G.Uhl. Dept. of Neurology and the Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Cholecystokinin (CCK) satisfies most of the criteria for a neurotransmitter: it is synthesized, stored, and released by neurons, it binds to specific receptors in the brain, and it can alter the firing rates of neurons. We are currently examining the dynamics of CCK in the brain from the aspect of modulation of gene expression. As an initial step, we have studied the distribution of neurons containing CCK mRNA within the rat brain using the techniques of in situ hybridizaton and Northern analysis.

Male Sprague-Dawley rats were used for all experiments. For in situ experiments, anesthetized rats were perfused with 2% paraformaldehyde /0.5 % glutaraldehyde /66% buffered lysine /0.2% sodium periodate, the brain removed and soaked overnight in diethylpyrocarbonate-treated sucrose. Coronal sections (10 um) were cut on a cryostat, thaw-mounted onto slides and subsequently treated with 0.2 N HCl followed by a proteinase K (1 ug/ml at 37° C) digestion. Sections were then incubated overnight with synthetic oligonucleotide probes. These probes are complementary to CCK mRNA sequences and are multiply-labeled with ^{35}S. The sections were then rinsed extensively, dehydrated and apposed to LKB ultrafilm in order to generate film autoradiograms of hybridization. Northern analysis was carried out by extracting total RNA using a centrifugation technique. Following phenol/chloroform extraction and ethanal precipitation, total RNA was run on a sizing denaturing agarose gel and transfered to a nylon membrane for detection. The probe used was an anti-mRNA sense cRNA (CCK cDNA insert generously provided by J. Dixon) labeled with ^{32}P. After an overnight hybridization period, the blot was washed at a level of high stringency and film autoradiograms produced after exposure to X-ray film for 1-48 hours.

Regional analysis of CCK mRNA content by in situ hybridization demonstrated a visual ranking of structures paralleling immunohistochemical findings: cortex=hippocampus > ventral tegmental area > hypothalamus > substantia nigra = striatum. A distinct laminar distribution in cortex and hippocampus was observed, with high grain densities found selectivley over the deep layers in the pyriform and entorhinal cortices. More moderate levels of hybridization were found over the ventral tegmental area, with mardkedly less densities overlying the substantia nigra and Edinger-Westphal nucleus. For Northern analysis, hybridization densities on autoradiograms were quantitated using computerized densitometry. The rank-order of CCK mRNA distribution detected with this method closely followed that observed with in situ hybridization: hippocampus = cortex > ventral mesencephalon > hypothalamus > spinal cord > striatum > cerebellum.

392.16 GLUTAMIC ACID DECARBOXYLASE (GAD) MESSENGER-RNA IN BRAIN AND PERIPHERAL TISSUES OF RATS

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Glutamic acid decarboxylase (GAD) catalyzes the production of GABA. The brain contains several electrophoretically distinct forms of GAD, which are thought to be entirely in neurons. Several groups have reported GAD enyzmatic activity and immunoreactivity in nonneural tissues, including oviduct, kidney, heart, liver, blood vessels, testis, and adrenal glands. This activity may result from the altered expression of the gene for neuronal GAD or the expression of a second GAD gene. We have recently characterized a cDNA clone containing most of the coding sequence for GAD from feline brain. (Kaufman, et al., Science, in press; Kobayashi, et al., in preparation) Using this cDNA, we have undertaken to examine the distribution and sizes of GAD mRNAs in several neural and nonneural tissues, as well as in several rat cell lines.

Among the brain regions tested the cerebellum contains the highest concentration of GAD mRNA. GAD RNA comprises about 3 parts per million of total cerebellar RNA, or about 0.015% of cerebellar poly (A) RNA. Cerebral cortex, striatum, and hypothalamus contain decreasing amounts of GAD RNA. In all brain regions examined, GAD RNAs migrate as a single component of 3.7 kb. A 3.7 kb GAD RNA is also present in PC12 cells induced with NGF, in C6 glioma cells, and in B103 neuroblastoma cells.

We also find detectable GAD RNAs in several peripheral tissues, although at concentrations 50-100 times less than in cerebellum. In liver, kidney, and testes, GAD cDNA hybridized to poly (A) RNAs of 2.3 and 1.0 kb at low stringency (2 X SSC, 20°). The former species is long enough to encode a full length (66 kD) GAD polypeptide; the latter is not. Only in the case of testis RNA did hybridization with GAD cDNA persist after washing at relatively high stringency (0.1 X SSC, 50°) We are now examining the nature of the two novel GAD RNAs in the testes.

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CELL-SURFACE MACROMOLECULES

393.1 NEUROBLASTOMA CELL LINES EXPRESS BAND 3 GLYCOPROTEIN PREFERENTIALLY DURING CELLULAR DIFFERENTIATION. M.F.D. Notter[1], M.M.B. Kay[2]* and J.F. Leary[3]*. [1]Dept. Neurobiology and Anatomy, and [3]Pathology, Univ. of Rochester School of Med. and Dentistry, Rochester, NY 14642 and [2]Lab. of Molecular and Clinical Immunology, and the Div. of Geriatric Med., Olin E. Teague Veterans' Center, Texas A & M Univ. Coll. of Med., Temple, Texas 76501.

Changes in the cell surface during development of the nervous system have been examined by using differentiating neuronal cell lines as models. In the present study, the amount of band 3 glycoprotein present on the surface membrane of mitotic and differentiated neuroblastoma cells was quantified by immunofluorescence and flow cytometry. Band 3 glycoprotein found in the membrane of red blood cells and, importantly, in neurons has been shown to be altered during aging. A mouse neuroblastoma line, N_2AB-1, was exposed to prostaglandin E_1 (10 ug/ml) and dibutyryl cyclic adenosine monophosphate (500 ug/ml) which has been shown to induce morphological and biochemical differentiation of these cells after four days. Mitotic and differentiated N_2AB-1 cells were labeled with specific antibody to band 3 protein and then stained with fluorescently labeled secondary antibody. Quantification of fluorescence per single, living cell indicated that differentiated cells expressed more band 3 antigen than mitotic cells. These cells were also examined for band 3 antigen expression through the cell cycle by double labeling experiments. Living cells were exposed to band 3 antibody followed by fluorescein labeled secondary antibody; cells were then fixed and treated with propidium iodide for DNA analysis. Results showed that after normalization for cell size, cells in G_0G_1 of the cell cycle or the resting stage expressed more band 3 protein than cells in any other stage.

A human neuroblastoma, IMR-32 was differentiated and treated with antibody to band 3. Fluorescence microscopy revealed that control cell cultures bound very little antibody while differentiated cultures showed intense staining of cell bodies and all neurites. Flow cytometric analysis of fluorescence antibody binding also indicated that mitotic human cells bound no antibody while differentiated cells expressed significant levels of this protein.

These studies suggest that differentiation of neuronal cells is accompaned by an increase in specific cell surface glycoproteins and that neural cell lines may be used for aging studies in the nervous system.

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393.2 THE REGULATION OF N-CAM IN MOUSE NEUROBLASTOMA, NG108-15, CELLS. J.R. Moskal, A.E. Schaffner, M. Giovanni* and G. Rougon*, LCB, NIMH, LNP, NINCDS, LBG, NHLBI, NIH, Bethesda, MD 20892 and Lab. Immunol., INSERM-CNRS, Marseilles, France.

The regulation of specific cell-surface glycoconjugates, which include the neural cell adhesion molecule, N-CAM, appears to be important for proper neural development. N-CAM has been suggested to play a role in neuron-myotube interactions and higher levels are found in embryonic, preinnervated muscle compared to innervated adult muscle. In order to further study the regulation and function of this and other glycoconjugates NG108-15 cells were chosen as a model system. This neuroblastoma x glioma hybrid is capable of both biosynthesizing N-CAM and synapse formation. In these experiments, an ^{125}I-labelled anti-N-CAM monoclonal antibody was used to analyze immunoblots of solubilized membrane proteins.

In one study NG108-15 cells were exposed to a variety of compounds for 48 hr and their effects on N-CAM expression was assessed. Retinoic acid (1.6 μM) and the potent tumor promoter, phorbol-12-myristate-13-acetate (0.16 μM), caused a 2 to 3-fold elevation in both the 180 kD and 140 kD forms of N-CAM. Phorbol (0.16 μM) had no effect. In contrast, dibutyrl cyclic AMP (1mM) had an inhibitory effect (>50%) under these conditions.

In a second study, dibutyrl cyclic AMP-treated (1mM: 7 days) NG108-15 cells were cocultured with fetal rat myotubes for 3 days. N-CAM levels were compared to identically treated, cultures of NG108-15 cells alone, muscle alone or NG108-15 cells cultured in the presence of muscle exudate (prepared from muscle cultures by scraping and washing in the presence of phosphate buffered saline). In the cocultures, the concentration of the 180 kD form of N-CAM was unchanged compared to muscle alone. The concentrations of the 140 kD and 120 kD forms of N-CAM were reduced 3 to 4-fold relative to NG108-15 or muscle cultured alone. Comparing NG108-15 cells cultured in the presence of muscle exudate with NG108-15 cells cultured alone, a 6-fold reduction in the concentration of 180 kD N-CAM was observed; the level of the 140 kD N-CAM remained unchanged and the 120 kD N-CAM increased in concentration greater than 10-fold. This was 4-fold higher than in muscle cultured alone and 10-fold higher than in cocultures.

These results suggest that the regulation of the expression of N-CAM is under complex control, perhaps involving glycosylation, cyclic AMP-dependent protein kinase and protein kinase C. Changes in the concentration of the different molecular forms of N-CAM, in both nerve and muscle, may be a prerequisite for synapse formation.

393.3 HISTOCHEMICAL LOCALIZATION OF HYALURONIC ACID IN DEVELOPING AND MATURE RAT CEREBELLUM J.A.Ripellino, R.U. Margolis and R.K. Margolis. New York University Medical Center, New York, NY 10016, and State University of New York, Health Sciences Center at Brooklyn, Brooklyn, NY 11203

We have recently developed a highly sensitive and specific biological probe for the histochemical detection of hyaluronic acid at the light and electron microscopic levels (J. Histochem. Cytochem., 33:1060, 1985). In this procedure, proteoglycan aggregates were isolated by CsCl density gradient centrifugation after extraction from a transplantable rat chondrosarcoma, and digested by clostripain to yield the hyaluronic acid binding region as a complex with hyaluronic acid and link protein. For the histochemical visualization of hyaluronic acid, the hyaluronic acid binding region/link protein complex was biotinylated in the presence of hyaluronic acid, and the biotinylated probe, which was separated from hyaluronic acid by gel filtration in the presence of 4 M guanidine HCl, was used in conjunction with avidin-peroxidase. For control sections, we have primarily relied upon the known ability of hyaluronic acid oligosaccharides of specified sizes to inhibit its binding to macromolecular hyaluronic acid. We have also demonstrated that treatment of tissue sections with Streptomyces hyaluronidase completely prevents staining by the HA-binding complex. This enzyme is specific for hyaluronic acid and shows no protease activity under our conditions.

Light microscopy of early postnatal rat cerebellum showed strong staining in the presumptive white matter, and to a lesser extent as a reticulum in the granular layer. During the first two weeks after birth, when the major developmental changes occur in rat cerebellum, there is also a band of intense staining which is initially present at the base of the molecular layer, near the apical tips of the maturing Purkinje cell dendrites. This band of staining progresses towards the pial surface as the Purkinje cell dendritic arbor shows normal branching and upward expansion, closely followed by the growing climbing fibers. In contrast to developing brain, in the mature cerebellum there appears to be intracellular staining of Purkinje cells as well as diffuse staining throughout the molecular layer, and the virtual disappearance of staining around myelinated axons. Electron microscopy demonstrated that the staining in early postnatal brain is almost entirely extracellular, and in this respect closely resembles that which we previously found in immunoelectron microscopic studies on the localization of chondroitin sulfate proteoglycan in developing brain (J. Cell Biol., 99:1117 and 1130, 1984). Also as in the case of the chondroitin sulfate proteoglycan, there is intra-axonal and membrane staining of parallel fibers. During the early stages of brain development, extracellular hyaluronic acid may provide a highly hydrated and easily penetrable matrix through which neuronal migration may take place, before the intercellular space decreases to its much smaller dimensions in mature brain.

393.4 MOLECULAR CLONING OF NILE GLYCOPROTEIN-RELATED cDNA PROBES. P. Sajovic, D.J. Ennulat, D.G.B. Leonard, K. Kaplan, A.L. Prochiantz, E.B. Ziff, M.L. Shelanski and L.A. Greene. Departments of Pharmacology and Biochemistry, New York University Medical Center, New York, NY 10016.

The NILE glycoprotein (GP) is an NGF-inducible surface component of PC12 cells that is also widely distributed in the nervous system, and that has recently been shown to be indistinguishable from the high-molecular-weight species of the brain antigens L1 and Ng-CAM. This molecule is thought to have a function in regulating cell adhesion in the developing nervous system.

We have used polyclonal anti-NILE antisera to screen a λgt11 cDNA library made from NGF-treated PC12 cells. Four molecular probes have been isolated that encode parts of the apoprotein and/or related proteins. These probes are 1600, 800, 330, and 300 bp long, respectively, and in Northern blots they recognize a family of 3 messages having lengths of about 3.4 kb, 2.4 kb, and 1.9 kb. Only the longest probe hybridizes to all three mRNA's, while the shorter probes bind to either one or two of them. These messages are found in PC12 cells and brain, but not in liver or other tissues that do not express the NILE GP or related smaller components. Moreover, two of the messages are found to be modestly but reproducibly up-regulated by NGF in PC12 cells.

The recombinant phages containing these inserts produce fusion proteins that share specific epitopes with the NILE GP (and a species which in PC12 cells has an SDS-PAGE M_r of 140-150 kDa) but not with other proteins. This was shown by using nitrocellulose filters coated with fusion protein to immunoabsorb specific activities from polyclonal and polyvalent antisera. Material eluted from the filters specifically immunoprecipitates NILE GP and a trace of the 140-150 kDa species from PC12 cells labelled with ^{35}S-methionine or ^{3}H-fucose. Other activities present in the original serum are not specifically absorbed by recombinant fusion protein. Filters made with nonrecombinant λgt11 do not absorb anti-NILE GP activity.

As a complementary approach, we have used the technique of DNA-mediated gene transfer to generate mouse L cells (a fibroblast line) transfected with rat genomic DNA and selected for expression of NILE GP by immunostaining. One such clone synthesizes substantial amounts of 200 kDa glycoprotein that is specifically immunoprecipitated by anti-NILE antiserum. The cells also contain DNA sequences that are recognized in Southern blots by the above described 1.6 kbp cDNA probe, but that are lacking in untransfected cells. These lines of evidence, taken in sum, indicate that we have isolated cDNA probes for the NILE GP and/or related proteins. Supported by NIH Grant NS 16839 (to M.L.S. and L.A.G.), CA 16239 (to E.B.Z.), NS 07178 (to P.S.), and NIGMS Training Grant 5T32 GM078308-11 (D.G.B.L.).

393.5 PC12 CELLS AS A MODEL FOR NEURON/EXTRACELLULAR MATRIX INTERACTIONS. K.J. Tomaselli*, C.H. Damsky* and L.F. Reichardt(SPON:D.Clegg) Dept. of Physiology and Depts. of Stomatology and Anatomy, UCSF, San Francisco,CA,94143.

Laminin substrates promote neurite outgrowth from a variety of central and peripheral neurons. We have shown previously that the neuron-like cell line PC12, after priming with nerve growth factor, regenerates neurites when passaged onto laminin coated substrates (Tomaselli and Reichardt (1984) Soc. for Neurosci. 10,40). This response is similar to that of neonatal rat sympathetic neurons grown on laminin. To study the interaction between PC12 cells and laminin, we have devised an assay that demonstrates dose dependent binding of PC12 cells to substrates coated with laminin derived from the murine EHS sarcoma. Maximal attachment is seen within two hours on substrates coated with 10 ug/ml laminin. This attachment is specific since it is blocked by antibodies to laminin but not by antibodies to collagen IV. In addition, an antiserum to a heparin-binding fragment of laminin (E3), produced by digestion with elastase, blocks attachment of PC12 cells to undigested laminin. Antibodies to this fragment previously have been shown to block the responses of peripheral neurons to laminin (Edgar et al. (1984) EMBO J. 3:1463-1468). Attachment to substrates coated with collagen IV is not blocked by either anti-laminin or anti-E3 serum, but is blocked by an affinity purified antiserum to collagen IV. We are currently characterizing molecules on the surface of PC12 cells that mediate attachment to these extracelluluar matrix adhesive proteins.
(Supported by NIH Grant NS 19090 to LFR)

393.6 DISTRIBUTION OF A "FIBRONECTIN" RECEPTOR, FIBRONECTIN, LAMININ AND HEPARAN SULFATE PROTEOGLYCAN DURING AVIAN NEURAL CREST MIGRATION. D. M. Krotoski, C. Domingo and M. Bronner-Fraser. Developmental Biology Center, University of California, Irvine, California, 92717

The CSAT antibody recognizes a 140 kD cell surface receptor complex involved in adhesion to fibronectin and laminin (Horwitz, et al., J. Cell Biol. 101: 2134-2144, 1985). Injection of CSAT antibody into the mesencephalon results in perturbation of cranial neural crest migration (Bronner-Fraser, Dev. Biol., in press). These results suggest that the CSAT antigen may play an important functional role during neural crest migration. Here, we describe the distribution of the CSAT antigen together with fibronectin (FN) and laminin (LM), the extracellular matrix (ECM) molecules to which it binds. In addition, we have examined the distribution of heparan sulfate proteoglycan (HSPG), since this molecules has been shown to complex with laminin.

The distribution of CSAT was examined during neurulation, neural crest migration, and gangliogenesis. In the head, CSAT immunoreactivity was visible surrounding all the cells within the neural tube prior to migration; later CSAT was visible on migrating neural crest cells. FN, LM, and HSPG were present in the matrix surrounding premigratory neural crest cells. During neural crest migration these molecules lined the pathways followed by cranial neural crest cells. CSAT immunoreactivity was also detected on the the basal surface of epithelial tissues such as the ectoderm, neural tube, notochord, dermomyotome, and dorsal aorta. FN, LM, and HSPG immunoreactivity were observed in the basement membranes surrounding these epithelial tissues. In the trunk region, high levels of CSAT immunoreactivity were seen on neural crest cells. During neural crest emigration, FN, LM, and HSPG were seen in the thin matrix between the dorsal neural tube and ectoderm. During neural crest migration, FN, LM, and HSPG were observed in the sclerotome of each somite. However, none of the ECM molecules were selectively distributed in the anterior half of the sclerotome where neural crest cells migrate.

After cessation of neural crest migration, CSAT, FN, LM, and HSPG were detectable on the basal surface of the neural tube, dermomyotome, notochord, and ectoderm. High levels of CSAT immunoreactivity were observed around cells within the dorsal root and sympathetic ganglia. In contrast, only low levels of FN, LM, and HSPG immunoreactivity were noted within the ganglia, though all three ECM molecules surrounded the ganglia. In the sclerotome, FN, LM, and HSPG immunoreactivity appeared to increase after ganglion formation in comparison to levels during the time of active neural crest migration. The dynamic levels of CSAT, FN, LM, and HSPG in the embryo suggest that a balance of surface-substratum adhesions may contribute to initiation, migration, and localization of neural crest cell populations. (Supported by USPHS HD-15527-01 and MOD 1-896).

393.7 ANALYSIS OF RETINAL SYNAPTIC MEMBRANE GLYCOPROTEINS. L. M. Snyder* and N. G. F. Cooper (SPON: B. J. McLaughlin). Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163.

Synaptic membrane oligosaccharides are implicated in neuronal interactions during the development and maturation of synapses. The objective of the present investigation is to determine the relative molecular weights (Mr), lectin binding specificities, and triton solubility of synaptic plasma membrane (SPM) associated oligosaccharides so that antibodies to specific, partially characterized oligosaccharides can be prepared. SPM enriched fractions from the retinas of hatched chicks are SDS solubilized and analyzed with polyacrylamide gel electrophoresis. Triton soluble and insoluble fractions are analyzed by electron microscopy, gel electrophoresis and also with lectin treated Western blots. Lectin affinity chromatography is used to obtain fractions enriched in oligosaccharides from the triton soluble component of SPM. The data demonstrates that the major Concanavalin-A (Con-A) binding oligosaccharide of the SPM fraction has an Mr of 42,000 (GP42). GP42 also binds succinylated Wheat Germ Agglutinin (sWGA) but not Peanut Agglutinin (PNA) or Limax Flavus (LFA) and therefore it is an asialoglycoprotein rich in mannose and glucosamine residues. LFA, a sialic acid specific lectin binds most avidly to high molecular weight proteins in SPM-blots. GP42 is a Triton soluble component of the SPM and is not, therefore, associated with the cytoskeletal substructure of synapses. Polyspecific antibodies raised against whole SPM recognize GP42 in western blots and monospecific antibodies to it are currently being prepared through use of Con-A affinity column enriched fraction of GP42. PNA binds to 3 protein bands with Mr's of 67,000 (GP67), 100,000 and 150,000, in Western blots of SPM. GP67 also binds sWGA suggesting that it contains both galactosamine and glucosamine residues. These PNA binding glycoproteins are triton insoluble. By electron microscopy the triton insoluble fraction is seen to be enriched with synapses. The PNA binding oligosaccharides may therefore be associated with the cytoskeletal substructure present in synapses. This synaptic fraction also contains 6 Con-A binding proteins with Mr's that range between 90,000 and 200,000. Affinity chromatography of the triton insoluble oligosaccharides is currently being investigated, so that monospecific antibodies can be prepared. Supported by NIH/NEI grant EY-02708.

393.8 **PROTEOGLYCAN SYNTHESIS IN PURIFIED CULTURES OF RETINAL NEURONS AND PHOTORECEPTORS.** L. Needham, R. Adler and A.T. Hewitt (SPON: J. Lindsey). Wynn Center, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

It is now clear that extracellular matrix (ECM) molecules play important roles in regulating neuronal survival and differentiation. However, there is much less information regarding the synthesis of these molecules by CNS neural cells in general and neurons in particular. The avascular, connective tissue-free neural retina of the chick embryo is a source of ECM molecules, although whether they are glial or neuronal in origin has not been determined (Morris, Arch. Biochem. Biophys., 235:127, 1984). We report here biochemical experiments showing that proteoglycans are actively synthesized in purified cultures of retinal neurons and photoreceptors which are free of contamination by glial, connective tissue, endothelial and pigmented epithelial cells.

Cultures prepared as described by Adler et al., (J. Cell Biol., 99:1173, 1984) and grown in polyornithine-coated, 100 mM dishes for 6 days, were labeled for 24-72 hrs with ^{3}H-glucosamine (7.5 µCi/ml) and $^{35}SO_4$ (25 µCi/ml). Metabolically labelled proteoglycans in the medium, associated with the cells, and attached to the substratum, were isolated by ion-exchange chromatography and characterized by gel filtration chromatography and susceptibility to degradation by enzymatic and chemical treatments. Nondialyzable, sulfated macromolecules eluted from a DEAE column at >0.35 M NaCl, a salt concentration suggestive of highly charged proteoglycans. CL-4B elution profiles indicate differences in the size distributions of proteoglycans found in the medium compared to those which are substratum-bound. In addition, while both chondroitin sulfate/dermatan sulfate and heparan sulfate are found in each compartment, the proportions of these glycosaminoglycans vary, with the medium containing predominantly chondroitin sulfate/dermatan sulfate. While further analysis of separate neuronal and photoreceptor populations (Politi and Adler, Inv. Ophthalmol. & Vis. Science, 1986, in press) is required to determine exact cellular origin, these studies show that cultures containing both neurons and photoreceptors are actively engaged in proteoglycan synthesis and secretion.

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393.9 CELLULAR EXPRESSION OF THY-1 IN THE AVIAN RETINA.

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The cell type/s which express Thy-1 in the retina has been the subject of much investigation, specifically, whether the retinal ganglion cells account for all the Thy-1 expression (Barnstable, C.J., and Drager, U.C., 1984, Neuroscience 11, 847-855) or whether amacrine and bipolar cells contribute to the staining present in the inner plexiform layer (IPL) (Perry, V.H., Morris, R.J., and Raisman, G., 1984, J. Neurocytol. 13, 809-824). To investigate which cell type express Thy-1 in the day old avian retina we have used drugs which are known to specifically ablate cell types of the retina in conjunction with monoclonal SB1-2D.11 specific for Thy-1 and an indirect immunoperoxidase staining technique (Sheppard et al. These Proceedings, 1986) to identify Thy-1 staining 7 days after intravitreal injection of the drugs.

Staining for Thy-1 in the IPL layer following intravitreal injection of colchicine (1.25 nmoles, which results in almost total destruction of ganglion cells) decreases to almost background over a three week period. The width of the IPL and inner nuclear layer (INL) remain constant. Injections of low doses of kainic acid (40 nmoles, resulting mainly in loss of amacrines, and half of the bipolar cells) and NMDA (2 µmoles, which only affects amacrine cells) result in a narrowing of the INL, due to cell loss, and a decreased staining together with a thinning of the IPL. There is also a decreased staining in the optic fibre layer (OFL).

High doses of kainic acid (120 nmoles, which removes horizontal cells as well as amacrine cells and half of bipolar cells) shows a further reduction in the intensity of staining and width of the IPL and INL. In addition, staining in the OFL is correspondingly reduced in intensity and accompanied by a further reduction in width.

One possible interpretation of the disappearance of staining after colchinine treatment is that Thy-1 has an exclusive ganglion cell localization. Furthermore, the reduction in staining after amacrine and bipolar ablation may represent a down regulation of Thy-1 expression due to removal of input to the ganglion cells.

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393.10 INCREASED SENSITIVITY OF THY-1 LOCALIZATION IN CHICKEN CENTRAL NERVOUS SYSTEM.

A.M. Sheppard, P.L. Jeffrey and P.W. French. The Children's Medical Research Foundation, The University of Sydney, N.S.W. Australia 2050

Studies on the localization and developmental expression of Thy-1 in the chicken central nervous system previously utilized a monoclonal antibody BSJ-1 on cryostat sections with a modified PAP immunohistochemical technique (P.L. Jeffrey et al. (1985) Society for Neuroscience, 11, 1105). In order to improve sensitivity in immunoblotting and immunohistochemical assays we have raised a panel of monoclonal antibodies of different subclasses to chicken Thy-1 (P.W. French and P.L. Jeffrey (1985) Aust. Soc. Immunol. 15th Ann. Meeting). In conjunction with a polyester wax embedding technique utilising the indirect immunoperoxidase method we have investigated further the expression of Thy-1 in cerebellum and retina.

In day old chick cerebellum, Thy-1 was localized principally to the molecular layer in association with Purkinje cell dendrites. In contrast to cryostat sections, intense intracellular staining of Purkinje cell bodies and dendrites was seen. A lesser degree of staining was apparent on the internal granular layer. Staining for Thy-1 in day old retina was localized to the optic fibre layer and the inner plexiform layer (IPL). This pattern of staining supports an association of the antigen with retinal ganglion cells, although additional association of Thy-1 with amacrine and bipolar cell processes in the IPL cannot be discounted.

At Ed15, in the cerebellum, the Thy-1 staining is intense within Purkinje cell bodies and in the apical extension that gives rise to the dendritic tree. The staining subsequently (Ed18) becomes stronger as the arborization of the Purkinje cell dendrites occurs. Concurrently, staining increases in the internal granule layer which corresponds to the time of formation of glomerula-like type contacts between granule cells, mossy fibres, golgi cells and Purkinje cell collaterals.

During development of the retina, Thy-1 expression is associated with the optic fibre layer and with the outgrowth and establishment of the area of contact between the ganglion cell dendrites and the cells of the IPL, namely, amacrine and bipolar cells.

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393.11 IMMUNO-LABELLING FOR FREEZE-FRACTURE: A NEW TECHNIQUE APPLIED TO A FIBRONECTIN AND LAMININ RECEPTOR. D. C. Quick and P. C. Letourneau. Dept. of Cell Biology & Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455.

A new technique was developed for electron microscopy that combines freeze-fracture with thin-sectioning. The technique has potential for a wide variety of applications, but the initial test of its usefulness was focused on identifying immuno-labelled fibronectin-laminin receptors in freeze-fracture replicas.

Chick heart cells were dissociated from 8-10 day embryos and plated on 4 mm round coverslips. After 2 days of culture, the cells were labelled with antibody to a glycoprotein complex that appears to be a fibronectin and laminin receptor (kindly provided by Dr. A. Horwitz, Univ. of Pennsylvania; Decker et al., 1984, J Cell Biol 99:1398). The cells were lightly fixed and a secondary antibody was applied, labelled with colloidal gold. Then, the cells were fixed in 2% glutaraldehyde, washed, and incubated in 30% glycerol overnight. The cells were fractured in a Balzers apparatus, using the technique for double replicas of cell monolayers (Yee et al., 1978, Proc Natl Acad Sci 75:3004). Immediately after fracturing, platinum (Pt) was evaporated onto the fractured surfaces at a 45 degree angle as is usual for freeze-fracture; however, no carbon film was applied. The coverslips carrying labelled, fractured, Pt-shadowed cells were submerged in buffer and glycerol was washed out of the cells overnight. At this stage, an optional treatment was to osmicate the cells in order to better see them in transmission electron microscopy. The cells were dehydrated and infiltrated with epoxy resin, still attached to the coverslips. Each coverslip was floated upside down on a small container of uncured epoxy, and the epoxy was then hardened. The coverslip was pried off, leaving the cells (with their gold-labelled antibodies and Pt shadow) embedded just below the surface of the epoxy stub. The specimens were thin-sectioned in a plane parallel to the cell monolayer and viewed by transmission electron microscopy (lead and uranium staining is optional). Where the plane of sectioning passed from the interior of a cell, across the fracture plane and into empty space, part of the fracture replica could be seen, looking very similar to a conventional freeze-fracture replica but superimposed on part of the thin-section image. Where the fracture plane passed through membrane containing immuno-labelled receptors, gold particles were seen superimposed with freeze-fracture surface.

Preliminary data reveal no freeze-fracture particles or pits that are congruent with gold-labelled fibronectin-laminin receptors. This might mean that the receptor is fractured in the same plane as the membrane bilayer, or that the labelled part of the receptor is not a trans-membrane structure.

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393.12 REINNERVATION OF ENDPLATE-FREE MUSCLES: ACETYLCHOLINE RECEPTORS AND PROTEOGLYCAN. Diana Card Linden. Occidental College, Biology Dept. Los Angeles, CA 90041.

The endplate zone was surgically removed from adult Rana pipiens cutaneous pectoris muscles to eliminate synaptic cell surface molecules. Then the operated mini-muscles were reinnervated with the original nerve. Sanes et al (1978) suggested that unidentified molecules in the synaptic basal lamina of muscle may be involved in providing chemotactic guidance for a reinnervating nerve. In the present research, reinnervation of the endplate-free muscle was done to study 1) the temporal relationship between functional innervation and, postsynaptic accumulation of acetylcholine receptors (AChR), heparan sulfate proteoglycan (HSP), cholinesterase (Che), and muscle fiber contraction; and 2) whether accumulations of HSP are important in determining the site of nerve attachment. The AChR and HSP were identified by using rhodamine-labeled α-bungarotoxin and a fluorescein-tagged monoclonal antibody to HSP, respectively. The antibody was a gift from Dr. M.J. Anderson, Univ. Calgary. Nerve terminal structure and Che were examined after fluorescent data were collected in each muscle, using the nitroblue tetrazolium technique of Letinsky & DeCino (1980).

The cut ends of the operated muscle fibers healed immediately, gradually lengthened, and the muscle was almost completely regenerated by 46 days. One week after surgery, the cut muscle fibers did not contract in response to nerve stimulation and did not have any new accumulations of AChR, HSP or Che. By 19 days some muscle fibers adjacent to the site of nerve implantation contracted in response to nerve stimulation up to 2 Hz. The nerve contacts had small clusters of AChR postsynaptically, but lacked dense accumulations of HSP and Che. Junctional AChR/HSP staining on the medial unoperated denervated junctions was normal. As the length of reinnervation increased more endplate-free muscle fibers became innervated and contracted in response to nerve stimulation. In addition, the muscle fibers with more mature synapses progressively responded to higher frequencies of nerve stimulation (up to 50 Hz). 25 and 46 days after reinnervation, HSP was colocalized with synaptic AChR, especially at larger AChR accumulations. These results suggest that in surgically altered endplate-free muscles, the motor nerve does not grow to pre-existing accumulations of HSP and/or AChR, but that the nerve induces AChR and then probably HSP. These experiments do not rule out other roles for HSP in the organization of synaptic molecules. It is possible that the nerve induces HSP, and then after the HSP has accumulated, there is a stimulation of further differentiation of the nerve, AChR clusters, HSP, and Che.

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393.13 PRELIMINARY IDENTIFICATION OF POLYPEPTIDES ASSOCIATED WITH THE SARCOLEMMAL ORTHOGONAL ARRAY. J.D. Hatton, G.F. Cox, A.L. Miller, J.A. Nichol* and M.H. Ellisman. Lab. Neurocytology, Dept. Neurosciences and Div. Neurosurgery, U.C.S.D., La Jolla, CA 92093.

Electron microscopy of freeze-fracture replicas from the sarcolemmas of fast-twitch muscle fibers reveals orthogonal arrays of particles. The biochemical nature of macromolecules associated with the sarcolemmal orthogonal array was investigated using muscle fragments and isolated sarcolemmal vesicles. Muscle fragments incubated in vitro with the lectin Concanavalin A (con A) exhibited a clustering of orthogonal arrays into local patches. Treatment with other lectins did not result in the clustering of arrays. Clustering was inhibited by the addition of α-methyl-D-mannoside, a ligand which also binds to con A. These results indicate that the orthogonal arrays (or associated components) specifically bind con A. Sarcolemmal vesicles from rabbit sacrospinalis (SAC) and rat extensor digitorum longus (EDL) (both primarily fast-twitch) and rat soleus (SOL) (primarily slow-twitch) were obtained by a combination of low-salt fractionation and sucrose density gradient centrifugation. SDS polyacrylamide gel electrophoresis (SDS-PAGE) of proteins and glycoproteins solubilized from these vesicles revealed several bands. Four of these bands were present in gels from both the rabbit and rat fast-twitch muscle sarcolemmal preparations (that contained arrays), yet were absent in gels from rat slow-twitch muscle sarcolemmal preparations (not bearing arrays). An enrichment in vesicles containing arrays was achieved by binding SAC sarcolemmal vesicles to con A-Sepharose 4B beads. SDS-PAGE analysis of array-enriched vesicles from the con A beads revealed enrichment of three major bands at M$_r$ 93,000, 54,000 and 49,000. These enriched bands correlate with three of the four bands common to fast-twitch EDL and SAC, yet are absent in slow-twitch SOL sarcolemmal preparations. Thus, several lines of evidence suggest that at least one of three proteins of approximately 49, 54 and 93 kD molecular weight are candidate components of the sarcolemmal orthogonal array. Since the α subunit of the Na-K ATPase is a sarcolemmal protein whose molecular weight is near that of one of the array candidate proteins (93 kD), we deemed it important to test for this possible identity. It was determined that no Na-K ATPase immunoreactivity was present within the array enriched fraction by both immunoblotting and enzyme linked immunosorbent assay. Antibodies against both purified guinea pig and rat ATPase failed to stain the 93 kD band in immunoblots. Further, antibodies raised against the array enriched material showed no significant binding to the ATPase by ELISA. Thus, we have defined possible candidate proteins that correlate with the orthogonal array, have determined that these are not Na-K ATPase related and have produced antibodies to this array enriched preparation that should be useful for further characterization.

393.14 UBIQUITIN-ANTIBODIES INHIBIT THE NEURON-SPECIFIC HIGH AFFINITY UPTAKE OF CHOLINE, NE, 5-HT, GABA, GLUTAMATE, AND ASPARTATE IN RAT BRAIN SYNAPTOSOMES. E.M. Meyer, C. West*, S.P. Baker, J.H. Judkins*, and V. Chau*. Depts. of Pharmacology, Biochemistry, and Anatomy, Univ. of Florida, Gainesville, FL 32610.

Ubiquitin is a 76 amino acid residue protein found in all eukaryotes. One of the most highly conserved proteins, ubiquitin is covalently linked to amino groups of acceptor proteins as a form of post-translational modification that may underlie many processes ranging from cellular interactions to rapid proteolysis. These cellular interactions in particular may involve enhanced recognition of specific cellular plasma membrane proteins that have been "ubiquinated". We therefore investigated whether ubiquitin was associated with several neuron-specific transport proteins, using antibodies to ubiquitin that were purified by a Sepharose-ubiquitin affinity column and further characterized by immunoblotting. These ubiquitin-antibodies bound to intact rat cerebral cortical synaptosomes in a saturable manner, with maximal binding observed (less than 0.5% of the total membrane protein) at 10-20 ug/ml after 30 min at 37°, using iodinated protein-A for quantification. Using similar incubation conditions, 10 ug/ml of the antibodies inhibited the synaptosomal high affinity uptake of labelled choline, NE, 5-HT, GABA, glutamate, and aspartate by 88%, 32%, 29%, 36%, 53%, and 49%, respectively. In the cholinergic system, which was the first to be studied more closely, this uptake-inhibition was time- and dose-dependent, with inhibition paralleling binding. Further, the antibody-induced inhibition of choline uptake and coupled ACh synthesis was blocked or reduced by pretreatment with pretreatment with 5% TCA or Sepharose-bound ubiquitin, respectively. Pre-immune IgG bound to synaptosomes in an apparently non-saturable manner and did not affect high affinity choline uptake and acetylation. Significantly, these ubiquitin-antibodies had no effect on muscarinic receptor binding to labelled QNB or on ACh release, demonstrating the specificity of the extracellular ubiquitin-binding for neuron-specific transporters as opposed to receptors or any of the proteins associated with transmitter release and maintenance of membrane potentials. These results: 1) show the potential of this antibody preparation for partially purifying membrane transporters; and 2) suggest a possible cell-recognition role for ubiquinated transporters.

393.15 CEREBELLAR GANGLIOSIDE ABNORMALITIES IN THE NERVOUS MUTANT MOUSE. T.N. Seyfried, D. Bernard* and R.K. Yu.^ Dept. of Biology, Boston College, Chestnut Hill, MA 02167, and Dept. of Neurology, Yale University, Sch of Medicine.

Gangliosides are a family of cell-surface glycosphingolipids that are most concentrated in the vertebrate CNS and are thought to play a role in neuronal differentiation and synaptogenesis. The distribution of cerebellar gangliosides was studied in adult (73 + 2 days) nervous (nr/nr) mutant mice which experience a degeneration of 50 to 90 percent of their Purkinje cells. This neuronal loss is associated with significant reductions in cerebellar weight and ganglioside concentration. The cerebellar dry weights (mg) and the ganglioside concentrations (ug N-acetylneuraminic acid per 100 mg dry weight) in nr/nr mice and age-matched normal littermates (+/?) are 7.4 + 0.3 mg and 13.2 + 0.4 mg, respectively; and 411.7 + 4.8 ug and 438.5 + 2.1 ug; respectively. Abnormalities were also observed for the concentration of certain ganglioside species. Most noteably, GT1a is significantly reduced by 42%, and GD3 is significantly increased by 29% in the nr/nr mice compared to the +/? mice. The nr/nr mice also express a slight but significant, reduction in GT1b. No ganglioside abnormalities were observed between the nr/nr and +/? mice in cerebral cortex. We previously found reduced cerebellar GT1a content in other mutants that also lose Purkinje cells, i.e., sg/sg, pcd/pcd and Lc/+. GT1a is not reduced, however, in wv/wv mice that lose mostly granule cells. The findings in nr/nr mice therefore support our hypothesis that GT1a is enriched in Purkinje cells. GD1a, which is enriched in mature granule cells, is not reduced in the nr/nr mice. Since we previously found that GD3 is a good marker for reactive glia in neurological disease, the elevated GD3 concentration in the nr/nr mice indicates a mild gliosis. Our findings with nr/nr and the other neurological mutants indicate that gangliosides can be used as cell-surface markers for monitoring changes in the cytoarchitecture of the mouse cerebellum. Supported by NSF grant (BNS8305449) and NIH grant 11853.

393.16 EXPRESSION OF THE LIMBIC SYSTEM ASSOCIATED MEMBRANE PROTEIN (LAMP) ON CULTURED NEURONS AND NEUROBLASTOMA CELLS. P. Levitt and V. Cooper*. Dept. Anatomy, Medical College of Penn., Philadelphia, PA 19129.

The limbic system associated membrane protein (LAMP) is a cell surface glycoprotein on the cell somata and dendrites of neurons comprising limbic system regions in mammals. Axons and fiber tracts are not immunoreactive in the adult, but are transiently stained during development. In order to assess the time course of expression and cellular distribution of LAMP during development, immunocytochemical staining with a monoclonal antibody was performed on primary monolayer cultures and N2A neuroblastoma cells at various stages of differentiation. The hippocampal formation was excised and dissociated for monolayer culturing at embryonic day 16, 3 days prior to in situ detection of LAMP. By forty-eight hours after plating, immunoreactive cell somata and short processes were evident in live cultures, indicative of the distribution of LAMP at the extracellular surface. Staining was spatially restricted on the membranes, found in distinct clusters. Ultrastructural immunocytochemical examination confirmed this patchy distribution. The pattern of cellular staining remained the same during the 14 day culture period. Increases in immunoreactivity were attributed to growth of neurite arbors in the culture system over time. In order to characterize the fiber class and cell types exhibiting the immunoreactivity, double immunofluorescence was performed with a monoclonal antibody to neurofilament (NF) protein and an antibody to glial fibrillary acidic (GFA) protein. Double labeled LAMP and NF processes were found throughout the cultures. No LAMP/GFA immunoreactive cells were present in the material. N2A cells exhibited identical patchy surface LAMP immunoreactivity. Both the somata of clusters of immature, flattened cells and large, differentiated process-bearing cells were immunostained, indicating that morphological differentiation is not a prerequisite for LAMP expression. The studies demonstrate that the epitope recognized by the LAMP monoclonal antibody is distributed on the extracellular surface of only neurons in the primary cultures and that during cell differentiation, membranes comprising the soma, axon and dendrite express the system-specific antigen.
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393.17 SEPARATION OF TWO DISTINCT POPULATIONS OF NEURONS FROM CHICK CILIARY GANGLION. D.L. Needels, M. Nieto-Sampedro, and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

It has been known for some time that ciliary ganglion neurons and nonneurons from 8 day chick embryo can be separated from each other, based on the greater adhesivity of the nonneurons to untreated tissue culture plastic. This separation is dependent on some as yet uncharacterized component(s) of fetal calf serum that differentially inhibits the attachment of neurons relative to nonneurons. As a class, all of the neurons in the ciliary ganglion are less adhesive than the nonneurons.

We report here that subpopulations of neurons can also be separated from each other on the basis of their adhesion to plastic in the presence of Serum Plus (a commercially available supplemented serum). Approximately half of the neurons plated in Serum Plus attach immediately to the plastic (within 15 minutes), but the other half do not attach for up to 90 minutes. Furthurmore, these two population of neurons have different properties in culture. Extract of injured rat brain contains a neurotrophic factor capable of supporting the short-term (24 h) survival of all of the neurons in ciliary ganglion. The less adhesive neurons can be quantitatively supported for greater than 2 weeks by such extracts, however the second population slowly dies during the first week in culture. Thus, the two populations of neurons show different sensitivities to brain-derived trophic factors. Secondly, the more adhesive neurons tend to aggregate in culture, whereas the less adhesive neurons remain isolated from each other.

The ciliary ganglion consists of two major classes of neurons, ciliary and choroid, that innervate different target muscles. While we have no direct evidence that the subpopulations separated here correspond to ciliary and choroid neurons, the relative proportions are consistent with this notion. Alternatively, we may be separating neurons that are in different developmental stages; if so, the differences in adhesivity and sensitivity to trophic factors may be functionally relevant. In principle, subpopulation of neurons from other sources could be separated from each other and from nonneurons by manipulating the relative levels of attachment factors and inhibitors (using either serum or purified components).

Supported by NIH postdoctoral fellowship 1F32 NSO7476-01A1 (DLN) and NIMH grant MH 19691-16 (CWC).

393.18 GADOLINIUM-LABELED MONOCLONAL ANTIBODIES PERMIT MAGNETIC RESONANCE IMAGING OF HUMAN T CELLS IMPLANTED IN CANINE BRAIN. S. Kornguth, P. Turski*, W. Perman*, R. Reale*, T. Kalinke*, and R. Schultz*. University of Wisconsin, Madison, Wisconsin 53706.

Two different murine monoclonal anti-human T cell antibodies, that were covalently coupled directly and indirectly to gadolinium (Gd), bind specifically to human T cells implanted in canine brain. This binding was at a concentration of Gd sufficient to delineate the implanted cells and to distinguish them from surrounding brain tissue with a 1.5 Telsa magnetic resonance (MR) imager. These Gd-labeled immunoglobulin preparations did not bind bovine T cells at a concentration sufficient to be detected on MR images. These Gd-labeled antibodies yield cell-specific contrast in MR images.

A protein solution containing the immunoglobulins was reacted with the dianhydride of DTPA; the DTPA acts as a metal chelator and as a protein crosslinking agent. As an example, a 1 ml solution (pH 8.6) containing 100 ug of monoclonal antibody, 2.0 mg gelatin and 2.5 mg bovine serum albumin was added rapidly to dry DTPA (1-2 mg). After 16 hours at 4°C, the protein-bound DTPA was separated from free DTPA by gel filtration. The DTPA-protein complex, in citrate buffer (pH 6.3, 0.1 M), was reacted with gadolinium chloride. In some cases, 153-Gd was added to the 157-Gd to determine the extent of protein modification. The free Gd was removed by gel filtration. There were approximately 10 DTPA residues per protein in the modified protein mixture.

Isolated human or bovine monocytes were implanted into the brains of anesthetized dogs. The cells were injected in a volume of 40 ul containing 12 million cells. Subsequently the blood-brain barrier was disrupted by the intraarterial injection of hyperosmotic mannitol (25%) through a catheter placed at the branch of the internal and external carotid. The Gd-labeled monoclonal antibody preparations were then introduced through the same catheter. Forty-eight to seventy-two hours later the brains were imaged. MRI revealed a markedly decreased T1(high signal intensity TE=25, TR=200 msec) related to the human T cell implants. There was no evidence of Gd activity at the site of bovine T cells. Neither control murine gamma-globulin coupled to Gd, nor murine anti-human T cell antibodies uncoupled to Gd contrasted the human T cells in the brain. As a consequence of binding Gd-labeled antihuman T cell immunoglobulins to T cells, the intracranial mass containing 10-12 million cells in a diameter less than 4 mm was readily visualized.

394.1 ROLE OF CENTRAL NORADRENERGIC AND PITUITARY ADRENAL SYSTEMS IN REGULATION OF BETA AND ALPHA ADRENOCEPTOR FUNCTION IN RAT CNS. E.A. Stone, B.S. McEwen, A.S. Herrera* and K.D. Carr, Dept. Psychiat., New York Univ. Sch. Med., New York, NY 10016 and The Rockefeller Univ., New York, NY 10021.

Catecholamines stimulate the formation of cyclic AMP (cAMP) in rat brain by activation of both beta and alpha adrenoceptors. Recent findings have suggested that the beta component of the response may be regulated chiefly by the noradrenergic system and the alpha component primarily by the pituitary adrenal system. The present study was undertaken to test this by determining how each adrenergic component is altered by chronic increases and decreases in the activities of these systems.

Central noradrenergic activity was chronically increased or decreased in separate groups of rats by repeated treatment with the NE uptake inhibitor, desmethylimipramine (DMI, 10 mg/kg, i.p., twice daily, 10 days) or by 6-OHDA induced lesion of the dorsal noradrenergic bundle (DNB, two weeks), respectively. Pituitary adrenal activity was chronically increased or decreased by repeated administration of $ACTH_{1-24}$ (50 U/kg/day, s.c., 10 days) or by hypophysectomy (2 weeks), respectively. The cAMP response to beta and alpha adrenergic receptor stimulation was assayed in cerebral cortical slices by previously published methods using isoproterenol to activate the beta response and 6-fluoro-norepinephrine the alpha.

It was found that the beta response was altered selectively by treatments affecting noradrenergic function whereas the alpha response was affected selectively by treatments affecting pituitary adrenal function. Thus DMI (noradrenergic increase) and DNB lesion (noradrenergic decrease) produced a decrease and increase respectively in beta receptor function whereas ACTH (pituitary adrenal increase) and hypophysectomy (pituitary adrenal decrease) produced a decrease and increase respectively in alpha receptor function. Preliminary findings suggest that the alpha response affected by pituitary adrenal activity is of the alpha-1 adrenergic subtype.

It is concluded therefore that the beta and alpha adrenergic components of the cAMP response to catecholamines are regulated by the noradrenergic and pituitary adrenal systems respectively. The respective roles of ACTH and corticosterone in the pituitary adrenal effects are currently under investigation. The finding that the noradrenergic system regulates beta and not alpha-1 adrenoceptor function may signify that these receptors have differential susceptibility to regulation by their agonist (norepinephrine) or are subject to different degrees of agonist exposure. (Supported by grants MH22768, MH08618 and NS07080).

394.2 **Structural Derivatives of Pindolol: Factors Influencing Interaction with Central Beta-Adrenergic Receptors In Vivo.** **S.M. Tejani-Butt* and D.J. Brunswick* (Spon.: P. Conway).** Dept. of Psychiatry, Univ. of Pennsylvania School of Medicine and Neuropsychopharmacology Unit, Veterans Administration Medical Center, Philadelphia, PA 19104.

Although (-)-^{125}I-iodopindolol (IPIN) can be used to label beta-adrenergic receptors in the central nervous system (CNS) in vivo (J. Pharm. Exp. Therap. 235:1, 1985) use of this ligand for receptor imaging studies in humans may be limited due to its relatively poor penetration into the CNS. A series of derivatives related to pindolol was therefore synthesized in an effort to determine the factors that might influence the penetration and interaction of these compounds with central beta-receptors in vivo. The synthesized compounds varied in the substituent at the terminal amino end of the molecule; their structures were confirmed using proton nuclear magnetic resonance, mass spectroscopy and elemental analysis. The affinity of these compounds for beta-receptors, as measured by their inhibition of the binding of IPIN to rat cortical or cerebellar membranes in vitro, ranged from 2 to 100 fold less than pindolol. The most potent compounds had IC_{50} values of approximately 5nM. These compounds varied widely in their lipophilicity (about 1000 fold), as measured by their distribution coefficients between phosphate buffer and n-octanol at pH 7.4. Evaluation of the ability of these derivatives to displace the binding of IPIN in the brain upon systemic administration provides an assessment of whether the derivatives penetrate and interact with central beta-receptors in vivo.

Approximately 2 uCi IPIN was injected intravenously into male Sprague-Dawley rats (200-250g) five minutes after the injection of either unlabelled derivative of pindolol or saline. Two hours later radioactivity was measured in cortex (CX), cerebellum (CB), and brainstem (BS). The potency of the compounds for displacing IPIN binding in vivo in the CX and CB were highly correlated with the IC_{50} values from competition experiments determined in vitro (r_{CX}= 0.87; r_{CB}=0.92). By contrast, increasing the lipophilicity of these compounds decreased the potency in vivo (r_{CX}=0.65 ; r_{CB}= 0.69). This result was somewhat unexpected as it was anticipated that lipophilicity would increase the penetration and interaction of these compounds with beta-receptors in vivo. It is concluded from this study of closely related derivatives of pindolol that high affinity for the receptor appears to be the most important property of the compounds influencing their interaction with central beta-receptors. After some limiting value of lipophilicity is reached increasing lipophilicity may actually decrease potency in vivo. **(Supported by Research Funds from the Vet. Adm. and USPHS MH 36761).**

394.3 A PROCEDURE FOR DETECTING α_2-ADRENERGIC RECEPTOR-MEDIATED INHIBITION OF ADENYLATE CYCLASE IN RAT BRAIN SLICES: INFLUENCE OF IMIPRAMINE. R.S. Duman, S. Nomura* and S.J. Enna. Depts. Pharmacology and Neurobiology and Anatomy, University of Texas Medical School, P.O. Box 20708, Houston, Texas 77025.

Although an α_2-adrenergic receptor-mediated inhibition of adenylate cyclase has been observed in peripheral tissues, cell cultures, and brain particulate fractions, it has not been detectable in brain slices. In fact, it has recently been reported that α_2-adrenergic agonists augment neurotransmitter receptor-stimulated cAMP accumulation in rat brain slices (Pilc, A. and Enna, S.J., J. Pharmacol. Exp. Therap., in press), suggesting that α_2-adrenergic sites may not be negatively coupled to adenylate cyclase in this preparation. On the other hand, it is conceivable that any inhibitory effect may be obscured by the augmenting action in brain slices. The present study was undertaken to address this issue by examining the effect of α-adrenergic agents on forskolin-stimulated cAMP accumulation in rat brain slices. For the study, forskolin-stimulated cAMP accumulation was measured in rat brain cerebral cortical slices using a prelabeling technique. It was found that 6-fluoronorepinephrine, clonidine and UK-14,304 caused a concentration-dependent inhibition of forskolin-stimulated cAMP accumulation in this preparation, whereas phenylephrine, an α_1-adrenergic agonist, was inactive in this regard. Moreover, α-adrenergic receptor antagonists were found to be more potent than α_1-adrenergic agents in reversing the inhibitory response to UK-14,304. Chronic (14 days) administration of imipramine (10 mg/kg, i.p.) significantly reduced the ability of UK-14,304 to inhibit forskolin-stimulated cAMP accumulation in rat cerebral cortical slices. The results suggest that rat brain slices possess α_2-adrenergic receptors that are negatively coupled to adenylate cyclase and indicate that the functional response to these sites can be examined in this preparation when the enzyme is stimulated directly, such as with forskolin. The data with imipramine indicated that the chronic administration of this antidepressant down-regulates these α_2-adrenergic receptors, lending biochemical support to behavioral and electrophysiological results suggesting that antidepressants may attenuate α_2-adrenergic receptor responsiveness in brain. (Supported in part by USPHS grants MH-36945 and MH-00501).

394.4 ACUTE AND CHRONIC STRESS AFFECT ALPHA$_2$-ADRENOCEPTORS IN SPECIFIC BRAIN REGIONS OF THE RAT. I. Nukina, G.B. Glavin and F.S. LaBella. Dept. of Pharmacology & Therapeutics, Univ. of Man. Winnipeg, Man. R3E 0W3

Various forms of stress promote decreases in levels of noradrenaline (NA) and an increase in NA turnover in brain. Brain adrenoceptors also appear to be influenced by stress, and subsensitivity of beta-adrenergic responses following repeated exposure to stress has been reported. But the effects of stress on alpha$_2$-adrenoceptors in brain are less clear. In the present studies, the effects of acute and chronic stress on the state of alpha$_2$-adrenoceptors in nine brain regions were examined using the specific alpha$_2$-adrenoceptor antagonist, (^{3}H)-rauwolscine (^{3}H-Rau) (Perry, B.D. and U'Prichard, D.C., European J. Pharmacol., 76:461, 1981).

The acute stressed rats were restrained in supine position for 3 hr at 4-6°C. The chronic stressed rats were restrained for 1 hr once daily for 10 days. Stressed and control rats were decapitated and amygdala, hippocampus, hypothalamus, cerebral cortex, pons plus medulla oblongata, basal ganglia, thalamus, midbrain and cerebellum were dissected on ice. Acute stress led to changes in ^{3}H-Rau binding in specific brain regions. A decrease in Bmax occurred in the amygdala and in the hippocampus, but an increase in the midbrain. The Kd was decreased in the amygdala and increased in the midbrain. Chronic stress led to an increase in Bmax in the amygdala and the midbrain but a decrease in the pons/med. The Kd was increased in the midbrain but decreased in the hypothalamus.

Thus, acute exposure to an endogenous agonist (stress-induced NA release) might cause desensitization of alpha$_2$-adrenoceptors but chronic exposure might cause normalization or supersensitivity of alpha$_2$-adrenoceptors in the hippocampus and amygdala. On the other hand, both acute and chronic stress increased the number of alpha$_2$-adrenoceptors in the mid brain. These differences in regional brain alpha$_2$-adrenoceptor responses to stress are puzzling. However, the midbrain may have an important role in physiological response to stress. We conclude that there is significant regional brain variability in the physiological regulation of alpha$_2$-adrenoceptors in response to stress and these regional discrepancies might be due to differences in the ratio of pre- and post-synaptic alpha$_2$-adrenoceptors, in time related differences in noradrenaline turnover and/or in the heterogeneity of brain alpha$_2$-adrenoceptors. (Supported by the Medical Research Council of Canada and Manitoba Health Research Council).

394.5 [^{3}H]UK14,304 BINDS AT MULTIPLE ALPHA$_2$-ADRENERGIC AFFINITY STATES IN HUMAN PREFRONTAL CORTEX:GTP INHIBITS BINDING AT THE HIGHEST AFFINITY STATE IN BOTH NORMAL SPECIMENS AND PRESUMPTIVE SUICIDES. R. C. Gilkeson*, M. A. Carlson*, A. C. Andorn* (SPONS: A. Halaris) Department of Psychiatry, Case Western Reserve University School o Medicine and Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109

[^{3}H]UK14,304 binds rapidly and reversibly to at least two high affinity states with alpha$_2$-adrenergic selectivity in particulate membrane fractions of human prefrontal cortex. Rate association studies at 21°C showed that the binding of [^{3}H]UK14,304 reached a steady-state at 30 min which was maintained for at least 2 hrs. In rate dissociation studies using 1 µM clonidine as the competitor, a quickly dissociating component ($t_{1/2}$ = 2.0 min) and a more slowly dissociating component ($t_{1/2}$ = 20 min) were demonstrated at 0.8 nM [^{3}H]UK14,304. Scatchard transformations of the saturation data obtained at 90 min incubation were biphasic. The high affinity component had a K_D of 88 pM and a B_{max} of 15 fmols/mg protein, and the low affinity component had a K_D of 850 pM and a B_{max} of 30 fmols/mg protein. The selectivity of [^{3}H]UK14,304 binding was characteristic of alpha$_2$-adrenergic binding sites. IC_{50}'s were: UK14,304 - 1.1 nM; clonidine - 1.7 nM; (-)-epinephrine - 1.7 nM; yohimbine - 9.2 nM, (+)-epinephrine - 240 nM; (dl)-propranolol - 1,000 nM; prazosin - 5,100 nM. In Scatchard transformations of saturation data in the presence of 1 mM GTP, the highest affinity component (K_D = 88 pM) was absent. We examined the density of the highest affinity state in normal brains and in those from presumptive suicides. No significant difference in the density of the high affinity state was noted between age matched normal (N=10) and presumptive suicides (N=13). GTP was an effective inhibitor of high affinity binding in both normal and suicide brains. A trend toward declining total receptor density with age was noted in both normals and suicides.

394.6 REVERSAL OF STRESS-INDUCED DELAYED RESPONSE DEFICITS IN RHESUS MONKEYS BY CLONIDINE AND NALOXONE Amy F.T. Arnsten and P.S. Goldman-Rakic, Section of Neuroanatomy, Yale Medical School, New Haven, CT 06510.

Humans exposed to loud noise stress exhibit cognitive deficits that are consonant with dysfunction of the prefrontal cortex (PFC) (J. Exp. Psychol. 102:62, 1974). In nonhuman primates, lesions of the dorsolateral PFC cause deficits on delayed response tasks. We reasoned that if noise stress disrupts PFC function, monkeys exposed to this stressor should display impaired delayed response performance. Accordingly, young adult rhesus monkeys were trained on the delayed response task with variable delays ranging between "0" sec and the temporal interval previously determined to yield performance of approximately 75% correct. Within this range, 5 different delays, determined individually for each animal, were quasi-randomly distributed over the 30 trials that made up a daily test session. Stress consisted of exposure to 100 db wide band noise either for 30 min immediately prior to testing or during the test session. For control sessions the sound was maintained at 60 db, the level of normal background masking noise.

Exposure to the noise stress either before or during testing reliably impaired delayed response performance at the longer delay intervals. Performance declined by at least 21%, and one monkey was reduced to chance levels of responding. As with frontal lesions, stress did not disturb performance after the "0" sec delays. These results suggest that errors at the longer delays did not arise solely from nonspecific changes in motivation or sensorimotor function, but rather involved alterations in cognitive processes related to the PFC. In order to investigate the possible involvement of opioid and noradrenergic (NE) systems in this stress response, animals were then injected with either the opiate antagonist, naloxone (0.5 mg/kg), or the alpha-2 adrenergic agonist, clonidine (0.04 mg/kg), prior to stress exposure. The alpha-2 NE system is critical for proper functioning of the PFC (Science 230:1273, 1985), and the dose of clonidine used here appears to act at post-synaptic receptors in this region (ibid). Neither clonidine nor naloxone altered the performance of young monkeys under normal noise conditions. However, when these drugs were administered prior to 100 db noise, stress-induced deficits in delayed response performance were ameliorated completely. These results suggest that (1) stress induces PFC dysfunction and (2) this deficit may be reversed by pharmacological agents that either protect NE from inhibition by opioid stimulation (naloxone), or compensate for NE loss by stimulating post-synaptic receptor mechanisms (clonidine).

394.7 QUANTITATIVE ASSESSMENT OF THE DEVELOPMENT OF α_2-ADRENOCEPTOR RECOGNITION SITES AND REGULATION IN RAT CEREBRAL CORTEX. E. Kunkel-Bagden*, J.M. Stolk*, and D. U'Prichard*. Dept. of Psychiatry, Univ. of Md., Baltimore, MD 21228.

The development of α_2-adrenoceptor recognition sites and their regulation by guanine nucleotides (GPP) and divalent cations (Mg^{+2}) was studied in rat cerebral cortex. A 2 x 2 matrix design was used to examine interactions between the agonists, p-aminoclonidine (PAC) and UK-14304 (UK), and the antagonist, rauwolscine (RAU), utilizing radiolabelled and unlabelled ligands. Detailed competition curves were done in the following combinations:

Hot agonist vs cold agonist
Hot agonist vs cold antagonist
Hot antagonist vs cold agonist
Hot antagonist vs cold antagonist

Analysis of the compiled data set by "Ligand" (Munson & Rodbard) enabled us to quantitatively estimate site densities for multiple states of the allosteric $\alpha_2$adrenoceptor. One site displayed high affinity for antagonist and low affinity for agonist (Site #1), while another (Site #2) displayed low affinity for antagonist and high affinity for agonist. K_D values for PAC and RAU were:

Parameter	N	Site #1	Site #2
K_D[RAU]	9	3.05±0.35nM	59.1±8.5nM
K_D[PAC]	"	21.5±2.5nM	0.70±0.10nM
B (fmol/mg)	"	70.3±5.1	69.1±6.7

GPP increased the relative abundance of antagonist-preferring sites (#1), while Mg^{+2} increased the abundance of agonist-preferring sites (#2), as the ratios of the binding site densities below illustrate.

Site 1:Site 2	1.08	no additions
Site 1:Site 2	1.72	+ 10^{-5}M GPP (NH)p
Site 1:Site 2	0.43	+3 x 10^{-4} Magnesium

The total number of sites (143 12.3 fmol/mg) was unaffected by GPP or Mg^{+2}.

At post-natal days 14 and 7 the antagonist-preferring site (#1) and agonist-preferring site (#2) had K_D values similar to those in adults for PAC (or UK) and RAU. Total site density in day 14 was 40% greater than adults, but regulation of the relative abundance of sites #1 and #2 by GPP and Mg^{+2} remained comparable to adults. Total site density in day 7 rats was 50% greater than adults; however while regulation of site distribution by GPP appeared normal, Mg^{+2} failed to alter the distribution of antagonist-and agonist-preferring sites in a predictable manner.
(Supported by USPHS MH 32842).

394.8 DIFFERENTIAL AUTORADIOGRAPHIC DISTRIBUTIONS OF (3H)RAUWOLSCINE AND (3H)IDAZOXAN ARE CONSERVED ACROSS RODENT AND PRIMATE SPECIES. C.L. Boyajian and F.M. Leslie, University of California, Irvine,CA 92717

We have previously reported that two α_2-adrenoceptor antagonists, (3H)rauwolscine (RAUW) and (3H)idazoxan (IDAZ), exhibit differential anatomical distributions and pharmacological binding profiles in rat brain (Boyajian, C.L. et al., Soc. Neurosci. Abstr. 11:1043, 1985). This report provides evidence that these radiolabeled binding sites are differentially localized in monkey brain (macaca fascicularis) in a manner that closely parallels the anatomical distributions observed in rat brain. Quantitative autoradiographic techniques were used to determine binding site densities for both radioligands in numerous monkey brain areas. Cryostat-cut tissue sections (20µm) were thaw-mounted onto subbed slides and incubated with (3H)RAUW or (3H)IDAZ in the presence and absence of 10µM phentolamine to define specific binding. Following incubation and washes, the sections were dessicated, then exposed to (^{3}H)-sensitive film for several weeks to visualize autoradiographic grains. Autoradiographic images were quantitatively analyzed by computerized densitometry. The radioligand binding sites were pharmacologically characterized by constructing dose-response curves for inhibition of the binding of either radioligand by various inhibitors. Preliminary data indicate that rauwolscine, idazoxan, prazosin and spiroperidol inhibit (3H)RAUW versus (3H)IDAZ binding with different relative potencies (Ki=1.94nM, 6.0nM, 55nM, 1440nM for (3H)RAUW and 14nM 1.68nM, 336nM, 3920nM for (3H)IDAZ), suggesting that these radioligands label heterogeneous populations of α_2-adrenoceptor sites in monkey brain. Quantitative autoradiographic analysis of saturation curves indicates that the density of high affinity radiolabelling is greater for (3H)IDAZ (K_D=0.73nM) in several monkey brain areas as compared to (3H)RAUW (K_D=1.40nM). (3H)IDAZ binding is most dense in hypothalamus, central grey, amygdala, dorsal pons and hippocampus (800-2300fmol/mg protein). Moderately dense labeling for (3H)IDAZ appears in frontal cortex, temporal cortex, caudate, putamen, antero-ventral thalamus, cerebellum and substantia gelatinosa (500-700fmol/mg). Less dense labelling is observed in reticular thalamus, ventral anterior thalamus, globus pallidus and spinal cord (150-400fmol/mg). In contrast, (3H)RAUW labelling is restricted to fewer brain areas, with highest grain densities apparent in caudate, putamen and pons (400-750fmol/mg). Hippocampus, frontal cortex, temporal cortex, cerebellum and substantia gelatinosa contain moderately dense (3H)RAUW labelling (100-250fmol/mg). Other brain areas exhibit lower levels of (3H)RAUW binding (<100 fmol/mg). The similarity of the anatomical localization patterns of (3H)RAUW and (3H)IDAZ labelled sites across rat and monkey species suggests that the relationship between α_2-adrenoceptors and the physiological functions they subserve is conserved in the phylogeny of mammalian brain. (Supported by NS 19319).

394.9 ALPHA-2 TYPE OF ADRENOCEPTORS MEDIATE THE RESPONSE OF RAT HIPPOCAMPAL PYRAMIDAL NEURONS TO NOREPINEPHRINE IN VIVO. O. Curet* and C. de Montigny (SPON: L. Vachon). Centre de recherche en sciences neurologiques, Université de Montréal, Canada H3C 3J7.

The present study was undertaken to characterize the nature of the adrenoceptor mediating the response of hippocampal pyramidal neurons to microiontophoretic application of norepinephrine (NE) in vivo in the rat.

Male Sprague-Dawley rats (270-290 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.). Five-barrelled micropipettes were used to record from CA_3 hippocampal pyramidal neurons. The following solutions were used for microiontophoresis: NE, 0.1 M; oxymetazoline, 0.01 M; clonidine, 0.1 M; idazoxan, 0.01 M; piperoxane, 0.1 M; yohimbine, 0.01 M; phentolamine, 0.01 M; acetylcholine, 0.02 M; γ-aminohydroxybutyric acid (GABA), 0.01 M; isoproterenol 0.1 M. All solutions were in 0.2 M Nacl, at pH: 4. Small currents of acetylcholine (~1 to 4 nA) were used to activate silent or slowly discharging units.

All agonists used had a suppressant effect on hippocampal pyramidal neuron firing activity. However, small currents of NE were consistently effective whereas much higher currents of clonidine, oxymetazoline and isoproterenol were required to obtain a similar degree of suppression. Systemic injections of idazoxan (2 mg/kg, i.v.), a selective α_2 adrenoceptor antagonist, completely blocked the effect of NE, whereas propanolol (4 mg/kg, i.v.) and prazosin (0.5 mg/kg, i.v.), which are selective β and α_1 adrenoceptor antagonists respectively, had no or little effect on the response of these neurones to NE. Only high doses of idazoxan (10 mg/kg, i.v.) blocked the effects of clonidine, oxymetazoline and isoproterenol, without altering the effect of GABA. To verify if the effect of intravenous idazoxan on NE was taking place at the level of the neuron recorded, idazoxan, piperoxane, yohimbine and phentolamine were applied microiontophoretically. All four antagonists readilly antagonized the effect of NE. Clonidine applied with a current which had no or little effect on the firing activity of pyramidal neurons also antagonized the effect of NE.

In conclusion, the results obtained with the antagonists suggest that the suppression of the firing activity of rat hippocampal pyramidal neurons by microiontophoretic applications of NE in vivo is mediated by an α_2 type of adrenoceptor. However, the results obtained with the agonists clearly indicate that this adrenoceptor is different from the α_2 adrenergic autoreceptor since: 1) clonidine and oxymetazoline are much less potent than NE; 2) clonidine antagonizes the effect of NE; 3) much higher doses of idazoxan are required to block the effects of clonidine and oxymetazoline than to block that of NE.

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394.10 IN-VIVO DIALYSIS MEASUREMENTS IN RAT THALAMUS: EFFECTS OF THE ALPHA-ADRENERGIC ANTAGONIST YOHIMBINE. C.W. BRADBERRY*, M.P. BRAZELL* and R.N. ADAMS. (SPON: R.BORCHARDT) Dept. of Chemistry, Univ. of Kansas, Lawrence, Kansas, 66045

Perfusion experiments were performed in rat thalamus, using a loop style dialysis probe. The experiments were performed on awake, freely moving animals the day following loop implantation. The effects of systemic administration of the alpha-2 adrenergic receptor blocker yohimbine (5 mg/kg) were investigated. Dihydroxyphenylacetic acid (dopac) levels in the extracellular space were enhanced 500% by this treatment. Basal levels of norepinephrine (NE) were not always measureable, but when they were, they increased by 300-400%. When yohimbine was administered into the thalamus via the dialysis loop, the increases in dopac were much less pronounced (125%). The changes in dopac levels in the thalamus are most likely reflecting the effects of yohimbine on NE neurons whose cell bodies are in the locus coeruleus (LC). It has been shown (Salzman and Roth, J. Pharmacol. Exp. Ther., 212: 64,1980) that tyrosine hydroxylase (TH) in NE neurons is rapidly activated by stimulating the LC, and can also be activated by systemic administration of alpha-adrenergic antagonists (Gysling and Roth, in Function and Regulation of Monoamine Enzymes. Basic and Clinical Aspects, *Usdin et al. eds., p. 83, 1981*). This increased TH activity could lead to increased dopamine levels in the cytoplasm, where mitochondrial monoamine oxidase could convert some to dopac.

Locally applied yohimbine could be acting on the presynaptic alpha-2 receptors which mediate an inhibition of NE release. Systemic administration allows interaction at both the presynaptic sites, and the sites on the cell bodies in the LC which regulate cell firing rate.

394.11 CLONIDINE'S SITE/S OF ACTION IN THE NEUROCIRCUITRY FOR THE NOCICEPTIVE JAW-OPENING REFLEX. A.L. Curtis and J. Marwah. Neuroscience Laboratory, Dept. of Pathol., Univ. Med. & Dent. of New Jersey, Camden, N.J. 08103.

The rat maxillary tooth pulp stimulation(TPS) evoked jaw-opening reflex(JOR) is an oral-facial reflex generally utilized as a nociceptive paradigm. The threshold for eliciting the electromyogram (dEMG) from the digastricus(one of the jaw opening muscles) was utilized as an index of nociception. Both clonidine(Curtis & Marwah, Exp. Neurol. 91(3):524, 1986) and morphine rapidly, reversibly and significantly increased the TPS-dEMG threshold. Yohimbine (an alpha$_2$ adrenoceptor antagonist) but not prazosin (an alpha$_1$ adrenoceptor antagonist) or naloxone (a mu receptor antagonist) antagonized the effects of clonidine. Conversely, naloxone but not yohimbine antagonized the effects of morphine. Neither naloxone or yohimbine antagonized the threshold elevating effects of pentobarbital. Alpha$_2$ adrenoceptor agonists (e.g. naphazoline, ST-91 and 4 hydroxyclonidine) that do not easily cross the blood brain barrier were devoid of effects on the TPS-dEMG threshold. Clonidine elicited a biphasic effect on mean arterial pressure (MAP)- a transient pressor response followed by a sustained depressor effect. The effects of clonidine on MAP and dEMG threshold were not directly related. In addition, clonidine had no effect on dEMG threshold as elicited by stimulation of the peripheral end of the motor nerve (cut or intact) to the digastricus or on the dEMG elicited by the stimulation of the curarized digastricus. It is concluded that clonidine increases TPS-dEMG threshold by specifically interacting with centrally located alpha$_2$ adrenoceptors.

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394.12 AMILORIDE AS A PROBE OF ION MODULATION OF α_1- AND α_2-ADRENERGIC RECEPTORS. M.J. Howard, M.D. Mullen*, and P.A. Insel*. Division of Pharmacology, University of California, San Diego, La Jolla, California 92093

Alpha-adrenergic receptors mediate a variety of neural and hormonal responses to catecholamines. The binding of agonist at α_1- and α_2-adrenergic receptors is modulated by sodium; in general, sodium has been shown to decrease the affinity for agonist binding. Amiloride, by virtue of its competitive interaction with several membrane-associated sodium transport sites has been useful as a tool in studies of sodium ion permeation. We have used amiloride to probe the interaction of sodium with alpha-adrenergic receptors.

We used rat renal cortical membranes and the Madin-Darby Canine Kidney (MDCK) cell line in tissue culture as model systems for examining α_1- and α_2-adrenergic receptors. Radioligand binding studies demonstrated that amiloride bound to both alpha-adrenergic receptor subtypes with an affinity lower than that of the electrogenic sodium channel but similar to that of the Na^+/H^+ exchanger. The order of potency for amiloride analogues in competitive binding studies indicated that interaction at adrenergic receptors was different than at the membrane-associated transport sites. Both benzamil and ethylisopropyl amiloride (EIPA) were more potent than amiloride. Benzamil was slightly more potent than EIPA at α_1-receptors but these two analogues were approximately equipotent at the α_2-receptor. Amiloride was a competitive inhibitor at the α_1-receptor as indicated by an increase in the Kd for [^{3}H]-prazosin binding without a change in the maximal number of binding sites (Bmax). Amiloride competed for [^{3}H]-rauwolscine binding at α_2-receptors with a mixed type of inhibition; both the Kd and Bmax were altered in the presence of amiloride.

Results of competitive binding studies indicated that amiloride binds to a single class of receptor binding sites with a Hill slope close to 1. The guanine nucleotide Gpp(NH)p was unable to decrease the affinity of amiloride indicating that amiloride is an antagonist at alpha-adrenergic receptors. Sodium appeared to increase α_2-receptor affinity for amiloride while having no effect on α_2-receptor affinity. The results show that α_2-receptors may be associated with and modulated by an amiloride-sensitive sodium transport site but that association with a sodium transport site is not likely to be a feature of α_1-adrenergic receptors. We conclude that amiloride might be a useful probe for membrane receptors associated with a discrete sodium transport mechanism.

This work was supported in part by GM31987 and HL35847.

394.13 VENTRAL BUNDLE TRANSECTION DECREASES LOCUS COERULEUS SENSITIVITY TO ADRENERGIC REUPTAKE BLOCKERS WHILE INCREASING RESPONSIVENESS TO NOXIOUS STIMULATION. P. G. Simson, P. E. Simson and J. M. Weiss. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

Alpha-2 adrenergic receptors on neurons of the locus coeruleus (LC) regulate (inhibit) the firing of the LC. The origin of terminals that supply transmitter to stimulate these receptors is not clear. Whereas it was originally thought that terminals were found on recurrent collaterals of LC axons (Aghajanian, Cedarbaum & Wang, Brain Res. 136:570-577, 1977), recent studies call this into question (Andrade & Aghajanian, J. Neurosci., 4:161-170, 1984). The present experiment examined whether afferents from the ventral noradrenergic bundle (VB) might supply terminals which regulate firing of the LC. Several groups have observed noradrenergic afferents to the LC arising from cell groups of the VB (Cedarbaum & Aghajanian, J. Comp. Neurol., 178:1-16, 1978; Sawchenko & Swanson, Brain Res. Rev. 4:275-325, 1982). The VB is comprised of noradrenergic fibers originating in A1 and A2 cell groups of the medulla and receives contributions from more rostral A5 and A7 cell groups as well (O'Donohue, Crowley & Jacobowitz, Brain Res. 172, 1979:87-100).

In order to ascertain whether the VB afferents to the LC play any functional role in regulating the firing of the LC, the VB was bilaterally transected caudal to the LC and extracellular recordings were obtained from LC neurons. Coordinates for bilateral knife-cut lesions aimed at the VB were derived from O'Donohue, Crowley and Jacobowitz (Brain Res. Rev. 172:87-100, 1979) and transected the VB where it contains ascending fibers from the A1 and A2 cell groups, but not those of A5 or A7. Single unit recordings of LC neurons were made between 12 and 20 days post-lesion.

If terminals on VB projections supply transmitter which stimulates alpha-2 receptors on LC neurons, then transection of the VB should produce predictable changes in LC firing. First, we compared responsiveness of LC neurons to noxious peripheral stimulation (paw compression [PC]). Previous results in our laboratory (Simson & Weiss, accompanying abstract) showed that pharmacological blockade of alpha-2 receptors increases responsiveness of LC neurons to PC. If VB supplies transmitter to stimulate alpha-2 receptors on LC, then transection of VB should produce effects similar to blockade of these receptors. VB-lesioned rats indeed were more responsive to PC than controls. Second, we examined the effectiveness of desmethylimipramine (DMI) in inhibiting LC activity. DMI blocks reuptake of norepinephrine and thereby heightens stimulation of alpha-2 receptors. Results indicated that DMI at all doses employed was significantly less effective in inhibiting firing of LC neurons in VB-lesioned animals than in controls, thereby indicating that VB-lesioned animals have decreased NE available to alpha-2 receptors in the LC.

Since alterations in the response of LC neurons to sensory and pharmacological manipulations were produced by transection of the VB, these results suggest that the VB plays a functional role in regulating the firing of the LC.

394.14 ALPHA-ADRENERGIC ANTAGONISTS INCREASE THE RESPONSIVENESS OF LOCUS COERULEUS NEURONS TO EXCITATORY STIMULATION WITHOUT ALTERING SPONTANEOUS ACTIVITY. P. E. Simson and J. M. Weiss. Dept. of Psychiatry, Duke University Medical Center, Durham, NC 27710.

The locus coeruleus (LC) is inhibited by adrenergic agonists acting through alpha-2 receptors. When this phenomenon was discovered by Aghajanian and his colleagues, it was initially believed that the activation of alpha-2 adrenoreceptors inhibited LC firing by producing a period of quiescence following a burst of LC firing (Aghajanian, G. K., Cedarbaum, J. M. and Wang, R. Y., Brain Research, 136:570-577, 1977). As evidence for this, blockade of alpha-2 adrenoreceptors reduced this post-burst quiescence. However, more recent studies from this same group (Andrade, R. and Aghajanian, G. K., J. Neurosci., 4:161-170, 1984) now suggest that quiescence following a burst of LC firing is not dependent on activation of alpha-2 adrenoreceptors, but rather results from inherent membrane changes in ionic conductance. Thus, the nature of the alpha-2 adrenoreceptor mediated inhibition of the LC remains unclear. Since the LC responds phasically to sensory inputs, the present study examined the possibility that alpha-2 adrenoreceptors inhibit the LC by regulating the initial excitability of LC neurons. To test this hypothesis, the effect of blockade of alpha-2 adrenoreceptors on the responses of LC neurons to excitatory stimulation was studied.

Extracellular recordings of LC neurons were obtained in fifty one male Sprague Dawley rats. Compression of the contralateral hind paw was found to produce a burst of LC activity followed by a quiescent interval. In the first experiment, the alpha-2 adrenergic antagonist idazoxan was intravenously administered. This drug markedly potentiated the responses of LC neurons to contralateral hind paw compression (PC) at doses far below those which altered the spontaneous activity of the LC. This increase in LC responsiveness after idazoxan treatment was accompanied by decreased suppression (i.e., reduced quiescence) in the initial period following the termination of PC. In the second experiment, a large increase in the response of LC neurons to PC was achieved when low doses of idazoxan were infused directly into the LC, thereby showing that the alpha-adrenergic antagonist increases the responsiveness of LC neurons to PC, rather than increasing the amount of input to the LC. In the third experiment, the alpha-adrenergic antagonist yohimbine also increased the responsiveness of LC cells to PC, demonstrating that the effects seen in the first two experiments are not limited to one particular alpha-adrenergic antagonist. In the final experiment, idazoxan was found to exacerbate the response of LC neurons to peripheral injection of nicotine, showing that the potentiation of LC responsiveness by alpha-adrenergic antagonists is not limited to noxious cutaneous stimulation.

The finding that alpha-2 adrenergic antagonists increase the responsiveness of LC neurons to excitatory stimulation at doses far below those required to alter baseline activity suggests that alpha-2 adrenergic receptors in the LC function to modulate the excitability of LC neurons to incoming stimuli.

DEVELOPMENT AND PLASTICITY: AGING II

395.1 QUANTITATIVE ASSESSMENT AND COMPARISON OF LHRH-IMMUNOREACTIVE NEURONS IN YOUNG, MIDDLE-AGED AND OLD FEMALE MICE. L.L. Don Carlos*, G.E. Hoffman and C.E. Finch. (SPON: J.A. Finkelstein) Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642 and Andrus Gerontology Ctr., USC, Los Angeles, CA 90089.

Reproductive senescence in female mice is associated with a cessation of the cyclic luteinizing hormone surge that may be due to age-related changes in luteinizing hormone releasing hormone (LHRH) regulation. Hoffman and Finch (Neurobiol. Aging 7:45, 1986) recently reported that no differences exist in the number of LHRH-IR (LHRH-immunoreactive) neurons in middle aged (15-16 mo) C57BL/6J female mice compared to young adults (5-6 mo). The present study was performed to extend these observations and to examine the possibility that, while numbers of LHRH-IR neurons do not change into middle-age, 1) numbers of LHRH-IR neurons may decrease after their function ceases; 2) age-related alterations in LHRH-IR neuron morphology occur, and 3) LHRH-LHRH interactions decrease with age. Immunocytochemically identified LHRH neurons were examined in young adult (5-6 mo, n=5), middle-aged (16-17 mo, n=5) and old (22-23 mo, n=6) C57BL/6J female mice. All animals were ovariectomized 2 mo prior to sacrifice to eliminate the effects of ovarian steroids on LHRH stores. Beginning 900 µm rostral to OVLT, every sixth section was examined through 4860 µm. LHRH-IR cells were examined by an observer blind to the age of the animal for 1) total numbers of LHRH-IR cells; 2) morphology (either smooth, irregular or unknown); and 3) LHRH-IR fiber apposition on LHRH-IR cells or dendrites. Estimated cell totals were obtained by multiplying cell counts by 6. The results are presented in tabular form, expressed as mean and standard error of the mean for each group, rounded to the nearest whole number (contacted cells = any cell morphology).

	Young	Middle-age	Old
LHRH Cells	1211 ± 103	1207 ± 114	1107 ± 58
Smooth	197 ± 33	130 ± 11	127 ± 15
Irregular	314 ± 51	293 ± 28	287 ± 46
Contacted	601 ± 57	488 ± 37	535 ± 47

The data were analyzed by analysis of variance. No statistically significant differences were obtained for any of the parameters examined. Categorization of cell morphology into smooth or irregular classes was difficult for the majority of cells. Hence, structural correlates of functional differences in LHRH regulation during aging may not be discerned at the light microscopic level. These studies may indicate that the LHRH system is resistant to neuron loss despite functional decrements. Supported by the NIA (AG 0046 and T32 AG107) and NIH (NS 13725).

395.2 ORAL ADMINISTRATION OF ESTRADIOL INDUCES AGE-LIKE REPRODUCTIVE ACYCLICITY IN C57Bl/6J MICE. S. Kohama*, P. May, C. Finch
Department of Biology, U.S.C., Los Angeles, CA 90089-0191

Exogenous estradiol (E2) given to intact female mice via depot injection or subcutaneous implants induces an age-like loss of cyclicity (Mobbs et al., Biol Repro 30:556, 1984). In order to better evaluate the dose-response relationship between E2 and cyclicity, E2 was administered orally via the drinking water. The oral route of E2 administration allows a non-invasive, stress-free and more controllable method for studying E2-dependent effects. The ingestion of E2 results in a diurnal pattern of exogenous E2 that is sufficient for stimulation of E2-dependent changes in physiological markers (Gordon et al., Neuroscience Soc.Abs. 164.7, 1985).

In Study I, 0 or 850 ug E2/kg wt/day was administered orally for 6 or 12 weeks to intact 4 mo old C57Bl/6J mice; post-treatment cyclicity was evaluated. Oral E2 progressively increased the occurance of permanent post-treatment acyclicity to 100% by 12 weeks of E2 exposure ($p<0.05$ chi-square). A single 0.2 mg sc dose of E2 valerate (EV) was given to another group of intact females as a positive control for the induction of acyclicity. The EV group as expected was acyclic within 4 weeks, while vehicle controls remained cyclic. These data were replicated in Study II. Studies I and II indicated that orally administered E2 was effective in inducing acyclicity in intact female mice, but did not provide information on the minimal time-dose level of E2 necessary for the effect.

In study III, ovariectomized (ovx) mice were treated for 6 weeks with 0, 50, 450 or 850 ug E2/kg wt/day. All groups then received ovarian grafts and vaginal cyclicity was assessed. Unlike Studies I and II, oral E2 did not induce significant acyclicity. Overall, cyclicity in mice with grafts declined with advancing age, as expected (MANOVA $p<0.001$), but E2 treatment had no effect. The presence of the ovary during oral E2 treatment may cause elevated steroidal levels, and/or an increased time period of steroid exposure not seen in ovx mice. Therefore, in Study IV ovx mice were treated with vehicle or 850 ug E2/kg wt/day for 12 weeks; then, were given ovarian grafts and cyclicity was monitored. The 12 weeks of E2 treatment caused 60% of the mice to become permanently acyclic by 3 months post-transplant, whereas 100% of the controls were cycling ($p<0.005$ chi-square). Thus, high but not pharmacological doses of E2 can cause an age-like reproductive deficit independent of the ovaries. However, intact animals treated with E2 go into an acyclic state much sooner than ovx animals, suggesting ovarian contributions accelerate the E2-induced acyclic state.

This study was supported by NIA Training Grant T32-AG00093.

395.3 ESTIMATION OF IN VIVO LHRH RELEASE IN MIDDLE-AGED AND YOUNG FEMALE RATS BY PUSH PULL PERFUSION OF THE MEDIOBASAL HYPOTHALAMUS. B.S. Rubin and R.S. Bridges. Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115.

Middle-aged (MA) females show attenuated LH surges on proestrus and following ovariectomy (ovx) and steroid treatment. Several lines of evidence suggest that alterations in LHRH release may be responsible, in part, for this age-related dimunition in gonadotropin secretion. We have recently reported (Neurobiology of Aging, 6:309, 1985) that the decreased LH surge induced in ovx'd steroid treated MA females does not result from decreased hypothalamic LHRH content; LHRH levels measured in MA females were as high or higher than levels in identically treated young (Y) animals. In the present study, we attempted to estimate LHRH release in the two age groups using this steroid primed model and the push pull perfusion method (PPP) pioneered by Levine and Ramirez for LHRH measurement (Endocrinology, 107:178, 1980). Regularly cycling Y (3-4 months old) and MA females (10-12 months) exhibiting lengthened estrous cycles were ovx'd and one week later were implanted with PPP guide cannulae directed toward the median eminence (ME). Three weeks after ovx, females were injected with estradiol benzoate (4 μg/100g BW; Day 0) and progesterone (0.8 mg/100g BW, Day 2, 10 am). At 1 pm on Day 2, inner cannulae assemblies were inserted and artificial CSF was pushed through the cannulae and drawn up at a rate of 20 μl/min. Samples were collected on ice for 12 minute intervals until 7 pm and LHRH was measured in the perfusates by RIA with anti-LHRH, EL-14 (Ellinwood, W.E., Peptides, 6:45, 1985).

Peak levels of LHRH measured in 7 MA and 9 Y females with verified cannulae placements in the medial basal hypothalmus (MBH) close to or in the ME did not differ (mean Y = 2.8±0.7 pg; mean MA = 4.6±1.8 pg; $p>0.20$). Overall LHRH profiles, however, did differ in the two age groups. A greater percentage of the samples collected from MA females (65%) contained levels of LHRH below the detectability of the assay (<0.2 pg) than did samples obtained from Y animals (21%; $p<0.001$). This difference is exemplified further when mean LHRH values of the samples from the 1 hour time period prior to the LHRH peak are compared in the two age groups (mean Y = 1.2 pg; mean MA = 0.3pg, $p<0.025$). The data collected thus far suggest that lower levels of LHRH may be released into the pituitary portal system in MA animals in the hours prior to the time of the LH surge. Such a decrease of LHRH release in MA females could result in reduced LHRH priming of the pituitary prior to the time of peak LHRH release and thereby contribute to the reduced LH surge observed in MA females. This research was supported by NIH Grant R23 HD 19174 awarded to BSR and NIMH Grant K02 MH 00536 to RSB

395.4 ACETYLCHOLINE RELEASE AND HIGH ENERGY METABOLITES IN NEOSTRIATAL SLICES FROM YOUNG ADULT AND AGED RATS. M.S. Weiler and C.H. Lee*. University of Wisconsin-Madison, School of Pharmacy, Madison, WI 53706.

Several neurochemical studies have addressed the question of age-related changes in cholinergic systems of central nervous tissue. In the neostriatum specifically, age-related changes in acetylcholine (ACh) synthesis (Sims et al., J. Neurochem. 38: 488, 1982) and drug-induced release of radioisotopically labelled ACh (Thompson et al., Brain Res. 299: 169, 1984) have been observed. The purpose of the present study was to determine whether endogenous as well as newly synthesized ACh release from rat neostriatum is changed in aged rats and whether differences in neostriatal slice energy metabolism could account for changes in ACh release.

Slices were prepared from the neostriatum of young adult (10-month) and aged (28-month) Fischer 344 rats, preincubated in Krebs Ringer (KR) bicarbonate buffer at 36°C for 1 hr, then transferred to fresh KR buffer or KR buffer with 25mM K^+ (each containing eserine, 30 μM) to monitor, respectively, spontaneous and potassium-stimulated ACh and choline (Ch) release. ACh and Ch were determined by gas chromatography mass spectrometry. Spontaneous release of ACh from neostriatal slices from aged rats was not significantly different from control, but potassium-stimulated ACh release was significantly depressed by 38% ($p<.001$; $n=4$). Spontaneous and potassium depolarized release of Ch and the tissue concentration of ACh and Ch were similar in both preparations. These results confirm the results of other investigations and demonstrate that endogenous as well as isotopically labelled ACh release is depressed specifically in the neostriatum of aged rats.

Since changes in high-energy metabolites could influence ACh release from the aged slices, tissue levels of high-energy metabolites, i.e., adenylate metabolites (ATP, ADP, AMP) and phosphocreatine (PCr), were measured over 8 hrs by the luciferin-luciferase technique (Lust et al., Anal. Biochem. 110: 258, 1981). ATP levels were 18-20% lower in aged slices during the first 90 min, but after 2 hr ATP concentrations were similar in both age groups. Also, PCr/ATP ratios and the energy charge of the slices exhibited similar rapid rates of recovery (30 min) and similar steady-state values after 2 hrs (PCr/ATP ratio: 2.38 ± 0.21, young adult, and 2.43 ± 0.08, aged group. Energy charge: 0.78 ± 0.04, young adult and 0.79 ± 0.02, aged group). This possible relation between decreased ACh release during decreased ATP levels should be guarded against in such assays. (Supported by PMA Foundation Research Starter Grant and NIH Grant AG05953).

395.5 AGE-RELATED DENDRITIC CHANGES IN THREE POPULATIONS OF STRIATAL NEURONS IN THE C57BL/6NNIA MOUSE. J.A.Rafols and T.H.McNeill. Dept. of Anatomy, Wayne State Univ., Sch. of Med., Detroit MI 48201 and Dept. of Neurology, Univ. of Rochester, Rochester, N.Y. 14642.

The Van der Loos modification of the Golgi-Cox method and morphometric analyses were used to examine age-related changes in dendritic parameters of three different populations of striatal (caudate-putamen) neurons. Six age groups (3,6,10,20,25 and 30 months) were represented in this study. In all age groups, the medium spiny neuron (spiny I) and two types of aspiny neurons (aspiny I and II) were analyzed (classification after DiFiglia et al., Brain Res. 114: 245, 1976). The spiny I neuron was characterized by a medium sized (11-17 um) soma, four to eight spine-free dendritic trunks and numerous spine-laden dendrites. Within this type, however, three subpopulations were distinguished in all age groups based on measurements of total dendritic lengths. With advancing age there was shift in the distribution of these subpopulations such that neurons with shorter dendritic arbors (< 700um) predominated in the older groups (20,25 and 30 months). Concomitant with decreases in dendritic length other changes observed in the older groups included: 1) irregular swellings occurring primarily on the distalmost segments of the dendrites, 2) changes in spine density (both increments and decrements) along the dendrites, and 3) presence of ectopic spines on the soma and dendritic trunks. In contrast the aspiny I neuron had a medium sized (13-22um) soma and thin varicose dendrites. With advancing age the total length of the dendritic arbor of some of these neurons increased due to both an increase in the number of dendritic branching points and the length of the distal dendrites. Finally the aspiny II neuron was the third type analyzed. This cell had a large, bulbous soma (23-35 um), 3-5 thick dendrites trunks and thin varicose dendrites many of which recurved around the soma. Some spines were found in all ages but primarily in the younger groups. With advancing age the total length of the dendritic arbor of some of these neurons also increased due to lengthening of the dendritic trunks and terminal dendrites. In addition, in the old mouse (30 month old) a few of these neurons exibited irregular swellings on the distalmost dendrites suggestive of early regressive changes. Our data shows that each population of striatal neurons undergo unique morphological changes with aging. It also indicates that with advancing age some but not all the neurons of a given population may undergo compensatory growth or regression of their dendritic arbors. This suggest in turn a selective sensitivity of some cells to the aging processes. Supported by PHS grant AG03254 and a grant from the Wayne State University Neuroscience Program.

395.6 CHOLINERGIC AND GABAERGIC PROJECTIONS FROM PALLIDAL AND PERIPALLIDAL REGIONS TO THE NEOCORTEX OF THE CAT: DEMONSTRATION BY DOUBLE PEROXIDASE LABELING OF THEIR NEURONS OF ORIGIN. R.S. Fisher, J.S. Asai*, B.H. Wainer, N.A. Buchwald and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024 and Department of Pharmacological and Physiological Sciences. University of Chicago, Chicago, Il 60637.

The existence of cholinergic and GABAergic afferents to the frontal and occipital pole neocortical regions of the cat were independently and directly demonstrated by the specific double peroxidase labeling of agranular neurotransmitter markers (choline acetyltransferase-CHAT and glutamic acid decarboxylase-GAD, the synthetic enzymes of acetylcholine and GABA, respectively) and a granular connectivity marker (HRP). Based on correlative light and electron microscopic identification, the transmitter markers and the connectivity marker were associated with different organelles within the perikaryal cytoplasm of the tract's neurons of origin.

Doubly labeled (CHAT + HRP; GAD+HRP) neurons were prevalent and located in overlapping anatomical regions in the medial septal nucleus, the vertical and horizontal limbs of the nucleus of the diagonal band, the substantia innominata and the globus pallidus in specifically stained sections obtained from 4 cases. Both types of projection neurons were polymorphic with medium to large somatic areas and fusiform to spherical somatic shapes. While neurons singly labeled by the connectivity marker were more frequent after HRP injections into the frontal pole, doubly labeled neurons containing HRP combined with CHAT or GAD were evident for both the frontal and occipital pole cases. These results indicated that basal forebrain projections to the neocortex are heterogeneous because they may be mediated by either acetylcholine or GABA. To our knowledge, this is the initial demonstration of an extracortical source of GABAergic inputs to neocortical target neurons. Supported by USPHS Grants AG01558, HD05958, NS17661 and HD04583.

395.7 BASAL FOREBRAIN NEURONS WITH AXON COLLATERALS TO WIDELY SEPARATED CORTICAL TARGETS: A DOUBLE RETROGRADE TRANSPORT STUDY IN THE CAT. M.K. Boylan*, C. Adams, C. Cepeda*, R.S. Fisher, B.H. Wainer, N.A. Buchwald and M. S. Levine. Mental Retardation Research Center, University of California, 760 Westwood Plaza, Los Angeles, CA 90024 and Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

The basal forebrain (BF) provides a widespread innervation of the cerebral cortex (CX). We have shown that BF neurons send axonal collaterals to adjacent sites in the neocortex in the cat. However, the existence and extent of axonal branching of BF neurons remains controversial.

The extent of cortical innervation provided by single BF branched axons was assessed using double retrograde labeling. Horseradish peroxidase (HRP) and the fluorescent marker nuclear yellow (NY) were pressure-injected into two different ipsilateral cortical sites in adult cats. One group of animals (N=3) received marker injections into the medial precruciate and anterior ectosylvian gyri (pre/ecto); a second group (N=4) received injections into the medial precruciate gyrus and the caudal marginal gyrus (pre/marg). After peroxidase histochemical processing (TMB method) single and double-labeled neurons were found within all areas of the BF (the globus pallidus (GP), the basal nucleus of Meynert, the vertical and horizontal limbs of the diagonal band of Broca, and the medial septal nucleus). For both injection combinations, single- and double-labeled neurons arose predominately from the substantia innominata and GP and less frequently from the other BF areas. Both branched and unbranched axons arose from similar populations of medium-sized polymorphic neurons located in overlapping BF regions. A larger proportion of HRP-NY cells projected to the pre/ecto than to the pre/marg cortices.

These results suggest that the axons of single BF neurons can innervate widely separated cortical targets over a relatively unrestricted intercortical distance. Within our series of experiments on BF collaterals to the CX, double-labeled BF cells have been seen in all cortical injection combinations tested. This indicates that collateralization of BF neurons may be a common occurrence although these collaterals may arise only from a small population of cells for each combination of cortical targets. Supported by USPHS Grants AG01558, HD05958, NS17661 and HD04583.

395.8 ELECTROPHYSIOLOGICAL PROPERTIES OF BASAL FOREBRAIN NEURONS: EFFECTS OF ANESTHESIA. C. Cepeda*, N.A. Buchwald, and M.S. Levine. Mental Retardation Research Center. University California, Los Angeles. 760 Westwood Plaza. Los Angeles, CA 90024.

These experiments were designed to compare the effect of variation of anesthetic on the neurophysiological activity of basal forebrain (BF) neurons. Cats were anesthetized either with a mixture of fluothane and nitrous oxide or with α-chloralose. Variation in the anesthetic produced a series of changes in spontaneous and evoked activity of BF neurons. In chloralose anesthetized cats neurons fired less rapidly and displayed more excitatory responses when their inputs were activated. Of more importance, however, proportionately more BF neurons were antidromically activated by cortical stimulation in chloralose anesthetized animals indicating that the anesthetic condition may determine which recorded neurons are considered BF projection cells.

A total of 82 neurons was recorded from 15 cats anesthetized with fluothane and nitrous oxide and 86 neurons in 9 cats anesthetizd with chloralose. In anìmals receiving fluothane and nitrous oxide the majority of cells (46%) exhibited firing rates of more than 5 spikes/sec. About 40% of the neurons had slower firing rates (interspike intervals between 200-1000 msec). The remainder displayed action potentials infrequently (10%) or were silent (4%). In cats anesthetized with chloralose BF neurons exhibited slower firing rates. Proportionately, more neurons were encountered with the slower firing rates (28%) or were silent (12%). In both types of preparations the patterns of spontaneous firing were diverse. Many neurons discharged in bursts. About 8% of the neurons exhibited rhythmic or semi-rhythmic patterns, firing at about 3 spikes/sec.

The responsiveness of BF neurons to afferent activation was assessed by electrical stimulation of amygdala and precruciate cortex. The properties of responsive neurons were similar in both preparations (44% and 80% of the neurons responded to cortical and amygdala stimulation, respectively, in fluothane anesthetized cats while 47% and 80% of the neurons responded to cortical and amygdala stimulation, respectively, in chloralose anesthetized animals). In contrast, excitatory responses were encountered more frequently in the chloralose anesthetized than in the fluothane anesthetized preparation (cortical stimulation: 46% chloralose vs 21% fluothane, amygdala stimulation: 53% chloralose vs 35% fluothane). The proportion of BF neurons that displayed antidromic responses to cortical stimulation was also greater in chloralose anesthetized animals (7.5% vs 1.5% in chloralose vs fluothane anesthetized preparations). Supported by USPHS AG 01558 and HD 05958.

395.9 ELECTROPHYSIOLOGICAL PROPERTIES OF BASAL FOREBRAIN NEURONS: RESPONSES TO MICROPHORETIC APPLICATION OF PUTATIVE NEUROTRANSMITTERS. M.S. Levine, C. Cepeda*, and N.A. Buchwald. Mental Retardation Research Center. University California, Los Angeles. 760 Westwood Plaza. UCLA Los Angeles, CA 90024.

These studies were designed to assess the effects of microphoretic application of putative neurotransmitters on the neurophysiological activity of basal forebrain neurons in chloralose-anesthetized cats (n=9). The effects of glutamate (1M), gamma-aminobutyric acid (GABA) (1M), carbachol (0.2M) and kainic acid (0.2M) were determined. Glutamate produced a sharp, short-latency excitatory response in 64% (32/50) of the neurons tested. GABA produced a variable latency inhibitory response in 91% (52/57) of the neurons tested. Changes in firing rates produced by either glutamate or GABA terminated rapidly after ejection currents were turned off. Carbachol, produced long-latency excitatory responses in 38% (15/40) of the neurons. In a majority of the neurons the increases in firing produced by carbachol continued after drug ejection was terminated. All neurons responded to kainic acid (8/8) with marked excitatory responses. As with carbachol spontaneous firing remained elevated in these neurons after kainic acid application was discontinued.

The responses to electrical stimulation of basal forebrain afferents from the precruciate cortex and amygdala were compared with the responses to the microphoretic application of glutamate, GABA and carbachol. The majority of the neurons (11/13) that responded to precruciate cortical stimulation with either excitation or excitation followed by inhibition were also excited by glutamate. Similarly most neurons that did not respond with excitation to the cortical stimulus were non-responsive to glutamate (9/13). Amygdala stimulation produced both excitatory and inhibitory responses. All neurons responding to stimulation of the amygdala were inhibited by GABA. About 65% (9/14) of the neurons excited by amygdala stimulation were also excitated by carbachol. These cells did not respond to cortical stimulation.

These results indicate that basal forebrain neurons may receive an excitatory glutaminergic input. These neurons also receive GABAergic inhibitory inputs. A proportion of them are also sensitive to carbachol, a cholinergic agonist. Supported by USPHS AG 01558 and HD 05958.

395.10 AGE-RELATED DECREMENTS IN THE POTENTIATION OF ENDOGENOUS DOPAMINE RELEASE FROM SUPERFUSED RAT STRIATAL SLICES BY MUSCARINIC AGONISTS. J.A. Joseph, T.K. Dalton* and W.A. Hunt*. The Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Previous experiments have indicated that the release of striatal dopamine (DA) is controlled by inhibitory DA autoreceptors (eg., Parker and Cebeddu, JPET, 232: 492, 1985), and that the activity of these DA autoreceptors is modulated via cholinergic heteroreceptors located on nigrostriatal DA nerve terminals. Thus, muscarinic agonists potentiate the K+ evoked release of both endogenous DA and [3H] DA release from the striatum (eg., Raiteri et al, JPET, 228: 209, 1984). Since diminution of striatal functioning seems to be a consistent finding in aged animals resulting in such changes as declines in psychomotor performance, it was of interest to determine if the relationship between DA autoreceptors and cholinergic heteroreceptors might be altered as a function of aging. Striatal tissue slices (300 mu) obtained from mature (6 mo) middle aged (12 and 18 mo), and senescent (Wistar rats) were placed in 50 ul superfusion chambers and superfused at the rate of 124 ul/min with a modified Krebs-Ringer basal release medium containing (in mM) NaHCO3 21, glucose 3.4, NaH2PO4 1.3, EGTA 1, MgCl2 .93, NaCl 127, KCl 2.5 (Ph 7.4) After a 30 min equilibration period, a 5 min baseline fraction was collected from each chamber. The medium was then switched to one containing 30 mM KCl, CaCl2.2H20 1.26 mM (in place of EGTA), and NaCl 57 Mm in addition to the components described above (Ph 7.4). To this "Hi KCl buffer" was added one of four concentrations (0, 100 uM, 500 uM, and 1mM) of a particular muscarinic agonist (i.e. oxotremorine, carbachol, pilocarpine and bethanecol). Six 5 min fractions were taken during depolarization with hi KCl. DA release was assessed in these fractions using high performance liquid chromatography coupled to electrochemical detection. Results showed that DA release during baseline was negligible for both young and old. No differences were seen among the striatal tissue obtained from the various groups of animals in KCl-induced release of DA without muscarinic agonists (eg., mature release = 58.09 + 10.66 pmoles/mg protein, 24 mo = 70.28 ± 14.40 pmoles/mg protein). However, when muscarinic agonists were added the KCl-induced release of DA was enhanced in striatal tissue obtained from all the groups but the 24 mo group (eg., peak carbachol enhancement mature 253%, 12mo 165%, 18 mo 172% 24 mo 5%). These findings suggest that some of the decrements in striatal functioning may be traced to age-related alterations in cholinergic-dopaminergic interactions such that DA autoreceptors are no longer under adequate inhibitory control by the cholinergic heteroreceptors.

395.11 REDUCTIONS IN NEUROTRANSMITTER RECEPTORS IN TEMPORAL CORTICES OF AGED RHESUS MONKEYS. M.V. Wagster, P.J. Whitehouse, L.C. Walker, K.A. Yagaloff*†, P.R. Hartig†, K.A. Marcus* and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2182; †Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218.

Little is known about age-related neurotransmitter receptor changes in the cerebral cortices of nonhuman primates. Using in vitro receptor autoradiography, we mapped the distribution of cholinergic ([^{3}H] N-methyl scopolamine and [^{3}H] pirenzepine) and serotoninergic ([^{125}I] lysergic acid diethylamide) receptor binding in temporal cortex in 17 rhesus monkeys (Macaca mulatta) ranging in age from 2-22 years. The number of monkeys in the four age groups and their approximate ages included: five animals, two years old; 2-3 animals, 6-7 years old; two animals, 13 years old; and 6-7 animals, 18-22 years old. [^{3}H] N-methyl scopolamine (using carbachol to displace high-affinity agonist sites and atropine to measure nonspecific binding sites) was selected to measure high- and low-affinity agonist muscarinic cholinergic receptor subtypes. A 36% decrease in low-affinity agonist binding concentration and a 31% decrease in high-affinity agonist binding concentration was demonstrated in 18-year old monkeys as compared to two-year old animals. [^{3}H] Pirenzepine, a novel antagonist that binds to a subtype of cholinergic receptor, showed a 21% decrease in binding in the older animals. [^{125}I] Lysergic acid diethylamide binding sites were diminished by 64% in the oldest monkeys. For all ligands, the oldest group of monkeys had significantly fewer binding sites than the youngest group (ANOVA, $p \leq 0.05$). Data from monkeys at intermediate ages suggest that the number of receptor binding sites declines throughout the age range studied, but additional subjects are needed to determine the rate of decline. Future studies of receptor populations will allow delineation of age-related alterations of receptors in brains of aged nonhuman primates. Tissues were obtained from the Regional Primate Research Center at the University of Washington, supported by NIH grant RR 00166.

395.12 AGONIST SUBTYPES OF THE MUSCARINIC CHOLINERGIC RECEPTOR IN FRONTAL CORTEX OF AGED RATS. N.W. Pedigo. Depts. of Pharmacology and Anesthesiology, Univ. of Kentucky Medical Ctr., Lexington, KY 40536

Previous studies have shown an age-related loss of muscarinic receptors in rat frontal cortex, as indicated by reduced numbers of ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) binding sites (i.e., decreased Bmax) without changes in the affinity (Kd) of ^{3}H-QNB for its receptor. However, it is not known whether this loss of ^{3}H-QNB binding sites represents a selective decline in agonist subtypes of the muscarinic receptor. Subtypes of the muscarinic cholinergic receptor in rat brain and other tissues have been characterized in radioligand binding experiments by shallow displacement curves for muscarinic agonist drugs, such as oxotremorine or carbachol. In these studies, oxotremorine inhibition of ^{3}H-QNB binding was analyzed by nonlinear, iterative curve-fitting procedures (SAS, NLIN) based on a model of two independent sites with high affinity (K_H) or low affinity (K_L) for the muscarinic agonist. Binding parameters derived from such data included K_H, K_L and A, A being the proportion of total binding sites having high affinity for oxotremorine. Values for K_H, K_L and A in frontal cortex (FC) of young (3 months old), adult (9 months old) and senescent (27 months old) Fischer 344 rats are provided in Table 1:

Age	A(%)	K_H(μM)	K_L(μM)
3 months	12.2	0.27	7.4
9 months	12.8	0.13	7.4
27 months	13.3	0.12	7.8

Analysis of variance statistics revealed no age-related changes in the binding parameters for high- and low-affinity agonist subtypes of the muscarinic receptor. Similar results were seen in hippocampus (HC), corpus striatum (CS) and hypothalamus (HY). K_H and K_L values were similar in the different brain regions, but the proportion of high-affinity sites varied significantly (HY > FC > CS = HC). These results indicate that agonist subtypes of the muscarinic receptor may not be selectively affected by aging, despite the overall loss of ^{3}H-QNB binding sites which occurs with advanced age. However, possible age-related alterations in the regulation of these sites or coupling of muscarinic receptors to second messenger systems requires further study. (Supported in part by grants from the American Federation for Aging Research and National Institutes on Aging, AG-06390-01).

395.13 EFFECTS OF AGE ON SERUM MELATONIN LEVELS AND BINDING SITES FOR MELATONIN AND GABA. O.M. Pulido* and L.P. Niles (SPON: G. Smith). Department of Neurosciences, McMaster University, Hamilton, Ontario Canada L8N 3Z5.

The biosynthetic capacity of the pineal gland appears to be significantly reduced in aged mammals and an age-related decrease in serum levels of the pineal hormone, melatonin, has been observed in aged male Syrian hamsters. In the present study we have maintained diurnal circulating levels of melatonin in young and old male Long Evans hooded rats. In order to determine the relationship between melatonin levels and binding, we have measured ^{3}H-melatonin binding at two points in the diurnal cycle in comparable groups of animals. High affinity binding of the tritiated γ-aminobutyric acid (GABA) agonist, ^{3}H-muscimol, was also determined.

Young (2-3 months old) and aged (21-23 months old) rats were maintained under a 14L:10D lighting cycle with lights on from 5 a.m. to 7 p.m. Serum melatonin, as measured by radioimmunoassay, exhibited a marked circadian rhythm with low levels during the daytime and elevated levels during darkness in young animals. A diurnal cycle in serum melatonin was also present in aged rats; however, their hormone levels were lower at all the times examined.

In young animals, ^{3}H-melatonin (20 nM) binding in hypothalamic membranes was significantly higher at 9 a.m. than at 10 p.m. In contrast, aged animals did not show significant differences in binding which remained elevated at the two times examined.

High affinity GABA receptor binding, as measured with ^{3}H-muscimol (5 nM), was significantly lower in the hippocampus of aged rats as compared with young animals. Conversely, aged animals exhibited higher binding in cerebellar membranes.

These findings are consistent with reports that circulating melatonin levels are reduced with age and that neurotransmitter receptors undergo age-related changes in the mammalian CNS. The significant difference in morning and nighttime ^{3}H-melatonin binding in young animals appears to be due to down-regulation of central binding sites for this hormone during darkness, when peak circulating levels occur, since melatonin readily crosses the blood-brain barrier.

Saturation experiments will be carried out in order to confirm the above findings and to determine whether changes in binding site density or affinity are involved.

(Supported by the Gerontoloty Research Council of Ontario and the Ontario Mental Health Foundation).

395.14 ALPHA-1-ADRENERGIC RECEPTORS ARE ALTERERED IN SPECIFIC BRAIN AREAS OF AGED FEMALE RATS. N.G. Weiland and P.M. Wise. Department of Physiology, School of Medicine, University of Maryland, Baltimore, MD 21201.

The progressive loss of estrous cyclicity in female rats is associated with first, an increase in irregularity of cycles, secondly, a stage of constant vaginal estrous (CE), and finally a period of persistent diestrous (PD). The noradrenergic system is an important regulator of luteinizing hormone (LH) secretion, and evidence suggests that activation of alpha(α)-1-adrenergic receptors is prerequisite to an LH surge. We measured α-1 receptors in old and young females to determine whether the loss of cyclicity in aging females is associated with a localized decline of α-1 receptors only in those regions which control LH release or a more generalized decline throughout the brain, and whether any observed changes are related to the vaginal smear patterns of the animals.

Four groups of female Sprague-Dawley rats; young estrous, young diestrous, old CE and old PD, were killed at 1000 h. Serum was assayed by RIA for estradiol and LH. The brains were frozen and sectioned at 20μ. We used 3H-prazosin, a specific α-1 antagonist, and computerized quantitative autoradiography to measure the density of receptors in specific brain nuclei.

The density of α-1 receptors in old PD rats declined in medial preoptic nucleus (MPN), the median eminence (ME), and the pineal gland, whereas the density increased in the ventral medial nucleus (VMN) and the premamillary nucleus dorsalis. In contrast, in CE rats the age-associated decline was observed only in the pineal gland and an increase only in VMN. No estrous versus diestrous differences were found in the young animals. No age- or vaginal smear-related differences were observed in the suprachiasmatic nucleus, arcuate nucleus, or caudate putamen. LH concentrations in serum were basal and equivalent in all groups. Serum E2 levels in old CE rats were elevated over those in PD rats but only to levels observed in young diestrous females.

In conclusion, the decrease in density of α receptors with age in PD rats occurs only in specific brain regions, MPN, ME, and pineal, which regulate LH secretion. An increase is observed in VMN, another estrogen responsive area which is involved with sexual behavior. In old CE rats, the alterations in the density of receptors are more limited. It is possible that constantly elevated levels of E2 may partially protect the animals from the age-associated alterations in α-1 receptor concentrations. Therefore, age-related changes in the density of α receptors do not involve a generalized decline in brain, but rather appear to be localized and progressive. These changes are more extensive in PD rats which are in a later stage of reproductive decline. Supported by NIH AG-0537 to NGW and AG-02224 to PMW.

395.15 AGE-RELATED CHANGES IN RAT CEREBELLAR NE RELEASE AND ADRENO-RECEPTIVITY EVALUATED WITH IN OCULO BRAIN GRAFTS. A.-C. Granholm, G.A. Gerhardt†, Å. Seiger*, L. Olson*, B.J. Hoffer and M.R. Palmer. Depts. of Pharmacology and †Psychiatry, Univ. of Colorado Health Sci. Cntr., Denver, CO, USA 80262 (ACG, GAG, BJH and MRP) and Dept. of Histology, Karolinska Institute, Stockholm S104 01 Sweden (AS and LO).

Changes in cerebellar noradrenergic transmission during senescence in the rat were measured using homologous brain grafts in oculo. The dynamics of presynaptic NE release were evaluated using in vivo electrochemistry with nafion-coated graphite-epoxy-capillary electrodes, which are highly selective for the monoamine neurotransmitters. Postsynaptic sensitivity of Purkinje neurons to catecholamine was determined by perfusing the anterior eye chamber with known concentrations of norepinephrine (NE) dissolved in a balanced salt solution. NE elicits a dose-dependent slowing of Purkinje neuron discharge in both young (3-6 months) and old (20-22 months) cerebellar grafts. Hill plots demonstrated that the dose-response relationships in both age groups were linear and parallel to one another. Aged transplant Purkinje neurons manifested a marked and highly significant subsensitivity to NE with an EC_{50} of 583 uM, as compared with an EC_{50} of only 15.9 uM in the young grafts. Collaterals of autonomic ground plexus of the iris innervate the grafts with presynaptic noradrenergic fibers which can be reflexly activated by altering illumination of the retina. As illumination is increased, the release of catechclamine from these sympathetic fibers decreases. A mean change in the extracellular electroactive species of 4.2 uM (from in vitro norepinephrine calibration curves) was found in young cerebellar grafts. Equivalent stimuli induced a mean change of only 2.3 uM in aged grafts. No difference in the time course of the electrochemical response was observed. Taken together, these results reveal both a marked postsynaptic subsensitivity to NE in neuronal targets and a more modest reduction in NE release with senescence. Insofar as these changes can be found in isolated cerebellar grafts in oculo, they apparently develop independent of extrinsic input from other brain regions.

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395.16 **QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF [125I]PINDOLOL BINDING IN RAT BRAIN: CHANGES IN β-ADRENERGIC RECEPTOR DENSITY WITH AGING.** J.A. Miller*, B.J. Hoffer and N.R. Zahniser (SPON: J.H. O'Brien). Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Responsiveness to catecholamines and the density of certain adrenergic receptors decrease with age. β-adrenergic receptor density decreases in rat pineal, striatal and cerebellar membranes (Greenberg and Weiss, Science 201: 61-63, 1978). Additionally, Maggi et al. (Life Sci. 24: 367-374, 1979) reported decreases in β-adrenergic receptor number in cerebellum and brainstem, but not cerebral cortex in aged rats. These reports suggest that age-related changes may be region-specific. Quantitative autoradiography provides the spatial resolution to examine region-specific receptor changes; we have used this technique to measure the binding of [125I]pindolol (IPIN), a β-receptor antagonist, to slices of brain tissue from young and old rats. Whole brains from young (4 mo) and old (19 mo and 25 mo) Fischer 344 rats were quickly frozen and sliced at a thickness of 10μm . The slices were incubated in a 20 mM Hepes buffer (pH 7.4) containing 154 mM NaCl and IPIN at final concentrations of 10-1000 pM (saturation isotherms) for 30 min at 37°C. Nonspecific binding was defined as the binding remaining in the presence of 1μM l-propranolol. The slices were washed in ice-cold Tris-HCl buffer for 10 min and then rinsed in ice-cold water for 10 sec. Slides and [14C]-labeled brain paste standards were apposed to LKB Ultrofilm for 24 hrs; a standard curve was generated for each sheet of film. The autoradiographs were then analysed with a Spatial Data Systems Eyecom Model 850 image analysis computer system using a new nonlinear curve fitting procedure to approximate the standard curve. In agreement with earlier reports, decreases in β-adrenergic receptor density in the cerebellum and brainstem, but no change in the cortex, from the aged animals were observed (Table 1). Additionally, there was a large age-related decrease in the Bmax for IPIN in the thalamus but no change in the hippocampus (Table 1). The receptor density for the young animals ranged from 55-66 fmol/mg protein in the different brain regions. The affinity of these receptors was not different in any of the brain areas examined in the young and old rats and ranged from 86-160 pM. The Scatchard transformation of these data displayed simple single-site binding in these areas. The region of the substantia nigra was also examined; however, it displayed curvilinear Scatchard plots. These results will be further analyzed using a multi-site nonlinear curve fitting program and pharmacological experiments to determine the nature of these binding sites. Localized changes within these brain regions, as well as other more discreet areas will be examined. The results demonstrate that with aging, β-adrenergic receptor density decreases in a nonhomgeneous manner in the rat CNS.

Table 1. Density of IPIN binding sites (Bmax; % of 4 mo values).

AGE (mo)	Cortex	Hippocampus	Thalamus	Cerebellum	Brainstem
4	100	100	100	100	100
19	109	108	133	87	65
25	89	86	54	71	51

This work was supported by grants USPHS AG 04418 and ES 02011.

395.17 **GLUTATHIONE REDUCTION MIMICS MPTP NEUROTOXICITY AND AGE-CORRELATED CHANGES IN DOPAMINE NEURONS IN SUBSTANTIA NIGRA OF THE C57BL/6NNia MOUSE.** T.H. McNeill, L.L. Koek*, J.W. Haycock and D.M. Gash. Depts. Neurology and Neurobiology and Anatomy, Univ. Rochester, Rochester, NY 14642 and Rockefeller University, New York, NY 10021.

Previous studies have reported age-correlated changes in dopamine (DA) perikarya of the substantia nigra (SN) in the C57BL/6NNia mouse. These changes are characterized by a progressive accumulation of lipofuscin, a markedly reduced DA content per cell and an increased number of large, fluorescent axonal dilations in DA fibers. Similarly, other studies have shown that MPTP treatment damages DA neurons in mice and causes a reduction in content of glutathione (GSH) in the SN. Since it is known that GSH is utilized as a scavenger of cellular free radicals and plays an important role in detoxification and cellular repair after exposure to neurotoxins such as MPTP, it was of interest to determine if the reduction of GSH would mimic the morphological changes found in DA neurons of the SN as a result of MPTP toxicity or normal aging.

For our study, male C57BL/6NNia mice (3 and 6 months-of-age) were treated with MPTP (2 x 50 mg/kg s.c.) or with the intracellular inhibitor of GSH, buthionine sulfoxine, (BSO) (3 mmol./kg, i.p.). Tissues were processed for light microscopic immunocytochemistry using antisera for tyrosine hydroxylase or prepared for histofluorescence examination of catecholamines. Results from our study suggest that depletion of GSH has a differential effect on selected populations of DA cells of the midbrain which project to different regions of the cortex and striatum and are similar in appearance to those morphological changes which have been reported previously with advancing age and following MPTP administration. In BSO treated mice dopaminergic perikarya of the pars compacta (A9) showed a markedly reduced DA content per cell as determined by microspectrofluorometry, an increased accumulation of lipofuscin and an increased number of large, fluorescent axonal dilations in dopaminergic fibers. However A9 neurons stained immunocytochemically for TOH appeared similar to controls. In addition, DA neurons in the ventral portion of the ventral tegmental area (VTA) were unaffected by drug treatment. These data suggest that morphological changes in DA neurons of the SN following GSH reduction are similar in appearance to those seen in normal aging and following MPTP treatment and support the notion that a common cellular defect may underlie all three conditions. In addition, these data provide support for the use of MPTP as a model to study normal aging of DA neurons of the SN in C57BL/6NNia mice.

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396.1 OLFACTORY MUCOSA TRANSPLANTED INTO THE SENSORY DEAFFERENTED OLFACTORY BULB IN ADULT HAMSTER. E.E. Morrison and R.M. Costanzo, Dept. Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298.

After olfactory nerve axotomy there is a retrograde degeneration of mature olfactory neurons followed by an increase in basal cell mitotic activity which generates new neurons. These new neurons develop and grow their axons centrally to reinnervate the olfactory bulb. Recently, it has been shown that neonatal olfactory neurons can survive transplantation into the eye and brain parenchyma of neonatal and adult rats. New olfactory neurons are produced, mature and grow axons into the host but do not form glomeruli. In the present experiment we have examined the interaction between olfactory neurons when transplanted into the sensory deafferented olfactory cortex (bulb) of adult (2-3 mo) hamsters. Following olfactory nerve transection a strip of teflon tape was placed between the bulb and cribriform plate blocking sensory reinnervation. Donor tissue, septal mucosa ($2mm^2$) from neonate hamsters (P 6-11) was transplanted via a glass rod within a mid dorsal incision on the bulb. Animals were fixed with Bouins and processed for LM examination at 25, 35, 45, and 50 days survival. Regenerating olfactory axons from the host nasal cavity were prevented from reaching the bulb by the teflon tape and formed neuromas. The host glomerular layer lacked olfactory axons, appeared pale and glomeruli were homogenous in texture and smaller (40-60 um) than on the control side. The transplants survived within the adult olfactory bulb and organized into vesicles lined by olfactory neuroepithelium containing mature neurons that resembled the in situ organization. In some regions of the transplant olfactory neurons migrated from the neuroepithelium into the lamina propria and into the host bulb tissue. We observed numerous mitotic figures within the transplant neuroepithelium and among the migrating olfactory neurons within the lamina propria. Unmyelinated axons from the transplant neuroepithelium formed large fascicles within the lamina propria. The transplant axon fascicles grew toward and entered the glomerular layer of the host bulb where they formed a tangled neuropile. Transplant axon bundles were often observed adjacent and in intimate contact with periglomerular cells and existing glomeruli.

The results have successfully demonstrated for the first time the survival of olfactory neurons when transplanted into adult hamster olfactory bulb. The deafferented environment provides target sites to the transplanted neurons and allows a unique opportunity to examine specific cell-target interaction. The degree of transplant-host connectivity are currently being examined at the EM level.

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396.2 OLFACTORY BULB GRAFTS INTO PREPYRIFORM CORTEX AND HIPPOCAMPUS. M. E. Gross,* J. N. Kott, L. E. Westrum and R. H. Schmidt (SPON: P. D. Swanson). Depts. of Neurological Surgery, Biological Structure and Psychology, Univ. of Washington, Seattle, WA 98195, USA.

Numerous models are being used in the rapidly expanding field of neural grafting. An excellent system with unique architecture and connectivity, which has received little or no attention in these studies, is the olfactory bulb (OB) and prepyriform or olfactory cortex. We are undertaking experiments aimed at investigating the morphological events associated with the implantation of dissociated fetal OB cells into adult rats.

Olfactory bulbs from fetal rats of known gestational ages are dissociated in trypsin. Subsequently, suspensions are implanted through a cannula either manually or using a stereotaxic instrument into adult hosts. Parallel experiments used whole OB, from similar donors. Areas receiving grafts include: hippocampus and anterior or posterior prepyriform cortex. Adult hosts were prepared, in the case of prepyriform preparations, by either small surface lesions of the cortex or by a deafferenting OB removal.

Preliminary light microscopy of cell and fiber stained material from the implanted sites shows cells with staining characteristics and morphological features of OB cell types. An especial finding in several of the preparations is the occurrence of apparent glomerulus-like formations, i.e. circular regions of neuropil (20 - 100 μm in diameter) surrounded by capsule-like cells and large neurons.

These observations suggest that a number of OB cell types remain "viable" and exhibit structural elements of the donor organ for several weeks after implantation. Details of this transplantation model are being pursued with emphasis on cell identification and synaptic organization.

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396.3 FETAL NEOCORTICAL TISSUE SURVIVES TRANSPLANTATION INTO ADULTS IN AN ISCHEMIC CORTICAL SITE. A. Justice, A. W. Deckel*, and R. G. Robinson. Dept. of Psychiatry, Johns Hopkins Univ. School of Med., Baltimore, MD 21205.

One of the most clinically important neuropathological conditions in man is that resulting from cerebral ischemia (CVA). Not only are CVAs the third leading cause of morbidity in the US but treatment is largely inadequate. One method that may someday prove useful for the treatment of deficits resulting from various types of neuropathology involves the transplantation of healthy neural tissue into damaged brain. While the transplant method has recently become widespread in animal models of clinical conditions, surprisingly little attention has been paid to its possible utility in the treatment of ischemic brain damage. We report here preliminary results indicating that fetal neural grafts will grow in an ischemic site in which host tissue dies.

The middle cerebral artery of adult rats was permanently tied with 6-0 silk on the lateral aspect of the brain. This procedure produces fairly discrete lesions (approximately 2 - 4 mm diameter) in the dorsolateral frontoparietal cortex, extending through all cortical layers. Four to sixteen days after ligation, 3 ul of embryonic day 16 neocortex was aspirated into a glass capillary tube and injected into the cortex in the area of the lesion. Two months later animals were sacrificed and their brains were processed for nissl staining. The transplants survived well and differentiated, partly replacing the necrotic tissue and filling in the cavity left by the infarct. Gliosis was generally apparent around the graft and often within it, but this response was variable.

It is encouraging for future research that transplants will survive in areas lacking a normal vascular supply and where the host tissue is dieing. Experiments are under way to discover what connections may have been made between graft and host and to determine if behavioral deficits resulting from the ischemic lesion can be reversed by the graft.

396.4 NEURAL TRANSPLANTS: INTERFACE AND CONNECTIVITY. J. Brasko and G.D. Das*. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Following transplantation, embryonic neural tissues are observed to grow, differentiate, and become integrated with the host brain. A developmental analysis of these events was performed to investigate the establishment of interface regions and the formation of connections between the transplants and the host parenchyma.

Neocortical tissue, 3.5 mm^3 in volume, from 17-day rat embryos was transplanted into the right cerebellar hemisphere of adult rat hosts (Das, '74, '79). One set of host animals was sacrificed at various times between 1 and 90 days following surgery and their brains were subsequently processed for C-V, H&E, and Bodian's Protargol histology. A second set of hosts received electrolytic lesions of the left pons between 1 and 90 days post-transplantation and the development of afferents to the transplants was studied by using the Fink-Heimer technique for degenerating axons.

Microscopic analysis indicated the presence of densely packed areas of viable transplant tissue in close apposition to the host brain 24 hours after surgery. Two days later, the transplant had achieved initial parenchymal attachment with the host brain. From days 4-5 onward, regions of true neuropil were seen to develop between the transplant and the host cerebellum, which became the interface between the two tissues. By day 15, transplants had grown large and contained aggregates of cells separated by bundles of fine caliber axons coursing through the transplant neuropil. These bundles comprised a system of intrinsic transplant connections, present during the whole 90-day period under study. In addition, extrinsic connections from the pons were seen to penetrate the transplants from the adjacent host granule cell or medullary layers, also beginning on day 15. Initially, diffuse patches of ingrowth were evident just inside the transplants, close to interface regions. In time, axons grew to progressively deeper areas of the transplants, often by fasciculating between clusters of transplant neurons or around the bundles of intrinsic transplant fibers. While the amount of such ingrowth to the transplants seemed to increase with time, not all integrated interface regions were traversed by growing axons. Thus, while an interface provides an essential substrate for the establishment of transplant/host brain connectivity, its mere presence does not guarantee that such connections will be formed. Instead, other factors, such as the nature of the interface regions, the degree of transplant differentiation, and the extent of transplant/host brain histopathology, are also seen to influence the establishment of connectivity between transplant and host brain tissues.

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396.5 NEOCORTICAL TRANSPLANTS IN THE SPINAL CORD: NATURE AND INGROWTH OF PERIVASCULAR FIBERS IN THEM. G.D. Das*, J. Brasko and M.S. Riedl*. (SPON: R.W. WALLACE). Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907.

Neural transplantation in spinal cord, particularly following an extensive lesion, involves an interaction between the growing transplants and various pathological reactions in the spinal cord. Of the various reactions affecting the survival and integration of transplants is regeneration of the damaged blood vessels with their fibers and their penetration into the transplants.

Adult Long-Evans hooded rats, under deep anaesthetic condition, received extensive surgical lesions in the spinal cord at T8-T9. The lesions created cavities in the spinal cord. The spinal cord tissue was removed from the cavities, and neocortical tissue from 15- and 16-day rat embryos was transplanted intraparenchymally. The host animals were killed in a developmental sequence between 1 day and 18 months after the surgery. One set of spinal cords with transplants was histologically processed for CV, H&E, and Bodian's protorgal stains, and the other set for Golgi-Cox impregnation.

Vascularization of transplants was seen to follow a well-defined time course. At first the damaged blood vessels retracted from the traumatized tissue. This was followed by proliferation and sprouting of new blood vessels of irregular caliber. They penetrated the transplants at a fast rate, providing a dense network of capillary beds. During further development there was an indication of reduction in the density of capillarization. In the final stage blood vessels appeared to penetrate into the transplants from all aspects, including the glial scar formations partially surrounding them.

Along with the penetrating blood vessels a large number of perivascular fibers also were seen to penetrate the transplants. They were of fine caliber, long, wavy, and closely adherant to the blood vessels. As they entered the transplants; they branched profusely, each branch of yet finer caliber. They penetrated the transplants very deep, and were seen to terminate around the neurons as fine baskets. The blood vessels providing such perivascular fibers were large arterioles and venules close to the interface between the transplants and the spinal tissue. Some perivascular fibers were seen to enter from glial scar formations that partially surrounded the transplants; and some penetrated into the white matter of the host spinal cord. On morphological basis they were evaluated as the autonomic fibers mainly from the sympathetic system.

These findings showed that the perivascular, rather autonomic, fibers are capable of extensive regeneration, and penetration into the transplants as well as the host brain tissue. It is possible that often they may be misidentified as the regenerating axons of the spinal cord. (Supported by N.I.H. Research Grant No. NS-08817).

396.6 FINE STRUCTURAL ASPECTS OF CAPILLARIES WITHIN TRANSPLANTED CEREBRAL CORTEX. J.M. Krum and J.M. Rosenstein. Dept. of Anatomy, George Washington Univ. Sch. of Med., Wash. D.C. 20037.

Angiogenesis, the formation of new blood vessels, is essential for survival of transplanted neuronal tissue and must occur rapidly to prevent ischemic damage. This study describes the temporal pattern of vascular development within grafted fetal CNS tissue at the ultrastructural level. Pieces of E17-E21 Wistar rat parietal cortex were placed in the fourth ventricle of conspecific neonatal recipients. After survival times of one day - six months, host animals were perfused with aldehydes and processed for electron microscopy. Some recipients were given an intravascular injection of horseradish peroxidase (HRP) which was allowed to circulate for 30 minutes prior to perfusion.

Within the first post-operative week, transplant vessels exhibited intact interendothelial junctions, intraluminal processes and discontinuous basal laminae. At branch points, "seamless" sprouts were often present, consisting of thickened endothelium lacking junctional complexes and containing large amount of rough endoplasmic reticulum. Astrocytic processes contacting the abluminal endothelium were rarely seen. HRP was readily taken up into transendothelial organelles and was present within developing neurites. Occasionally, fragments of degenerating vessels were apparent; perivascular macrophages were frequently observed.

At later post-operative times, capillary endothelium became more mature in appearance, with fewer luminal processes and well-defined basal laminae. However, HRP was still present in endothelial vesicles and basal lamina, suggesting that these cells may not be metabolically normal. Perivascular cuffs of inflammatory cells were apparent around some transplant capillaries in addition to abnormal formations of astrocytic end feet. These results indicate that vessels within fetal cortical transplants do not show completely normal development especially with regard to perivascular cellular relationships and, possibly, endothelial transport mechanisms. (Supported by NS-17468).

396.7 **STIMULUS-EVOKED INCREASE IN GLUCOSE UTILIZATION IN TRANSPLANTED RAT FETAL SOMATOSENSORY CORTEX.** **B.E.Levin,C.Wilmot,A.Dunn-Meynell. Neurol. Serv. VA Med. Ctr., E. Orange, 07019, Neurosci. Dept., NJ Med. Sch., Newark, NJ, 07103.**

Fetal (E15-16) somatosensory cortex was unilaterally transplanted into the barrel receptor field (BRF) of adult male (250-300g) rats ("homotypic transplant"). Three to 6 mo later, swaddled awake rats were given 50 μCi ^{14}C-2-deoxyglucose (^{14}C-2DG; 50 Ci/mmole), iv, while having their vibrissae stroked bilaterally at a rate of 3-4 Hz for 45 min. This procedure increases local cerebral glucose utilization (LCGU) over the BRF in normal rats. Following decapitation, the brains were processed for ^{14}C-2DG autoradiography by standard procedures. Controls included: 1) bilateral vibrissal stimulation in rats with fetal cerebellar implants into either host BRF, occipital or frontal cortex or fetal somatosensory cortex implants into host frontal or occipital cortex; 2) fetal somatosensory cortex into host BRF or frontal cortex in unstimulated rats. Autoradiograms were read by outlining the transplant from the thionine-stained section and reading the ^{14}C-2DG uptake (LCGU) over the comparable autoradiographic image. Average LCGU in transplants from 8 controls did not differ significantly from each other in any parameter and results were thus pooled. Average LCGU values integrated over the entire transplant in the 4 rats with homotypic implants and bilateral vibrissal stimulation (121 ± 2) was 15% higher than controls (105 ± 5 μmol/100g/min; p<0.05). The areas of most intense transplant uptake were defined as those transplant LCGU values which were greater than hippocampal LCGU+1 Std dev in the same section of brain. There was 40% greater LCGU in these areas in transplants from vibrissal-stimulated rats with homotypic transplants (167 ± 15) than in transplants from control rats (119 ± 4 μmol/100g/min; p <0.01). These data suggest that fetal somatosensory cortex can become integrated into the BRF of adult rats to the degree that afferent thalamic impulses generated by vibrissal stimulation will increase glucose utilization in the transplanted tissue if it is located within the appropriate cortical receptive area.

396.8 REMOVAL AND REIMPLANTATION OF SOMATOSENSORY CORTEX IN MICE: CONSEQUENCES TO THE BARRELFIELD. F.L. Andrés* and H. Van der Loos* (SPON: G. Innocenti). Institute of Anatomy, University of Lausanne, Switzerland.

The vibrissal follicles in the mouse whiskerpad are topologically connected to the barrels in the contralateral somatosensory cortex. The barrels develop on postnatal day 3 through 6. Recently, we have observed that barrelfields still develop in pieces of parietal cortex that were removed and reimplanted, in the same place and with the same orientation, on the day of birth (P0) or on postnatal days 1 or 3 (P1 or P3) (Andrés, F.L. and Van der Loos, H., Dev. Brain Res. 20:115-121, 1985). We now wanted to know whether a critical age existed after which no barrelfield would form in the somatosensory cortex, after similar operations. To answer this question we have removed and reimplanted a piece of parietal cortex in the same place and with the same orientation, in 79 mice from a C3H strain. The operations were made on P0, P1, P2, P3, P5, P6, P7, P8 or P9. Fifty one mice survived and were processed for histology. Their brains were cut coronally to facilitate the identification of the limits of the reimplanted cortex and of its lamination. In 29 cases the reimplanted cortex could be identified and in 17 cases barrel-like structures had developed. The "barrelfields" were obtained from the coronal sections, by means of a computer program which permitted the reconstruction of the reimplanted pieces of cortex and their rotation in space. In this way the barrel-like structures and the formed "barrelfields" could be visualized, as if obtained from sections tangential to the parietal cortex. "Barrelfields" were found in the pieces of somatosensory cortices reimplanted at different ages up to postnatal day 9. In the cases operated up to postnatal day 3, the barrelfields may have developed as a consequence of regrowth of interrupted thalamocortical axons or ingrowth of new ones. However, the "barrelfields" of the cases operated at ages in which the barrels had already been formed, may have remained without receiving new thalamic projections. We do not know if the whiskers in the corresponding whiskerpad re-establish functional connections with the "barrelfields". We are presently performing experiments to answer this question. Support: Swiss NSF, grant 3.158.

396.9 **SYNAPTIC COMPOSITION OF NEOCORTICAL TRANSPLANTS IN MICE.** L.M. Smith, M. DiPrete, and F.F. Ebner, Brown University, Providence, R.I. 02912.

When embryonic neocortex differentiates in the environment of adult neocortex there is almost no ingrowth of axons from the host thalamus or corpus callosum. Nonetheless, many synapses still develop in the donor cortex, even in the absence of sensory fiber activity (Smith & Ebner, Neurosci. Abst. 1983). In this study we have used transplants to ask whether the density of synapses and the ratio of round vesicle-asymmetrical (RA) and flat vesicle-symmetrical (FS) contacts onto cortical neurons is altered in the absence of normal sensory fiber activation. The fine structure was examined in neocortical transplants taken from E14 and E18 embryos after short term (one week) and long term (six weeks) survival in the neocortex of adult host mice. Six animals were analyzed from each donor group, from each survival period, and from age-matched controls.

The numerical density of axon terminals in transplants of both donor groups was decreased by 20% when compared with control cortex. Asymmetrical membrane differentiations in these transplants were decreased by 20% of control values, as were contacts on spines. These differences between transplants and controls may reflect the absence of specific afferents in the transplants since it has been estimated that approximately 20% of the innervation of neocortex comes from extrinsic fiber systems that form asymmetrical synapses upon dendritic spines. The normal 8.5:1.5 ratio of RA to FS synapses, however, was indistinguishable in transplant and control cortex. Comparisons between the two age groups showed that E18 transplants contained 30% more F terminals than E14 transplants. This difference is consistent with previous light microscopic findings showing glutamic acid decarboxylase immunoreactive neurons in E18 transplants comparable to the host cortex, but no such labeled neurons in E14 tranplants.

Qualitative observations indicate that 1) dendritic spines in the transplants rarely contain a spine apparatus, 2) R terminals and dendritic spines are smaller and more irregular in shape, 3) F terminals are usually larger than controls with long, well defined symmetrical membrane differentiations, and 4) polyribosomes are frequent in transplant dendritic spines and in dendrites subjacent to synapses after short survivals.

We conclude that cortical neurons exert precise control over the number and the ratio of excitatory to inhibitory synapses made on their receptive surfaces even in the absence of normal extrinsic sensory fiber inputs. The atrophy in the transplants of the RA contacts and the hypertrophy of the FS contacts in the transplants suggests, however, that the extrinsic sensory inputs may be necessary for the final regulation of synaptic maturation (supported by NIH grant #NS-13031)

396.10 FETAL CORTEX TRANSPLANTS ATTENUATE THALAMIC ATROPHY CAUSED BY NEONATAL FRONTAL CORTEX LESIONS: WGA-HRP AND AMINO ACID STUDIES OF CONNECTIONS BETWEEN MOTOR CORTEX AND THALAMUS OF NEONATALLY LESIONED RATS WITH AND WITHOUT TRANSPLANTS. F.R. Sharp and M.F. Gonzalez, Dept. Neurology, UCSF and SFVAMC, S.F., CA 94121.

Removal of frontal cortex at birth causes ipsilateral thalamic atrophy in the adult brain. Moreover, stimulation of the intact motor cortex in these neonatally lesioned subjects metabolically activated thalamus bilaterally, whereas similar stimulation of juvenile lesioned subjects activated thalamus unilaterally (Sharp, F. R., and Evans, K., Develop. Brain Res., 6:1, 1983). Since it was not known how the thalamic atrophy and bilateral activation occurred following neonatal frontal cortex lesions, connections between rat motor cortex and thalamus were examined in neonatal and juvenile lesioned subjects.

WGA-HRP and amino acids were injected into the intact motor cortex of adult rats following neonatal or juvenile removal of the opposite cortex. The results show that motor cortex projects bilaterally to thalamus in subjects with one motor cortex removed at birth or at 30 days of age. In addition, motor cortex receives afferents only from ipsilateral thalamus in normal newborns and in adults following removal of one motor cortex at 1 or 30 days of age. Thalamic atrophy appears to develop because thalamic neurons ipsilateral to neonatal motor cortex lesions die after their normal cortical target has been removed. These neurons cannot sprout into the contralateral cortex because it is already partially innervated at birth.

To test the hypothesis that fetal cortical transplants might decrease the thalamic atrophy observed, two groups of rats had one frontal cortex removed at birth. One group had fetal frontal cortex from 18 day old embryos placed in the cavities and the other had gelfoam placed in the cavity. Ninety days following surgery, both groups were sacrificed and thalamic areas measured at three levels. The thalamic areas ipsilateral to the surviving fetal transplants were significantly greater than in subjects which had cortical lesions and received no transplants. This could be due to transplant trophic effects on the neonatal host thalamus, host cortex, or both. Injections of WGA-HRP into the host thalamus demonstrated anterograde and retrograde labeling in the transplants. The demonstration of host thalamic projections to the transplant suggests the transplant serves as a target for developing thalamic neurons which might have otherwise died following neonatal cortical lesions. These results also indicate that the neonatal cortical injury might be ameliorated by the fetal cortical transplants, and that this system could be useful for assaying thalamocortical trophic factors in vivo.

396.11 **ADULT BRAIN TRANSPLANTS FACILITATE BEHAVIORAL RECOVERY.** J.P. Kesslak, L. Calin*, A Walencewicz*, C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717

Transplants of adult neurons have not been shown to survive well or facilitate behavioral recovery. Survival of transplants may be enhanced by introducing a delay between host injury and implant of donor tissue (Gage et al, Nature, 1984; Nieto-Sampedro et al, PNAS, 1982). Embryonic frontal cortex transplants have been found to survive and facilitate behavioral recovery on a reinforced alternation task (Kesslak et al, Exp Neurol, 1986; Labbe et al, Science, 1983). We now demonstrate that transplants of adult frontal cortex also survive and accelerate recovery.

Male Sprague-Dawley rats received frontal cortex ablations followed by embryonic or adult frontal cortex transplants. Animals were then tested on a reinforced alternation task until reaching a criterion of 19/20 correct on 2 consecutive days. Embryonic frontal cortex transplanted immediately after the ablation showed poor survival and did not increase the rate of recovery on the alternation task ($p > .10$). Both adult and embryonic transplants, when introduced after a delay, survived and increased the rate of recovery comparable to undamaged controls ($p < .05$). Functional recovery may be due to establishment of neuronal connections or biochemical interactions between host and transplant. Establishment of neuronal connections may not be necessary to increase the rate of recovery after frontal cortex ablation, since transplanted glial cells also have been shown to significantly increase recovery (Kesslak et al, Exp Neurol, 1986).

The hippocampal formation was used to further examine the role of transplants in a system where specific connectivity may be more essential. Kainic acid lesions were used to selectively eliminate CA3 pyramidal cells, and were followed by delayed (10 days) injectons of adult or embryonic hippocampal suspensions. Animals were tested on a forced-choice alternation task to a criterion of 5 consecutive days of at least 7/8 correct choices. Both embryonic and adult transplants survived and accelerated the rate of recovery ($p < .05$), but neither completely ameliorated the behavioral deficit on the alternation task. These results suggest that relatively mature neurons can survive transplantation and enhance the rate of recovery after brain injury.

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396.12 FETAL CORTICAL TRANSPLANTS INTO PARTIALLY HEMISECTED MEDULLA OF ADULT RAT: PRELIMINARY STUDIES. M.F. Jacquin, W.E. Renehan, B.G. Klein & B.H. Hallas. Depts. of Neuroscience & Anatomy, New York College of Osteopathic Medicine, Old Westbury, NY 11568.

We have begun to address 3 questions: 1) Can implanted fetal brain tissue provide a "bridge" for regeneration of centrally axotomized trigeminal (V) axons? 2) What are the structural and functional characteristics of transplanted neurons, and of host V axons which innervate a transplant? Do they assume properties of brainstem V neurons, or do they express predetermined morphologies? 3) What are the projections of transplanted neurons? The V system was chosen since a great deal is known regarding its normal adult organization, development and response to injury.

Twenty adult rats had a 1X1 mm piece of 16-day fetal somatosensory cortex transplanted into the site of a fresh partial medullary hemisection at the level of the obex. In all cases, the lesion severed most, if not all, of the descending V spinal tract and nucleus. Twenty-four to 60 days later, transplant structure and function were assessed. Cortex continued to develop and differentiate in the medulla, filling the lesion site. Anterograde HRP transport from the V ganglion revealed a large number of labeled primary afferent fibers descending through the lateral edges of the transplant, many of which resumed their descent in the caudal V spinal tract to innervate the caudal medulla and cervical spinal cord. Diffuse terminal label was observed in the transplant, most notably in its outermost portions. Labeled "growth cone-like" structures were also visible in this region. When WGA-HRP was used, transneuronally labeled cells were also observed in donor tissue. Transplants stained heavily for cytochrome oxidase with some regions showing more activity than others; however, a barrel-like staining pattern characteristic of normal cortex and brainstem was never observed. Intraaxonal recording and HRP labeling experiments revealed low-threshold, fast-conducting V primary afferents terminating in the transplant. Their arbors appeared somewhat more tortuous than normal, with a smaller number of terminal boutons. Injections of 2 axons in one transplant also provided strong evidence for alterations in V primary afferent topography. Intracellular recording and HRP labeling experiments revealed a large number of spontaneously active donor cells without V afferent input. Some of these were successfully stained and had stellate-like morphologies with small-medium sized somata and restricted, multipolar dendrites. Their axons did not exit the transplant. A small number of cells were recorded that received convergent V inputs, though they were not stained and, therefore, may not be of donor origin. Retrograde HRP and fluorescent tracing experiments showed a very small number of transplant cells projecting to host cervical spinal cord or thalamus. Cerebellar or pontine injections did not label any cells in transplanted tissue. Support: DE07662, AOA.

396.13 TRANSPLANTATION OF SEROTONERGIC NEURONS INTO THE TRANSECTED SPINAL CORD OF ADULT RATS. A. Privat°, H. Mansour°,*, M.Geffard° and F.Sandillon°, U-249 INSERM, Institut de Biologie, 34060 Montpellier, and *IBCN CNRS 33000 Bordeaux, FRANCE

Serotonergic neurons of the raphe nuclei project to the dorsal and ventral horns and the intermediolateral columns of the spinal cord. The aim of the present study was to restore this innervation after spinal cord trans ection by transplantation of foetal raphe neuron suspension below the section.

Twenty adult Sprague-Dawley male rats underwent a complete section of the spinal cord at lower thoracic level. One week later 2-5µl of a cell suspension of serotonergic (5-HT) neurons were injected 2 mm below the section. The primordium of the raphe nuclei was dissected from 14 days foetuses, mechanically dissociated in glucose enriched buffer, and concentrated to 30,000 cells per µl . After a survival of 10 days to 8 months, the animals were sacrificed by intracardiac perfusion of aldehyde fixative, and the spinal cord was processed for the immunocytochemical detection of 5-HT. Control spinal animals without transplants displayed a total absence of 5-HT immunoreactive material. Conversely, all the transplanted animals showed many immunoreactive pericarya, whose axons projected over more than 20 mm in the grey matter, exhibiting an innervation pattern similar to that of intact animals. Ultrastructural 5-HT immunocytochemistry disclosed typical synapses in the anterior horn and intermediolateral columns, morphologically similar to those of controls.

Recent studies (Mas M. et Al. Brain Res. 342, 128 , 1985) have shown that erection and ejaculation in rats are dependant upon the integrity of the 5-HT innervation of the cord. Using the 5-HT uptake inhibitor Zimelidine, via intraperitoneal injection, we have confirmed that spinal rats were unable to ejaculate, whereas transplanted animals showed ejaculation reflexes qualitatively and quantitatively similar to those of intact animals.

These results demonstrate that transplanted serotonergic neurons are able to survive and mature in a transected spinal cord, to establish synaptic contacts with their presumptive targets and to restore specific reflexes. Work is now in progress to evaluate similar parameters after transplantation of dopaminergic and noradrenergic neurons.

(Supported by a DRET grant 84-126)

396.14 FLECTROPHYSIOLOGICAL ACTIVITY OF LARGE NEURAL TRANSPLANTS IN THE MICRENCEPHALIC RAT. M.H. Lee, M. Donadio,* A. Rabe, J. Majkowski* and H.M. Wisniewski*. New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Using the micrencephalic rat as a model, we are seeking to determine whether congenital brain abnormalities and associated functional deficits could be corrected by neural transplants. Severe cerebral hypoplasia was produced in the offspring of Long-Evans rats by injecting them with a 30 mg/kg dose of methylazoxymethanol acetate on gestation day 15. Such animals, though grossly normal, are known to show behavioral, electrophysiological and neurochemical abnormalities. Tissue from almost the entire cerebral cortex (10-30 mm^3) of 17-18 d old normal feti was placed into the posterior cortex of 10-12 d old micrencephalic rats. At 6-12 weeks after transplantation the solid transplants had occupied the intracranial space over the midbrain tectum. Their size and appearance varied from animal to animal. Even with the largest transplants (about 200 mm^3), there was no evidence of compression in the host brain. The transplants consisted of a dense population of normal-appearing neurons, myelinated fiber bundles, neuroglial elements and neuropil. Cortical laminar organization was present in a few loci. The transplants often interfaced with the cerebral cortex, hippocampus, colliculi, posterior thalamus, and cerebellum.

EEG and evoked potentials were recorded from the transplanted rats at 3-4 months after transplantation using 10 stainless steel epidural screws (diameter of 0.6mm) chronically implanted over the host cerebral cortex and the transplant. Normal-appearing EEG, consisting of various frequency bands, was recorded from bipolar and monopolar configurations located over the transplant. Furthermore, during behavioral change from awake to sleep state, a concomitant EEG transition occurred in the transplant with an increase in high voltage slow wave activity similar to that seen in the host brain. Visual evoked potentials with prominent early components were recorded from the transplant, similar to those seen in the ungrafted micrencephalic rat. In some animals, the EEG contained spontaneous epileptiform discharges, which were localized in the transplant without spreading to the host brain. These observations suggest that the large mass of transplanted normal fetal brain tissue, while displaying electrical activity of its own, was also functionally related to the host brain.

396.15 **THE EFECTS OF FETAL SEPTAL CELL TRANSPLANTS IN RATS WITH SELECTIVE FIMBRIA-FORNIX LESIONS: A BEHAVIORAL AND HISTOLOGICAL STUDY.** C.KELCHE, J.C.DALRYMPLE-ALFORD, J.C.CASSEL and B.E.WILL, (SPON:G.Gmelin). Lab. de Neurobiologie des Comportements, Université Louis Pasteur, 67000 Strasbourg, France.

The aim of our study was to investigate 1) whether hippocampal grafts are able to compensate for behavioral deficits induced by selective lesions of the septo-hippocampal pathways and 2) whether such selective deafferentations of the hippocampus could affect survival and development of the grafts.

Thirty-one day-old female rats of the Long-Evans strain sustained either a bilateral lesion of the medial fimbria (Fi), a lesion of the dorsal fornix (Fo), a combination of these (FF), or sham surgery (Sha). Ten days later, the rats with lesions were allocated to equally sized subgroups receiving either intrahippocampal injections (2x2ul per side) of dissociated fetal (ED17) septal cells (FiT, FoT, FFT) or a second sham operation. Five and ten months after grafting, behavioral performance was evaluated in a 8-arm radial maze. Three dependent variables were taken into account: the number of errors, the number of arms visited before committing the first error and choice sequence. Five months after surgery, all non-grafted rats with lesions made more errors and visited fewer arms before the first error than sham-operated rats. These deficits were exacerbated in all grafted rats.

Ten months after surgery, Fi and FF (but not Fo) rats still made more errors than sham-operated rats. The "negative" effect of the graft was observed only in FiT and FoT rats. The number of arms visited before the first error was not signtficantly different between groups. These results were complemented by an analysis of choice sequence strategies classed as either "orientation", "mapping", "adjusting" or "no decision".

After behavioral testing, the rats were sacrificed for anatomical and histochemical analyses (Nissl staining, acetylcholinesterase- and immuno-histochemistry). Preliminary results showed that one year after tansplantation: 1) all grafted rats had surviving implants, mostly in both injection sites, 2) all grafts exhibited marked acetylcholinesterase-positive staining, however 3) the size of the graft was lesion-locus-dependent (FiT>FFT>FoT). More detailed histological analyses are underway and will be taken into account for the discussion of the present findings.

396.16 SEROTONIN NEURON TRANSPLANTS: ELECTROPHYSIOLOGICAL UNIT ACTIVITY OF INTRAHIPPOCAMPAL RAPHE GRAFTS IN FREELY MOVING CATS. M.E. Trulson, A. Hosseini and T.J. Trulson. Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

Several studies have examined grafting of serotonin (5HT)-containing raphe neurons into adult host brain. Even though grafted 5HT neurons may be viable in the host brain, it is not certain whether these neurons display activity similar to that found in a normal, intact adult brain. A recent study demonstrated that grafted 5HT neurons displayed many of the same electrophysiological membrane properties of non-grafted neurons. Using intracellular recording techniques, Segal and Azmitia (Brain Res., 364, 1986, 162-166) reported that grafted 5HT neurons in rat brain display slow membrane time constants, high input resistance, a potent 4-aminopyridine-sensitive transient outward rectification, and lacked inward rectification in response to hyperpolarization current pulses. While grafted neurons may possess the membrane properties characteristic of intact non-grafted cells, no studies have been conducted to determine whether they display the same physiological properties as found in an intact brain. We recorded the electrophysiological activity of 5HT neurons grafted from fetal cat brain into a host cat brain in freely moving cats. Fourty day old cat fetuses served as the source of immature 5HT neurons. Pregnant females were anesthetized by i.v. pentobarbital and the fetuses were surgically removed under sterile conditions, their brains removed and the mesencephalic midline, containing the dorsal (RD) and median (NCS) raphe nuclei, was dissected free. The grafting procedures were a modification of the cell suspension method of Björklund et al. (Acta Physiol. Scand. Suppl. 533, 1983, 1-10). The tissue suspension was injected into the dorsal hippocampus of young adult female cats (2.0-2.3 kg). After a 4-week recovery period, the cats were anesthetized and received macro- and micro-electrode implants, as previously described (Brain Res. 163, 1979, 135-150). After a 2-week recovery period electrophysiological unit recordings were made from the grafted 5HT neurons. These recordings revealed that the grafted 5HT cells displayed the characteristic rhythmic discharge pattern found in the RD of anesthetized rats or freely moving cats. However, grafted neurons recorded from the hippocampus showed no change in activity across the sleep-waking cycle, in dramatic contrast to the profound changes that occur in the RD in intact cats, in which the cells are virtually silent during REM sleep. Also, these neurons were not responsive to phasic auditory or visual stimuli, or as are RD cells in intact cats. Fluorescence histochemistry revealed large numbers of 5HT cell bodies in the hippocampus of grafted cats. These data question whether such tissue grafts can restore functional activity in the central 5HT system.

396.17 ELECTROPHYSIOLOGICAL PROPERTIES OF SEPTAL NEURONS GRAFTED INTO HIPPOCAMPUS. L.J. Reece* and P.A. Schwartzkroin (SPON: T. Kennedy). Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195

The technique of grafting fetal CNS neurons has raised a host of questions about properties developed by these neurons after relocation. The focus of many studies has been the evaluation of behavioral recovery, expression of appropriate neurotransmitters and corresponding synthesizing enzymes, and the demonstration of establishment of functional synaptic contact. The electrical properties of the grafted cells themselves, however, have not been closely examined. Since these cells not only develop and differentiate in a new environment, but are deprived of their normal afferent connections, it is important to study their cellular properties.

Much of this recent transplant work has been performed in the septo-hippocampal system. Using the in vitro hippocampal slice preparation, we have examined the electrophysiology of rat fetal septal neurons grafted into the hippocampus. Septal cells were dissected from embryonic day 13-15 fetuses, digested in trypsin and dissociated by trituration. 1-2 ul were then injected stereotaxically into the right hippocampus of adult female Sprague-Dawley rats which had received unilateral transections of the fimbria/fornix. The hippocampal and graft cell physiology were examined at 4 to 12 weeks after the surgery.

Grafts were often visible in the slice, but alternate slices were taken for histological verification of graft location by Nissl, AChE or ChAT staining. Slices used for electrophysiology were also taken for histological analysis. Stimulation of the graft resulted in a depolarization of several millivolts, an increase in R_{IN}, and an increase in firing frequency of hippocampal pyramidal cells. The grafts, therefore, appear to function similarly to normal septal innervation, which is known to be primarily cholinergic.

Recordings from the graft itself revealed that the transplanted cells probably receive a variety of inputs from the host hippocampus. The graft can be effectively stimulated from several locations in the host, depending on the placement of the graft within the hippocampus. Cells of the medial septal nucleus have been reported to be cholinoceptive; this ACh sensitivity was also seen in the grafted cells. Application of cholinergic agonists resulted in cell depolarizations of several millivolts, an increase in R_{IN}, and an increase in firing frequency. In several cases, a brief hyperpolarization preceded the depolarization. Since these hyperpolarizations were accompanied by a decrease in cell input resistance, it is likely that this effect was mediated by inhibitory interneurons.

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396.18 LOCUS COERULEUS TRANSPLANTS REVERSE CENTRAL ANGIOTENSIN II DRINKING RESPONSE DEFICITS IN 6-HYDROXYDOPAMINE LESIONED RATS. J.T. Cunningham*, A. McRae-Degueurce, S. Bellin, S. Landas*, L.D. Wilkin, & A.K. Johnson. Depts. of Psychology & Pharmacology & the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242 & INSERM U-259, Bordeaux, France.

Intraparenchymal injections of 6-hydroxydopamine (6-OHDA) into the ventral median preoptic nucleus (vMnPO) and the organum vasculosum of the lamina terminalis (OVLT) which produce noradrenergic depletions will block drinking and pressor responses produced by intraventricular (i.v.t.) or systemic angiontensin II (ANG II) (Bellin, Landas, Bhatnagar, & Johnson, Fed Proc. 43: 1070, 1984). Furthermore, noradrenergic neural transplants into the ventral lamina terminalis have been shown to reverse the drinking response deficit to peripheral ANG II (McRae-Degueurce, Bellin, Landas, & Johnson, Brain Res. in press). The present experiment was conducted to determine if locus coeruleus (LC) transplants into 6-OHDA lesioned vMnPO would restore drinking responses to centrally administered ANG II as well as to peripherally administered ANG II.

Normal male Sprague-Dawley rats were prescreened for water consumption of at leat 2.5 ml following subcutaneous (s.c.) ANG II injections (1.5 mg/kg). The rats then received injections of either vehicle (2 ul) or 6-OHDA (4 ug/2 ul) into the vMnPO. Two weeks after surgery, the rats were retested with s.c. ANG II and the 6-OHDA lesioned rats were divided into 3 groups. One group (LC-TRANS) received injections in the vMnPO with cell suspensions that were prepared from the LC of 17 day old rat fetuses. The second group (Cx-TRANS) received injections of dissociated cerebral cortical cells from the same donors, and the third group (CONT) served as time controls. Two months post-transplantation, all animals were tested with s.c. ANG II and then instrumented with cannulae into their right lateral ventricle. Following recovery from surgery, they were tested for drinking responses to i.v.t. ANG II (100 ng/2 ul) and carbachol (250 ng/2 ul). In the post-lesion drinking test, all 6-OHDA lesioned rats drank significantly less than sham-lesioned controls to s.c. ANG II. After transplantation, the LC-TPANS group drank significantly more than either the Cx-TRANS group or the CONT group in response to both s.c. and i.v.t. ANG II administration. The drinking responses of the LC-TRANS group were comparable to the s.c. and i.v.t. ANG II drinking responses of the sham-lesioned rats. There were no between-group differences observed for carbachol induced drinking. These results indicate that LC transplants can reverse drinking deficits to both systemic and central ANG II produced by 6-OHDA lesions of the vMnPO.

TRANSPLANTATION V

397.1 PRIMATE NEURAL TRANSPLANTS: I. NEUROCHEMICAL CHARACTERIZATION OF 1-METHYL- 4-PHENYL- 1,2,3,6-TETRAHYDROPYRIDINE (MPTP) EFFECTS IN AFRICAN GREEN MONKEYS. J.D. Elsworth, A.Y. Deutch, D.E. Redmond, Jr., J.R. Sladek, Jr., and R.H. Roth. Depts. Pharmacol. & Psychiat., Yale Univ., New Haven, CT 06510; Dept. Neurobiol. & Anatomy, Univ. Rochester, Rochester, NY 14642.

Motor abnormalities induced by MPTP in monkeys are remarkably similar to those suffered by patients with Parkinson's disease (PD). In both cases the nigrostriatal pathway is damaged. However, in PD the biochemical deficits are not uniform within this system and other areas such as the ventral tegmental area (A10) and its projections are affected. Thus, the biochemical changes in the MPTP-treated monkey have now been more carefully compared with those known to occur in PD.

Four male Cercopithecus aethiops sabaeus were treated with 5 doses of 0.4 mg/kg IM MPTP over 5 days. They were sacrificed two months later, together with seven untreated controls. Regions were punched from frozen coronal slices. Dopamine (DA) and norepinephrine (NE) were measured by HPLC and homovanillic acid (HVA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) by GC-MS.

HVA concentration was slightly reduced (30%) in the central region of substantia nigra (A9) by MPTP; although in the most disabled monkey the loss of DA was marked in both central and medial (90%), but not lateral regions of A9. However, even including asymptomatic animals there was gross depletion of striatal DA concentration (75-99%) at all analyzed sites, indicating that toxicity is initially exerted at the terminal level. The putamen and caudate were equally affected, but DA loss was more marked dorsolaterally than ventromedially. DA concentration in the lateral region of the retrorubral field (A8), which projects to the striatum, was reduced by 45%, while the medial part of A8 was spared. DA content in the dorsal region of A10 was decreased (60%), but the ventral A10 region appeared slightly elevated. DA depletion in the septum was substantial (63%), compared with that in the nucleus accumbens (31%), which may reflect innervation of the septum by dorsal A10. As in PD, the HVA to DA ratio was elevated after MPTP, which may reflect increased turnover in surviving neurons. NE concentration was elevated in the medial A8 region by MPTP, although non-significant decreases were noted in the dorsal A10 region and cerebellum. CSF MHPG levels were lower after MPTP, but this was not so marked as the drop in CSF HVA concentration (75%).

These changes in DA and NE concentration and metabolism indicate that the MPTP-treated monkey resembles PD better than originally supposed and provides an excellent model for testing the functional effects of transplanted dopamine neurons.

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397.2 PRIMATE NEURAL TRANSPLANTS. II. HISTOFLUORESCENCE CORRELATES OF DEGENERATION OF THE NIGROSTRIATAL DOPAMINE SYSTEM FOLLOWING MPTP. V. Bruemmer*, T.J. Collier, D.E. Redmond, Jr., R.H. Roth, and J.R. Sladek, Jr. (SPON: M.L. Blair). Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642; Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 86510.

As a corollary to the neurochemical analyses presented in the preceding abstract by Elsworth and colleagues, we have examined catecholamine histofluorescence in some of the same African green monkeys reported therein. Additionally, asymptomatic monkeys treated with MPTP as well as untreated control monkeys were examined. Brain tissue was prepared for fluorescence analysis with the Falck-Hillarp technique and dopamine (DA) systems were analyzed qualitatively with respect to the effect of MPTP on cell bodies and axons. Histofluorescence also was examined with quantitative microspectrofluometric techniques to assess intraneuronal DA content within perikarya of the substantia nigra and terminals in the striatum. Untreated monkeys exhibited typical patterns of DA fluorescence in the nigrostriatal system. Perikarya were moderately intensely fluorescent, ascending preterminal axons appeared as delicate profiles and the striatum displayed intense, diffuse fluorescence. Symptomatic, MPTP-treated monkeys, in contrast, showed a 72% reduction in number of fluorescent DA neurons in the substantia nigra. Ascending axons appeared swollen and intensely fluorescent which is consistent with the appearance of degenerating axons. Asymptomatic, MPTP-treated monkeys displayed a transitional picture, with a 62% reduction in number of DA neurons in the substantia nigra and the presence of swollen axons as they coursed through the ventral tegmental area. Fluorescence in the striatum also was reduced. All MPTP-treated monkeys, whether symptomatic or asymptomatic, also exhibited increased fluorescence in perikarya of remaining nigral DA neurons. This suggests either an accumulation of transmitter proximal to the site of axonal degeneration in damaged neurons, or the possibility of increased neuronal activity in a population of nigral neurons which may be undamaged following MPTP intoxication. In addition, numerous nigral neurons showed an apparent displacement of DA fluorescence by granular, yellow, lipofuscin fluorescence. Several stages of lipofuscin accumulation were seen ranging from low levels within neurons of moderate DA intensity to neurons with only lipofuscin fluorescence. These neuroanatomical data support the view that MPTP induces nigrostriatal degeneration by initially affecting terminals with retrograde degeneration ensuing in ascending axons. This degeneration of the DA system is reminiscent of that observed in Parkinson's disease. Supported by the Axion and St. Kitts Biomedical Research Foundations, AG 00847, and an ADRDA Faculty Scholar Award.

397.3 PRIMATE NEURAL TRANSPLANTS. III. SURGICAL PROTOCOL FOR FETAL CNS GRAFTS INTO MPTP-TREATED ADULT MONKEYS. T.J. Collier, D.E. Redmond, Jr., D.R. Feikin*, P.N. Foster*, L. Kaplowitz*, V. Lewis*, L.M. Salzer*, R.H. Roth, and J.R. Sladek, Jr. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642; Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 86510.

Three adult male African green monkeys (Cercopithecus aethiops sabaeus) received grafts of fetal brain derived from the substantia nigra (SN), hypothalamus, or dorsolateral pons; the latter containing the region of the locus coeruleus (LC) and subcoeruleus (SC). Each monkey had been treated with MPTP 3-4 weeks previously and exhibited signs of parkinsonism prior to neural grafting as reported in the accompanying abstract by Redmond and colleagues. Graft recipients were given routine supportive care for surgery. Predetermined stereotaxic coordinates were utilized for placement of fetal CNS into the recipient striata or cerebral cortex.

Dissection of fetal tissue was performed following hysterotomy under sterile conditions. Fetal brains were held in oxygenated Eagles medium and the selected sites were microdissected. Tissue to be transplanted was minced, drawn into 19 gauge cannulae and stereotaxically delivered to the host brains. Two hosts (S092 and S054) simultaneously received SN from a single African green monkey fetus (17cm CRL). Solid tissue grafts were implanted bilaterally into the head and body of the caudate nucleus. Three injections were made at each of six penetration sites. Initial implants were performed at 30 minutes after removal of the fetus; final implants occurred 80 minutes later. The third host (S114) received control grafts from an early gestational fetus (1.3cm CRL). The SN was implanted bilaterally into the cingulate cortex; hypothalamus was placed into the left striatum - lateral ventricle and LC-SC was implanted into the right striatum - lateral ventricle. Routine post-operative care was provided for each animal. Immunosuppresant drugs were not utilized in this experiment.

The anatomical, biochemical and behavioral correlates of these fetal CNS grafts as reported in the accompanying abstracts support the interpretation that these grafts survived, grew, and interacted with the host brain in a functional sense. Supported by the Axion and St. Kitts Biomedical Research Foundations, AG 00847, and an ADRDA Faculty Scholar Award.

397.4 PRIMATE NEURAL TRANSPLANTS: IV. BEHAVIORAL CHANGES IN AFRICAN GREEN MONKEYS FOLLOWING MPTP INTOXICATION AND TRANSPLANTATION OF FETAL NEURONS. D.E. Redmond, Jr., J.P. Fenerty*, R.H. Roth, J.R. Sladek, Jr. Dept. Psychiat., Yale Univ., New Haven, CT 06510; Dept. Neurobiol. & Anatomy, Univ. Rochester, Rochester, NY 14642.

The biochemical and histopathological effects of MPTP reported in previous studies in monkeys and in accompanying reports from our program support MPTP effects as an experimental Parkinson's Disease model. The fact that the dopamine precursor L-DOPA produces substantial improvement in MPTP-induced and idiopathic PD further suggests that replacement of missing dopamine neurons by transplantation might produce improvement in function. This motor and behavioral model therefore might serve to demonstrate possible functional consequences of integration of grafted neurons with the host brain.

Nineteen male Cercopithecus aethiops sabaeus were treated IM with 0.3 to 0.4 mg/kg doses of MPTP administered 5 or 6 times over a 5 day period, with appropriate saline-treated control monkeys. Behavioral ratings were made by observers who did not know which monkeys received MPTP or which grafts had survived in three of these monkeys. Ratings measured tremor, effect of intention, motor freezing, eating problems, delayed initiation of movement, speed of movement, and responses to threats; eighteen spontaneous behaviors were also scored per unit time.

There was variability in MPTP effects on behavior. With identical doses and treatment schedules, some animals were asymptomatic, while others showed significant and persistent behavioral changes, and some died, without weight loss, associated with profound motor freezing and incoordination. All animals with any behavioral impairment were given adequate food and water by hand to maintain nutrition and hydration.

Behavioral changes began after 2 or 3 doses in some monkeys, and most changes progressed over the next 2 weeks following MPTP administration. All three animals grafted with fetal neurons, as described in the accompanying presentation by Collier et al were severely parkinsonian prior to grafting and required hand feeding. The two with large numbers of TOH-positive cell grafts in the striatum (S092 & S054) showed behavioral improvements correlating with increased CSF HVA concentrations. These improvements increased until sacrifice 70 days after grafting. The third grafted animal (S114) remained profoundly impaired and died 56 days after grafting. Another monkey, which was not grafted, remained parkinsonian during this time period. These data suggest that fetal neuronal grafts may reverse MPTP effects in monkeys, but longer term studies are needed to confirm these promising preliminary observations.

Support by Axion/St. Kitts Biomedical Research Foundations, RSCDA DA00075 and RSA MH00643 to D.E.R.

397.5 PRIMATE NEURAL TRANSPLANTS. V. NEUROANATOMICAL ANALYSIS OF SURVIVAL AND GROWTH OF FETAL CNS GRAFTS IN MPTP-TREATED ADULT AFRICAN GREEN MONKEYS. J.R. Sladek, Jr., T.J. Collier, S.N. Haber, R.H. Roth, and D.E. Redmond. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642; Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 86510.

Immunohistochemical staining for tyrosine hydroxylase (TOH - Eugene Tech, Allendale, New Jersey) was performed according to established procedures (Haber and Nauta, Neurosci. 9:245, 1983) in each monkey which received fetal neuronal transplants described in the preceding abstracts. Non-grafted monkeys also were examined. Monkeys receiving fetal substantia nigra (SN) into the caudate nucleus (i.e. S092 and S054) were perfused with paraformaldehyde at 10 weeks after transplantation. The control monkey (i.e. S114), in which SN was grafted into inappropriate cortical sites, was perfused following death on day 56 after grafting. Neuroanatomical maps were prepared in each animal depicting the location of fetal grafts as well as the number and position of TOH-positive neurons in the host striatum and cerebral cortex. S092 and S054 possessed numerous TOH neurons throughout the striatum, on the order of 60,000 and 15,000, respectively. Beaded processes of these neurons ramified extensively throughout the neuropil and were often more extensively branched in S054. TOH-positive cells were found in clusters most apparent in S092 in the vicinity of the cannula tracks, and were seen in high densities at the rostral boundaries of the striatum which suggests either a migration, perhaps along nigrostriatal routes, or an induction of TOH from an endogenous neuronal pool. These neurons were not seen in the monkey (S114) which failed to show behavioral improvement. Plugs of grafted tissue were seen within the striatal neuropil in each monkey; in S054 they extended bilaterally into the lateral ventricles and contained TOH-positive neurons and fibers. SN grafts were found bilaterally in cingulate cortex in the control (S114). Each graft contained dense clusters of branched TOH-positive neurons. Neuronal processes extended throughout the grafted tissue, but did not cross the graft-host interface. Grafts containing TOH neurons and fibers from hypothalamus and LC-SC were seen in the caudate and lateral ventricle in S114 and exhibited features reminiscent of donor regions with respect to neuronal geometry and spatial relationships, e.g. the pontine graft contained large, multipolar TOH neurons adjacent to smaller, densely packed TOH neurons comparable in appearance to the LC-SC region in primates. These findings provide evidence for the survival and growth of catecholaminergic neurons from fetal brain grafted into neurologically impaired adult monkeys and support the behavioral observations of improvement of parkinsonism in MPTP-treated monkeys (S092 and S054) presented in the accompanying abstract. Supported by the Axion and St. Kitts Biomedical Research Foundations, and grant AG 00847.

397.6 FETAL DOPAMINE CELL TRANSPLANTATION AS A TREATMENT FOR PARKINSON'S SYNDROME IN BONNET MONKEYS. C.R. Freed, J.B. Richards*, E. Alianiello*, R. Peterson*, L. Ruppe*, S. Singh*, and M. Reite, Depts. of Med., Pharm., Ob/Gyn., and Psych., Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

The Parkinsonian syndrome produced by N-methylphenyltetrahydropyridine (MPTP) in man and in other primates has provided a useful model for studying treatment of Parkinson's disease. Preliminary reports indicate that transplantation of fetal dopamine cells may improve neurologic performance in Parkinsonian monkeys (Bakay et al., Soc. Neurosci. Abstracts 11: 1160, 1985; Redmond et al., Lancet, in press, 1986). We have studied the behavioral effect of fetal substantia nigra tissue transplanted unilaterally into caudate and putamen of MPTP lesioned monkeys which had been pretrained to perform a motor task. Five male Bonnet monkeys (maccaca radiata) were food deprived and trained to pull a lever for a food reward following a light cue. They were trained to an FR 10 schedule with each arm. Performance was alternated from side to side randomly under computer control. In addition to the lever pulling task, animals were timed during reaching tasks and other fine motor control tasks. A total of ten trials with each arm made up a daily session. Following optimization of motor performance, animals were lesioned with MPTP hydrochloride. They were injected with 0.5 mg/kg i.m. of the drug every other day and motor performance was monitored on the day following each injection. An effort was made to control the severity of the lesion so that some performance in the trained task persisted. Motor behavior was followed for four weeks after stabilization of the MPTP syndrome. Fetal tissue was obtained from monkeys 12 to 20 weeks gestation as determined by ultrasound. This age range corresponded to biparietal diameters of 22 to 50 mm and crown-rump lengths of 7 to 18 cm. Substantia nigra was dissected as a block from the ventral third of the mesencephalon using caudal hypothalamus and anterior pons as the rostral caudal boundaries. The tissue was minced into pieces smaller than 1 mm in size and then drawn into an 18 gauge stainless steel needle for injection. Under pentobarbital anesthesia, the recipient animals were injected with tissue unilaterally into caudate, putamen, and globus pallidus at a total of 24 sites through 7 tracts. Tissue was injected via an infusion pump at 2 µl per min with a 2 µl injection at each site. Improvement in motor performance was assessed both with the lever pulling task and with the reaching and fine motor tasks. Two months after transplant, animals were anesthetized and sacrificed by cardiac perfusion with formaldehyde-glutaraldehyde and dopamine cells in striatum were identified by histofluorescence.

397.7 FUNCTIONAL DOPAMINE TRANSPLANTS IN THE STRIATUM OF BILATERALLY DOPAMINE-DEPLETED NEONATAL RATS. R.K. Carder*, A.M.Snyder-Keller, R.D.Lund, (SPON:V.K. Neilsen), Dept. of Neurobiology, Anatomy and Cell Science and Center for Neuroscience, Univ. of Pittsburgh, Sch. of Medicine Pittsburgh, PA 15261

Dopamine (DA)-depleting lesions of the nigrostriatal pathway have devastating behavioral consequences when made in adult rats, but minimal effects when performed on neonates. These neonates are insensitive to DA receptor antagonists (Bruno et al., Behav. Neurosci. 771-775, 1985) and have an enlargement of the serotoninergic projection from the raphe nucleus to the striatum (Snyder et al., J. Comp. Neurol. 245: 274-281, 1986). Embryonic nigral cells placed in mature striatum have been shown to ameliorate many of the deficits caused by DA-depleting lesions in adult rats (Bjorklund, et al., Brain Res. 199: 307-333, 1980). We have begun to investigate the impact of DA-rich grafts placed into the immature striatum on the plastic responses following DA depletion in neonates.

Unilateral grafts of DA-rich cell suspensions were injected into the striatum of 5 day old bilaterally 6-hydroxydopamine lesioned rats as well as intact animals. Immunohistochemistry for tyrosine hydroxylase revealed that embryonic DA neurons were able to survive in both cases. Cells were often highly dispersed throughout the striatum, ventricle and septum, and appeared to survive equally well in all these locations. The DA neurons showed extensive fiber outgrowth into the striatum, at times innervating over half of the striatum.

In order to examine the functional significance of these grafts, an amphetamine rotation test was employed. Amphetamine elicited a turning away from the side with the transplant in bilaterally lesioned animals. Turning was most intense in the case of more dorsally located transplants. No turning was seen in the non-lesioned animals with unilateral transplants nor in lesioned animals without transplants.

These data demonstrate that unilateral DA transplants can survive in neonates and send out extensive processes into the striatum. These grafts appear to be functional in that these animals turn in response to amphetamine. Experiments are now underway to determine if the DA transplant alters the development of compensatory changes in the striatum of animals DA-depleted as infants.

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397.8 **REINNERVATION OF THE DOPAMINE DEPLETED STRIATUM OF ADULT WEAVER MUTANT MICE BY GRAFTED MESENCEPHALIC DOPAMINERGIC NEURONS.** L.C. Triarhou, W.C. Low and B. Ghetti. Depts of Pathology and Physiology & Biophysics, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

A progressive depletion of striatal dopamine (DA) has been shown in the weaver mutant mouse (Schmidt et al., J. Neurosci., 2: 376-380, 1982). In the present study, we transplanted embryonic grafts of substantia nigra (SN) into the lateral ventricle of adult weaver mice to test whether genetically normal grafts survive in the mutant host environment and innervate the host brain parenchyma. Moreover, we examined the SN of the weaver recipients and of age-matched controls (+/+) by tyrosine hydroxylase (TH) immunocytochemistry to determine whether the striatal DA deficiency is due to an abnormality of axon collaterals or to a specific nigral depletion. Donor tissue was obtained from the ventral mesencephalic flexure of normal mouse embryos at gestational day 15 and stereotaxically implanted into the lateral ventricle of 18 mutants. Recipients were perfused 36-51 days postoperatively. TH immunoreactive neurons in the SN pars compacta of mutant hosts were approximately 80% fewer than in controls at comparable anatomical levels. Grafts were found within the ventricle, attached to the caudate-putamen complex of the host. DA neurons were identified by immunocytochemical labeling with antiserum against TH. The same neurons were negative for DA β-hydroxylase (DBH). The diameter of such neurons ranged from 8 to 18 μm. Grafts contained an estimated 130-830 TH positive neurons. Fine TH immunoreactive fibers were detectable within the host caudate in the vicinity of the mesencephalic grafts, while they were not detectable in the contralateral, non-grafted caudate. The depth of reinnervation of the host caudate was up to 250 μm. These findings (a) demonstrate a severe decrease of DA neurons in the SN of the weaver mutant, (b) provide evidence for a graft-host integration, and (c) suggest that grafting of mesencephalic DA neurons may be effectively applied to nigrostriatal degenerations of genetic etiology. (Supported by PHS grant RO1-NS14426 to B.G., and SO7-RR5371 and RO3-AG5575 to W.C.L.).

397.9 DOPAMINE NEURON TRANSPLANTS: ELECTROPHYSIOLOGICAL UNIT ACTIVITY OF INTRASTRIATAL NIGRAL GRAFTS IN FREELY MOVING CATS. V.M. Trulson and M.E. Trulson. Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

Much research has been devoted to transplanting fetal brain tissue into adult host animals. One of the most commonly studied systems is the dopamine (DA)-containing substantia nigra. Most of these studies utilized fetal substantia nigra tissue grafted into the DA innervated caudate nucleus of adult recipient animals. These grafts have been shown to be capable of re-establishing DA innervation of a host caudate, forming ultrastructurally normal synapses within a previously denervated caudate. These grafts have also been shown to restore a variety of motor and sensorimotor deficits associated with destruction of the DA pathway. Because the behavioral deficits are reversed by nigral grafts it is assumed that the grafted DA neurons are spontaneously active in the host brain. However, evidence for spontaneous activity of grafted DA neurons has not been presented. We examined the activity of single DA neurons after grafting from fetal cat brain to adult host cat brain. Adult female cats received a unilateral 6-hydroxydopamine (6-OHDA) injection into the right nigrostriatal bundle (12 ug free base in 6 μl of saline-ascorbic acid). Cats were selected on the basis of their bias to turn towards the ipsilateral side of the 6-OHDA injection in response to a 2 mg/kg (i.p.) dose of d-amphetamine. The grafts were administered as a cell suspension, using a modification of the method of Björklund et al. (Acta Physiol. Scand. Suppl. 522, 1983, 1-10). Four grafted deposits (each 5 μl) were given into the denervated right caudate nucleus. Five months after grafting, the amphetamine-induced rotational test was repeated. Grafted cats showed no turning bias, whereas 6-OHDA-lesioned non-grafted cats continued to show a strong bias for ipsilateral turning. Microelectrodes were implanted into the tissue graft, using techniques previously described (Neurosci. Biobehav. Rev., 9, 1985, 283-297). Single units recorded from the intrastiatal nigral grafts revealed that these neurons displayed some of the characteristic parameters of DA neurons, i.e., long duration action potentials (>2 msec) and tri-phasic waveform. However, these neurons discharged at a faster rate and never displayed the decremental burst pattern seen in intact, non-grafted animals. Thus, these neurons behaved more like DA neurons recorded in vitro (Neuroscience, 12, 1984, 793-801) than DA neurons recorded in vivo in an intact animal. Grafted DA neurons were inhibited by low doses of apormorphine or amphetamine, and these responses were blocked by haloperidol. Therefore, DA autoreceptors appear to be intact on grafted neurons. Fluorescence histochemistry revealed large numbers of DA-containing cell bodies in the recording site within the caudate.

397.10 NEURONAL XENOGRAFTS FROM HUMAN FETUSES TO THE EYE AND BRAIN OF IMMUNOSUPPRESSED RATS: STRUCTURAL AND FUNCTIONAL DEVELOPMENT IN OCULO AND DOPAMINERGIC REINNERVATION OF HOST STRIATUM. I. Strömberg*, M. Bygdeman*[1], B.J. Hoffer[2], R. Freedman[2], M. Goldstein[3], L. Olson*, A. Seiger* Dept. of Histology, Karolinska Inst., [1]Dept. of Gynecology, Karolinska Hosp., 104 01 Stockholm, Sweden, [2]Dept. of Pharmacology, Univ. of Colorado Med. Ctr., Denver, CO 80262, [3]Dept. of Psychiatry, New York Univ. Med. Ctr., NY, NY 10016.

Syngeneic grafts of fetal brain tissue in rodents are powerful tools in studies of brain development and connectivity. Such grafts can also ameliorate experimentally induced impairment of central nervous function. We now report initial results from grafting human fetal tissues to the anterior chamber of the eye and to the brain of adult rats immunosuppressed with cyclosporin A. Material was obtained from non-viable fetuses using a protocol approved by the Karolinska Institute Ethical Committee. Tissues from week 8-11 were prepared for grafting. Locus coeruleus, raphe pallidus, parietal cerebral cortex, hippocampus, cerebellum, spinal cord, dorsal root ganglia, adrenal medulla and heart muscle all survived grafting to immunosuppressed hosts. In spite of daily cyclosporin (10 mg/kg), approximately 50% of the intraocular grafts deteriorated, while the rest became vascularized and grew in the eye. Central monoamine neurons were often able to innervate adjacent areas of the host iris. Human fetal parietal cerebral cortex grafts increased their size several-fold. The presence of tyrosine hydroxylase immunoreactive nerve terminals in such grafts suggested ingrowth of noradrenergic fibers from the host iris. Electrophysiological recordings from cortical transplants one month after grafting revealed spontaneously active neurons with immature electrophysiological characteristics. Chromaffin cells were strongly catecholamine-fluorescent, had elongated shapes, and tended to form nerve fibers on a sympathetically denervated host iris. Heart muscle from a 9-week fetus showed spontaneous rhythmic contractions in oculo. Sympathetic nerve fibers had innervated the heart graft from the host iris. Fetal substantia nigra was also grafted to cavities overlying striatum in animals with a unilateral 6-hydroxydopamine-induced dopaminergic denervation of striatum. Such grafts grew and tyrosine hydroxylase-immunohistochemistry revealed large numbers of cell bodies in the graft as well as many immunoreactive nerve fibers both in the graft neuropil, and invading the dopamine-denervated host striatum. Apomorphine-induced rotational behavior in these animals was reduced by the human substantia nigra grafts. We conclude that human fetal brain tissue can be successfully grafted to immunosuppressed rat hosts. The technique opens new possibilities to study human brain development, maturational disturbances and neurodegenerative diseases.

397.11 DOPAMINE METABOLITES IN THE VENTRICULAR CSF OF RATS STUDIED WITH MICRODIALYSIS. F.S. Adams*, J.B. Becker, and T.E. Robinson (SPON: C.M. BUTTER). Dept. of Psychology, University of Michigan, Ann Arbor, MI 48104-1687.

Dopamine (DA) and serotonin metabolites can easily be detected in the ventricular CSF of rats by using push-pull techniques, but the exact source of these metabolites has not been well characterized. The present study employs microdialysis and selective lesion techniques to assess the amount and source of these metabolites.

To determine the relative contribution of the nigrostriatal DA system to monoamine metabolites found in the lateral ventricles, rats were given unilateral 6-OHDA lesions of the substantia nigra (SN). Two weeks later, rotation in response to the DA agonist, apomorphine, was used as a prelimenary assessment of striatal DA denervation. The CSF was then sampled in rats anesthetized with ethyl urethane, by stereotaxically inserting microdialysis probes into both lateral ventricles near the medial surface of the striatum. Artificial CSF was infused at a rate of 1 ul/min and samples collected over 15 min intervals into tubes containing perchloric acid to stabilize the metabolites, and dihydroxybenzylamine (DHBA) as an internal standard. After a 2 hour baseline collection period, animals received an injection of haloperidol and samples were collected for an additional 2 hours. The concentration of homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindole-3-acetic acid (5-HIAA) in the dialysate was analyzed by HPLC-EC and expressed in units of pmol/ul in CSF. The concentrations of metabolites from the intact side were compared to those from the denervated side, as well as to the levels from a group of animals that had received sham lesions of the SN. Following dialysis, the animals were killed by decapitation and their brains rapidly removed and dissected on ice. Bilateral tissue punches from the striatum, nucleus accumbens, and olfactory tubercle were taken, and DA and DOPAC assayed by HPLC-EC after alumina extraction to determine the extent of DA depletion. Placement of the dialysis probes was confirmed histologically. Studies to date have shown that although there is a great reduction in DA metabolites on the side of a 6-OHDA lesion, easily measurable concentrations remain, even after extensive striatal DA depletion. Research is in progress to identify the source of the remaining metabolites.

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397.12 CATECHOLAMINE RELEASE FROM TISSUE GRAFTS IN THE LATERAL VENTRICLE STUDIED BY MICRODIALYSIS IN FREELY MOVING RATS. Jill B. Becker, Terry E. Robinson, and William J. Freed. Department of Psychology and Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, MI 48104 and St. Elizabeth's Hospital, The National Institute of Mental Health, Washington, D. C. 20032.

It is well established that grafts of catecholamine (CA)-rich tissue into the lateral ventricle or striatum can alleviate some of the symptoms associated with unilateral nigrostriatal dopamine (DA) depletion. It has been assumed that the release of DA from these gratfs is responsible for the recovery of function, but this has not been explicitly demonstrated. In the study to be presented we correlate the release of CA from adrenal chromaffin cells grafted into the lateral ventricle with changes in rotational behavior in freely moving rats.

Female Long-Evans rats received unilateral 6-hydroxydopamine lesions of the substantia nigra and 2 guide cannulae were chronically implanted above the lateral ventricle in the lesioned hemisphere. The efflux of CA and indolamine metabolites into the lateral ventricle was measured in freely moving rats by introducing a microdialysis probe into the ventricle through the caudal cannula. Animals were placed in a large bowl and artificial cerebrospinal fluid (CSF) was infused at a constant rate of 1 ul/min. After 1 hour of dialysis, to allow release to stabilize, samples were collected at 15 min intervals in collection vials that contained 0.05 N perchloric acid (to stabilize the compounds in the dialysate) and dihydroxybenzylamine (as an internal standard). Two baseline samples were collected, then each animal received 3 mg/kg d-amphetamine sulfate (s.c.) and ventricular dialysate samples were collected for an additional 2 hours. Rotational behavior was simultaneously recorded on videotape for subsequent analysis. Samples were assayed the same day by HPLC-EC. Values in CSF were calculated from the percent recovery that had been determined for the probe in vitro prior to the dialysis.

After this initial screening, animals received an implant of adrenal chromaffin cells by injection through the rostral cannula. The dialysis/rotational behavior procedure was repeated at various time intervals after tissue implant. Experiments are still in progress. Results will be presented at the meeting.

(Supported by NIH #NS-22157 and The American Parkinson's Disease Foundation).

397.13 SUBSTANCE P-LIKE AND TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITY IN FETAL SUBSTANTIA NIGRA GRAFTED TO CATECHOLAMINE DENERVATED STRIATUM: A DUAL-LABEL ULTRASTRUCTURAL STUDY. Thomas J. Mahalik[1], Ingrid Strömborg[2], Lars Olson[2] and Thomas E. Finger[1]. [1]Dept. Cell. & Struct. Biol. U. Colo Med Sch, Denver CO. and [2]Dept. of Histology, Karolinkska Institute, Stockholm, Sweden.

Grafts of fetal substantia nigra to the striatum can reduce the severity of a number of behavioral deficits produced by depletion of striatal catecholamines. In addition, graft-derived catecholaminergic axons form synapses and release dopamine within the host CNS. These and other observations suggest that, to a certain extent, fetal nigral grafts become integrated into the host CNS. In order to determine the specificity of graft-host interconnections, the distribution of substance P-like (SPLI) and tyrosine hydoxylase-like (THLI) immunoreactive material in the substantia nigra of the adult rat was compared with the distribution of THLI and SPLI material in fetal substantia nigra grafted to the denervated striatum of the adult rat. Since the primary antisera were generated in different species, peroxidase label was used to identify regions immunoreactive fer substance P, while a colloidal gold label was used to localize tyrosine hydroxylase immunoreactivity.

Sprague-Dawley rats were used for studies of normal adults. In the substantia nigra of these rats, we found that SPLI axon terminals make synaptic contacts with THLI dendrites and cell bodies. Our findings support the idea that striatal substance P-containing neurons directly influence the activity of nigral dopaminergic neurons in the normal rat CNS.

For studies of grafted nigral material, adult female Sprague-Dawley rats were given unilateral injections of 6-OHDA (4 ug/8 ul) into the right substantia nigra, followed 6 weeks later by a 'delayed cavity' graft of fetal substantia nigra. After an additional 6 to 9 month period, the rats were prepared for ultrastructural, dual-label immunocytochemistry. In fetal substantia nigra grafts we observed many SPLI terminals and numerous THLI dendrites, axons and cell bodies. We also observed SPLI terminals in contact with THLI cell bodies and proximal dendrites. These contacts were morphologically similar to the synapses that we observed in normal adult substantia nigra.

These observations suggest two possible hypotheses. First, nigral grafts may contain intrinsic SPLI neurons that synapse upon dopaminergic cells. Alternatively, substance P neurons from the host striatum may send axons into nigral grafts. In either case, it appears that both normal and grafted dopaminergic neurons of the substantia nigra receive direct input from substance P neurons.

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397.14 TRITIATED THYMIDINE ANALYSIS OF NEOSTRIATAL PRIMORDIA TRANSPLANTED TO ADULT NEOSTRIATA. J.P. McAllister II and A. Norelle*. Departments of Anatomy and Neurosurgery, Temple University School of Medicine, Philadelphia, PA 19140.

Previous identification of embryonic neostriatal tissue transplanted into either intact or lesioned host neostriata has been based purely on cytoarchitectural criteria. The possibility remains that neurons could have translocated from the adjacent host into the transplant, or that transplanted cells may integrate with the adjacent host tissue. To unequivocally determine the origin of the transplanted neurons, we have tagged either the donor or host tissue with tritiated thymidine and examined the transplant and host neostriatum for labelled cells. Pregnant rats received 2 intraperitoneal injections of ^{3}H-thymidine (spec. act. 6.7Ci/mM, dose 5 uCi/g body wt.) on days 12 and 13 of gestation (E12,E13), day 0 being the day of sperm positivity. For experiments in which the transplanted cells were labelled, E14 striatal ridges were dissected from the embryos of donors that received ^{3}H-thymidine. These neostriatal primordia were dissociated in 0.1% trypsin and transplanted into either the intact neostriatum of adult hosts, or into neostriata that had been lesioned 5 days previously with kainic acid (3 nmol). In order to determine if some cells within the transplant arise from the host, a second series of animals were prepared in which only the hosts received ^{3}H-thymidine. Hosts were sacrificed 30-80 days post-transplantation; their brains were embedded in paraffin, sectioned at a thickness of 15 um and processed by routine autoradiographic methods. At present, only the analysis of labelled donor tissue transplanted into intact neostriata is complete. Transplants up to 2mm in diameter were located in the middle of the host neostriatum and within the overlying cerebral cortex along the needle track. These transplants were usually separated from the adjacent host tissue by a region of intense gliosis. Intensely and moderately labelled neurons were found in all parts of the transplants. These cells were similar to host neostriatal neurons by being round or polygonal in shape, 12-25 um in diameter and containing a thin rim of cytoplasm. Approximately 40% of all neurons within the transplant contained some label. Occasionally labelled neurons were located within the surrounding gliotic region, but never in the adjacent host neostriatum or cerebral cortex. Glial cells were seldom labelled within the transplant or in any parts of the host brain. However, in the glial scar, label was present in cellular fragments and within glial cells, and may represent debris from transplanted cells that had died. These results demonstrate that at least part, if not all, of the neurons comprising neostriatal implants are derived from embryonic donors.

397.15 EVIDENCE SUGGESTING MINIMAL CONNECTIVITY BETWEEN NEOSTRIATAL GRAFTS AND HOST BRAIN. P.D. Walker*, J.S. Way*, and J.P. McAllister II (SPON: J. Kenning). Departments of Anatomy and Neurosurgery, Temple Univ. Sch. Med., Philadelphia, PA 19140.

Neostriatal (NS) primordia transplanted into adult NS have been shown to reduce behavioral deficits resulting from intrastriatal excitoxic lesions (Deckel et al., 1983; Isacson et al., 1984). However, it is unclear if this recovery is mediated by direct axonal connections with the transplant. Lectin peroxidase (WGA-HRP) labelling was employed to determine if afferent and efferent connections exist between NS grafts and the host brain. Adult rats received unilateral or bilateral intrastriatal injections of dissociated E14 NS five days after kainic acid (KA) lesions as described previously (McAllister et al., 1985). One to two months later, transplanted animals received WGA-HRP injections directly into the transplant or ventral midbrain of the host. Injection sites within the transplant were restricted to the graft itself. Retrogradely labelled neurons and axonal labelling were found within regions of the transplant rostral and caudal to the injection site. However, no label was found in the host brain. These data suggest intrinsic connections exist within NS grafts, but no extrinsic connectivity is established with the host brain. In animals that received ventral midbrain WGA-HRP injections, retrogradely labelled neurons were found in the intact host NS. Anterograde axonal labelling was located within regions of intact and KA damaged NS adjacent to the grafts. Anterograde reaction product was found in peripheral locations within the graft. Label in these regions was extremely sparse and did not extend more than 200 μm into the transplant. The labelling pattern was characterized by fine grains typical of axon terminals, as well as linear arrays considered to be preterminal axons. In many instances, this anterograde label was located in regions populated predominantly by glial cells. Rarely, retrogradely labelled neurons were located in these peripheral zones. In contrast, nearly all regions of the grafts contained an abundance of neurons, and these areas were devoid of antero- or retrograde reaction product. These results indicate that afferent and efferent connections between the transplant and host brain after 1-2 months are extremely sparse or absent. It is possible that peripheral regions containing reaction product might represent transition zones between host brain and transplant. The origin of labelled cells and whether the labelled axons are terminating within host or graft tissue remains in question. Therefore, mechanisms other than transplant-host connectivity may be responsible for behavioral recovery after NS grafting.

397.16 LOCATION OF FETAL STRIATAL TRANSPLANTS AS AN IMPORTANT DETERMINANT FOR BEHAVIORAL RECOVERY OF STRIATAL LESIONED RATS. M. Giordano, S.H. Houser, K.H. Russell* and P.R. Sanberg. Behavioral Neuroscience Lab., Ohio University, Athens, Ohio 45701, and Div. of Psychobiol., Univ. of Cincinnati Medical Center, Cincinnati, Ohio 45267.

Fetal neural tissue transplants have been shown to reverse behavioral and biochemical deficiencies produced by brain lesions in a variety of animal models. In particular such transplants have been shown to reverse the hyperactivity that follows kainic acid (KA) lesions of the striatum. The results, however, have been variable possibly due to the location of the transplant. The present study examined the efficacy of placing the transplants directly into the lesioned striatum.

The effect of fetal striatal ridge (day 17) transplants on the locomotor behavior of striatal kainic acid lesioned rats (Sprague-Dawley) was examined. Several locomotor activity variables were measured using.Digiscan-16 monitors (Omnitech Electronics). In order to avoid the placement of the transplant within the ventricles, 4 ul of fetal tissue were injected more lateral to the midline (2.6 mm) than in previous studies. In addition, HRP histochemistry was used to detect connections between transplant and host brain.

Preliminary comparisons of the data showed that three weeks after the KA lesions, the lesioned animals appeared hyperactive with respect to the control group. Ten weeks after the transplant, those animals receiving the fetal tissue reduced their activity to the level of the control subjects. In contrast, the lesioned only group remained hyperactive.

Histological examination of the brains, showed that the majority of the transplants were small and deeply embedded in the anterior portion of the striatum, none were found to grow within nor near the lateral ventricles. It has indeed been difficult to find clear cut boundaries between the host brain and the transplant. Those brains which did not receive the fetal transplant, showed ventricular dilation. HRP histochemistry showed some evidence of connectivity between host brain and transplant.

These results appear to support the idea that anatomical interaction between host brain and transplant is necessary to obtain behavioral recovery. They also suggest that a more dramatic behavioral recovery can be obtained when the transplant is completely embedded in the host tissue, rather than near or within the ventricles.

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397.17 TRANSPLANTATION OF DIFFERENT VOLUMES OF FETAL STRIATUM: EFFECTS ON LOCOMOTION AND MONOAMINE BIOCHEMISTRY. A.W. Deckel and R.G. Robinson. Dept. of Psychiatry, Johns Hopkins Univ. Sch. Med., Balt., MD 21205.

Kainic acid lesions, in female rats, cause long-lasting changes in locomotor behaviors that are partially remediated by transplanting fetal striatum (str) into the lesioned area. Previous work suggested that the number of transplanted fetal str cells positively correlated with the extent of transplant-induced recovery. This experiment examined if different volumes of fetal striatal transplants differentially affected the behavior or brain biochemistry of male rats at extended time periods post surgery.

Forty-eight male Sprague-Dawley rats, weighing 200-300 grams, were assigned to one of five groups. Four groups received bilateral lesions (0.8 ugs kainic acid in 0.4 uls PBS, 0.1 ul/90 sec) of the anterior medial str followed, one week later, by transplants of E16 str placed directly into the lesioned str. Transplanted animals received different volumes of nontrypsinized donor tissue, including; 1) 6.0 ul, 2) 3.0 ul, 3) 1.5 ul, and 4) 0 ul of donor striatum. The fifth group recieved sham lesions/sham transplants. Beginning 25 weeks post-lesion, spontaneous locomotion during the dark period and during a period of food deprivation was assessed in an animal activity monitor. In addition, daytime locomotion was measured for 2 hour periods following injection of saline (1.0 ml/100 kg), amphetamine (1.0 mg/100 gms), apomorphine (0.2 mg/100 gms), and scopolamine (0.8 mg/100 gms). Animals were sacrificed by decapitation, and NE, DA, and DOPAC HPLC biochemistry was done in frontal cortex, striatum, and substantia nigra via the method of electrochemical detection.

In contradistinction to female rats, which demonstrate continued hyperactivity at comparable periods following kainic acid str lesions, and acutely lesioned male rats, which also demonstrate an increase in locomotive behavior, the kainic acid lesioned animals were hypoactive compared to controls across the different conditions. Under the saline condition, differing volumes of transplants had a dose response-like effect on locomotive behavior, with activity being greatest (i.e., similar to controls) in the 1.5 group, less in the 3.0, and significantly reduced in the 6.0 group. The 6.0 group also was hypoactive compared to controls in the various other conditions of locomotion, while the 1.5 and 3.0 groups variably remediated the lesioned-induced hypoactivity. As the volume of the transplant increased, cortical levels of NE increased in a dose-response manner, while str DOPAC/DA levels nonsignificantly declined.

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397.18 HOST NIGRAL FIBERS MAY GROW INTO STRIATAL GRAFTS. F.C. Zhou, C.D. Hull, M.S. Levine and N.A. Buchwald. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024.

In several brain-graft procedures, replacement of experimentally destroyed or inherently lacking neuronal structures, has resulted in alleviation of pathological symptoms. For example, rats in which neostriatal lesions are made may exhibit a considerable degree of hyperactivity. This can be alleviated by grafting embryonic neostriatum (NS) into the lesioned host brain. The extent to which this alleviation is dependent on newly formed connections between the graft and the host brain was investigated.

Ibotenic acid lesions were made unilaterally in the host NS or substantia nigra (SN) region. Fetal (gestational day 14) NS tissue was then transplanted into the NS or SN region of the lesioned host brain. Six to 7 weeks later, 300nl of 5% WGA-HRP were injected into the host SN (n=5). The animals were then sacrificed and processed. HRP-TMB reaction products were observed all along the nigral injection sites. Retrogradely marked neuronal somata were observed in all areas of the host striatum and adjacent pallidum. No neurons in the transplant were marked. This result was taken to mean that axons of transplanted NS neurons did not reach the host SN. The behavioral recovery after striatal transplant cannot be due to new striato-nigral connections.

To examine host-graft nigro-striatal connectivity animals receiving striatal grafts in the NS (n=4) or adjacent to (above) the SN (n=3) were processed for immunocytochemical staining of nigral dopamine neurons. Antisera to the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH) were used to produce a marker. TH positive fibers innervated the host NS heavily. There was also some evidence of sprouting of host striatal axons. These sprouts were sparse if the grafted NS was placed in the host NS. They were heavier, however, if the grafted embryonic tissue was placed in or near the SN. Whether or not the host SN neurons make synaptic contact with NS cells is still not clear, although Walsh et. al. (adjacent poster) have electrophysiological evidence that neurons in NS grafts may respond synaptically to extra-graft electrical stimulation.

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397.19 INTRACELLULAR STUDY OF TRANSPLANTED STRIATAL NEURONS. J.P. Walsh, C.D. Hull, M.S. Levine, F.C. Zhou and N.A. Buchwald. Mental Retardation Research Center. University California, Los Angeles. 760 Westwood Plaza. Los Angeles, CA 90024.

Intracellular records were obtained from fetal neostriatal (NS) neurons grafted into adult rat brain. NS cells obtained from fetuses (gestational age=14 days) were inserted into the NS of adult host rats. Three weeks after grafting, brain slices (350 to 500μm thick) were prepared for intracellular recording. Upon visual inspection, the graft appeared as a separate, encapsulated structure within the host NS. Neurons in the graft had resting membrane potentials of -55 to -70 mV and could generate action potentials of 60 to 80 mV. The neuronal membrane responses to intracellularly applied current pulses provided input resistances of 32±2 MΩ and time constants of 13.5±7 msecs (n=4).

Electrical stimulation of host NS bordering the transplant produced both EPSPs and IPSPs in the grafted NS neurons. In three neurons, stimulation evoked EPSP-IPSP complexes with latencies of 1,2 and 4 msecs. In 2 of these, the excitatory component seemed to be evoked at lower stimulus intensity than the inhibitory component. In 5 neurons the EPSP was evoked without an accompanying inhibitory component and in another 5 neurons, stimulation evoked inhibitory responses without EPSPs. Mean latencies in all 13 cases were 2.3±1.2 msecs. In the 5 cases in which EPSPs appeared alone, potential durations varied from 15-30 msecs. At threshold, action potentials similar in amplitude to those occurring with current injection were generated. When IPSPs appeared alone, their durations varied from 30-37 msecs. In one case the IPSP was reversed by hyperpolarizing currents at a membrane potential consistent with the equilibrum potential for Cl^-. The fact that potentials were evoked in graft neurons by stimulation of the host tissue may indicate that connections were formed between the host brain and the graft, possibly by ingrowth of host axonal elements. Verification of this possibility requires further careful physiological and anatomical observations. (Supported by USPHS HD 05958 and HD 07032).

397.20 THE USE OF COLLOIDAL GOLD-LABELLED CELL SUSPENSIONS AS A MARKER FOR INTRACEREBRAL TRANSPLANTATION. S.C. Ardizzoni*, G.W. Arendash, and A. Michaels* (SPON:S. Swihart). Department of Biology, University of South Florida, Tampa, FL 33620.

A new technique has been developed to monitor the survival and movement of transplanted neurons. This procedure has significant advantages because it is simple, rapid, and apparently a permanent cellular label with no obvious harm to transplanted cells. The labelled cells can be observed in the light or electron microscope.

Cells were labelled with colloidal gold (which is inert in cells) by encapsulating the gold in Sendai virus envelopes. The gold-containing membrane vesicles were fused with previously frozen or freshly prepared nucleus basalis magnocellularis (nBM) cell suspensions from dissected fetal rat brains (D18). After the cells were washed by centrifugation, the cholinergic-rich, labelled nBM cell suspensions were then transplanted into the cortex and corpus striatum of neonate rats or into several fronto-parietal cortex sites of nBM-lesioned adult rats. Recipients were sacrificed at either 10 or 30 days following transplantation and their brains processed for light microscopy.

In neonate recipients, gold-labeled cells were present at both cortical and striatal infusion sites. In adult nBM-lesioned recipients, large numbers of gold-labeled transplant cells were found in the vicinity of cortical infusion sites at both 10 and 30 days after transplantation, especially if the cell suspensions had been freshly prepared on the day of transplantation. However, nBM cell suspensions previously frozen for 6 months prior to gold-labelling showed excellent survival. Additionally, a dramatic migration of gold-labeled neurons from cortical infusion sites in adult recipients to the excitotoxin-lesioned nBM (a distance of 3-4 mm) was observed.

These results suggest that gold-labeling of neuronal cell suspensions will be a powerful new tool for: 1) investigating the development (migratory patterns, morphology, and synaptic connectivity) of transplanted cells, and 2) monitoring the viability of transplanted cells in vivo.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM II

398.1 INCREASED HEART RATE AND PLASMA LEVEL OF β-LIPOTROPIN DURING A COMPETITIVE ORAL EXAMINATION. J.L. Meyerhoff, M.A. Oleshansky, E.H. Mougey*, H.R. Smith*, S.R. Hursh*, L.K. Wittig*, H.E. Wood*, C.R. Glass* and D.B. Arnkoff*. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, and Dept. of Psychology, Catholic University, Washington, D.C. 20064.

β-Lipotropin (β-LPH) and ACTH are derived from the same precursor molecule, stored in the same granules and thought to be concomitantly released from the anterior pituitary gland in response to stress. We have previously reported increased plasma levels of β-LPH after vigorous physical exercise. The present study was undertaken to determine whether β-LPH would also rise during the purely psychological challenge of a competitive oral examination.

A "Soldier of the Month" contest is held regularly and is entered by young, healthy enlisted U.S. Army personnel. Each contestant is interviewed courteously but rigorously for 30 min by a panel of six senior non-commissioned officers. The winners are chosen on the basis of apppearance and bearing, as well as knowledge of Army procedures, protocol and regulations. Some of the contestants volunteered to be studied during this competition and gave informed consent for heart rate monitoring (N=8) and/or blood sampling (N=5) during the interview. Heart rate was monitored using a PMS-8 portable physiological monitoring system (Vitalog Corp., Redwood City, CA). Blood was collected through an intravenous line attached to a Cormed pump (Medina, N.Y.) which withdrew blood continuously at 0.1 ml/min between samples and at 5.0 ml/min at discrete sampling times. Samples were collected: (A) after 30 minutes of sitting in a quiet waiting room prior to the interview; (B) 5 minutes after beginning the interview; (C) 20 minutes after beginning the interview; (D) 5 minutes after finishing the interview and returning to the waiting room; and (E) after sitting in the waiting room for another 15 minutes. Plasma levels of β-LPH and cortisol were determined by radioimmunoassay. β-LPH was separated from β-EP with Prep Pak 500/C18 (Waters Associates, Milford, MA).

	A	B	C	D	E
β-LPH (pg/ml)	130±18	190±19	181±30	130±24	121±22
Cortisol (μg%)	15.0±2.1	15.2±1.2	18.2±1.2	17.7±2.7	13.7±0.7
Heart Rate	88±3.8	123±9.2	113±9.3	93±4.5	84±3.3

Prior to the interview, resting heart rate as well as plasma β-LPH and cortisol levels were elevated compared to resting values in other studies from our laboratory. We attribute this to apprehension about the upcoming interview. Five minutes after beginning the interview, the heart rate and plasma β-LPH level increased 40% over point A. These values remained elevated for fifteen minutes and returned to initial levels after the subjects returned to the waiting room. The plasma cortisol level tended to increase toward the end of the interview. The present study demonstrates that acute psychological stressors can produce rapid increases in heart rate and plasma β-LPH levels over the elevated baselines associated with apprehension.

398.2 PLASMA CATECHOLAMINE RESPONSES TO MENTAL AND PHYSICAL STRESSORS. M. Oleshansky, J. Zoltick*, J. Meyerhoff, K. Kennelly*, J. Knapik* and F. Drews*, Dept of Medical Neurosciences, Div of NP, Walter Reed Army Institute of Research, Washington, D.C. 20307 and Army Physical Fitness Research Institute, Carlisle Barracks, PA 17013.

We are currently undertaking a study of the relationship of Type A behavior with acute responsivity to stress. We have completed a series of pilot experiments to determine the magnitude of physiological and hormonal responses to a variety of physical and mental stressors, including moderate and maximal exercise as well as two cognitive tasks. Preliminary findings for plasma catecholamines are presented below.

In the first study, seventeen healthy male subjects, age 39.8±2.5 years (mean ± S.E.M.), exercised on a treadmill until exhausted at a constant rate of 3.3 mile/hr with a 5% increase in grade every three min. Mean maximal exercise treadmill test (MAX ETT) time was 15.2±0.7 min. In a second study, fifteen additional male subjects, age 43.6±1.2 years, were studied during and after either four min of cognitive testing or twenty min of moderate treadmill exercise (MOD ETT). Cognitive testing consisted of mental arithmetic (n=10) or the Stroop Color-Word Dissociation Task (n=4). The MOD ETT consisted of walking on a treadmill at 3.3 mile/hr at a grade sufficient to exercise the subjects at 60% maximal VO_2 based on a previously determined maximal VO_2 (n=6). Blood was collected for two min with a Cormed blood withdrawal system: (REST) thirty min after resting supine (before maximal ETT) or sitting (cognitive tests); (STAND) fifteen min after standing, just prior to exercise; (TEST) upon exercising to a heart rate of 140 beats/min or during the second two min of cognitive testing; (MAX) immediately following maximal exercise; and (RECOVERY) fifteen min after the stressor. Plasma was assayed for norepinephrine (NE) and epinephrine (EPI) utilizing HPLC with electrochemical detection.

NE (pg/ml)	REST	STAND	TEST	MAX	RECOVERY
MAX ETT:	290±39	588±69	1357±138	1991±241	650±70
MOD ETT:		511±52	1242±183		334±31
MENTAL MATH:	416±34		512±34		455±56
STROOP TASK:	352±45		351±20		387±25
EPI (pg/ml)	REST	STAND	TEST	MAX	RECOVERY
MAX ETT:	81±9	115±15	255±36	333±47	141±26
MOD ETT:		81±16	141±34		68±14
MENTAL MATH:	68±11		130±24		82±13
STROOP TASK:	94±31		114±41		75±36

Standing resulted in a doubling of plasma NE levels over resting supine levels while sitting appeared to give intermediate NE levels. Moderate treadmill exercise produced a doubling of NE and EPI levels and further increases were seen after a maximal ETT. The cognitive tests appeared to produce disproportionately larger increases in plasma EPI levels than in NE levels as previously reported for psychological stressors. Mental math appeared to be a substantially better psychological stressor than the Stroop task. Future studies are directed at investigating the relationship of Type A behavior with these acute stress responses.

398.3 ELEVATED SERUM DEHYDROEPIANDROSTERONE SULFATE LEVELS IN OLDER, EXPERIENCED PRACTITIONERS OF THE TRANSCENDENTAL MEDITATION AND TM-SIDHI PROGRAMS. J.L. Glaser*, J.L. Brind*+, M. Eisner*, R. K. Wallace. Dept. of Physiological and Biological Sciences, Maharishi Intl. Univ., Fairfield, IA 52556 and Orentreich Foundation for Adv. of Sci., NYC, NY 10021+.

Serum levels of the adrenal androgen, dehydroepiandrosterone sulfate (DHEAS) are closely correlated with age in man (Orentreich N, Brind JL, et.al. 1984, J Clin Endocrinol Metab 59:551), and higher levels of DHEAS or DHEA may contribute to the prevention of age-related disease. Long-term administration of DHEA to various mouse strains inhibits the development of spontaneous and chemically induced tumors. In vitro, DHEA inhibits superoxide radical production in human polymorphonuclear leukocytes, which could be a mechanism for this anti-tumor activity and its inhibition of autoimmune diseases in mice.

Since previous studies have shown that older individuals practicing the Transcendental Meditation (TM) technique tend to have lower scores on a standardized test designed to estimate physiological age, we hypothesized that older TM practitioners would have elevated serum DHEAS levels characteristic of a younger population.

Serum DHEAS levels were measured in 252 men and 74 women who were experienced practitioners of TM and the TM-Sidhi programs, and compared according to sex and five-year age grouping to 799 men and 173 women who were not TM practitioners. The mean DHEAS levels in the TM group were higher in all five of the age groups measured in women. There were no systematic differences in younger men, but elevations were observed in TM practitioners in six of seven five-year age groups over 40 years. The mean percentage of DHEAS elevation over normal values was 23% for men over 40 and 47% for women over 45. ANOVA revealed significant systematic effects due to age and TM practice accounting for differences in group mean DHEAS levels. For TM practice, $p=.0001$ for all women and $p=.0026$ for all men. Identifiable differences in diet, obesity, and exercise in the TM group were not significant covariates in accounting for differences in DHEAS levels.

For men and women, DHEAS was highest in the youngest age groups and declined progressively with age to reach levels in the seventh and eighth decades which were only 20% of peak levels. The mean levels measured in the older meditators were comparable to levels in non-meditators five to ten years younger. The conclusion that regular practitioners of TM have higher DHEAS levels suggests that some characteristics of the TM group may be modifying the usual age-related deterioration in DHEAS secretion. It also suggests that there may be preventive health benefits accruing from the regular practice of TM; we have elsewhere found subnormal DHEAS levels in a population with endemically poor health.

398.4 PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF NON-PHARMACOLOGICALLY INDUCED PANIC ATTACKS. S.W.Woods*, D.S.Charney, C.A.McPherson*, A.H Gradman*, G.R.Heninger (SPON: M.H.Sheard) Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT., 06508.

A panic attack is a severe form of pathologic human anxiety. The neurobiologic mechanisms underlying panic attacks are important but relatively obscure. Most previous investigations in this field have studied drug-induced panic attacks. In order to investigate the pathophysiology of non-pharmacologically induced panic attacks, 18 drug-free agoraphobic patients and 13 matched healthy subjects were studied.

METHODS: Each patient underwent structured exposure to an individualized phobic stiuation. The duration of exposure ranged from 30 to 55 minutes. Heart rate and blood pressure were recorded using ambulatory monitors. Blood samples for determination of plasma free 3-methoxy-4-hydroxyphenylglycol (MHPG), cortisol, growth hormone, and prolactin were obtained 30 and 5 minutes before, at the mid-point of, and 15, 30, 45, 60, 90, 120, and 150 minutes after exposure. Each healthy subject was matched to a patient for age, sex, and exact procedure before, during, and after exposure.

RESULTS: Fifteen patients experienced panic attacks lasting an average of 20.5 minutes during exposure. Behavioral measures documented a greater increase in anxiety in the patients than in healthy subjects. Patients experiencing panic attacks displayed significantly greater peak increases (mean $\pm$ SEM) in heart rate (23 ± 3 vs. 12 ± 2 beats/min, $p < .01$) but not blood pressure or plasma free MHPG (0.8 ± 0.1 vs. 0.9 ± 0.1 ng/ml, N.S.). Cortisol (5.8 ± 1.5 vs. 2.4 ± 0.9 mcg/dl, $p < .08$), growth hormone (7.5 ± 1.7 vs. 13.8 ± 4.7 ng/ml, N.S.), and prolactin (0.7 ± 0.4 vs. 3.2 ± 1.1 ng/ml, $p < .05$) increases also were not significantly greater in patients than in healthy subjects.

COMMENT: Increased noradrenergic activity occurring during non-drug induced panic attacks may be too brief or too restricted in regional localization to affect plasma MHPG. The neuroendocrine effects of panic attacks in agoraphobic patients appear to be less marked than neuroendocrine effects of acute fear in healthy subjects reported in previous studies. The effects of panic attacks on heart rate, blood pressure, and hormone secretion may be diminished by "adaptation" or desensitization of neural systems subserving these functions as a result of the recurrent panic attacks which characterize agoraphobia. Recent advances in the assessment of neurobiologic functioning in humans may help elucidate the mechanisms underlying panic attacks. Supported by: MH25642, MH30924, and MH38007.

398.5 FOOD-THWARTING AND NALOXONE EFFECTS ON THE BEHAVIOR OF DOMESTIC CHICKENS. N.S. Williams and P. Elias*. Biology Dept., Simmons College, Boston, MA 02115.

Food-thwarting has been shown to increase stereotyped pacing in domestic chickens. It has also been shown that stress-induced grooming in rats and chickens can be blocked by naloxone.

In the present study, two experiments were conducted to determine if stress-induced pacing could be blocked by pretreatment with naloxone. Heavy-hybrid chickens (3-5 Kg) were trained to expect feeding after food deprivation. In the first experiment, they were trained for 10 days and then exposed randomly to four treatments: no food-thwarting/saline, food-thwarting/saline, food-thwarting/naloxone, no food-thwarting/naloxone. In the second experiment, birds were trained for 14 days and the fourth treatment was omitted. Food-thwarting consisted of presenting hungry birds with food covered with a clear plastic plate. Naloxone HCl doses were 0.05, 2.0 and 4.0 mg/Kg and were injected s.c. in the first experiment and i.p. in the second.

The results of these studies indicated that while food-thwarted chickens paced and moved their heads in a stereotyped manner significantly more than non-thwarted controls, pretreatment with naloxone had no significant effect on pacing or stereotyped head movements.

398.6 SEX DIFFERENCES IN THE EFFECTS OF GONADECTOMY ON SENSITIZATION TO REPEATED, INTERMITTENT RESTRAINT STRESS. Dianne M. Camp* and Terry E. Robinson (SPON: R. E. Davis). Department of Psychology and Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, MI 48104-1687.

The repeated, intermittent administration of amphetamine (AMPH) produces a progressive and enduring enhancement in many AMPH-stimulated behaviors. It has been suggested that this behavioral sensitization may be due to AMPH's action as a stressor, partly because repeated, intermittent stress also enhances the behavioral response to a subsequent AMPH injection. We have shown previously that the development of AMPH sensitization is influenced by sex and endogenous gonadal hormones. Females show more robust sensitization than males, and castration of males enhances the development of sensitization to AMPH. The present experiment was designed to determine if sensitization to stress is similarly influenced by sex and gonadal hormones, as would be predicted if the sensitization to AMPH is due to AMPH's action as a stressor.

Two weeks after gonadectomy (GDX) or a sham operation, adult male and female Holtzman rats were restrained by wrapping them in towels for 3 hrs daily for either 5 or 15 days, or were left undisturbed. Ten days after the last restraint session the animals were given an i.p. injection of AMPH, and AMPH-induced stereotyped behavior and locomotion were quantified. To control for sex differences in the metabolism of AMPH intact males received a higher dose (3.5 mg/kg) than GDX males, intact females and GDX females (3.0 mg/kg).

Five daily sessions of restraint stress produced an enduring enhancement in overall stereotypy scores and repetitive head/limb movements in intact females, GDX females and GDX males, but not in intact male rats. After 15 restraint sessions these AMPH-induced behaviors were enhanced in all groups, although the increase in overall stereotypy in intact males did not quite reach statistical significance. In addition, prior stress produced changes in the pattern of AMPH-induced locomotion in all groups, but the pattern in intact males differed from that of the other three groups. In contrast, previous stress enhanced repetitive sniffing behavior to the same extent in all 4 groups.

It is concluded that the presence of a testicular hormone either directly or indirectly attenuates the development of sensitization produced by either repeated AMPH treatment or repeated restraint stress. Interestingly, the enhancement in plasma corticosterone produced in response to daily footshock stress is affected by GDX in the same manner as reported here (Hennessy et al., JCPP, 91:770, 1977). These findings suggest that the sensitization produced by AMPH and stress may be similar, and support the idea that some of the enduring effects of AMPH on brain and behavior are related to its action as a stressor.

398.7 NEURAL AND ENDOCRINE RESPONSES TO FOOTSHOCK STRESS IN RATS SENSITIZED TO AMPHETAMINE. T.E. Robinson, J.B. Becker, E. Young*, H. Akil and E. Castaneda. Departments of Psychology and Psychiatry, Neuroscience Laboratory Bldg. and Mental Health Research Institute, The University of Michigan, Ann Arbor, MI 48104-1687.

The repeated intermittent administration of amphetamine (AMP) produces an enduring hypersensitivity to the motor stimulant effects of a subsequent AMP 'challenge', and this behavioral sensitization is accompanied by enduring changes in brain dopamine (DA) systems. However, animals sensitized to AMP may be hypersensitive to many other stimuli that activate catecholamine systems, including stressful stimuli. For example, prior AMP treatment enhances the behavioral response to subsequent tail pinch stress, and stress has been reported to precipitate psychotic episodes in former AMP addicts. To determine if prior experience with AMP alters selected brain DA or endocrine responses to subsequent footshock stress ovariectomized female rats received either: (1) one injection of 3.0 mg/kg AMP, once every 3-4 days for a total of 10 injections (AMP-pretreated); (2) a series of saline injections (SAL-pretreated); or (3) were left undisturbed (nonhandled controls; NH). Seven days after the last injection the animals were killed by decapitation following either 0 min, 5 min (30 shocks) or 30 min (180 shocks) of discontinuous footshock (1.3 mA shocks of 0.5 sec duration). Selected brain regions were assayed for DA and its metabolites. Plasma was assayed for N-acetylated-beta-endorphin (N-END; as an index of pituitary intermediate lobe activity).

It was found that: (1) In some brain regions AMP pretreatment altered the response of DA neurons to footshock. For example, in the medial frontal cortex (MFC), hypothalamus (HYP) and dorsolateral striatum (STR) 5 min of footshock produced a greater decline in DA concentrations and a greater elevation in DOPAC/DA ratios in AMP-pretreated rats than in NH controls. (2) In some brain regions repeated SAL pretreatment also altered the response to footshock. For example, in STR and nucleus accumbens 5 min of footshock produced a greater decline in DA concentrations and a greater elevation in DOPAC/DA ratios in SAL-pretreated rats than in NH controls. (3) The dopaminergic response to footshock varied greatly over time in a regionally specific manner. For example, after 30 min of footshock DA concentrations had returned to control levels in the MFC, but were significantly elevated in the HYP. (4) Footshock elevated plasma N-END concentrations in all groups, but to a significantly greater extent in AMP-pretreated than in control rats.

It is concluded that sensitization to AMP is accompanied by enduring (at least 7 days) changes in some neural and endocrine responses to subsequent footshock stress in a time-dependent and regionally specific manner, and that in some instances repeated stressful experiences (SAL injections) can produce comparable effects.

398.8 IS AMPHETAMINE-INDUCED STEREOTYPY A COPING RESPONSE TO STRESS? G.H. Jones*, G. Mittleman*, and T.W. Robbins* (SPON: B.J. Sahakian) University of Cambridge, Department of Experimental Psychology, Cambridge, U.K.

The neural and neurochemical bases of amphetamine-induced stereotypy are relatively well understood, but there has been relatively little research into the functions, if any, of this abnormal behavior. One possibility is that amphetamine (AMPH) stereotypy may represent a coping response to stress. Evidence for this hypothesis includes: 1) AMPH causes physiological and neurochemical changes similar to those observed during stress, and cross-sensitization between AMPH and a variety of stressors has been reported. 2) The intensity of AMPH stereotypy is influenced by behavioral coping contingencies (Maclennan and Maier, Science, 219:1091, 1983). 3) Possibly related behavior such as schedule-induced polydipsia appears to regulate plasma corticosterone (CCS) levels (Brett and Levine, JCPP, 93:946, 1979). This experiment examined the time course of the relationship between AMPH-induced stereotypy and hyperactivity and a neuroendocrine index of stress, plasma CCS both in intact rats and in animals with selective, bilateral dopamine (DA) depletions in the caudate (CD) or nucleus accumbens (N.ACC). Rats were injected with 5 mg/kg d-AMPH or saline (i.p.) and placed in photocell activity cages. After either 15, 30, 60, 90 or 120 min post injection, stereotypy was rated; rats were sacrificed and blood was collected for determination of plasma CCS levels. Following AMPH injection, rats with 6-hydroxydopamine lesions of the CD (DA depletion 70-80%) and unlesioned controls both showed significant CCS elevation, maximal at 30 min post injection. However, the CD group CCS response remained significantly elevated at 60, 90 and 120 min post injection. This lesion significantly attenuated stereotypy and also significantly increased locomotor activity at all time points. N.ACC lesions (DA depletion 85-99%) failed to increase CCS levels relative to controls following AMPH injection, but lengthened the latency of the CCS response. This lesion reduced locomotor activity at all time points and stereotypy was unaffected. These results indicate that CCS levels are increased in rats showing reductions in behavioral stereotypy but that no clear relationship exists between amphetamine locomotor activity and the CCS response. This is consistent with the hypothesis that stereotypy may represent, in part, a coping response to stress.

398.9 SENSITIZING EFFECTS OF AMPHETAMINE ON STRESSOR-INDUCED ALTERATIONS IN INTRACRANIAL SELF-STIMULATION IN RATS. W.J. Bowers and Z. Amit. Dept. Psychology, Concordia University, Montreal, Canada H3G 1M8.

Exposure to uncontrollable stressors has been shown to alter the rewarding/motivational value of intracranial self-stimulation (ICSS) in mice. These alterations are region-specific (i.e., occur only in brain regions where stressors alter dopamine (DA) turnover) and can be eliminated by chronic desipramine administration. To date, however, these alterations in ICSS have been reported only in mice, while the neurochemical effects of uncontrollable stressors have been noted in a variety of species. The present investigation examined the effects of two stressors (restraint and inescapable footshock) on ICSS from brain regions where DA activity is either sensitive (the ventral tegmental area, VTA) or insensitive (substantia nigra, SN) to the effects of stressors. Following surgery response rate-frequency curves were obtained for ICSS from the SN or VTA. Once stable responding was obtained, the rats were placed in wire mesh restrainers for 1 hr or were placed in a similar environment for 1 hr and were then tested for ICSS. No consistent alterations in responding were noted from either brain region. A separate group of rats, implanted and trained in the same manner, were exposed to either 30 or 60 inescapable footshocks (500 uA, 6 sec duration, 60 sec ITI) or placed in identical chambers for the same period of time but received no shock and were tested immediately thereafter. Once again, no alterations in responding for ICSS in either brain region were found. These results suggest that either the stressor-induced alterations in ICSS noted in mice do not generalize to rats or, more likely, rats are more resistant to the behavioral effects of these stressors. If the latter is indeed the case, then manipulations which modestly alter DA activity should sensitize animals to the effects of stressors. To examine this possibility rat were implanted and trained as above and were exposed to either 30 inescapable footshocks or no shock. Immediately following the stressor animals were injected with d-amphetamine (.10 or .25 mg/kg) and tested for ICSS 15 min later. Regardless of the electrode site or the dose, administration of the drug alone did not produce any significant changes in responding. However, the combination of .25mg/kg of amphetamine and shock treatment produced a significant shift in the curve for rats with electrodes in the VTA but not the SN. The .10 mg/kg dose combined with shock treatment failed to alter ICSS regardless of the electrode site. Thus rats appear to be less susceptable to the effects of these stressors on ICSS than mice. However, the present data suggest that prior sensitization of the appropriate neurochemical system with amphetamine predisposes rats to the shock-induced alterations in ICSS.

398.10 MEASUREMENT OF ECCRINE SWEAT IN UNANESTHETIZED RATS. J.F. Ross* and E.A. NEWMANN* (SPON: WM. C. DEGROAT) Procter and Gamble Co., Miami Valley Labs, Cincinnati, OH 45247.

Reported methods (see below) for assessing eccrine (stress related) sweating in rats are difficult to perform and to interpret because they require anesthesia/surgery and/or chemical/electrical stimulation to induce/measure sweating. We report here a simple method for inducing and measuring sweating in conscious rats without pharmacologic pretreatment. Both hind-feet of adult Sprague-Dawley rats were placed into plastic "boots", and sweat from the plantar foot pads collected on filter paper liners during 45 min of restraint stress in a Fisher rodent restraining device. Sweat on liners was weighed to nearest 0.1 mg, and the mean of both feet used to calculate group (generally N=8) $\bar{X}$ + SD. Electrolytes were extracted from boot liners in 0.5 ml hot deionized, distilled water and measured by atomic absorption spectroscopy (Na^+) or flame photometry (K^+).

Baseline sweat values for control male rats (350-450 gm) from different control experiments were 8.0 mg compared with 5.2 mg from females (250-300 gm). Average coefficient of variation was .25. These data compare with predicted values for maximum (chemically induced) sweat of 5-8 mg/paw-hr calculated from in-vitro studies of single sweat glands (2.8 nl/gland-hr x 2500 glands/paw) or direct measurement of sweat wt. (5 mg/40 min) obtained in anesthetized rats by pipetting sweat drops from feet coated with mineral oil (Sato & Sato, J. Physiol.: 274, 37-50, 1978; Quatrale & Laden, J. Invest. Dermatol.: 51, 502-4, 1968). Estimates ($\bar{X} \pm$ SE) of Na^+(38.6 + 4.8 meq/l) and K^+(151 + 14 meq/l) concentration also compared with previous estimates from sweat obtained by more laborious techniques, and total K^+ correlated with sweat wt. (r=0.87). Sweating was not significantly affected (4.7±0.8 vs. 5.3±1.0 mg) by dramatically increasing environmental temperature (23.3° vs. 37.7°C) suggesting that sweating was stress-related and not thermoregulatory. Sweating did not habituate during four repeated tests, suggesting that the restraint procedure is psychologically stressful and may elicit maximal sweat rates. As expected, scopolamine HBr (0.008-0.5 mg/kg SQ) reduced sweating by up to 65%. However, pilocarpine (1.0, 10.0 mg/kg SQ) failed to increase sweating, again suggesting that sweating was already maximal.

This model offers significant advantages (i.e., technically simple, conscious rats, eliminate need for electrical/chemical stimulation) relative to previous techniques, but further modifications may be required to reduce baseline sweat rates so that procedures which increase sweating can be evaluated.

398.11 EMOTIONAL RESPONSIVITY AND PAIN PERCEPTION IN RATS WITH AMYGDALOID LESIONS. C.V. Grijalva, E.D. Levin, M.M. Morgan, and B. Roland. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

It recently has been proposed that the medial amygdala participates in an ancillary pain system while the lateral region functions as an inhibitory area during stressful experiences. In support of this hypothesis, it was demonstrated that the severity of stress-induced gastric pathology can be attenuated or increased by either lesions of the medial or lateral amygdala, respectively (Henke, Neurosci. Biobehav. Rev. 6: 381, 1982).

To further examine the role of the amygdala in emotional behavior and pain perception, two experiments were conducted in a group of control male albino rats (N = 8) or groups with either medial (N = 7) or lateral (N = 8) amygdaloid lesions. In the first experiment rats were tested for exploratory locomotion in an unbaited elevated 8-arm radial maze. The arms were each 10 cm wide and 80 cm long and had no sides. The propensity of rats to journey down arms of an elevated maze without walls has been used as a measure of anxiety (Pellow, et al. J. Neurosci. Methods 14: 149, 1985). Three preoperative sessions were conducted by permitting animals to freely explore until they had entered all eight arms, slipped off the maze or five minutes had elapsed. Choice behavior was measured as the number of arms entered until the first time an arm was re-entered (entries to repeat). Response speed was measures as the number of seconds taken for each arm entry (sec/entry). Three weeks after the lesions, rats were tested for two sessions on the maze. The medial group made choices at a faster rate than controls while the lateral group made choices at a slower rate. In the orthogonal contrasts for the sec/entry measure, the comparison of the controls (28.7 $\pm$5.0) vs the lesioned groups was not significant, but the comparison of the lateral (113.1$\pm$30.3) vs the medial group (79.0$\pm$24.8) was significant ($p<.025$). Rats in the lateral group did not make enough choices to calculate entries to repeat. The control group did not significantly change from pretest to postest, while the medial group showed a mariginally significant increase in entries to repeat (3.1$\pm$0.3 to 4.2$\pm$.3, $p<.051$).

In a second experiment rats were subjected to two measures of pain sensitivity: (1) tail-flick test to assess nociception as measured by a spinal reflex or (2) hot-plate test as a measure of a supraspinal response. No significant differences were found between groups.

Although no differences were found between groups on the tests for pain perception, the differences in maze performance suggest that the lateral and medial amygdaloid area may play reciprocal roles in stress perception. The hesitancy of rats in the lateral group in making arm entries suggested that they were more intimidated by the maze, whereas the faster rates of arm entries and increased number of entries into novel arms by the medial group may indicate that these animals are less anxious or intimidated.

398.12 MODIFICATION OF BIOCHEMICAL AND NEUROENDOCRINE EFFECTS OF PHYSOSTIGMINE IN RATS BY ALTERATIONS IN ENVIRONMENTAL TEMPERATURE. D.R. Kinney*, M.B. Nichols* and R.P. Maickel. Dept. of Pharmacology & Toxicology, School of Pharmacy & Pharmacal Sciences, Purdue Univ., West Lafayette, IN 47907.

A previous report from this laboratory (Nichols, M.B., et al., The Pharmacologist, 1986, in press) has presented data on the time course of effects of single doses of physostigmine (0.25, 0.50, 0.75 mg/kg, s.c.) on a number of biochemical and neuroendocrine parameters of adult, male Sprague-Dawley rats maintained at an environmental temperature of 23°C. The present paper extends these studies to similar dosage in rats maintained at environmental temperatures of 16°C and 4°C. Groups of 4-6 rats were dosed with vehicle (distilled water) or physostigmine and decapitated at time points ranging from 0.5 hr to 96 hrs post-dosage. Body temperatures were measured by rectal probe just prior to dosage and again just prior to decapitation. The magnitude of physostigmine-induced hypothermia was greater at the lower environmental temperatures, although the duration of hypothermic action of the drug was prolonged only at the highest dose. The effects of the drug dosage on plasma corticosterone were also modified, since the lower environmental temperatures per se acted as stressful stimuli over the first 2 hours of exposure. The greatest AUC differential between physostigmine-dosed and vehicle-treated rats was seen at the intermediate dose (0.50 mg/kg) of the drug in rats at a 4°C environmental temperature, in contrast to the results at 23°C which showed a virtually linear dose-effect relationship. Plasma fatty acids over the first 2 hours of exposure had somewhat of an inverse dose-effect relationship in rats at both the 23°C and 4°C environmental temperatures, with the overall effects being greatest at the highest environmental temperature. Plasma glucose level dose-effect relationships in response to physostigmine were not significantly altered by varying environmental temperatures. Thus, the effects of physostigmine, a short-acting reversible inhibitor of acetylcholinesterase, are reflected in : (I) an hypothermia which is related to the environmental temperature, (II) stimulation of the hypothalamus-pituitary-adrenocortical axis which is additive to that produced by lowered environmental temperature, (III) elevation of plasma glucose levels caused by adrenal medullary discharge, and (IV) an inverse dose-effect relationship in terms of plasma fatty acids. (Supported by USAMRDC contract DAMD 17-85-C-5099.)

398.13 EFFECT OF ETHANOL ON HEART RATE, ECG AND BLOOD PRESSURE IN ANESTHETIZED AND STRESSED AND NONSTRESSED CONSCIOUS MALE RATS. M. Graffy Sparrow*, H. Roggendorf*, and W.H. Vogel, (Spon: T.A. Hare), Dept. of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

The influence of ethanol on the cardiovascular system was studied in anesthetized and conscious nonstressed rats. In anesthetized animals (ketamine/acepromazine or pentobarbital), ethanol showed no effect on heart rate or ECG. In conscious nonstressed rats, moderate doses of ethanol (1 g/kg, ip) increased heart rate significantly (345±9 vs. 461±38 bpm). The increase was unaffected by atropine pretreatment but was reduced by propranolol and reserpine. Thus, ethanol-induced tachycardia is apparently due to direct stimulation of the sympathetic nervous supply to the heart. Blood pressure was only slightly reduced by ethanol (118±3 vs. 110±2 mm Hg). Immobilization caused behavioral excitation, marked tachycardia (327±12 vs. 506±8 bpm) and increased blood pressure (117±4 vs. 135±5 mm Hg). Ethanol-pretreated rats were considerably calmer during the stress period, and the stress-induced rise in blood pressure was antagonized by ethanol (121±3 vs. 128±3 mm Hg). However, increases in heart rate during the 30 min immobilization period were unchanged by ethanol pretreatment (342±28 vs. 499±27 bpm). No major changes in ECG were noted during these studies. These data demonstrate the differential effects of ethanol in anesthetized and conscious rats, in the absence or presence of a stressor.

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398.14 THE EFFECT OF ALCOHOL ON PLASMA CATECHOLAMINE STRESS RESPONSES IN THE SAME INDIVIDUAL MALE AND FEMALE RATS: A CORRELATION STUDY. G.T. Livezey, N. Balabkins, and W.H. Vogel, Dept. Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107

Individual male and female Sprague-Dawley rats were implanted with indwelling jugular catheters, allowed to recover, and subjected to restraint stress both with and without pretreatment with 1g/kg alcohol (i.p., 20% w/v in water). Plasma norepinephrine (NE) and epinephrine (E) were determined prior to restraint (baseline, time 0) and at 5, 15 and 30 minutes after the onset of immobilization. The area under the stress response curve (AUC) was calculated for each catecholamine in each individual both with and without ethanol.

Great individual and sex-dependent differences were noted. Females displayed greater plasma response levels and interindividual variability (NE more than E), as well as relatively delayed response peaks, with or without alcohol, when compared to males.

In general, alcohol pretreatment resulted in significant increases in the baseline levels and decreases in the total stress response of both catecholamines. The increases in baselines did not significantly contribute to the decreases in the total responses. Alcohol pretreatment also resulted in a redistribution of the responses (delay of the peak) in many animals, particularly among the females.

Without alcohol, the single time point most predictive of the entire response (AUC) was at 5 minutes. Following alcohol, this predictive value was found at later times (15 or 30 minutes).

Among the more significant correlations noted, we found that those animals displaying the highest stress responses without alcohol also displayed the greatest stress response reducing effects of alcohol. Thus, our data support the "tension reduction hypothesis" of alcohol consumption (H. Capell and P. Herman, Q.J. Stud. Alcoh. 33:33-64, 1972), and also places those individuals with the highest stress responses at greatest risk of increased use and abuse of alcohol, since they will derive the most "benefit" from alcohol's stress response reduction effect.

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398.15 ADRENAL RELEASE OF MET-ENKEPHALIN AND OTHER NEUROPEPTIDES DURING HYPOTENSIVE HEMORRHAGE IN HALOTHANE ANESTHETIZED CATS. D. Gaumann* T. Yaksh*, D. Roddy*, S. Michener*, D. Nelson* and D. Lucas* (SPON: J. Daube). Crit. Care Ser., Dept. of Anesthesiol. and Med.; Dept. of Neurosurg. Res.; and GI Hormone Research Laboratory, Mayo Clinic, Rochester, MN 55905.

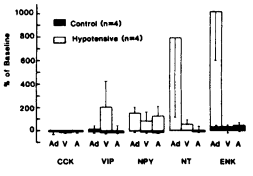
Hypotensive hemorrhage (HTH), a strong activator of the sympatho-adrenal axis, was induced in cats, to evaluate the adrenal outflow of different neuropeptides: met-enkephalin (ENK), neurotensin (NT), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK).

Two groups of cats (group I, n=4, HTH; group II, n=4, parallel, non-bled control) were studied under halothane anesthesia (0.8 vol%). 1 hr after cannulation of the femoral artery (A), femoral vein (V) and adrenal vein (Ad), baseline samples for ENK, NT, NPY, VIP and CCK were taken. 1 hr later, following induction of HTH in group I, samples were repeated at the three collection sites. All neuropeptides were detected by selective radioimmunoassays. Except for NPY, that showed higher adrenal than venous and arterial levels, the plasma baseline levels for the different neuropeptides were practically the same at all collection sites. HTH (34% blood loss, MABP 46 mm Hg) led to a marked increase of ENK, NT and NPY in the Ad, and of VIP in the V. The percent increase of ENK was highest (Fig. 1). No changes were observed in the control group (MABP 118 mm Hg) at any time point. Based on AV differences, NPY, ENK and NT are secreted by the adrenal; and HTH evokes their release in a differential way. In contrast, VIP is secreted by extra-adrenal sources which are also activated by HTH, plasma CCK is not modulated by HTH.

Baseline Neuropeptide Levels (pg/ml ± SEM) in Cats (n = 8)

Source	CCK	VIP	NPY	NT	ENK
Ad	23.5±2.4	34.5±6.0	1406±114	35.8±6.4	52.6±7.6
V	22.5±2.1	34.8±5.9	985±60	30.5±5.6	39.9±7.0
A	23.3±2.5	36.1±5.9	987±43	34.4±6.3	38.0±5.5

Figure 1. % increase of baseline values of CCK, VIP, NPY, NT and ENK in Ad, V, A during HTH (□). Black bars (■) represent control animals which were not subjected to hemorrhage.



(Supp. by NS16541, TY; Burroughs Wellcome Res. Fellow for 1986, DG)

398.16 THE B-ENDORPHIN AND MONOAMINERGIC RESPONSE TO STRESS IN THE DEVELOPING RAT PUP. L.J. Iny*, D.H. Aitken*, C. Gianoulakis*, R. Palmour and M.J. Meaney. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill Univ., Montréal H4H 1R3 Canada.

Apart from the hypothalamic-pituitary-adrenal (HPA) system, little is known about the response to stress in the developing animal. During the first two weeks of life in the rat, pups exhibit a considerably dampened HPA response to a variety of stressors, as indicated by reduced levels of both corticosterone and adrenocorticotropin. This period of HPA quiesence has been referred to as the «stress hyporesponsive period», and may be considered as an adaptive response to the metabolic needs of the developing animal, thus protecting it against exposure to the catabolic glucocorticoids.

In order to examine the generality of this phenomenon, we have compared the stress response in other neuroendocrine systems of Long-Evans rats 3, 7, 14, 21, and 35 days of age with that of adult animals. Control animals were quickly removed from their home cages and sacrificed. Stressed animals were removed from their home cages and either exposed to ether fumes for 2 minutes, followed 3 minutes later by decapitation, or handled by the experimenter for 5 minutes followed by decapitation. Following decapitation, trunk blood was collected and the brains were quickly removed and placed on dry ice. All samples were stored at -70°C until assayed.

Plasma B-endorphin (unextracted) and corticosterone levels were determined by radioimmunoassay. At each age studied, both ether and handling stress resulted in significant ($p < .05$) elevations of B-endorphin-like immunoreactivity (B-EPLIR) compared to controls. Moreover, basal B-EPLIR levels were higher ($p < .01$) in animals 3, 7, and 14 days of age than in adults. In contrast, basal corticosterone titers were low during the first two weeks of life, and not until the second to third week was there evidence for a stress-induced increase in HPA activity. These data suggest that adrenocorticotropin and B-endorphin secretion under both resting and stress conditions differ in the developing animal.

Monoaminergic activity was analyzed in microdissected brain regions using high-pressure liquid chromatography with electrochemical detection. The results, thusfar, reveal a pattern that is strikingly similar to that seen in the HPA system. Thus, for example, serotonin levels in the frontal cortex are low during the first two weeks of life, and not until Day 21 is there evidence for a serotoninergic response to stress in this region.

These results indicate that there is considerable variation in the maturation of the stress response across neuroendocrine systems.

398.17 LONG-TERM EFFECTS OF EXPOSURE TO MAGNETIC RESONANCE IMAGING: NO SIGNIFICANT CHANGES IN SURVIVABILITY, BODY GROWTH, ORGAN WEIGHTS AND STRESS RELATED HORMONE LEVELS IN MALE RATS. F.S. Prato*, G.C. Teskey, K.-P. Ossenkopp, N.K. Innis*, and P. Harris* (SPON: F.S. Tepperman). Research Institute, St. Joseph's Hospital and Department of Psychology, University of Western Ontario, London, Canada N6A 5C2.

Although exposure to a variety of magnetic field conditions has been shown to influence a number of biological systems, the long-term effects of such exposure have received minimal experimental examination. Nuclear Magnetic Resonance Imaging (MRI) is a powerful noninvasive diagnostic procedure that is growing in popularity. Although a number of laboratory studies have found no immediate adverse effects of acute exposure to the magnetic and radio-frequency fields associated with MRI, the possibility that long-term effects might occur has not been examined in animal models.

The present study examined the effects of repeated acute exposure to MRI on the survivability and stress related physiology in 151 Long-Evans male rats. Subjects in the experimental group were exposed daily to the MRI procedure for 23.2 minutes on five successive days (Treatment 1) or on twenty-one successive days (Treatment 2). Sham exposed animals received identical treatments except that the magnetic and radio-frequency fields associated with MRI were not present. A group of untreated control rats was also maintained in the animal colony. After 13 to 22 months post-exposure the surviving animals (n = 120) were euthanized and their whole body, spleen, heart, thymus, and adrenal weights were obtained. Blood samples were taken for assay of adrenocorticotropic hormone (ACTH) and corticosterone levels. Red and white blood cell counts and hemoglobin content were also recorded. Statistical analysis of these data failed to provide any evidence for changes in survivability and stress related physiology in the MRI exposed rats relative to sham-exposed and control animals. These results fail to challenge the view that MRI is a safe diagnostic procedure.

(Supported by a National Health Research and Development Program grant with F.S. Prato as principal investigator)

399.1 SUPPRESSION OF NOCTURNAL CEREBROSPINAL FLUID MELATONIN BY HIGH IRRADIANCES OF BROADBAND AND MONOCHROMATIC LIGHT IN THE CYNOMOLOGUS MACAQUE. G.C. Brainard, B.E. Northrup*, P.L. Podolin* and M.D. Rollag*. Departments of Neurology and Neurosurgery, Jefferson, Med. Col., Philadelphia, PA 19107 and Department of Anatomy, USUHS, Bethesda, MD 20814.

The purpose of the following study was to develop a model for examining how photic input regulates neuroendocrine parameters in a color-sighted animal species which is phylogenetically close to humans. Specifically, cerebrospinal fluid (CSF) melatonin content was used as a marker for neuroendocrine and circadian responsiveness to photic stimuli. Four adult male Cynomologus macaque monkeys, weighing 3-4 kg each, were used in this study. Each animal was habituated to a light:dark cycle (L:D 14:10), routine handling and short-term chair restraint. For CSF sampling, monkeys under Ketamine anesthesia had a catheter inserted by lumbar puncture (L_{4-5}) and advanced cephalad to the foramen magnum. Catheter position was confirmed radiographically. Animals were then placed in a primate chair and given free access to food and water. CSF was continuously withdrawn (1 ml/hr) by a peristaltic pump and automatically collected by a fraction collector for 48 to 72 hours. Melatonin was quantified by radioimmunoassay. Normal circadian rhythms of melatonin production were documented in each animal, with peak levels occurring during the night and low levels during the day (Brainard et al., Anat. Rec. 211:25, 1985). During the night, on separate occasions, individual animals were exposed to a one hour pulse of monochromatic green light (λ max 500 nm, 10 nm half peak bandwidth) at irradiances of 0.1, 12, 24 or 60 uW/cm^2. The monochromatic light was produced by a tungsten bulb and collimated with condensing lenses. The light was filtered with a glass infrared filter, a glass narrow band interference filter and glass neutral density filters (Oriel). Surprisingly, the above irradiances caused only 30% or less CSF melatonin suppression. To ascertain if higher intensity light could produce a greater suppression of CSF melatonin, 2 of the animals were exposed to a one hour pulse of broadband white light (approximately 2,000 uW/cm^2). The white light was produced by the same apparatus described above without an interference filter. Bright white light suppressed melatonin by at least 60%. These data indicate that relatively high light irradiances are required to perturb CSF melatonin in the Cynomologus macaque. Since high intensity light is required to suppress melatonin in humans, the Cynomologus may be a good animal model for understanding photic regulation of neuroendocrine and circadian parameters in humans. Supported by the Lighting Research Institute, The Pfeifer Foundation, The Light Research Foundation and USUHS Protocols R07039 and C07049.

399.2 EEG CHANGES IN CORTEX, AMYGDALA AND HIPPOCAMPUS, DURING LIGHT AND DARK, AFTER PINEALECTOMY IN THE RAT. J.H. Peck, G.Miller*, M. Davis*, and L.J. Grota. Dept. of Psychology, Ithaca College, Ithaca, NY 14850.

The pineal gland has been implicated in a variety disorders including epilepsy, parkinson's disease and seasonal affective disorders. A number of investigators have shown behavioral and EEG effects of pinealectomy followed by injections of pineal concentrate or melatonin. Our past work attempted to study these findings by using an antibody to melatonin. Unfortunately, control problems prevented a clear interpretation of the data. This study was an attempt to replicate and extend studies which showed a relationship between pinealectomy and brain activity.

Male Charles River rats (CD) 90-120 days old were either pinealectomized or sham operated using a suction technique. One pair of stainless steel bone screws served as cortical electrodes on each hemisphere. Depth electrodes were also implanted in the amygdala and hippocampus contralateral to each other. The rats recovered for 1 week and EEG was recorded during the light and dark phases of their daily cycle. No efforts were made to prevent the rats from sleeping and hence generating low frequency high voltage outbursts or "sleep spindles."

Results showed that low frequency high voltage outbursts were present in all records except the sham night records. In the sham rats, high voltage activity was simultaneously present in both cortexes, the amygdala and hippocampus. In the pinealectomized rats, the amygdala, and to a lesser extent the hippocampus, did not generate high voltage activity along with the cortex. Recordings showing the range of results will be presented.

399.3 STUDIES OF ARGININE VASOTOCIN IMMUNOACTIVITY IN OVINE PINEAL GLAND AND SUBCOMMISSURAL ORGAN.
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The biological or immunological activity of arginine vasotocin has been found in rat, hamster (Prechel et. al., J. Pin. Res. 1:175, 84), bovine (Pavel, Endocrinology 89:613, 71) and human (Geelan, et. al., Peptides 2:459, 81) pineal glands, and also in rabbit (Rosenbloom and Fisher, Endocrinology 96:1038, 75) and bovine (Pavel, Endocrinology 89:613, 71) subcommissural organs (SCO). In two species of laboratory-raised rodents (Prechel et. al., J. Pin. Res. 1:175, 84) arginine vasotocin immunoactivity (iAVT) exhibited a circannual rhythm with elevated levels present only in August (Prechel et. al., Endocrinology 112:1474, 83). The purpose of this study was to look for AVT immunoactivity in the pineal and SCO of sheep, and to determine whether the pineal iAVT content of these domestic animals, housed out of doors, also varied during August.

All tissues were collected from lambs 6-9 months old, at Chiapetti Lamb Packing Co. (Chicago, IL, USA) from July to Sept. 1983. Pineal glands were dissected into anterior and posterior halves. Subcommissural organ samples consisted of the ependymal lining of the roof of the cerebral aqueduct just caudal to the pineal stalk. Tissues were frozen on dry ice within 30 minutes after death of animals and then stored at -80°C. The glands were later weighed, homogenized in 0.1 N acetic acid containing 10^{-6} M pepstatin (an acid protease inhibitor) and then heat denatured to precipitate large proteins. Supernatants were assayed for iAVT by radioimmunoassay (Fernstrom et. al., Endocrinology 106:243, 80).

AVT-immunoactivity was present in all SCO and pineal samples assayed to date. SCO samples contained higher activity per mg wet weight of tissue than pineal gland. While there was no difference in iAVT concentration between anterior and posterior pineal regions the hormone was distributed non-uniformly through the ependyma containing the SCO. Levels of ovine pineal iAVT increased in mid-August and then declined again by September; values varied from 500 to 4500 pg iAVT/gland. The dilution curve of the ovine pineal extract did not parallel the AVT standard curve.

The results of this study indicate that AVT and/or similar neuro hypophyseal peptides are present in ovine pineal glands and SCO-containing tissue. Also, pineal peptide levels increase 9-fold for a short time in August. Thus, the August pineal iAVT peak seen previously in laboratory rats and hamsters (Prechel et. al., J. Pin Res. 1:175, 84) is also characteristic of sheep, a short-day breeding species, maintained in a natural habitat.

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399.4 PARAVENTRICULAR CONTROL OF HAMSTER PHOTOPERIODISM: DESCENDING PATHWAYS AND RESPONSE TO MELATONIN. E. L. Bittman and M. N. Lehman, Dept. of Zoology, University of Massachusetts, Amherst, MA 01003 and Dept. of Anatomy and Cell Biology, Columbia University, New York, N.Y. 10032

Short days arrest hamster reproduction through a mechanism which involves the suprachiasmatic nuclei (SCN), paraventricular nuclei (PVN), superior cervical ganglia (SCG) and pineal gland. Projections from the retina to the SCN, the SCN to the PVN, and the the PVN to the intermediolateral spinal cord are believed to regulate sympathetic preganglionics which control pineal melatonin secretion. To determine whether descending influences of the PVN are exerted through a crossed or uncrossed pathway, we removed the left SCG and produced unilateral PVN lesions on either the left (IPSI) or right (CONTRA) side. After 10 weeks of 10L:14D, testis weights (TW) were sharply reduced in IPSI hamsters (0.91±0.38 g, mean±SEM), suggesting the pathway is at least partly uncrossed. In contrast, CONTRA lesions, bilateral PVN destruction, and bilateral ganglionectomy were comparably effective in blocking gonadal regression (TW of 2.89±0.20, 3.24±0.07, and 2.50±0.27g, respectively). Thus the pathway is predominantly uncrossed.

We measured pineal melatonin content in groups of hamsters killed at 4h intervals 11 weeks after bilateral SCN or PVN lesions and transfer to 10L:14D. Low levels (less than 150 pg/gland) were found throughout the day and night in both groups. Does PVN control of melatonin rhythms fully account for its role in the photoperiodic response? To find out, we challenged long day hamsters with exogenous melatonin for 8 weeks. Three daily injections (25 ug at 3h intervals beginning 6.5h before dusk) produced gonadal regression despite destruction of the SCN (TW 0.99±.18g), PVN (TW 0.45±.05g) or SCG (TW 0.49±.05g). Thus neither the SCN nor the PVN plays a critical role as a melatonin target. Damage to the retrochiasmatic area, however, prevented gonadal responses to 3 daily injections (TW 2.65±.45g). Testes of ganglionectomized and SCN lesioned hamsters injected once daily with melatonin 0.5h before dusk remained large (TW 2.96±.19g and 3.01±.20g). Surprisingly, gonads of 9 of 12 PVN lesioned hamsters regressed completely in response to such treatments (group mean TW 1.13±.39g vs. 0.67±.25g for intact controls). Thus lesions which disrupt melatonin rhythms and reproductive photoperiodism are not equivalent in their influence on responsiveness to melatonin. Supported by NIH grants NS20279, HD20018, and HD20463.

399.5 PINEALOCYTE GRANULAR VESICLES IN RATS AND MICE ARE NOT CORRELATED WITH SEROTONIN AND MELATONIN LEVELS OVER A LIGHT:DARK CYCLE. J.A. McNulty*, L.M. Fox*, S.J. Lisco* (SPON: F. LaVelle). Dept. of Anatomy, Loyola Univ. Stritch School of Med., Maywood, IL 60153.

Granular vesicles (GV) have been implicated in the storage/release of pineal products (e.g., indoles and peptides/proteins). In the present study, we applied quantitative morphological and biochemical techniques to the pineal gland of both mice and rats to determine whether numbers of GV are correlated with physiologic changes in indole compounds over a light:dark cycle. These species were selected because the frequency of GV in pinealocytes of the mouse is several fold greater than in the rat. Male, young adult mice (outbred ICR strain) and Sprague-Dawley rats were sacrificed at mid-light and mid-dark of a 12:12 L:D cycle. Glands were processed either for routine TEM or frozen for assay of indoles by reverse-phase liquid chromatography with electrochemical detection as previously described (McNulty J.A., et al. Endocrinology 117: 1035, 1985). Number of GV were counted in 6 grid spaces (300 mesh) in the distal and proximal thirds of each gland. Number of GV/unit area were divided by mean diameter (=100 nm) x section thickness (=80 nm) to estimate the volume density of GV. These data were multiplied by the volume density of pinealocytes x total volume of gland (calculated from serial thick (2um) sections) to estimate total number of GV/gland. The results of morphometric and biochemical analyses are presented below:

		GV/gland (x10⁷)	5HT/gland (ng)	Melatonin/gland (ng)
RAT	Day	3.90 (+0.3)	95.0 (+4.9)	nd
	Night	9.12 (+1.2)*	27.8 (+3.2)*	2.75 (+0.3)*
MOUSE	Day	5.64 (+1.5)	9.01 (+1.3)	nd
	Night	2.05 (+0.3)*	7.83 (1.09)	nd

n = 5-6 for each time point; nd = not detectable;
* = $p<0.01$ compared to daytime levels.

The biochemical data indicate that indole levels in the mouse are not cyclical over a 24 h photoperiod and we confirm by direct measurement with HPLC the observation that the pineal gland in certain strains of mice does not synthesize significant amounts of melatonin (Ebihara, S. et al., Science 231: 491, 1986). The latter finding suggests that GV do not contain melatonin, at least in the mouse. This correlative study further suggests that pinealocyte GV do not contain physiologically important stores of 5HT since 1) the mouse gland contains the same number of GV as the rat gland during the daytime, but only 1/10 the levels of 5HT, 2) day/night 5HT levels do not vary in the mouse gland, but there is a significant nocturnal decline in GV numbers and 3) 5HT levels in the rat gland decline at night when GV numbers increase.

399.6 EVIDENCE FOR NEURAL MEDIATION OF VIP ACTIVITY IN THE DOGFISH RECTAL GLAND. S. R. Chipkin*, J.S. Stoff*, N. Aronin. Univ. of Massachusetts Medical School, Worcester, MA 01605.

The rectal gland of the spiny dogfish, Squalus acanthias, secretes sodium chloride at a concentration close to that of seawater to maintain salt balance. Vasoactive intestinal peptide (VIP) has been shown to increase chloride secretion into ductal fluid by a cyclic AMP-mediated Na^+-K^+ ATPase mechanism. Immunoreactive VIP (iVIP) is detectable by radioimmunoassay in the venous effluent of the isolated perfused gland. The localization of iVIP in the dogfish rectal gland is not known. We therefore studied by immunohistochemistry the distribution of iVIP at the light and electron microscopic level in this model system of ion transport.

Rectal glands perfused with either buffered 5% acrolein or 4% paraformaldehyde containing 0.1% glutaraldehyde were sectioned (20 μm) on a vibratome and processed for immunohistochemistry by either avidin-biotin complex (ABC) or peroxidase anti-peroxidase (PAP) methods using VIP antiserum at a dilution of 1:800. Processed tissues were either mounted with gelatin for light microscopy, or prepared for electron microscopy by treatment with 2% osmium tetroxide (1 hr) and uranyl acetate (2 hr) and then embedded in Epon. Ultrathin sections were cut serially and mounted on formvar-coated slot grids. Specific staining was detected in all specimens using both fixatives and both techniques. The combination of acrolein perfused glands prepared by the ABC method yielded the most immunoreactivity. Preabsorption with VIP antibody (100 μg/ml) resulted in loss of specific immunoreactivity. At the light microscopic level, iVIP was observed in thick fibers which traversed the fibro-membranous capsule of the rectal gland and formed extensive arborizations in the capsule. In the parenchyma of the rectal gland, smaller iVIP-containing fibers were noted within connective tissue and in close approximation to tubule cells. These smaller fibers often ended in bouton terminals. At the ultrastructural level, iVIP axons in the fibro-membranous capsule were unmyelinated and entered the rectal gland within a connective tissue bundle, where some iVIP terminals were found. Immunoreactive fibers that entered the parenchyma frequently terminated on the basal side of tubule cells. There was no evidence of termination on the apical side of the tubule. The boutons in the capsule within the connective tissue bundle and parenchyma contained vesicles of different shape and size, with reaction product in dense core vesicles (60-120nM).

These findings suggest that the VIP regulation of chloride secretion by the tubule cell in the dogfish rectal gland is neurally mediated. Supported in part by NIH AM 01126-04, 2T32AM-07302, AHA81-164, and BRSG from UMMS.

399.7 CENTRAL CHANGES IN NOREPINEPHRINE TURNOVER IN RESPONSE TO REDUCED EXTRACELLULAR FLUID VOLUME. L.D. Wilkin, K. Patel*, P. Schmid* and A.K. Johnson. Depts. Anatomy, Psychology, Pharmacology and Internal Medicine, Univ. of Iowa, Iowa City, IA 52242.

Brainstem noradrenergic cell groups participate in the neural circuitry serving to relay cardiovascular information to preoptic and anterior hypothalamus. Projections from these noradrenergic regions to the basal forebrain areas are thought to be involved in feedback of blood pressure and blood volume information. Pathways have been demonstrated from the A1 and A2 cell groups to the paraventricular nucleus (PVN), the supraoptic nucleus (SON), and the median preoptic nucleus (MnPO), regions involved in control of body fluid homeostasis. Central depletion of catecholamines has been shown to disrupt fluid balance regulation via impaired drinking responses and vasopressin secretion in response to systemic challenges. However, the role of noradrenergic inputs to the anterior hypothalamus has remained unclear as intracerebral injections of noradrenergic agonists have been shown both to stimulate and to inhibit vasopressin secretion. In the present study, the role of noradenergic input to the anterior hypothalamus was studied by examination of norepinephrine turnover during reduction of systemic extracellular fluid volume.

Extracellular fluid volume was decreased iso-osmotically by subcutaneous 5 ml injections of 30% polyethylene glycol (PEG), a concenration known to increase thirst and vasopressin release. NE turnover was assessed by measuring the decline of NE concentration in brain micropunches four hours after administration of 300 mg/Kg α-methyl tyrosine alone or PEG and α-methyl tyrosine. Several hypothalamic areas were investigated, including the MnPO, PVN, SON, subfornical organ, preoptic area, ventromedial hypothalamus, and posterior hypothalamus. Volume depleted animals showed significantly increased NE turnover in the MnPO, an important area for integration of fluid balance information. The preoptic area and the SON showed trends toward increased NE turnover in these animals. All other areas showed no difference in NE turnover between volume depleted and normal animals. These results are consistent with previous findings that NE terminals in the MnPO are important in control of fluid balance and support the hypothesis that basal forebrain NE projections facilitate thirst and vasopressin secretion.

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399.8 DIURETIC AND NATRIURETIC PHENOMENA IN THE CONSCIOUS SPINAL RAT INDUCED BY EXPERIMENTAL INFUSIONS OF DISTILLED WATER AND ISOTONIC GLUCOSE SOLUTIONS INTO THE THIRD CEREBRAL VENTRICLE. A. Guevara-Rojas*, M.P. Rosas-Arellano*, B. Barrera-Mera and P. Vergara-Aragón*. Depto. de Fisiología, Facultad de Medicina, UNAM. Apdo. Postal 70250 and Depto. de Neurociencias, Instituto de Investigaciones en Fisiología Celular, UNAM. México 04510, D. F.

Evidence for a sodium sensor involved in water drinking are in the sense that it is located in close vecinity of the third cerebral ventricle. On the other hand, the existance of a sodium hypothalamic sensor responsible of the severe reduction of the renal excretion of water and sodium in response to intense over hydration by stomach tube of female dogs (Guevara-Rojas 1974), has also been postulated. In order to explore the role of the central nervous system on the neuroendocrine mechanisms controlling the extracellular fluids, we studied the modification of sodium and water excretion in conscious unrestrined rats. That response was studied under the effects of the microinjection of distilled water or isotonic glucose solution (5μl in 10 min.). The cannula (No.21) had been stereotaxically implanted into the third cerebral ventricle of the rats. Female Wistar rats were used. A vesical poliethylene tube (pp 190) fixed into the bladder of these animals. This cannula permitted to obtain all the urine at fixed intervals of time. The animals were given free access to water and food until the begining of the experiment. Then they received by stomach tube distilled water, isotonic NaCl solution or 5% NaCl solution in a volume equivalent to 3% b.w. The urine was collected every 15 min. until its volume showed a definite decreasing tendency. Destilled water and isotonic glucose 5% were injected into the third ventricle as soon as the diuresis became increased. Shortly after the hydration with distilled water the urinary volume progressively increased (0.01-0.19 ml/min.) as classically described by E.B. Verney. A discrete natriuresis was also noted 30 min. after the hydration. And it was followed by a clear and energic retention of sodium. As soon as the ventricular perfusion started astrong antinatriuresis contrasted with the increased output of water which also followed the ventricular microinjection. In order to suppress any peripheral reflex influence, a spinal cord transection (T8) was performed and the tests repited one or two days later. A delay and decrease in the renal excretion of water and sodium from 15 to 60 min. followed to the application of distilled water in the third cerebral ventricle of the spinal preparations.

399.9 EFFECT OF INTRACISTERNAL INJECTION OF 16-PROSTAGLANDIN E_2 (PGE_2) AND INDOMETHACIN ON GASTRIC SECRETION IN RATS. G. Kauffman*, Y. Tache and E. Kolve* (SPON: A. Yuwiler). Milton S. Hershey Medical Center, Division of General Surgery, Hershey, Pennsylvania 17033 and Center for Ulcer Research and Education, VA Medical Center, Department of Medicine, UCLA, Los Angeles, CA 90073.

Growing pharmacologic evidence indicates that the central nervous system (CNS) regulation of gastric function may involve brain neuropeptides as well as PGE_2 (Science 222, 935-937, 1983; Europ. J. Pharmacol. 91, 245, 1983). We further investigated the possible role of prostaglandins in the CNS regulation of gastric secretion. In 24 h-fasted rats under urethane anesthesia for the duration of the experiment, the pylorus was ligated and a double lumen cannula was inserted in the forestomach. The inlet and outlet of the double cannula was continuously perfused with 3 ml/min saline solution adjusted to pH 5.5. The perfusate was titrated with 0.02 M NaOH to the end point of 5.5 using an automatic titrator. The acid output was recorded every 2 min by the use of a zero suppressor adaptor. The low basal acid secretion (0.16 ± 0.1 µmol/10 min) was stimulated by baclofen (4 mg/kg s.c.). The peak response of acid (5.62 ± 1.06 mol/10 min), was observed within 58 ± 3 min, and maintained at this level for 90 min in rats injected intracisternally with saline every 30 min. When step doses of PGE_2, 1, 3 and 10 µg or 16,16 dimethyl PGE_2, 10, 30 and 100 ng were injected intracisternally at 30 min intervals a dose dependent inhibition of baclofen stimulated gastric acid output was observed. Suppression of acid secretion was observed within 3.7 ± 0.5 min following intracisternal injection of PGE_2 or the analog. The 10 µg dose of PGE_2 given intracisternally completely suppressed gastric acid secretion and infused intravenously had no effect. Intravenous doses of PGE_2 significantly inhibiting baclofen stimulated gastric secretion were 30-100 µg. Intracisternal indomethacin (0.5 mg, -1 h) modify neither basal nor baclofen stimulated gastric secretion and higher doses (0.75- 1 mg) induced acute lung vascular injury and lethality within 10 min. Intracisternal indomethacin (0.1-0.5 mg) induced a dose dependent inhibition of PGE_2 generation (ng/g wt tissue) in gastric fundus from 285 ± 6 (saline group) to 3.9 ± 0.5 (indomethacin, 0.5 mg group) and decreased PGE_2 content in the cerebrospinal fluid (saline: 3.6 ± 0.7; indomethacin 0.2 ± 0.1 ng/ml n=5). These results demonstrated that intracisternal PGE_2 acts centrally to inhibit baclofen stimulated gastric acid secretion. No evidence suggests that endogenous PG may exert a tonic inhibitory effect on gastric secretion.

399.10 BEHAVIORAL AND ENDOCRINE EFFECTS OF INTRATHECAL CAPSAICIN IN FEMALE RATS. T.R. King*, P.W. Nance and D.M. Nance. Depts. of Anatomy and Medicine, Faculty of Medicine, Dalhousie University, Halifax N.S. B3H 4H7, Canada.

Neural feedback from the gonads and the reproductive tract is believed to play an integral role in normal reproductive function. In order to assess the role of primary sensory afferents in endocrine control, the effects of a single intrathecal injection of capsaicin into the lumbosacral region on estrous cycles, ovarian compensatory hypertrophy, female sexual behavior, fertility and pseudopregnancy were tested. The effects of cervical probing on lordotic behavior and immobilization were also tested in capsaicin treated and control rats. Additionally, the long-term effects of capsaicin on noxious heat and pressure stimulation were tested as well as any possible interactions with the analgesic effects of cervical probing. Neurotoxic effects of the capsaicin were assesed by immunocytochemical localization of substance P in the spinal cord of the animals. Ovarian function and sexual receptivity were unaltered by intrathecal capsaicin. Despite their appafent normal mating behavior, only 13% of the capsaicin treated females delivered pups compared to 100% of the control rats. In keeping with this, cervical probing or vaginal stimulation was less effective in inducing pseudopregnancy in the capsaicin treated rats. As measured by the development of a deciduoma by the uterus in response to local trauma induced four days following cervical probing at proestrous, less than 25% of the capsacin treated rats showed a positive decidual response vs 100% of the controls. Consistent with the loss in the endocrine response to cervical probing, the facilitation in the lordotic response and immobilization typically produced by cervical probing was diminished or absent in the capsaicin treated rats. Intrathecal capsaicin attenuated responsiveness to noxious heat as shown by near maximal tailflick latencies. There were no group differences in response to the pressure algometer tests on the hind paws. The analgesic effects of cervical probing on the pressure algometer tests were normal in the capsacin treated rats. The immunocytochemical results indicated a substantial reduction in substance P in the dorsal horns of the capsacin treated rats, relative to the vehicle injected controls. These results indicate that intrathecal capsacin has an effect in adult female rats that is specific to the endocrine and behavioral consequences of cervical or vaginal stimulation. The long-term analgesic effects of intrathecal capsaicin were specific to the tailflick tests. Whether substance P mediates any of the endocrine or behavioral effects of cervical stimulation remains to be established. Research supported by MRC of Canada and the Dalhousie Medical Research Foundation.

399.11 LONG-TERM DISRUPTION OF HORMONAL RHYTHMS FOLLOWING A SINGLE EXPOSURE TO SOMAN. G.J. Kant, T.-M. Shih, J.R. Leu, T.G. Raslear*, V.R. Jimmerson and E.H. Mougey*. Dept of Medical Neurosciences, Walter Reed Army Institute of Research , Washington DC 20307 and Basic Pharmacology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010.

Exposure to organophosphorus (OP) compounds has been reported to cause long-term physiological and behavioral effects. Animal studies have shown that a single exposure to soman, an OP that irreversibly inhibits acetylcholinesterase, may produce marked neuronal degeneration. It has also been reported that a single exposure to the organophosphate, DFP, may disrupt circadian rhythms of eating and motor activity in rats for more than two weeks. Disruption of behavioral rhythms was observed even after the animals had resumed eating normal total amounts of food. These studies suggest that exposure to OPs may affect neuronal pathways involved in the regulation of biological rhythms. Since some hormones display marked circadian rhythms, the present study was conducted to assess the effects of a single exposure to soman on hormonal rhythms.

Rats were allowed two weeks of acclimatization to the animal housing facility and lighting regimen (lights on from 0600 to 1800) prior to soman administration. Rats were injected with 100 ug/kg soman s.c. (0.9 LD_{50}) or vehicle and replaced in their home cages. Two weeks following soman administration, control and soman-treated rats were killed every two hours for a 26 hr period. Trunk blood was collected in heparinized beakers and Trasylol was added to plasma to inhibit peptidases. Rectal temperatures were recorded from each carcass as an additional index of circadian rhythm.

Plasma corticosterone, prolactin, and growth hormone were measured by radioimmunosassays. Substantial differences in levels of these hormones were seen in soman-treated rats as compared to controls. Levels of prolactin were suppressed at all time points relative to controls. Growth hormone levels were also suppressed in soman-treated rats at most time points. Both control and soman-treated rats displayed marked rhythms in corticosterone but the usual late afternoon rise in plasma corticosterone levels was shifted to earlier time points in the soman-treated rats. In addition, afternoon temperatures were elevated in soman-treated rats as compared to controls.

The results of this experiment demonstrate that a single exposure to soman may have long-lasting effects on neuroendocrine function. The mechanisms for these effects are not known. However, the observed changes in levels of the hormones measured are similar in direction to reported effects of cholinergic agonists, e.g. i.v.t. administration of acetylcholine inhibits prolactin release. Thus the changes seen in the present experiment may reflect increased central levels of acetylcholine at central synapses involved in regulation of neuroendocrine function.

399.12 DESCENDING SEROTONERGIC PROJECTION TO THE CAUDAL NEUROSECRETORY COMPLEX. S.L. Cohen and R.M. Kriebel. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

Several brainstem nuclei that project to the caudal neurosecretory complex (CNC) of poecilids have been identified with HRP tracing techniques. Immunohistochemical studies showed many serotonergic fibers in the CNC, and the reduction in fiber number that followed transection of the spinal cord indicated that the origin of some of these fibers was rostral to the CNC. This report describes the specific brainstem origin of the descending serotonergic projection to the neurosecretory nucleus. Fish were anesthetized with MS-222 and wrapped in moist gauze for protection. The spinal column was visualized by transillumination of the caudal peduncle, and an incision was made through the musculature overlying the 3rd -5th preterminal vertebrae. An insect pin coated with Fast Blue (FB) was inserted into the exposed spinal cord. After 2 min., the pin was removed, and the wound was packed with gelfoam. The fish were anesthetized after 10 days and perfused with 4% paraformaldehyde in phosphate buffer. The brainstem was transversely sectioned on a freezing microtome, and immunofluorescent staining for serotonin, (anti-5HT; Immunonuclear, GAR-FITC; Cappel) was carried out at 4°C on free-floating sections. Neurons containing both FB and FITC labels were seen only in the nucleus reticularis inferioris of the lower medulla. These cells had a long cell soma axis of 20-30um, and were spheroidal in shape. Their specific location was usually within 300um of midline, and a similar distance beneath the inferior border of the medial longitudinal fasciculus (MLF). About twice as many retrogradely labelled cells that were not serotonin positive were counted in the same region as the double labelled cells, indicating that the reticular projection to the CNC is not solely serotonergic. The results of these experiments with FB generally confirmed our HRP tracing studies with one exception. Another population of projection neurons was noted with the FB method in the lower and middle medulla. This group of cells was intermingled with the fascicles of the MLF in the superior aspect of this fiber bundle. These cells had a long axis of 15-20um, but were only 4-6um wide. The size and shape of these neurons may have prevented the accumulation of sufficient quantities of cytoplasmic HRP to be detectable. Affinity of the fluorescent marker to both nuclear and cytoplasmic components makes this label more readily demonstrable in spindle-shaped cells. The neurotransmitter of these cells is unknown. The presence of serotonergic projection neurons to the CNC in regions of the medulla believed to project to the hypothalamus suggests that these brainstem neurons may coordinate the output of the cranial and caudal neurosecretory systems.
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399.13 ELECTRICAL PROPERTIES OF CELL MEMBRANES IN THE CORPORA ALLATA OF THE COCKROACH, DIPLOPTERA PUNCTATA. C.S. Thompson and S.S. Tobe*. Dept. of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

Recent studies of the biosynthesis and release of juvenile hormone (JH) by the corpora allata (CA) of the cockroach, Diploptera punctata have indicated that intracellular calcium levels are important in the regulation of these processes. The small cells of the CA are electrically coupled, thus permitting current to be injected into one cell and membrane responses to be recorded from other cells of the gland. In normal saline current-voltage relations are linear in the hyperpolarizing direction and show a decrease in membrane resistance (and/or gap junction conductance) in the depolarizing direction. In the presence of the potassium channel blockers tetraethyl ammonium ions and 4-aminopyridine, positive current injection elicits secondary membrane responses. These responses are blocked by cobalt and lanthanum ions and by calcium-free saline they are not blocked by tetrodotoxin or sodium-free saline. Also, a pronounced after-hyperpolarization follows secondary membrane responses. These observations are consistent with the hypothesis that there are voltage-sensitive calcium channels present in the CA cell membranes.

Lucifer-yellow injections show that neurosecretory neurons in the pars lateralis of the protocerebrum send axons to the CA by way of nervi corporis cardiaca II. Electrical stimulation of this nerve hyperpolarizes CA cells as does bath application of octopamine. In both cases the effect is blocked by phentolamine. Radioenzymatic assay of the CA and the brain reveals the presence of octopamine. Electrical stimulation of other axon tracts innervating the CA elicits depolarizing responses resembling conventional excitatory postsynaptic potentials. Thus, neurons innervating the CA may regulate the biosynthesis and release of JH by controlling the membrane potential of CA cells which, in turn, determine the calcium conductance of the CA cell membranes. Supported by operating grants to SST from Natural Sciences and Engineering Research Council of Canada and USPHS.

399.14 CHARACTERIZATION OF INSULIN BINDING IN HYPOTHALAMUS OF THREE GENOTYPES OF ZUCKER RAT BY IN VITRO QUANTITATIVE AUTORADIOGRAPHY. B.J. Wilcox, E.S. Corp*, D.P. Figlewicz*, D.M. Dorsa, M.R.C. Greenwood, D. Porte, Jr.*, D.G. Baskin. University of Washington and Veterans Administration Medical Center, Seattle, WA 98108 and Vassar College, Poughkeepsie, NY, 12601

Physiological and behavioral evidence supports functional roles for insulin as a neuromodulator in the hypothalamus. Among these are regulation of food intake and body weight. Specific insulin binding has been localized by autoradiography to several hypothalamic areas including the arcuate, dorsomedial and paraventricular nuclei. This evidence suggests the hypothesis that altered hypothalamic insulin binding may be a contributing factor in obesity. Therefore, we have now measured insulin binding in individual hypothalamic nuclei of the genetically obese Zucker rat using in vitro quantitative autoradiography, a method which allows measurement of binding in small, discrete areas. For this study, brains from 4 animals from each of the three Zucker genotypes (Fa/Fa, homozygous lean; Fa/fa, heterozygous lean; fa/fa, obese) were perfused with 0.9% saline. The brains were removed and frozen on dry ice. Slide-mounted 10 µm frozen sections of the hypothalamus were labeled with (125-I)-monoiodinated human insulin (0.1nM; S.A.=237 µCi/µg) to determine total binding. Nonspecific binding was determined with addition of 1µM unlabeled porcine insulin. Radiolabeled sections were then apposed to LKB Ultrofilm for five days. The autoradiographic images produced were examined by computer digital image analysis and the amount of radioactivity bound per $mm^2$was determined for each hypothalamic area. Preliminary results indicated a 20% increase of insulin binding associated with the dorsomedial (DMN) and arcuate (ARC) nuclei of the obese rat hypothalamus as compared to the heterozygous and homozygous lean littermates. No difference in concentration of insulin binding was detected in association with the ventromedial nucleus or cingulate gyrus (an extrahypothalamic area of high insulin binding). This result indicates that the increase of specific insulin binding in the brain of the obese animal is not a result of an overall binding disturbance but is localized to discrete nuclear areas. Because the DMN and the ARC are nuclear areas implicated in control of numerous neuroendocrine functions including body weight regulation, the results of the present study support the hypothesis that altered insulin binding in the DMN and ARC may contribute to the obesity observed in the obese Zucker rat. (Supported by NIH Grants Am 17047, AM 17844, AM 12829, NS 20311, and the Veterans Administration).

399.15 CENTRAL NOREPINEPHRINE DEPLETION ATTENUATES HYPERGLYCEMIA CAUSED BY CHRONIC TREATMENT WITH DEXAMETHASONE. R.J. Bialik, B.S. Stevenson* and D.C.S. Roberts. Dept. of Psychology, Carleton University, Ottawa, Canada. K1S 5B6

Recent evidence indicates that central norepinephrine (NE) systems are involved in the control of blood glucose. We have investigated the effect of brain NE depletion on blood glucose changes caused by glucocorticoids. Chronic treatment with glucocorticoids is known to produce hyperglycemia in experimental animals and man. We now report that depletion of brain NE attenuates the rise in blood glucose caused by implantation of a dexamethasone (DEX) pellet in male rats.

Lesions of the dorsal and ventral NE systems were achieved by infusions of 2 ul (containing 4 ug of 6-hydroxydopamine, free base) into the ascending fibers of each NE system. The lesion reduced levels of NE to 30% in the hypothalamus, 15% in the cortex and 7% in the hippocampus.

Glucocorticoid pellets (25% DEX & 75% cholesterol, 100 mg each) were implanted under the skin at the back of the neck during light ether anesthetic. Blood glucose was measured in approximately 100 ul of blood collected from a small incision in the tail. Blood glucose levels were determined prior to implantation of the DEX pellet and on each of the next four days.

In the Table it can be seen that NE depletions did not affect baseline blood glucose levels (Day 0) but attenuated the hyperglycemia caused by the DEX pellet. This effect was most evident four days following implantation of the pellet.

Mean (SEM) blood glucose levels (mg/dl) and body weight (grams) for NE depleted and sham lesioned rats before and four days following implantation of DEX pellets.

Group	Blood Glucose Levels					Body Weight				
Day	0	1	2	3	4	0	1	2	3	4
6-OHDA	89	105	116	151	152	348	332	316	309	302
(SEM)	(4)	(5)	(10)	(20)	(26)	(9)	(10)	(8)	(8)	(9)
SHAM	95	122	131	170	205	339	318	303	296	286
(SEM)	(2)	(4)	(6)	(15)	(26)	(12)	(11)	(11)	(11)	(11)

It is presumed that DEX causes hyperglycemia by effects entirely in the periphery. The effects of DEX that would affect blood glucose levels are 1) the liberation of fatty acids and amino acids from peripheral tissues (which causes weight loss, See the Table) and 2) the facilitation of hepatic gluconeogenesis (which causes an increase in blood glucose).

In order for brain NE lesions to have reduced DEX hyperglycemia, the lesions either reduced the effects of DEX on peripheral tissue catabolism or enhanced the utilization of glucose. In the table it can be seen that NE depletion did not affect the weight loss caused by DEX, therefore it is assumed that peripheral tissue catabolism was not affected. The data are then suggestive that NE depletions lead to facilitation of glucose utilization. It would be of interest to determine whether NE depletions cause general metabolic alterations or whether these effects are specific to the situation studied here.

These findings may have behavioral relevance since we have reported that similar treatment with DEX pellets impairs acquisition of a shuttlebox avoidance response but this impairment was not observed in NE depleted rats (Bialik, Pappas & Roberts, Hormones & Behavior, 1984, 18, p. 12).(Supported by MRC)

400.2 CRH SYSTEMS IN NOVELTY-INDUCED BEHAVIORS. N.H. Kalin* and J.E. Sherman* (SPON: L. Heystrand). Psychiatry Service, William S. Middleton Memorial Veterans Hospital, Madison, WI 53705, and Dept. of Psychiatry, Univ. of Wisconsin School of Medicine, Madison, WI 53792.

There is evidence implicating corticotropin-releasing hormone (CRH) in both hypothalamic and extrahypothalamic mechanisms underlying the stress response. Intracerebroventricular (ICV) CRH produces pituitary–adrenocortical activation, autonomic activation, and behaviors hypothesized to be stress related. To pursue the role of CRH systems in the mediation of stress-related behaviors, we studied the effects of ICV-CRH in rats exposed to novelty. We selected environmental novelty as a stressor because it potently elicits hormonal and behavioral changes.

ICV-CRH (0, 30, 300, and 3000 ng) was administered to rats in the same environment. One group of rats had been habituated to this environment before testing; for the other group, the environment was novel. Novelty significantly increased rearing, walking, and latency to respond on the hot-plate test, and significantly decreased sleeping and grooming. In both the novel and familiar test situations, CRH (300 and 3000 ng) significantly increased grooming, walking, self-gnawing, and burrowing and decreased sleeping and rearing. CRH did not affect hot-plate responding. Thus CRH had the same qualitative effect as novelty on sleeping and walking, but in the doses tested CRH did not selectively enhance these behaviors in the novel condition. In contrast, CRH had effects opposite to those of novelty on rearing and grooming.

These data demonstrate that, in our paradigm, CRH does not selectively enhance novelty-induced behaviors. For some behaviors, CRH had effects opposite to those induced by the stressor.

400.1 WITHDRAWN

400.3 INFLUENCES OF CORTICOTROPIN-RELEASING FACTOR (CRF) ON SHOCK INDUCED FIGHTING IN RATS. A. Tazi*, R. Dantzer*†, M. LeMoal†, and G. F. Koob. Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd, La Jolla, CA USA; †INSERM U-259, Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saeus, 33077, Bordeaux, France.

Corticotropin releasing-factor is a 41-amino acid polypeptide released by the hypothalamus that stimulates pituitary ACTH production and thus triggers the classical neuroendocrine response to stress. Several studies have demonstrated that infusion of CRF into the rat brain potentiates the behavioral response to stressful stimuli, as measured in conflict and startle paradigms.

We have recently studied the effects of CRF on shock-induced fighting (SIF) in rats. In the SIF paradigm, two rats exposed to inescapable shock exhibit a series of behavioral changes, culminating at high shock intensities with aggressive attacks. Since CRF has been reported to potentiate the behavioral reponse to stress, we predicted that intraventricular infusion of CRF should potentiate SIF.

Twenty-two male Wistar rats were implanted with indwelling steel cannulae aimed at the lateral ventricle. One week later, animals were grouped in pairs; both animals in each pair received intraventricular (icv) injection of either rCRF (0.1 μg/2 μl) (n=6 pairs) or saline vehicle, (n=5 pairs) and 30 min later each pair was placed in a box with an electrified grid floor. Electric shocks (0.5 mA intensity, 500 msec duration) were administered in groups of 10 pulses, with a 1 sec inter-pulse interval. Each train of 10 shocks was separated by 45 sec without shock. Three mutually exclusive behaviors were recorded according to their high occurrence in each train of shocks: fighting (upright position and strikes exchange between the rats); boxing position (upright position with no strikes exchange); and no interaction (mainly freezing and escape attempts). Sessions were repeated on 5 consecutive days. No differences were detected between CRF- and saline-injected animals on the first test day, but CRF-injected animals exhibited significantly more SIF days 2 - 5; ANOVA with repeated measures on sessions revealed a significant main effect of CRF treatment.

Our results confirm previous reports that CRF potentiates the behavioral response to stress. Our current investigations are aimed at parametric studies of CRF dose and shock intensity. SIF is a sensitive animal model with which to study the neuroendocrine substrates of stress-induced behavioral changes in the rat.

400.4 **BAM-18-INDUCED ANALGESIA AND HYPERALGESIA IN THE RAT TAIL-FLICK TEST.** K.E. Stevens, F.M. Leslie, C.J. Evans, J.D. Barchas, J.D. Belluzzi and L. Stein. Department of Pharmacology, University of California, Irvine, CA 92717 and Departments of Psychiatry and Behavioral Science, Stanford University, Stanford CA 94305

A new opioid peptide containing 18 amino acid residues has recently been isolated from bovine adrenal medulla (Evans, *et al.*, Fed. Proc., 44:422, 1985). Known as BAM-18, it is a derivative of the pro-enkephalin A molecule (Pittius, *et al.*, Br. Res., 304:127, 1984), and is found in high concentrations in many areas of the central nervous system including striatum, hypothalamus, pons/medulla and spinal cord (Evans, *et al.*, Fed. Prod., 44:422, 1985). We have found that BAM-18 binds with high affinity to both μ and κ receptors (k_i=0.29 nM and 0.84 nM, respectively) and might therefore be expected to exhibit potent analgesic activity. To test this idea, we implanted cannulas in the lateral ventricle of male Sprague-Dawley rats. One week after surgery, the animals were placed in restrainers for 30 minutes and then were tested in the D'Amour tail-flick paradigm. The tip of the rat's tail was blackened. Light intensities were adjusted for each animal to yield baseline latencies of 7-9 seconds. After baseline readings, the animals received an ICV injection of either BAM-18 (0.1, 0.5, 1.0, 5.0, 10 or 50 μg) in 20 μl of modified Ringer's solution or Ringer's alone. Tail-flick latencies were determined every 2 minutes for 30 minutes. Statistically significant analgesia was observed only with the 50 μg dose from the 8th-20th minute of testing (see Fig.). However, comparable analgesia is obtained with only 0.5 μg of morphine, ICV (McGregor, *et al.*, Life Sci., 23:1371, 1978). Hyperalgesia was observed at 2 min for all doses but was most pronounced at 5 μg. BAM-18-induced (10 μg) analgesia was reversed by injection of 10 mg/kg naloxone HCl, but the hyperalgesia was not blocked. This suggests that the hyperalgesia is not mediated through opiate receptors.

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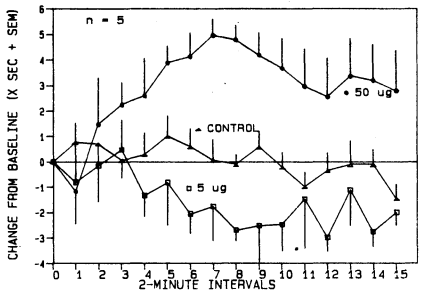

400.5 CCK-8-S ANTAGONIZES THE ANALGESIC ACTIVITY OF MORPHINE IN THE TAIL FLICK PROCEDURE, BUT NOT IN THE HOT PLATE NOR THE ACETIC ACID-INDUCED WRITHING ASSAYS USING THE MOUSE. B. S. Barbaz. N. R. Hall* and J. M. Liebman. Neurosci. Res., Res. Dept., Pharma. Div., CIBA-GEIGY Corp. Summit, NJ 07901.

The sulfated octapeptide form of cholecystokinin (CCK-8), when administered systemically at microgram doses, has been reported to antagonize morphine-induced elevations of tail flick latencies in rats (Faris et al., Science 219: 310-312, 1983). Paradoxically, however, higher doses of CCK-8-S cause analgesia in irritant-induced writhing tests and in the hot plate test (Zetler, Neuropharmacology 19: 415-422, 1980; Barbaz et al., Neuropharmacology, in press, 1986). These observations raise the question of whether the CCK-induced antagonism of morphine occurs only in the tail flick assay or whether it can be demonstrated in other models of nociception and with other opioid analgesics. The present experiments compared the antagonism of morphine and U-50,488H by CCK-8-S, using different assays of nociception.

In all assays, the doses of morphine and other analgesics were selected to produce a 60% minimum effect or > 60% increase over control latencies. Confirming previous studies using rats, CCK-8-S (1.25, 2.5 and 5.0 ug/kg i.p.) significantly antagonized elevations of tail flick latencies by morphine (23 mg/kg i.p.). Higher and lower doses of CCK-8-S were ineffective, indicating an inverted U-shaped function. However, CCK-8-S (2.5 and 5.0 ug/kg i.p.) did not antagonize the analgesic activity of morphine (2.6 mg/kg i.p.) in the acetic acid-induced writhing assay, nor the morphine-induced elevation (3.0 mg/kg i.p.) of mouse hot plate jump latencies.

The effects of CCK-8-S were assessed upon tail flick response changes induced by U-50,488H, a putative kappa agonist and meptazinol, a reputed mu-1 agonist. At doses of 2.5 and 5.0 ug/kg i.p., CCK-8-S did not antagonize the elevation of tail flick latencies induced by U-50,488H (14 mg/kg i.p.), but did antagonize meptazinol-induced (30 mg/kg s.c.) increases in tail flick latencies.

The present studies, therefore, have extended the reported antagonism of morphine by CCK-8-S from the rat to the mouse, but they indicate that this effect is not demonstrable in all analgesia assays, nor with all opioid drugs. The action of CCK-8-S may be selective for neuronal substrates that are regulated by mu receptors and are involved in spinal nociceptive reflex responding.

400.6 SELECTIVE ANTAGONISM BY PROGLUMIDE AND CR-1409 OF THE ANTI-WRITHING ACTIONS OF CCK-8-S, CCK-4 AND RELATED PEPTIDES IN THE MOUSE. F. G. Ambrose*, W. L. Autry*, J. M. Liebman and B. S. Barbaz (SPON: J. J. Welch). Neurosci. Res., Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Antinociceptive effects are produced in mice by three forms of cholecystokinin (CCK), namely the sulfated octapeptide (CCK-8-S), the unsulfated octapeptide (CCK-8-U) and the C-terminal tetrapeptide (CCK-4) (Zetler, Neuropharmacology 19: 415-422, 1980; Barbaz et al., Neuropharmacology, in press, 1986). In various analgesia assays, these three peptides produce unique antinociceptive profiles that differ from those of other gastric neuropeptides (neurotensin and bombesin) or morhpine (Barbaz et al., Neuropharmacology, in press, 1986). If these actions of the various forms of CCK are indeed mediated by CCK receptors, then they should be blocked by CCK antagonists, but the analgesic effects of other peptides should be unaffected. Proglumide has been characterized as a CCK antagonist but as having relatively low potency (Hahne, et al., Proc. Natl. Acad. Sci. USA 78: 6304-6308, 1981). Recently, another glutaramic derivative, CR-1409, has been reported to be approximately 10,000 times more potent than proglumide as a CCK antagonist in peripheral tissue (in vitro) (Makovec, et al., Arzneim.-Forsch. 36: 98-102, 1986). Using proglumide and CR-1409, the present experiments assessed the specificity for CCK receptors of the analgesic actions of various forms of CCK, as compared with other neuropeptides.

The mouse phenylquinone writhing test was used, and doses of peptides and morphine were selected to have equivalent anti-writhing efficacy. By themselves, neither proglumide (1 - 100 ug) nor CR-1409 (1 - 30 ug), administered intracerebroventricularly (ICV), had any appreciable anti-writhing activity. The anti-writhing effects of CCK-8-S (10 ug) and CCK-4 (1 ug) were blocked by CR-1409 (ED50 approximately 1.7 ug for antagonism of either peptide). However, CR-1409 did not block the antinociceptive actions of CCK-8-U (100 ug) nor of morphine (0.3 ug) in PQW. Proglumide, like CR 1409, antagonized the anti-writhing activity of ICV CCK-8-S or CCK-4 (ED50 = 37 ug ICV against CCK-8-S and 14 ug against CCK-4) but not that of CCK-8-U. Surprisingly, proglumide also blocked the analgesia produced by morphine (antagonism ED50 = 8 ug ICV). Proglumide did not antagonize the anti-writhing actions of either bombesin or neurotensin. These results indicate that the anti-writhing (and by implication, at least some of the antinociceptive) actions of CCK-8-S and CCK-4 are mediated by CCK receptors. Other receptors apparently mediate the anti-writhing effects of CCK-8-U and other gastric neuropeptides.

400.7 CHOLECYSTOKININ OCTAPEPTIDE (CCK 8) ANTINOCICEPTION - POTENTIATION BY ENZYME INHIBITORS. K.M. Pittaway*, R.G. Hill and J. Hughes*. Parke-Davis Research Unit, New Addenbrooke's Hospital Site, Hills Road, Cambridge. CB2 2QB, U.K.

There is conflicting evidence in the literature concerning the antinociceptive properties of CCK 8. The actions of CCK 8 have therefore been investigated in a number of analgesia tests after subcutaneous, intracerebroventricular and intrathecal administration. Results from drug treated animals were compared with the appropriate vehicle controls and statistical significance tested using the Mann Whitney U test.

Subcutaneous administration of CCK 8 produced significant antinociception 10 minutes after injection in the rat tail immersion, and paw pressure tests and in the mouse acetylcholine induced writhing test, the lowest effective doses being 0.05 mg/kg, 3.33 mg/kg and 1.11 mg/kg respectively. After intrathecal administration, CCK 8 (30 and 300 ng) produced analgesia and 3 µg and 15 µg produced hyperalgesia in the rat paw pressure test, both effects being maximal 5-10 minutes after injection. CCK 8 analgesia (30 ng) was also detected in the rat tail immersion and tail flick tests. When CCK 8 was given intracerebroventricularly to mice, doses of 3-30 µg produced significant antinociception in the hot plate test, the maximal effect being observed within the first 10 minutes after injection. The antinociceptive effect of CCK 8 under any of the test situations was not accompanied by motor incapacitation or any other overt side effects. The finding that the maximum antinociceptive effect of CCK 8 occurs within 5-10 minutes after injection (by any of the routes tested) suggests that its action may be limited by metabolism and indeed the level of antinociception produced by CCK 8 after intracerebroventricular injection in the mouse hot plate test could be increased by coadministration of a mixture of peptidase inhibitors (captopril, bestatin and thiorphan, 25 µg each). Each inhibitor alone had little effect on CCK 8 analgesia and the inhibitors themselves were not analgesic. The inhibitors used are likely to protect against the degradation of both CCK 8 and endogenously released opioids and it is likely that both opioid and non-opioid mechanisms are responsible for CCK 8 analgesia.

400.8 FURTHER EVIDENCE FOR VAGAL INVOLVEMENT IN THE PERIPHERAL PEPTIDERGIC MODULATION OF NOCICEPTION. J.L. Steinman, P.L Faris* and J.W. Olney (SPON: J.H. Thurston). VA Medical Center, Sepulveda CA 91232 and Washington Univ, St. Louis, MO 63110.

Peripherally circulating opioid peptides may have antinociceptive effects. With McLaughlin and Baile, we have demonstrated that sequestration of peripherally circulating β-endorphin attenuates the antinociceptive effects of morphine (M) (Science 226, 1215, 1984). Adams et al (Fed Proc 45, 916, 1986) found an increase in plasma opioid concentrations following M administration. Collectively, these findings suggests that peripheral opioids, released in response to M, are one component involved in determining the magnitude of the antinociceptive response to M. In addition to opioids, non-opioid peptides may also act peripherally to influence nociception. We have previously demonstrated that neutralization of peripheral cholecystokinin (CCK) potentiates morphine analgesia.

Randich and Maixner (PB & B 21, 441, 1984) have suggested that peripheral opioids may act on vagal receptors to influence nociception. They reported that cervical vagotomy (VAG-X) reduces the antinociceptive effects of both an enkephalin analog and footshock. Since VAG-X attenuates the effects of CCK on other behaviors, it is possible that the vagus is also involved in mediating the attenuation of M antinociception by intraperitoneal (IP) CCK (Science 219, 310, 1983). The present study was undertaken to assess the effect of VAG-X on both M antinociception and CCK attenuation of the antinociceptive action of M.

Male rats received bilateral sub-diaphragmatic VAG-X (n=14) or sham surgeries (n=14). Within each surgical group, rats were given CCK (5ug/kg) or saline (S) IP and 20 min later, all animals received 10mg/kg morphine IP. Tail flick latencies were recorded at 20 min intervals after the first injection. As in previous studies CCK attenuated M antinociception in sham controls. VAG-X significantly attenuated M antinociception. However, CCK did not further reduce the M suppression of a nociceptive reflex in VAG-X rats. These findings support vagal involvement in nociception and suggest that the effects of peripheral CCK on food intake and antinociception may be mediated by a common vagal pathway.

		Mean Tail Flick Latencies (±SEM)			
Drug	Surgery	40	60	80	100
S-M	Sham[A,B]	4.5(.45)	5.4(.39)	4.5(.5)	4.5(.57)
S-M	VAG-X[B]	3.1(.18)	4.0(.45)	3.9(.33)	2.7(.15)
C-M	Sham[A]	3.3(.17)	4.3(.5)	4.0(.46)	2.9(.3)
C-M	VAG-X	3.3(.16)	3.6(.4)	3.0(.2)	2.9(.37)

[A] $p<0.03$ [B] $p<0.04$

[RSDA MH00595 (PLF), a Monsanto Co grant and RSA MH38894 (JWO)]

400.9 BEHAVIORAL AND BIOCHEMICAL ANALYSIS OF CR1409, A NEW CHOLECYSTOKININ ANTAGONIST. Maria-Th. Kaltwasser,* Barbara Petrack and Jacqueline N. Crawley. Unit on Behavioral Neuropharmacology, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892 and Ciba-Geigy Corp, Summit, NJ 07901.

Our understanding of the functional significance of endogenous cholecystokinin (CCK) is hampered by the dearth of potent and specific CCK antagonists. A new derivative of proglumide, CR1409 (Rotta Research Laboratorium, Milano, Italy), was tested for its ability to block the "satiety" syndrome induced by peripherally administered CCK and for its activity in displacing ^{125}I-CCK-8 binding in both brain and pancreas in the rat.

CR1409 was administered five minutes before CCK-8-sulfate in both mice and rats, which were then tested for five minutes in an open field exploration paradigm, and for thirty minutes in a palatable food consumption paradigm (Crawley, Stivers, Hommer, Skirboll and Paul, JPET 236:320, 1986). CR1409 significantly blocked the reductions in feeding and approaches to a novel object, and the cumulative duration of pauses, induced by CCK (5 ug/kg, i.p.), at doses of CR1409 1-10 mg/kg i.p. in mice and 10 mg/kg i.p. in rats. CR1409 had no effect when administered alone on exploration or feeding at doses of 10 mg/kg i.p. in mice or rats, but at a dose of 25 mg/kg i.p. in rats, CR1409 reduced feeding and exploratory parameters, suggesting a mixed agonist-antagonist profile.

Displacement of ^{125}I-CCK-8 binding to mouse cortical membranes and mouse pancreatic membranes by CR1409 was assayed at concentrations of 2-200 nM for pancreas and 0.2-2 µM for cerebral cortex (Wennogle, Steel and Petrack, Life Sciences 36:1485, 1985). The IC_{50} values were found to be 13.7 nM for pancreas and 2.6 µM for cortex. Hill plots yielded values of approximately 1.2 in both tissues. Our results suggest that CR1409 is a potent competitive inhibitor of CCK-8 in the gut, but may be considerably less active in brain. This finding that CR1409 is selective for peripheral-type CCK receptors, further supports the hypothesis that cortical and pancreatic receptors for CCK are pharmacological different.

400.10 INTRA-ACCUMBENS INJECTIONS OF CCK BLOCK THE HYPERLOCOMOTION AND POTENTIATE THE STEREOTYPY PRODUCED BY D-AMPHETAMINE. F.Weiss, J.C.Horvitz, J.G.Mann* and A. Ettenberg. Department of Psychology, University of California, Santa Barbara, CA 93106.

Although it is well established that the octapeptide cholecystokinin (CCK) coexists with dopamine (DA) within a subpopulation of mesolimbic neurons, the precise functional significance of this coexistence remains unclear. Last year, we reported that ICV application of CCK produced a reduction in the locomotion and a potentiation in the stereotypy induced by the indirect DA agonist, d-amphetamine (AMP)(Ettenberg & Weiss, Soc. Neurosci. Abstr.,1985,11, 618). Such results might be accounted for by a differential action of CCK in the nucleus accumbens (NAS) and the caudate nucleus; the DA terminal areas believed to be responsible for AMP-induced locomotion & stereotypy, respectively. One might, for example, propose that CCK acts as an antagonist of mesolimbic and as an agonist of nigrostriatal DA function. However, CCK was completely ineffective to induce either ipsi- or contraleteral rotation in AMP-treated rats following unilateral application directly into the caudate (Weiss & Ettenberg, Soc. Neurosci. Abstr.,1985, 11, 618). Together these data suggest that both the locomotor-attenuating and stereotypy-potentiating effects of CCK in AMP-treated rats resulted from a peptide-DA interaction within the NAS. To test this hypothesis, we examined the effects of CCK on AMP hyperlocomotion and stereotypy following bilateral peptide administration into the NAS.

Rats underwent bilateral stereotaxic implantation of guide cannulae into the NAS. Each rat received either a 1 or 3mg/kg s.c. injection of D-AMP followed, 15 min later, by a central application of CCK (0, 20ng, 200ng, 2ug) or haloperidol (5ug). Stimulant-induced locomotion was measured, beginning 1 min post-infusion, in individual photocell-equipped chambers. Stereotypy was assessed using a 4-pt rating scale by a trained observer who was unaware of the treatment conditions of the rats. Behavioral measures were taken at 5 min intervals over a single 90 min session. CCK produced an inverse dose-dependent effect on both AMP behaviors. However, drug-induced locomotion was attenuated by the peptide while stereotyped behaviors were reliably increased by CCK. In both cases, maximum effects were observed with the smallest (20ng) dose. Haloperidol also reduced locomotion and potentiated stereotypy. These data suggest that CCK serves as a DA antagonist within the NAS and that the potentiation of stereotyped behavior observed following ICV application of the peptide does not require a DA agonist action in the caudate -- the area presumed to mediate the stereotypic response to AMP. Such results might be accounted for by the Robbins & Everitt Response Competition Hypothesis which postulates a mutually competitive functional relationship between the mesolimbic and nigrostriatal DA systems. Additional studies are currently underway to more closely examine this hypothesis with respect to CCK's actions.

400.11 THE HYPERACTIVITY PRODUCED BY INTRA-MEDIAN RAPHE (MR) INJECTIONS OF MUSCIMOL AND SP5-11 ARE MEDIATED BY DISTINCT NEURONAL MECHANISMS. J.M. Paris and S.A. Lorens. Dept. Pharmacology, Stritch School of Medicine, Loyola Univ. of Chicago, Maywood, IL 60153

Intra-midbrain MR injections of the GABA-A agonist, muscimol (25-200 ng in 0.5 ul vehicle; Pharm. Biochem. Behav. 17:973, 1982), and of the metabolically stable substance P (SP) analog, (pGlu-5,MePhe-8,Sar-9) SP5-11 (10-1000 ng in 0.5 ul vehicle), produce dose-dependent increases in locomotor activity (LMA) as measured in photocell chambers. Ibotenic acid (8.0 ug in 2.0 ul buffer) lesions of the MR blocked the hyperactive effects of both muscimol (100 ng) and SP5-11 (1.0 ug) indicating that the LMA effects of these agents are dependent on intact MR perikarya and are not due to their spread outside the MR. Intra-MR injection of the serotonin (5HT) neurotoxin, 5,7-dihydroxytryptamine (6.0 ug in 1.5 ul vehicle) after pretreatment with the dopamine and norepinephrine (NE) uptake inhibitor, nomifensine maleate (15 mg/kg,ip), blocked the LMA effect of SP5-11 (1.0 ug) but not muscimol (100 ng). In contrast, intra-MR injection of the catecholamine neurotoxin, 6-hydroxydopamine (6.0 ug in 1.5 ul vehicle) after pretreatment with the NE uptake inhibitor, desipramine (15 mg/kg ip), eliminated the LMA effects of both SP5-11 and muscimol. Intra-MR injections of the 5HT-1A agonist, 8-OH-DPAT (6.0 ug in 0.5 ul or 100 ng/0.025 ul/min for 60 min) also led to increased LMA. These observations suggest that 1) acute suppression of the firing rate of intra-MR 5HT neurons can produce hyperactivity; 2) activation of intra-MR SP receptors can suppress the activity of 5HT neurons and thereby elevate LMA; 3) the increases in LMA produced by activation of MR GABA-A receptors are mediated by non-5HT neurons; and, 4) the LMA effects of both muscimol and SP5-11 are mediated directly or indirectly via dopamine terminals or perikarya located in the MR.

400.12 EFFECTS OF NALOXONE AND CHOLECYSTOKININE ON FOOD AND WATER INTAKE IN THE BRATTLEBORO RAT. R. Yirmiya*, M. D. Holder*, and J. Garcia* (SPON: B.M. Wenzel). Mental Retardation Research Center, Neuropsychiatric Institute, University of California, Los Angeles, CA 90024.

One site in which both naloxone and cholecystokinine (CCK) were shown to suppress feeding is the paraventricular nucleus (PVN) of the hypothalamus. Since neurons in the PVN contain vasopressin and project to limbic and brainstem nuclei involved in visceral and gustatory functions, an efferent vasopressinergic system may be involved in this suppression. We investigated this hypothesis using Brattelboro rats (HODI), which are homozygous for diabetes insipidus, lacking the ability to synthesize vasopressin. In the first experiment, 10 HODI and 15 age-matched Long-Evans (LE) rats were food deprived for 14 hours, and then injected with naloxone (2.5 mg/kg) or saline. Thirty minutes later the food was returned and food and water consumption were measured after 1, 3 and 4 hr. This procedure was repeated 5 days later, with the rat to injection assignments reversed so that each rat received both naloxone and saline. Naloxone suppressed the food consumption of both HODI and LE but the suppression was greater for the HODI ($F(1,23)=4.19$, $p<0.05$). Water consumption was equally reduced in both HODI and LE. In the second experiment, conducted 2 weeks after the first, the same rats were food deprived for 6 hours and then injected with CCK-8 (2.5 μg/kg) or saline. Food was returned one minute after the injection and food and water consumption were measured 30 and 60 minutes later. This procedure was repeated 2 days later, so that each rat received both injections. Food intake was reduced equally for both HODI and LE. Water intake was not reduced. The results suggest that the suppression of feeding by both naloxone and CCK does not require an intact vasopressinergic system. The reason for the greater effect of naloxone on suppressing food intake in HODI is not clear. One possibility is that vasopressin modulates the effect of opiates on feeding in normal rats.

400.13 MICROINJECTIONS OF RAT HYPOTHALAMIC GROWTH HORMONE-RELEASING FACTOR (rhGRF) INTO THE SUPRACHIASMATIC NUCLEUS (SCN) STIMULATES FOOD INTAKE IN FREE-FEEDING RATS. F. J. Vaccarino and M. Hayward* Departments of Psychology and Psychiatry, University of Toronto, 100 St. George Street, Toronto, Ontario M5S 1A1

Research from our laboratory indicates that intracerebroventricular (icv) administration of rhGRF in picomole doses can stimulate food intake in both food-deprived and non-food-deprived rats (Nature, 314:6007, 1985; Soc. Neurosci. Abstr., 1985). Further findings demonstrating that peripheral injections of rhGRF or growth hormone do not influence feeding, suggest that the feeding-enhancing effects of rhGRF may be centrally mediated. Consistent with this notion are recent immunocytohistochemical findings (J. Comp. Neur., 237:100-115, 1985; Peptides, 5:1071-1075, 1984) indicating that in addition to being present in the median eminence, proximal to the portal vessels, rhGRF immunoreactive terminals are also present in various central sites distal to regions directly associated with the portal blood system. In an effort to elucidate possible central sites mediating the stimulatory effects of rhGRF on feeding we examined the effects of rhGRF microinjections into the SCN (a region containing rhGRF immunoreactive terminals) on food intake in free-feeding rats.

The food (Purina Rat Chow) and water intake of adult male Wistar rats with cannula implants aimed at the SCN was measured for a 90 minute period (15:30 - 17:00) on six consecutive days following a 60 minute exposure to fresh food. On the seventh day, rats were tested for food and water intake following intra-SCN microinjections of one of the following rhGRF doses: 0.0 (vehicle), 0.01, 0.1 or 1.0 picomoles. RhGRF was administered in a 0.5µl volume of 0.01% ascorbic acid vehicle over 60 seconds using a 30 gauge injector.

The results demonstrate that intra-SCN microinjections of rhGRF produce a significant dose-dependent increase in food intake. The mean food intake (grams) ± S.E.M. following microinjections of 0.0, 0.01, 0.1 and 1.0 picomoles was 0.83 ± .31, 1.64 ± .44, 2.22 ± .49 and 3.13 ± .35, respectively. Microinjections of rhGRF into regions outside the SCN did not appear to influence food intake. No significant effect of rhGRF on water intake was observed. There were no significant differences in baseline food intake across the four SCN groups tested.

The present results suggest that the SCN may be an important site in the mediation of the central facilitatory effects of rhGRF on food intake.

This research was supported by NSERC grant #U0443 to FJV.

400.14 EFFECTS OF CHRONIC NALOXONE ON MACRONUTRIENT SELECTION AND CIRCADIAN FEEDING IN THE ZUCKER RAT. J.R. Andrade* and M.J. Lewis, Dept. of Psychology, Howard University, Washington, D.C. 20059.

Elevated levels of the opiate beta-endorphin have been implicated in the development of hyperphagia and hypertrophy in the genetically obese mouse and Zucker rat. Acute injections of the opiate receptor blocker naloxone have been shown to abolish overeating and suppress weight gain in both obesities. The purpose of this research was to examine the effects of chronic administration of naloxone on macronutrient selection and circadian feeding in the Zucker rat.

All animals were maintained on an isocaloric self-selection diet regimen which consisted of protein, carbohydrate, and fat rations. Following baseline measures of the macronutrient diets, lean and obese Zucker rats were matched on body weight and were implanted subcutaneously with either 5 naloxone (10 mg/pellet) or 5 lactose control slow release pellets. On Day 1 measures of diet selection were recorded at 2, 4, and 6 hours. On Days 2-6 measures of diet selection were recorded during the light (beginning 0900) and dark (beginning 1700) of the 24 hour cycle. Analysis of the data showed that in obese and lean rats naloxone significantly suppressed all diets during the first 2 hours; however, fat consumption returned to baseline during the second 2 hr. period (four hour measurement) in lean rats and only during the last 2 hr. (six hour measurement) in obese rats. Over the five day period, naloxone significantly suppressed all diets in obese rats during the light and dark cycles. In lean rats, naloxone also significantly decreased carbohydrate consumption during both cycles; however, there was an increase in fat consumption in both cycles.

These data are consistent with other data suggesting that naloxone may produce its anorexic effects by primarily suppressing carbohydrate intake. These data also support our previous research indicating that naloxone has differential effects on light versus dark cycle feeding.

Supported in part by grant DHHS RR08016 and Howard University, GSAS, Terminal Fellowship and Trustee Scholarship.

400.15 ANESTHETIC MODIFICATION OF ELECTROCONVULSIVE SHOCK (ECS) EFFECTS ON THYROTROPIN RELEASING HORMONE (TRH) IN RAT FOREBRAIN REGIONS. A. Sattin and M.J. Kubek. R.L. Roudebush VA Medical Center and Depts. of Psychiatry and Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

We have previously characterized an apparent seizure-dependent increase in TRH in specific limbic and cortical regions following single and repeated ECS (Abstr. Soc. Neurosci. 11: 622, 1985; Life Sci. 34: 1149, 1984 and 36: 315, 1985). The increase in TRH is maximal after 3 alternate-day ECS. In pyriform cortex (PYR) a significant intermediate TRH increase is seen 2 days after only one ECS and by 2 days after 3 ECS it is 8-fold higher than in sham ECS controls. TRH remains significantly elevated in PYR for up to 12 days after the third ECS. Thus, of all regions studied PYR appears to be the most sensitive and responsive to the effects of ECS on TRH.

During the first decade of clinical application of ECT no anesthesia was used. At present, the peripheral motor effects of the seizure are reduced or eliminated by i.v. pre-treatment with muscarinic blockade, methohexital (ME) and succinylcholine (SDC) with no apparent diminution of the clinical antidepressant effect. Therefore, if the documented effects of ECS on limbic TRH were relevant to human antidepressant treatment the TRH response should not be eliminated by anesthetic modification in rats provided that a seizure discharge occurs in the brain.

Young male S-D rats were injected via tail vein with full anesthetic and neuromuscular blockade doses of glycopyrillate, ME and SDC then intubated and mechanically ventilated for 15-20 min with 97% O_2-3% CO_2. Following insertion of subdermal EEG scalp electrodes they were given transauricular ECS, 30 mA (a.c.) x 1.5s or sham ECS. One technically adequate EEG recording showed increased amplitude x 6s, then decreased x 20s. Two days later all rats were sacrificed and TRH was determined by specific RIA in eleven forebrain regions.

Preliminary data showed no significant difference between control and experimental TRH levels in any region except for PYR where Sham ECS and ECS values were 1.72 + 0.18 (n=4) and 2.93 + 0.34 (n=4) pg/mg ww, respectively ($p<0.02$, student-t). The lack of effect in hippocampus, amygdala and cortex is consistent with our previous results in unanesthetized rats wherein TRH content of PYR was also increased following a single ECS.

Since anesthesia and motor paralysis did not eliminate the PYR response to one ECS we believe that the tonic-clonic motor activity during ECS is not responsible for the TRH response.

Further work will be required to confirm this finding, determine the effect, if any, of anesthesia alone and extend these observations to repeated ECS. Supported by VA Research Service.

400.16 BOMBESIN: EFFECT ON THE ETHOGRAM OF ETHANOL SATIATION AND BLOOD ETHANOL LEVELS. G.W. Glazner* and P.J. Kulkosky* (SPON: P. Schnur). Dept. of Psychology, Univ. of Southern Colorado, Pueblo, CO 81001

Experiments have recently shown that peripheral injection of bombesin-like peptides potently and specifically inhibits consumption of ethanol solutions in the rat. The effect of bombesin on the ethogram of ethanol satiation and blood ethanol levels was characterized for bombesin tetradecapeptide (BBS-14) and nonapeptide (BBS-9). Nine naive male Sprague-Dawley rats were deprived of water for 23 hours. Rats were injected with i.p. saline and allowed access to 5% w/v ethanol for 45 minutes daily, followed by 15 min access to water. During ethanol access behavioral observations were made with the technique of Gibbs et al. (Soc. Neurosci. Abstr., 6: 530, 1980). Observations of behaviors were made at tone-cued 1 min intervals and classified into categories of ethanol drinking, feeding, standing, grooming, resting, and a category including all other behaviors. After an adaptation period of 8 days, rats were randomly assigned to receive i.p. injections of either saline or BBS-14 (1, 2, or 4 μg/kg). Bombesin significantly reduced ethanol intake at all doses, ($ps < 0.05$). Analysis of observational data revealed significant decreases in total observations of feeding and drinking, and a significant increase in resting behavior after 4 μg/kg BBS-14. These rats were then adapted to a schedule of 30 min access to 5% w/v ethanol followed by 30 min access to water. Rats received i.p. injection of saline or BBS-9 (4 μg/kg) prior to ethanol. At the conclusion of ethanol access and at 30 min intervals to 150 min after ethanol presentation, a 0.1 ml blood sample was taken from the tail of each rat and enzymatically assayed for ethanol content using the method described by Cornell and Veech (Anal. Biochem., 132: 418, 1983). BBS-9 significantly reduced ethanol intake as did BBS-14 ($ps < 0.05$). Bombesin significantly reduced blood ethanol levels ($p < 0.05$). These results are consistent with the idea that a bombesin-like peptide may act as an endogenous ethanol satiety factor. (Supported by NIH Grant No. RR-08197).

400.17 SUBSTANCE P ANTAGONISTS BLOCK CARBACHOL-INDUCED "BOXING" BEHAVIOR AT THE POSTSYNAPTIC SITE OF A TRIPLE COEXISTENCE IN THE RAT PREFRONTAL CORTEX. Jill A. Stivers* and Jacqueline N. Crawley (SPON: K. Heidenreich). Unit on Behavioral Neuropharmacology, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892.

Substance P (SP), corticotropin releasing factor (CRF) and acetylcholine (Ach) coexist in a population of neurons in the dorsolateral tegmental nucleus (NTDL) in the rat (Crawley, et al, Peptides, 1985). Microinjection of the cholinergic agonist, carbachol, 1-5 ug bilaterally into the terminal region of the NTDL in the medial anterior cerebral cortex, produced an unusual motor behavior resembling boxing, in which the rat stands in an upright posture and repetitively moves the forepaws. Electroencephalographic analysis revealed high amplitude, synchronous sharp wave spiking from bifrontal and fronto-occipital cortical electrodes, temporally correlated with the "boxing" behavior, suggesting that this motor pattern is an unusual form of seizures. "Boxing" was blocked by intraperitoneal treatment with standard anticonvulsants, including diazepam (2mg/kg), clonazepam (0.5 mg/kg) and pentobarbital (10 mg/kg). Microinjection with Ach (80ug) + physostigmine (20 ug) mimicked carbachol-induced "boxing", and intraperitoneal injections of atropine (5 mg/kg) but not mecamylamine (3 mg/kg) blocked carbachol-induced "boxing", suggesting a muscarinic cholinergic mechanism. Topographical analysis demonstrated a decline in "boxing" at sites more than 1 mm rostral, caudal, lateral, dorsal and ventral to the medial anterior cortex site of bilateral carbachol microinjection. Unilateral microinjection of carbachol (6-8 ug) induced more contralateral than ipsilateral "boxing".

Coinjection of coexisting peptides and cholinergic agonists revealed a modulatory action of SP and CRF on carbachol-induced "boxing". SP (1 ug) significantly potentiated carbachol-induced "boxing", while CRF (100 ng) significantly inhibited carbachol-induced "boxing". Neither peptide induced "boxing" when microinjected alone. Two peptides also localized in the medial anterior cortex but not coexisting with Ach, galanin (up to 400 ng) and calcitonin gene related peptide (up to 200 ng), had no effect on carbachol-induced "boxing".

Two antagonists of substance P, [D-Pro^2,D-Phe^7,D-Trp^9]-Substance P (500 ng), and [D-Pro^2,D-$Trp^{7,9}$]-Substance P (500 ng) significantly blocked carbachol-induced "boxing". These results suggest an endogenous function for SP in facilitating cholinergic seizures in the prefrontal cortex, circumscribing the postsynaptic site for an Ach-SP-CRF coexistence.

401 SPECIAL LECTURE. MULTIPLE TYPES OF CALCIUM-CHANNEL IN NEURONS AND MUSCLE CELLS AND THEIR MODULATION BY NEUROTRANSMITTERS. R.W. Tsien, C.D. Benham, A.P. Fox, P. Hess, D. Lipscombe, E.W. McCleskey, D.V. Madison, R.L. Rosenberg. Department of Physiology, Yale Univ. Sch. of Med. New Haven, CT 06510.

Calcium entry through voltage-gated Ca channels is a vital link between membrane depolarization and diverse cellular responses. We have studied properties of multiple types of Ca channel, their co-existence within individual cells, and their expression across the spectrum of excitable tissues.

Patch clamp recordings on cell bodies of cultured chick sensory neurons reveal three types of Ca channel with different kinetics and conductance properties (Nowycky et al., Nature 316, 440). A channel with large Ba conductance (L) contributes long-lasting current at strong depolarizations; a relatively tiny conductance (T) underlies a transient current activated at weak depolarizations; a third type of channel (N) with unitary Ba conductance intermediate between T and L also contributes decaying current. N-type Ca channels require strongly negative potentials for complete removal of inactivation (unlike L) and strong test pulses for activation (unlike T). L-type channels differ from the other types in their relative sensitivity to dihydropyridines. T-type channels stand out in certain other respects: they are more resistant to block by Cd than N and L-type channels, and are only weakly and reversibly inhibited by the peptide toxin ω-CgTx VIA while N and L-type channels are persistently blocked (McCleskey et al., Biophys. J. 49, 431a). The toxin binds with high affinity to brain synaptosomes (Cruz & Olivera, 1986), and to detergent-solubilized synaptosomal membranes.

Some examples of the varied expression of Ca channel types:

chick DRG	T	N	L	Nowycky et al., 1985
rat DRG	T	N	L	Perney et al., this vol.
rat hippocampal	T	?	L	(D.V.M.); Brown et al. 1985
rat sympathetic*	-	N	L	Hirning et al., this vol.
frog sympathetic*	-	N	L	(D.L.)
rat type 2 astrocyte	T	-	-	Barres et al., 1985
guinea pig/dog heart	T	-	L	Nilius et al.,1985; Bean,1985
rabbit artery smooth	T	-	L	Aaronson et al., 1986

*In sympathetic neurons, "N" labels a Ca current component that inactivates much more slowly than N-type Ca currents in chick DRG neurons but is similar in several other respects. This current plays a dominant role in mediating K-induced norepinephrine (NE) release (Hirning et al., this volume).

Examples of adrenergic modulation of N and L-type Ca channels have been found. Isoproterenol enhances L currents in heart cells; NE acting on α-receptors increases L currents in arterial smooth muscle cells; NE decreases the inactivating current (presumably N) in frog sympathetic neurons.

402 SYMPOSIUM. THE NEUROBIOLOGY OF SLEEP: IMPLICATIONS FOR SLEEP DISORDERS MEDICINE. W.C. Dement, Stanford Univ. Sch. Med. (Chairman); M.H. Chase, J.M. Siegel, UCLA Sch. Med.; C.A. Czeisler, Harvard Sch. Med.; J.M. Orem, Texas Tech Sch. Med.; A. Rechtschaffen, Univ. Chicago; H.P. Roffwarg, Univ. Texas Health Sci. Cntr.

It is now a safe estimate that 100 million Americans suffer from a diagnosable sleep disorder. Furthermore, the most prosaic and ubiquitous events, snoring and dozing off in the daytime, can be the crucial signs of a life threatening sleep disturbance. In the past decade, a new brain-related clinical discipline, sleep disorders medicine, has been established, and skilled practitioners are currently dealing with sleep problems in over a thousand medical centers. Two fundamental principles of sleep disorders medicine are death, sleep is a kinsman of death, that is we are more likely to die when asleep than when awake of all causes, and that habitual sleep time is one of the best, if not the best, predictor of longevity.

Sleep disorders medicine owes a considerable debt to basic sleep research. More germane to this occasion is that the practice of sleep disorders medicine is a collective, strong voice calling for an acceleration of basic sleep research. Today nearly 5 billion people sleep or attempt to sleep with each rotation of the earth. Even though understanding of this most fundamental of human characteristics remains a tremendous challenge, it is axiomatic that the sleep states are active and actively induced, and that they are purposeful.

This symposium was organized by the Clinical Sleep Society and the Association of Sleep Disorders Centers to present new and interesting trends in understanding the sleeping brain and its impact on the waking brain and to underline strongly the relevance of basic research for better treatment and cures of patients suffering a wide range of sleep related illnesses.

The fundamental question is "Why does the sleeping brain fail to perform its tasks in so many people?" One such task is to maintain the vital processes, breathing and cardiovascular functions. The way in which the sleeping brain handles such tasks is different from the waking brain. Failure is much more likely to occur during sleep. Dr. John Orem will discuss studies of the CNS regulation of respiration and his studies of the firing patterns of respiratory cells across the sleep/wake cycle and their relevance to the avirulent and serious problem of sleep apnea syndromes.

Dr. Michael Chase will discuss the latest work on motor inhibition during REM sleep and the relevance of these mechanisms to the cataplectic seizures of narcolepsy.

Dr. Jerome Siegel will discuss studies of medullary cells that fire only during REM sleep in both cats and narcoleptic dogs. These cells may mediate normal REM sleep motor inhibition.

Dr. Allan Rechtschaffen will present an up-to-date account of his ongoing comprehensive studies on the physiological effects of sleep deprivation. These studies provide compelling evidence that sleep serves a vital function -- that we cannot survive without it.

Dr. Howard Roffwarg will discuss the role of REM sleep in the developmental maturation of the mammalian central nervous system. This work begins to answer the ontogenetic riddle, "Why is there so much REM sleep in the newborn organism?"

Dr. Charles Czeisler will discuss fundamentals of the organization of the circadian system in man and its relevance to jet lag and rotating shift work. The organizers hope that the symposium will expose a new vista of the sleeping brain to neuroscientists, and reveal the multiple research opportunities of the basic mechanisms of these mysterious states. Such research will provide knowledge vital and necessary to the practice of medicine.

Dr. William Dement will chair the symposium and will present both an overview of sleep disorders and a discussion of recent work on neurochemical abnormalities in the quintessential sleep disorder of narcolepsy.

403 SYMPOSIUM. SPINAL CORD NOCICEPTIVE PATHWAYS. G.J. Giesler, Jr., University of Minnesota (Chairperson); G.J. Bennett, National Institute of Dental Research; C.J. Vierck, University of Florida; W.D. Willis, Marine Biomedical Institute, R.P. Yezierski, University of Mississippi.

For more than 75 years the spinothalamic tract in primates has been thought to be the pathway primarily responsible for conveying nociceptive information to the brain, and ultimately to consciousness. As a result, the spinothalamic tract has been, and continues to be, the focus of a large number of anatomical and functional studies. However, it is becoming increasingly clear that several other spinal pathways perform important roles in nociception, roles distinct from those of the spinothalamic tract. These other pathways are thought 1) to underlie the recovery of pain that occurs following disruption of the spinothalamic tract, 2) to provide input to neurons at the origin of pathways that descend to control pain processing in the spinal cord, and 3) to underlie nociception in non-primates. The goal of the proposed symposium is to present an overview of our current understanding of these pathways and to assess the contributions of each to pain processing. Willis will summarize the current understanding of the spinothalamic tract in cats and monkeys. Bennett will describe his anatomical and physiological studies of the post synaptic dorsal column pathway in the cat. In addition, Bennett will discuss his recent studies of the large number of lamina I neurons that project to analgesia-producing areas of the midbrain. Yezierski will present findings from his recent series of studies on the spinomesencephalic tract. Giesler will discuss the contrasting roles in nociception of the post synaptic dorsal column and spinocervicothalamic systems in different species. Vierck will discuss the effects of disrupting various spinal pathways on nociception. He will also discuss the role of various cord areas in the recovery of nociception following cordotomy.

404.1 DIFFERENT MODES OF NPY RECEPTOR ACTIVATION MAY REGULATE LH SECRETION AND FEEDING BEHAVIOR. S.P. Kalra, W.R. Crowley and P.S. Kalra, Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610 and Dept. Pharmacology, Univ. Tenn, Memphis, TN.

Neuropeptide Y (NPY) is abundant in the rat hypothalamus and a large body of evidence shows that it may participate in the regulation of gonadotropin secretion and food intake. To determine the modality of NPY discharge that may normally evoke gonadotropin and feeding responses, the effects of bolus and continuous infusions of NPY into the third ventricle of the rat brain were studied. A bolus intraventricular injection of NPY (0.1-2.2 nmol) stimulated LH release in a dose-related fashion in estradiol benzoate, progesterone (EBP)-primed ovariectomized (ovx) rats, but inhibited LH release in steroid-unprimed ovx rats. However, similar NPY administration uniformly stimulated vigorous feeding in a dose-related fashion in both these treatment groups. The effects of continuous long-term (3 hr) infusion of NPY on these responses were different. In unprimed ovx rats, NPY infusion at the rate of 0.23 and 1.17 nmol/h promptly suppressed LH release as seen after bolus NPY injection; after 100 min of infusion complete cessation of episodic LH secretion occurred. Lower concentrations of NPY (0.04 nmol/h) either produced a similar degree of suppression (2 rats) or drastically reduced only the amplitude of LH episodes (4 rats, 10.0 $\pm$ 2.0 ng/ml) as compared to that in control, saline-infused rats (35.0 $\pm$ 5.7 ng/ml). Quite unexpectedly, while bolus injections stimulated LH release, continuous long-term NPY infusion in EBP rats at the rate of 0.23, 1.17 or 3.52 nmol/h failed to stimulate LH release. On the other hand, regardless of previous treatment history (ovx or ovx + EBP) these rats displayed feeding behavior during the course of continuous NPY infusion in a dose-related fashion. Infusion of 0.23 nmol/h produced significant increase in food intake over that seen in saline infused rats (saline = 0.4 $\pm$ 0.2, EBP = 3.6 $\pm$ 0.7, unprimed = 1.1 $\pm$ 0.4 g/3h) and 1.17 nmol/h evoked maximal responses (EBP = 9.0 $\pm$ 1.8, unprimed = 7.5 $\pm$ 1.0 g/3h). Therefore, collectively these studies show that differing modalities of NPY receptor activation in the hypothalamus may be involved in the regulation of LH secretion and feeding behavior. Since NPY administration either as a bolus dose or continuous infusion uniformly suppressed LH release in unprimed, ovx rats, we suspect that hypothalamic NPY receptor activation may be normally curtailed drastically to allow postovariectomy LH hypersecretion; in EBP-treated and perhaps in intact rats, excitation of LH release may require intermittent NPY receptor activation. Stimulation of food intake can be triggered by each of the two modalities of administration and increased feeding behavior can be sustained in satiated rats for a long time with continuous NPY receptor activation. (Supported by NIH HD 08634 (SPK) and 13703 (WRC)).

404.2 ANGIOTENSIN RECEPTOR-MEDIATED CATECHOLAMINE SECRETION FROM CULTURED BOVINE ADRENAL CHROMAFFIN CELLS V. L. Boyd* and M. P. Printz. Division of Pharmacology M-013H, Univ. of Calif. at San Diego, La Jolla, CA 92093.

Angiotensin II (AII), the active peptide of the renin angiotensin system (RAS), acts on receptors on a variety of tissues. These sites include peripheral tissues such as liver, anterior pituitary and kidney, as well as the central nervous system and adrenal medulla, neural crest derivatives. Our laboratory has recently shown that the AII receptor differs between these two groups of tissues with respect to gunanine nucleotide sensitivity. In order to investigate the neural AII receptor at the cellular level studies have been carried out on primary cultures of bovine adrenal chromaffin cells.

Cells were isolated using the method of Levitt (Levitt B.G. Phys. Rev. 64:1103 1984) including cell enrichment with Percoll gradient centrifugation. Primary cultures of bovine adrenal medullary cells were maintained in DME/F12 medium containing 10% FCS for 5-7 days before being used. We have recently reported that these cells possess AII receptors with a Kd of 0.7 nM and a Bmax of 36,000 sites/cell (Boyd V.L. and Printz M.P. Fed. Proc. 45:789 1986 [abst]). Additionally, an increase in Bmax can be seen with percoll purification. In order to extend these studies receptor autoradiography was performed on both crude (i.e., no percoll purification) and purified cell preparations after crosslinking the [(125)-I]AII ligand to its receptor. Autoradiography provides further evidence that the percoll gradient purifies cells that possess AII receptors. Morphological analyses do not predict binding ability since not all cells with the same morphology bind AII equally well. Therefore, our previous report of 36,000 sites/cell is an underestimate.

The functional response of the cells to AII was also studied. In the adrenal medulla it has been shown, in vivo and in vitro, that AII stimulates release of catecholamines. However, AII stimulated catecholamine secretion from cultured chromaffin cells has yet to be described. Our preliminary studies reveal that AII is capable of inducing a 2-fold increase in epinephrine (EPI) over basal levels, which is approximately 50% of carbachol stimulated EPI release. AII does not increase carbachol-induced release. Norepinephrine secretion is not significantly stimulated by AII alone, but the peptide does increase carbachol-induced secretion.

These cells are easily obtained in large numbers. Therefore, having a cell system that possesses AII receptors that are functionally responsive makes this an attractive system for studying the neural AII receptor.

404.3 **NEUROTRANSMITTER REGULATION OF RELEASE OF HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR (CRF).** **M.J. Owens,* M.A. Smith and C.B. Nemeroff (SPON: E. Busse). Depts. Pharmacol. and Psychiat., Duke Univ. Med. Ctr., Durham, N.C., 27710.**

Considerable controversy exists concerning the role of various neurotransmitters in modulating activity of the hypothalamic-pituitary-adrenal (HPA) axis. Norepinephrine, acetylcholine and serotonin have been reported to stimulate HPA activity by some investigators, whereas others have reported that these neurotransmitters inhibit HPA activity. Naloxone increases plasma ACTH and cortisol concentrations in man, yet some investigators have reported that morphine stimulates CRF release from rat hypothalami. These early studies were conducted using a bioassay in which a perfusate thought to contain CRF was used to release ACTH from pituitary cell cultures. Bioassays such as these lack specificity because ACTH can be released by a variety of substances (e.g., vasopressin, oxytocin, epinephrine). In the present study, we have determined the effects of several substances on CRF release using a specific and sensitive (1.25 pg CRF/tube) radioimmunoassay (RIA) that utilizes ^{125}I-Tyr^{0}-rCRF as a tracer.

Male Sprague-Dawley rats were decapitated and a hypothalamic block was dissected using rostral-caudal boundaries of the optic chiasm and mammillary bodies, respectively, and dorsally to a point just above the third ventricle. Two such blocks were immediately placed in a test tube- containing 0.6 ml of a modified Kreb's-Henseleit buffer which was gassed with 95% O_2/5% CO_2 and the pH adjusted to 7.4 at 37°C. At ten minute intervals the buffer was removed and stored at -70°C until assayed for CRF. The buffer was replaced by either fresh buffer in the case of basal release, or by buffer containing the neurotransmitter or drug under investigation. Basal release was measured for five consecutive ten minute intervals, and then the test substance was added during the 50-60 minute interval and once again restimulated during the 80-90 minute interval.

By the 40-50 minute epoch, basal release had stabilized to 6.8 pg CRF/500µl incubation buffer. High potassium concentrations [40mM KCl] stimulated CRF release 1100% above basal levels. Serotonin [10µM], naloxone [10µM] and carbachol [10µM] stimulated CRF release 500%, 325% and 300% above basal levels, respectively.

These studies are concordant with the hypothesis that serotonin, acetylcholine and opioid-containing neurons regulate CRF release. The absence of effects in previous studies using minced hypothalami suggest that the projections of CRF neurons from the PVN to the median eminence must be intact to respond to serotonin and acetylcholine.

404.4 INTRACEREBROVENTRICULAR INFUSION OF OXYTOCIN MODIFIES NEURONAL-GLIAL AND SYNAPTIC RELATIONSHIPS IN THE ADULT HYPOTHALAMUS. D.A. Poulain*, C. Montagnèse*, F. Rodriguez*, J.D. Vincent and D.T. Theodosis*. INSERM, U.176, rue Camille Saint-Saëns, F-33077 BORDEAUX-Cedex, France.

Glial envelopment of neurons and the establishment of synaptic circuitry are thought to occur during development and then to remain unchanged under normal conditions. However, in the supraoptic (SON) and paraventricular nuclei of the adult hypothalamus, containing neurons that secrete the hormones, oxytocin and vasopressin, both these parameters are modifiable, under physiological stimuli. Upon stimulation of the oxytocin-secreting system, glial coverage of oxytocin neurones diminishes and contiguous cell bodies and dendrites become directly juxtaposed ; the neurones also receive an increased synaptic input, from terminals that contact two adjacent post-synaptic elements simultaneously (Theodosis et al., 1986, Neuroscience, 17, 661-678). The modifications are reversible and glial reinsertion occurs once the system is no longer activated. In order to see whether oxytocin itself is responsible for these anatomical changes, we studied the effects of intracerebroventricular administration of oxytocin upon the structure of the SON. Using miniosmotic pumps delivering fluid at 0.5 µl/h, synthetic oxytocin, its analogue, 4-Thr-oxytocin, vasopressin (each at 2µg/ml) or vehicle (artificial CSF) were infused continuously into the 3rd ventricle of non-lactating rats for 7 days. Ultrastructural analyses of the SON indicated that infusion of oxytocin, or its analogue, altered the morphology of the nucleus to an extent similar to that observed after normal stimulation (e.g. lactation) : close to 45% of all neurosecretory cell bodies were directly apposed to each other or to adjacent dendrites, as compared to 10% in the controls (vasopressin- or vehicle-infused rats). The proportion of cell bodies sharing the same synapse also increased only in the SON of animals infused with oxytocin or its analogue (from 6 to 14%). Immunocytochemical analysis showed that the anatomical changes affected almost exclusively oxytocinergic neurones and not adjacent vasopressin cells. Our observations suggest that the neuroanatomical changes in the magnocellular nuclei are under hormonal control and provide evidence that a neuropeptide can act centrally to modify the relationship of particular neurones and their surrounding glial cells.

404.5 8-GLYCINE-des-16-LEUCINE-24-*D*-ARGININE-SALMON CALCITONIN AFFECTS THE FIRING RATE OF NEURONS IN RAT HYPOTHALAMUS. M. J. Twery, R. L. Moss, and R. C. Orlowski[1]*. Dept. Physiology, Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75235 and [1]Armour Pharmaceutical Co., Kankakee, IL 60901.

Calcitonin (CT) is capable of altering the firing rate of individual rat forebrain neurons. In order to determine whether the serum calcium-lowering biological activity of the hormone is important in its action on neurons, the effects of salmon CT (sCT) and an analogue, 8-glycine-des-16-leucine-24-*D*-arginine-salmon CT (CTA), on neuronal electrical activity were compared. Experiments were performed on 13 male, Long-Evans rats (312-532 g) anesthetized with urethane (1.3 mg/kg, IP). *In vivo* extracellular recording of individual neurons and the application of chemicals were accomplished through a single multibarrel electrode (center recording barrel 4-15 mΩ). Synthetic salmon CT (4413 MRC U / mg, 0.1 mM) and CTA (2.5 MRC U/mg, 0.1 mM) were prepared in distilled water, and iontophoretically ejected (10-30 nA, 30 sec). The effects of peptide application were categorized based on percent change in firing rate calculated from the mean firing rate (Hz) during treatment and immediately prior to application of peptide.

Iontophoretic application of CTA altered the firing rate of 15 out of 34 neurons localized to the hypothalamus. CTA depressed the firing rate in 5 of 8 ventromedial, 4 of 7 dorsomedial, 3 of 6 anterior, and 1 of 4 arcuate hypothalamic neurons. In addition, 2 of 9 neurons localized to the posterior hypothalamus were excited by treatment with CTA. Further testing of 29 hypothalamic neurons determined that the majority (19 of 29) responded to sCT in a manner which resembled their response to CTA. One neuron was excited, 7 neurons inhibited, and 11 neurons unaffected by application of either peptide. In addition, 4 neurons appeared to possess membrane sensitivity only to CTA and 6 neurons appeared to possess membrane sensitivity only to sCT. No neurons were observed to respond in an opposite manner to CTA and sCT. These results confirm a previous report from this laboratory that a subpopulation of neurons in the rat hypothalamus possessed membrane sensitivity to sCT. The results also show that CTA is capable of affecting neuronal activity in a manner similar to sCT, even though this analogue lacks the α-helical secondary structure of intact sCT which is important for high potency in lowering serum calcium. Further studies using additional analogues of CT are needed to characterize the neuronal membrane action of this hormone and determine which regions of the peptide are responsible for its central actions.

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404.6 CALCITONIN GENE-RELATED PEPTIDE MIMICS SLOW SYNAPTIC EXCITATION IN MYENTERIC NEURONS OF GUINEA-PIG SMALL INTESTINE. J.M. Palmer*, M. Schemann*, K. Tamura* and J.D. Wood. Dept. of Physiol., College of Medicine, Ohio State University, Columbus, Ohio 43210

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide encoded by the calcitonin gene and produced in neural tissue by specific RNA processing. CGRP-like immunoreactivity is distributed extensively in the myenteric and submucosal plexuses of the enteric nervous system suggesting a role for CGRP as a neurotransmitter or neuromodulator. Our aim was to investigate the effects of CGRP on the electrical behavior of enteric ganglion cells from guinea-pig small intestine. Conventional intracellular methods with 3M KCl-filled microelectrodes were used to record and inject electrical current in neurons from myenteric plexus preparations superfused with carboxygenated Krebs solution in vitro. CGRP and thyrocalcitonin, which is co-transcribed from the same gene, were applied either in the superfusion solution or by microejection from fine-tipped pipettes with nitrogen pulses of controlled amplitude and duration. Application of synthetic human and rat CGRP (0.1nM-1uM) to AH/Type 2 myenteric neurons mimicked slow synaptic excitation (slow EPSP). The excitatory actions of CGRP consisted of membrane depolarization ranging from 5-30 mV that was dose-dependent (ED_{50} = 10nM), decreased membrane conductance, decreased amplitude of post-spike hyperpolarizing potentials, and increased probability of action potential discharge with occurrence of spontaneous spikes and stimulus-evoked repetitive spike discharge. Decreased membrane conductance was indicated by an increased slope of ohmic current-voltage relationships. Lines on these plots before and during application of CGRP intersected at a membrane potential near the estimated K^+ equilibrium potential. This suggested that the excitatory action of CGRP involved closure of K^+ channels. Application of synthetic human thyrocalcitonin did not alter electrical behavior of AH/Type 2 neurons. Electrical behavior of S/Type 1 neurons was unaffected by CGRP and thyrocalcitonin. CGRP exerted its effects after blockade of axonal spike conduction and synaptic transmission in tetrodotoxin (0.3-1uM), and during superfusion of 16 mM Mg^{2+}-1.25 mM Ca^{2+} Krebs solution indicating CGRP had a direct action on the impaled neurons. Our results are consistent with a neurotransmitter/neuromodulatory role for CGRP in the enteric nervous system and suggest that it may participate in local neurohumoral regulation of gastrointestinal effector systems. (Supported by NIH grant AM26742 to J.D.W., NRSA AM07308 to J.M.P., DFG award Sche-267/1-1 to M.S., and Overseas Visiting Researcher Program of Tokai University to K.T.)

404.7 BOMBESIN: PREFERENTIAL DEPRESSION OF DORSAL HORN NEURONES IN THE SPINAL CORD OF THE CAT. Y. De Koninck* and J.L. Henry, Depts. Physiol. and Anaes. Res., McGill Univ., Montreal, Que., H3G 1Y6

The presence of bombesin-like immunoreactivity and bombesin binding sites in laminae I-IV of the dorsal horn of the cat spinal cord as well as the depolarising effect of this peptide on primary afferent terminals in the toad spinal cord implicate this amphibian peptide or a peptide of similar nature as a possible chemical mediator of sensory transmission. The present study was done to determine the effects of iontophoretic application of bombesin on dorsal horn neurones in vivo. Thirty-one cats were anaesthetized with alpha-chloralose (60mg/Kg i.v.)and spinalised at the L_1-L_2 level. Spinal segments L_5-L_6 were exposed for recording. Multibarrelled micropipettes were used to record extracellular single unit spike activity and to apply the following by iontophoresis: bombesin (1mM in 165mM NaCl, pH 5,5), Na-L-glutamate (1M; pH 7,4), substance P (1mM in 165 NaCl, pH 5,5) and other peptides. Selected sites were marked with Pontamine Sky Blue for histological identification of positive sites of recording. Of 20 neurones found at depths of less than 1 500μm, 13 were depressed by bombesin, 1 was excited and the remaining 6 were unaffected. Of 24 neurones found at depths greater than 1 500μm, 1 was excited and 23 remained unaffected (even though all were excited by glutamate and 12 of the 18 tested were excited by substance P). Typically, the depression started at 40-60s after the beginning of the application, reached a maximum 60-80s later and outlasted the application by up to 6min. This depression was observed on on-going activity (n=10) as well as on excitatory responses induced by application of noxious heat (n=3) and by low threshold mechanical stimulation (n=2) to the receptive field. In three trials, naloxone (up to 0,3mg/Kg, i.v.) failed to block this depression. These results indicate that bombesin has a depressant effect on neurones in the dorsal horn, in particular upon those in the upper laminae. These results thus provide physiological evidence to support the possible involvment of a bombesin-like peptide as a chemical mediator of synaptic transmission in spinal sensory pathways.

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404.8 BIOCHEMICAL IDENTIFICATION OF NEUROPEPTIDE Y AND PEPTIDE YY RECEPTORS IN GUT AND BRAIN. M.W. Walker* and R.J. Miller (SPON: J. Cowan). Dept. of Pharmacol. & Physiol. Sciences, Univ. of Chicago, Chicago, IL 60637.

Neuropeptide Y (NPY) and peptide YY(PYY) are homologous members of the pancreatic polypeptide family. NPY is found in neurons throughout the central and peripheral mammalian nervous system, including the small intestine. PYY is found primarily in endocrine cells of the gut. NPY was a potent inhibitor of short circuit current (Isc) when added to the serosal side of rabbit ileal mucosa mounted in Ussing chambers: ΔIsc max = -50μA cm^{-2} and EC_{50} = 3 x 10^{-8}M. Isotopic flux studies showed that 1 x 10^{-7}M NPY increased mucosal-to-serosal Na^+ and Cl^- flux and decreased serosal-to-mucosal Cl^- flux. Norepinephrine exerts a similar effect through binding to the α_2-adrenergic receptor. We tested whether NPY stimulated the release of norepinephrine by pre-treating the tissue with yohimbine, an α_2-adrenergic blocker. Yohimbine had no effect, however, on the NPY response. This suggests the presence of a unique receptor for NPY-like structure on the enterocytes. PYY was even more potent as an inhibitor of Isc: ΔIsc max = -60μA cm^{-2} and EC_{50} = 2 x 10^{-9}M. Rat pancreatic polypeptide (RPP), another member of the pancreatic polypeptide family, caused no response at concentrations up to 10^{-6}M. Receptor binding studies in rabbit ileum were performed with PYY-I^{125} as the labeled ligand. The peptide was iodinated with chloramine T and purified to homogeneity with HPLC. Receptors were detected throughout the ileum, including the submucosa plus circular and longitudinal muscle (6.3 fmol/mg), the mucosa (0.12 fmol/mg), and the enterocytes (0.01 fmol/mg). (Weight refers to protein). Receptors were also detected in rat ileum, whole gut (3.1 fmol/mg). Studies are currently underway with NPY-I^{125} as the labeled ligand. Rat brain synaptosomes and cultured cell lines were also used as receptor binding assays for using both PYY-I^{125} and NPY-I^{125}. As with PYY, NPY was iodinated with chloramine T and purified to homogeneity with HPLC. Specific binding of PYY-I^{125} was > 95%. Scatchard plots using PYY-I^{125} revealed two classes of binding sites: K_{d1} = 2 x 10^{-11}M and K_{d2} = 3 x 10^{-9}M. PYY-I^{125} was displaced by NPY (IC_{50} = 7 x 10^{-10}M), RPP (IC_{50} = 1 x 10^{-7}M) and SCMC-MNPY-1, a synthetic NPY analog (IC_{50} = 6 x 10^{-9}M). Specific binding of NPY-I^{125} was > 85%. Scatchard plots using NPY-I^{125} again revealed two classes of binding sites: K_{d1} = 5 x 10^{-11}M and K_{d2} = 1 x 10^{-9}M. NPY-I^{125} was displaced by PYY (IC_{50} = 3 x 10^{-10}M) and RPP (IC_{50} = 1 x 10^{-7}M). NPY-I^{125} also bound specifically to dorsal root ganglion cells cultured from two day old rat pups. No binding was observed on F-11 (undifferentiated and differentiated) or on PC12 (differentiated) neuronal cell lines, or on HSWP fibroblast cells. Thus, we have demonstrated that NPY and PYY are potent modulators of ileal Isc and we have developed high affinity labeled ligands to facilitate the investigation of receptor interactions.

404.9 **EVIDENCE THAT CHOLECYSTOKININ IS A NEUROTRANSMITTER IN THE INFERIOR MESENTERIC GANGLION OF GUINEA PIG.** M. A. Schumann* and D. L. Kreulen (SPON: P. Consroe). Dept. of Pharmacology, Coll. of Med., Univ. of Arizona, Tucson, AZ 85724.

Cholecystokinin (CCK)-like immunoreactivity has been localized in neurons with cell bodies residing in the intramural ganglia of the colon and axons terminating in the inferior mesenteric ganglion (IMG) of guinea pig. The physiological significance of neuronal CCK in sympathetic prevertebral ganglia is unknown. The purpose of these experiments was to determine whether CCK functions as a neurotransmitter producing slow depolarizations in the IMG, especially those slow depolarizations produced by colon distension. In vitro IMG preparations with a segment of the colon attached were utilized to conduct intracellular microelectrode recordings of potentials elicited in ganglionic neurons by nerve stimulation, colon distension and application of CCK_8. CCK_8 delivered by pressure-ejection from a single pipette adjacent to the recording electrode triggered a slow depolarization with 1-5 sec onset and rate of rise (1.6 ± 0.4 mV/sec) in 95% of the neurons tested. The ED_{50} for depolarization averaged 1.1 ± 0.5 pmoles (9 cells). The nonsulfated form of CCK_8 was twice as potent as the sulfated form and equipotent with CCK_{30-33}. In 59% of the cells the depolarization was associated with a decrease in input resistance (R_{in}) and in 20% with an increase. The remaining cells showed no change in R_{in}. CCK_8-evoked slow depolarization was similar to the slow depolarization produced either by colon distension or by electrical stimulation of lumbar colonic nerves in terms of changes in R_{in}, amplitude, and time course. CCK_8 also increased the frequency (300%) and amplitude (200%) of continuous fast nicotinic excitatory postsynaptic potentials evoked by contractions of the colon. Upon repeated (<2 min intervals) or prolonged administration of CCK_8, neurons became desensitized to the further application of CCK_8. However, there was no cross-desensitization between CCK_8 and substance P (SP) or vasoactive intestinal peptide. In addition, spantide (SP antagonist) blocked the response to pressure-ejected SP in 5 out of 6 neurons, while leaving the same cells fully responsive to CCK_8. In 50% of cells tested the slow depolarization induced by either nerve stimulation or colon distension was blocked by previous desensitization to CCK_8. These results support the hypothesis that CCK_8 or a related peptide is a neurotransmitter mediating peripheral reflex activity involving the colon and the IMG. Support HL01136, HL27781, AM36289.

404.10 **NEUROTENSIN FACILITATES SYNAPTIC TRANSMISSION IN GUINEA PIG INFERIOR MESENTERIC GANGLION BY POST- AND PRESYNAPTIC MECHANISMS.** W.H. Stapelfeldt* and J.H. Szurszewski. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905.

Immunohistochemical studies in guinea pigs have demonstrated neurotensin(NT) like immunoreactivity in nerve fibers originating in the spinal cord, leaving it via the ventral roots and forming nerve terminals around cells of the inferior mesenteric ganglion (IMG) (Reinecke, M., Neurosci. Lett., 37:37, 1983). The aim of the present study was to determine a possible physiological role of NT in this sympathetic ganglion. The IMG and its nerve trunks were dissected from adult male guinea pigs, pinned out in an organ bath and superfused with an oxygenated Krebs solution at 37°C. Intracellular recordings were made with 3M KCl filled glass micropipets (35-55 MΩ) and nerve trunks were stimulated with bipolar platinum electrodes. Superfusion of NT (1-5 μM) caused a depolarization in 74% of examined neurons (42 of 57 cells, n = 35). Two different types of depolarizing responses were observed. In the first, in 31% of responding cells, depolarization reached steady state during the presence of NT (3.3 ± 0.5 mV, range 2-6 mV). This effect was not blocked by either hexamethonium (10 μM), atropine (10 μM), low calcium (1 mM Ca^{++}, 15 mM Mg^{++}) or leucine-enkephalin (50 μM) indicating a postsynaptic site of action. In the second type of response, in 69% of responding cells, the depolarization was transient (max 5.1 ± 0.5 mV, range 2-10 mV, duration 2.5 ± 0.1 min), even in the maintained presence of NT. The transient response was not blocked by hexamethonium (10-100 μM) or atropine (10 μM). However, it was blocked by low calcium, leu-enkephalin (50 μM) and capsaicin (3 μM) suggesting a presynaptic site of action involving a noncholinergic opiate- and capsaicin-sensitive mechanism. Therefore, the effect of NT on slow EPSPs was studied. In 4 of 5 neurons tested, electrically evoked slow EPSPs in the presence of NT were greater in amplitude and longer in duration compared to slow EPSPs evoked in normal Krebs solution. These data further support the concept that NT acted presynaptically to facilitate slow synaptic transmission. Both types of depolarizing responses to NT were associated with an increase of input resistance, a production of action potentials in response to intracellular injection of subthreshold depolarizing pulses and a conversion of electrically evoked subthreshold fast EPSPs to spikes. Thus both responses facilitated synaptic transmission. In conclusion, these findings demonstrate a facilitating action of NT upon synaptic transmission in IMG by post- and presynaptic mechanisms. The NT-ergic fibers may thus represent a central efferent pathway that facilitates ongoing synaptic activity in the IMG. (Supported by AM 17632 and DFG Sta 252/1-1.)

404.11 **DIFFERENTIAL PARALYTIC ACTIONS OF INTRATHECAL VASOPRESSIN, OXYTOCIN AND OTHER PEPTIDES IN RATS.** J.B. Long, E.E. Echevarria* and J.W. Holaday. Neuropharm. Br., Dept. of Med. Neurosci., Div. of Neuropsych., Walter Reed Army Inst. of Res., Wash., D.C. 20307-5100.

Arg^8-vasopressin (AVP) and oxytocin (OXT) are distributed throughout the rat CNS and, following their central administration, elicit a variety of responses unrelated to their pituitary functions. Both AVP and OXT induce a loss of posture and rotational behavior ("barrel rolling") following i.c.v. injection. Additionally, following intrathecal (i.t.) injection, AVP produces a transient hindlimb (HL) flaccidity. Several other peptides, including dynorphin (DYN), somatostatin (SOM), Substance P antagonists, Neuropeptide Y, bacitracin and the delta opioid receptor antagonist ICI 174864 (ICI) also produce "barrel rolling" and HL paralysis following i.c.v. and i.t. injection, respectively. These similarities suggest that these diverse peptides may share common mechanisms of action. In these studies, we characterized and compared the motor effects of i.t. AVP, OXT, lys^8-vasopressin (LVP) and several related molecules with the i.t. actions of SOM, DYN and ICI.

Peptides were injected through PE 10 catheters into the spinal subarachnoid space of male S-D rats at the lumbar enlargement. HL motor function was evaluated using a 0-3 grading scale and tail-flick latencies were recorded. AVP and LVP (0.6 pmoles to 1.8 nmoles) produced a dose-related HL paralysis, with loss of motor function appearing within 2-3 min of injection and generally lasting 10-30 min. OXT significantly elevated tail-flick latencies and disrupted motor function, but only with doses exceeding 1 nmole. Pressinoic acid and AVP fragment (4-9) ([$pGlu^4$, Cyt^6]-AVP (4-9)) failed to alter motor or nociceptive function with doses as high as 25 nmole. In contrast to SOM, DYN and ICI, the paralytic actions of AVP, LVP and OXT were always transient. The short durations of these actions were not due to rapid enzymatic degradation of these peptides since: 1) effects were not prolonged by peptidase inhibitors, and 2) effects of the aminopeptidase-resistant analog deamino [arg^8]-vasopressin were similarly short-lasting. The paralytic actions of AVP, LVP and OXT, but not DYN, SOM and ICI, were absent in rats injected 10 min earlier with V_1 receptor antagonists (0.4 nmoles, i.t.), suggesting that effects of AVP, LVP and OXT, but not DYN, SOM and ICI, were mediated through V_1 receptors. However, we subsequently observed that 1) pretreatment with non-paralytic doses of OXT also selectively blocked paralytic actions of AVP, LVP and OXT, but not DYN, SOM or ICI, and 2) these V_1 antagonists alone produced paralysis at higher doses. Additionally, immediately following recovery from AVP, LVP or OXT-induced paralysis, rats were refractory to paralytic actions of AVP, LVP and OXT but not DYN, SOM or ICI. Similar desensitizations were not seen after recovery from SOM or DYN-induced paralysis. Results indicate that AVP, LVP and OXT induce HL paralysis: 1) with relative i.t. potencies paralleling i.c.v. potencies for barrel rolling, and 2) through recognition sites and/or mechanisms not shared with SOM, DYN, or ICI. These effects of AVP, LVP and OXT are also distinguished by their transient nature, antagonism by V_1 antagonists and tachyphylaxis to repeated injections.

404.12 **THYROTROPIN RELEASING HORMONE (TRH) FAILS TO ANTAGONIZE THE PARALYTIC EFFECTS OF INTRATHECAL DYNORPHIN A (1-13) AND A SUBSTANCE P ANTAGONIST.** A. Martinez-Arizala*, J.B. Long, E.E. Echevarria* and J.W. Holaday. Neuropharm. Br., Dept. of Med. Neurosci., Div. of N.P., Walter Reed Army Inst. Research, Washington, DC 20307-5100.

Improved neurological recovery following TRH treatment in traumatic spinal cord injury was proposed to involve actions of TRH as a physiological opioid antagonist. Endogenous opioids were implicated in the pathophysiology of spinal cord injury since the opioid antagonist naloxone improved neurologic outcome following spinal trauma. Among the opioids, Dynorphin A (Dyn A)-related peptides have been suggested as the mediators of spinal cord injury since Dyn A, but not other opioid peptides: 1) increases progressively at the injury site following spinal cord trauma, and 2) produces hindlimb (HL) paralysis following intrathecal (i.t.) injection in rats. While unique among opioids, these paralytic actions of Dyn A are shared by several neuropeptides, including a number of Substance P antagonists. Recently, the neuronal damage produced by i.t. injection of the Substance P antagonist Spantide was shown to be eliminated by TRH (Freedman et al, Exp. Br. Res.-62:175,1986). If: 1) Dyn A is a principle mediator in the pathophysiology of spinal cord injury, and 2) therapeutic effects of TRH in spinal cord injury involve opposition of Dyn A actions, then TRH may oppose the deleterious actions of i.t. Dyn A, similar to that reported for Spantide. Therefore, we examined the ability of TRH to antagonize HL paralytic actions of i.t. Dyn A (1-13) and the SP antagonist ($D\text{-}Arg^1D\text{-}Pro^2D\text{-}Trp^{7,9}Leu^{11}$ SP) (SPA) in rats.

Male S-D rats (350g) were prepared with external jugular catheters (PE 50) and i.t. catheters (PE 10) terminating in the spinal subarachnoid space at the level of the lumbar enlargement. The next day, motor function was evaluated using a 0-3 point scale at 0.25, 1, 2, 4, and 24 hrs following i.t. injection of Dyn A (1-13) (25 nmoles) or SPA (0.7 and 1.7 nmoles), and either i.t. or i.v. injection of TRH or vehicle. TRH was injected i.t. (10 μg) 5 min before injection of Dyn A (1-13) or SPA. For i.v. studies, TRH was injected 60 min (5 mg/kg) and immediately (2 mg/kg) before injection of Dyn A (1-13) or SPA, as well as hourly (2 mg/kg) for 4 hrs following these injections.

Dyn A (1-13) and SPA produced an immediate flaccid HL paralysis and a pronounced respiratory impairment with pulmonary edema which resulted in 30 and 50% mortality, respectively. Saline and TRH alone (i.t. and i.v.) did not alter HL motor function, although TRH produced tail-rattling (i.t.) and wet-dog shakes (i.v.). TRH (i.t. or i.v.) failed to improve neurological function or mortality following Dyn A (1-13) or SPA. Thus, although TRH was reported to decrease morphological damage produced by the closely related SP antagonist Spantide, we were unable to demonstrate any effects of TRH on neurologic outcome or survival using a similar protocol. The disparity in the therapeutic effects of TRH in injury-induced paralysis versus the absence of TRH antagonism of i.t. Dyn A (1-13) paralysis suggests that: 1) different pathophysiological mechanisms or species differences are involved in these two models of spinal cord injury, and 2) salutary effects of TRH in spinal cord injury do not involve an antagonism of endogenous Dyn A actions.

405.1 Pattern Responses from Monkey Primary Visual Cortex: Retinotopic map and receptive field properties. B. van Dijk*, G. Dagnelie* and H. Spekreijse* (spon: ENA). Netherlands Ophthalmic Research Institute, P.O.Box 12141, 1100 AC Amsterdam the Netherlands.

The projection of the central 5 degrees of visual field onto Rhesus primary visual cortex (V1) was determined with a subdurally implanted stainless steel electrode array. A complex log map gave an adequate description both of the position of maximum sensitivity for patterned stimuli, and of the element size that yields optimal responses. On the other hand the smallest element size that yields recordable responses proved to be nearly independent of eccentricity.

Each electrode was found to sum passively activity from a $3x3mm^2$ patch of V1. The area of visual field from which responses could be elicited at each electrode was estimated using narrow stripes of patterned stimuli. We observed that the widths of these so called "receptive fields" could also be described by a complex log map. For electrodes close to the midline of the visual field the input from the ipsilateral half field was substantial, extended up to 3 degrees.

The sensitivity profile of these receptive fields was found to be dependent on stimulus configuration. If two regions of about equal sensitivity within the receptive field of a single electrode are stimulated with different contrasts, then the total response is close to the response from that region which is stimulated most strong. On the other hand, if simultaneously the center and a less sensitive region are stimulated, then the response from the central area is modulated: if the two regions are close the response is diminished; if they are spatially separated the response is enhanced.

From these experiments the conclusion can be drawn that the responses in Rhesus V1 evoked by the onset of pattern are driven by a relatively wide area for small patterned fields and by a smaller area for larger patterned fields. This suggests a form of spatial opponency at the level of local mass responses in V1.

405.2 VISUAL MOTION PROCESSING IN PRIMATE AREA 17: EVIDENCE FROM MASS NEURONAL ACTIVITY. G. Dagnelie*) B.W. van Dijk* and H. Spekreijse* (SPON: ENA). Netherlands Ophthalmic Research Institute, Amsterdam, Netherlands*).

Evidence for early visual motion processing in higher animals has thus far been found in two types of studies: recordings of directionally selective (DS) cells in retina, lateral geniculate and cortex of mammals, and psychophysical studies in man. The absence of explicitly DS cells whose DS character is not dependent on the type of pattern presented as a stimulus, prior to the cortical level in primate, has directed the attention of electrophysiologists to primate cortex as the initial stage in motion processing. Within cortex, the identification of area MT in monkey as an area specialized in motion processing may have led some scientists to overlook the possibility that also in V1 genuine motion detection is taking place. Extensive experiments in our laboratory in man (scalp electrodes) and alert monkey (scalp and subdural electrodes) have shown, however, that this area contains an early local motion processing mechanism, and that this mechanism can be studied elegantly through Visually Evoked Potentials (VEPs).

The evidence that we have collected for the existence and the nature of this mechanism can be summarized as follows:
- local motion onset and offset each elicit a characteristic VEP waveform
- through projection studies in monkey, as well as via dipole localization in man, the sources of these potentials have been located unequivocally in area 17
- these VEPs show a simple and consistent dependence on stimulus parameters
- these VEPs can be elicited by certain types of motion illusion, whereas a variety of closely related stimuli that do not lead to a motion percept do not yield similar VEPs either
- the mechanism underlying these VEPs is strictly monocular
- in all our experiments, correlators of the Reichardt type are best suited to account for the data.

Therefore we feel free to claim that our experiments yield solid evidence for the existence of motion detecting units in primate V1, and for their working principle of Reichardt correlators.

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405.3 FUNCTIONAL AND MORPHOLOGICAL IDENTIFICATION OF DOUBLE AND SINGLE OPPONENT COLOR CELLS IN LAYER IVC_b OF THE MONKEY'S STRIATE CORTEX. Charles R. Michael, Dept. of Physiology and Vision, Yale Medical School, 333 Cedar St., New Haven, CT. 06510.

Using tungsten microelectrodes I have re-examined layer IVC_b with long, oblique penetrations. In agreement with my earlier studies, I found clusters of non-oriented double opponent color cells which were separated by regions containing on and off center Type III neurons. In a color cluster all of the cells had the same red/green double opponent field organization. A few cells were single opponent Type I units; they always had on centers. Having confirmed the presence and the properties of the opponent color cells in IVC_b, I then studied them with micropipette electrodes filled with horseradish peroxidase (HRP). I recorded intracellularly from the cells, functionally characterized them and then filled them with HRP. All double opponent cells recovered histologically were found to be spiny stellate neurons with spherical, non-oriented dendritic arbors confined to IVC_b. Their axons projected upward to lower layer III where they formed terminal fields similar in size to the supragranular cytochrome oxidase (CO) blobs. A combined staining procedure for HRP and CO revealed that the double opponent spiny stellate cells projected to the blobs. Most often they terminated in a single blob; in three instances their axons branched to end in two adjacent blobs. It seems reasonable to assume, then, that the properties of the red/green double opponent color cells which Livingstone and Hubel found in the blobs may be generated primarily by inputs from the spiny stellate cells in layer IVC_b. When the single opponent Type I color cells were recovered, they always were smooth stellate neurons. Their beaded axons arborized locally, close to their own dendritic fields in IVC_b. Since both spiny and smooth stellate cells in IVC_b receive excitatory contacts from the axons of opponent color neurons in the parvocellular geniculate layers, these cells could represent the earliest stages in cortical color processing. The smooth stellate cells may make inhibitory contacts on the spiny stellates to produce double opponency from single opponent color inputs. The spiny stellates could then pass this information up to the color cells in the blobs. Supported by NIH Grant EY 00568.

405.4 **RELATIONSHIPS BETWEEN COLOR-SPECIFIC CELLS IN CYTOCHROME OXIDASE-RICH PATCHES OF MONKEY STRIATE CORTEX.** D.Y. Ts'o, C.D. Gilbert, and T.N. Wiesel. Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021

Using multiple vertical penetrations of two recording electrodes into the superficial layers of the cytochrome oxidase-rich blob regions of the monkey striate cortex, we have studied the functional architecture and, through cross-correlation analysis, the intrinsic connections within and between the blobs. We found a predominance of unoriented cells, including Type I (color opponent center-surround), Type II (color opponent center-only), Type III (broadband center-surround), and Type IV (broadband, tonic red surround), confirming much of the earlier report of Livingstone and Hubel*. However, few true double opponent cells were found. Instead a large proportion of color cells exhibited modified Type II properties, having an antagonistic broadband surround. All color cells in individual penetrations were of the same color opponency, red/green or blue/yellow. Multiple closely spaced penetrations into the same blob also yielded color cells of the same color opponency, suggesting that an individual blob is color system specific. Along individual penetrations, clusters of cells having the same receptive field type were encountered. Some color and non-color cells were found that were much more responsive to moving stimuli (of any orientation and direction) rather than stationary flashing stimuli. Oriented cells having color selectivity have been found in penetrations of blob regions, suggesting a close proximity between oriented and unoriented color cells.

Cross-correlation analysis demonstrated interactions only between blob cells of the same color opponency. In addition, these interactions tended to occur between cells of the same receptive field type, and were observed within a single blob and between cells in two different blobs. For example, we have found peaked correlograms between two red-ON green-OFF Type II cells within a single blob and in two different blobs, but not between a red-ON green-OFF and a blue-ON yellow-OFF Type II cell in two different blobs. These correlograms reflected monosynaptic and common input interactions. Some correlations have been found between color selective and non-color selective oriented cells having the same orientation. The interactions between color cells in the blob regions are another example of the specificity of horizontal connections in the striate cortex.
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*Livingstone and Hubel (1984), J. Neurosci. 4: 309-356.

405.5 LOCAL CIRCUITS AND OCULAR DOMINANCE COLUMNS IN MONKEY STRIATE CORTEX. L.C. Katz, C.D. Gilbert & T.N. Wiesel. Laboratory of Neurobiology, The Rockefeller Univ., 1230 York Ave., NY NY 10021.

In macaque striate cortex, geniculo-cortical afferents are rigidly segregated into alternating, parallel, eye specific bands in layer 4, which form the anatomical basis of ocular dominance columns (ODC). Cells in layer 4 are exclusively monocular, while binocular cells occur in both supra- and infragranular layers. We examined where intrinsic cortical circuits first integrate visual information from the two eyes by simultaneously visualizing ocular dominance columns in layer 4 and the dendritic and axonal arbors of individual layer 4 cells.

Geniculate laminae relaying information from one eye were injected with a 5% solution of the fluorescent, anterogradely transported tracer, tetramethylrhodamine isothiocyanate (TRITC) in DMSO; subsequently brain slices were prepared from the striate cortex. Under epifluorescence illumination, ocular dominance columns were clearly visible in living tissue as sharply defined patches of TRITC label in layers 4A, 4Cα and 4Cβ. A total of 30 spiny and aspinous neurons in layer 4Cβ were then intracellularly injected with lucifer yellow.

Most cells near ODC borders had asymmetric dendritic fields. Dendrites either turned vertically at borders, recurved back to their "home" ODC, or extended shorter processes in the direction of the border. A few cells, at the border between two columns, had distinct clumps of dendrites in each column. These results are consistent with physiological findings that reveal only monocular cells in 4Cβ, but further suggest that a small population of binocular cells (those with dendrites in both columns) exists at column borders.

In contrast to the dendrites, most axons crossed ODC borders and arborized within the adjacent column. The arborization in layer 4 was usually denser in the "home" column; however cells with dendrites in two columns had more evenly distributed axons. The axons of many 4Cβ cells ascended, and, as previously noted (Lund & Boothe JCN 159:305, 1975) arborized in layer 3. These collaterals had a greater tangential extent than those in layer 4.

These observations suggest that binocular interactions, either excitatory or inhibitory, can occur even within layer 4, via collaterals of cells in layer 4Cβ. However, the relatively sparse connections imply that the principle locus for generating binocular cells is within layer 3, via the more widespread tangential arbors in layer 3. If the horizontal collaterals crossing columns in layer 4 primarily contact inhibitory cells, this cross-columnar innervation could sharpen, rather than reduce, the segregation between the two eyes at early stages of cortical processing.

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405.6 COLUMNS IN STRIATE AND PRESTRIATE MONKEY CORTEX USING 2-DEOXY-D-GLUCOSE AND CYTOCHROME OXIDASE MARKERS. M.L.J. Crawford and J-T. de Faber. Sensory Sciences Center, U. of Texas, Houston, Texas and the Eye Hospital Rotterdam, The Netherlands.

Stimuli from different sensory systems are processed in cortex by clusters of cells, or columns. Using the combined 2-DG autoradiography and cytochrome oxidase staining techniques, we show how different visual stimuli activate a variety of typical patterns in both striate and prestriate cortex of macaque.

In V1, black and white, drifting luminance contrast (LC) gratings having multiple orientations produce a continuous 2-DG band in Layer 4Ca, while the same grating of a single orientation produces an interrupted band in the same layer. Cytochrome oxidase staining verifies the laminar location indicating a magnocellular LGN input to this layer. Color contrast (CC) gratings produce continuous, but density-modulated, bands in Layers 4A and 4Cb, the main parvocellular LGN inputs to visual cortex. The spatial correlation between 2-DG and CO densities in Layers 2-3 differed with the kind of stimulus. With CC pattern stimulation there was > 70% spatial overlap, with < 25% overlap with LC stimulation. This is in agreement with electrophysical data suggesting more color coded cells within CO blobs than between. In experiments using bi-directional drifting gratings, the 2-DG pattern appears more crowded; the 2-DG hot spots tending to merge or appear interconnected.

In V2 cortex, CC stimuli produced 2-DG densities with a high spatial correspondence with CO densities, both forming a stripe-like pattern. Multiple orientations of CC gratings seem to activate cells within every other CO stripe, while a CC grating of single orientation activated cells in every CO stripe. The spatial correspondence between 2-DG and CO was less with LC stimulation.

In cortical area V4, CC gratings produce 2-DG densities, while LC gratings do not.

In cortical area V5, a LC grating of multiple drift rates produce differentiated 2-DG uptake.

These findings are in accord with electrophysiological and neuroanatomical findings from other laboratories. (Supported by NS19342).

405.7 A THEORY FOR THE USE OF VISUAL ORIENTATION INFORMATION WHICH EXPLOITS THE COLUMNAR STRUCTURE OF STRIATE CORTEX. M.A.Paradiso. Neurobiology Group, University of California, Berkeley, CA 94720

Most cells in the striate cortex of higher mammals are orientation selective and their orientation preferences change progressively through "columns" running perpendicular to the cortical surface. Hubel and Wiesel define a "hypercolumn" as a block of cortical cells in which all orientation preferences are represented for a small portion of the visual field.

I have analyzed the performance of a hypothetical neural network modeled after a visual hypercolumn. Limits on the precision of orientation estimation and discrimination are calculated for a network which extracts orientation information from the pattern of activity in the hypercolumn with maximum efficiency. These limits are functions of single unit response properties such as orientation tuning width, response amplitude and response variability as well as the degree of organization in the network. The analysis demonstrates that a network of modest size is capable of reliably discriminating orientation with a precision exceeding human performance (about 1/2 deg) even though the individual cells are responsive over a range of tens of degrees. The orientation discrimination threshold is a roughly linear function of orientation tuning width, the variance of responses, and the degree of disorder in the network. In contrast, discrimination threshold is a highly nonlinear function of the number of cells in the hypercolumn. One finds that performance depends more critically on the number of cells than on the single unit properties. This finding predicts changes in orientation discrimination threshold with eccentricity which have been confirmed experimentally. The model system's performance is also consistent with psychophysical data in two situations in which human performance is not optimal. First, interference with orientation discrimination occurs when multiple stimuli activate cells in the same hypercolumn. Second, systematic errors in the estimation of orientation can occur when a stimulus is composed of intersecting lines. The magnitude of these "tilt illusions" is a function of the enclosed angle between the lines.

In summary, performance of the model hypercolumn parallels human performance under both optimal and non-optimal conditions. This suggests that the mammalian brain may efficiently extract orientation information on the basis of overall hypercolumn activity, thus overcoming the unreliability of single units. The relationships between network parameters and the precision of orientation estimation and discrimination may serve as a first approximation to the performance of a real hypercolumn.
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405.8 PRONOUNCED ARCHITECTONIC DIFFERENCES BETWEEN MONOCULAR AND BINOCULAR SEGMENTS OF THE MONKEY'S STRIATE CORTEX. R.W. Williams and P. Rakic. Section of Neuroanatomy, Yale Medical School, New Haven CT 06510.

Primary visual cortex (area 17) of primates is considered a homogeneous field made up of a few thousand essentially identical hypercolumns. However, the number and types of connections within functionally distinct subregions of area 17 vary, particularly with respect to the relative number of thalamic afferents subserving the two eyes. Thus, part of area 17 receives an input only from the ipsilateral eye (this region corresponds to the optic disc of the other eye), while a larger area representing the far periphery of the visual field receives an input only from the contralateral eye. We have assessed the size and cell composition of area 17 with reference to regional variation. Brains of 5 rhesus monkeys were embedded in celloidin, cut at 35 µm, and stained with cresyl violet. The area, width, and volume of area 17 were measured. Neuron and glial cell densities were assessed in regions representing monocular and binocular periphery, the blind spot, and central visual fields. Cells were counted from pia to subplate at 2500X using DIC optic and a high resolution camera attached to an image analysis computer. Neurons were distinguished from glia by staining, size, and shape. Nuclei that intersected any one of three of the six surfaces of the sampling box (including one of the cut surfaces) were excluded from the counts thus abolishing a need for the Abercrombie correction.

The area of striate cortex ranged from 500 to 700 mm^2; volume from 650 to 900 mm^3. Neuron number varied between 280 million and 440 million, on average twice as high as previously reported. Regions of area 17 subserving monocular and binocular vision differ markedly in thickness and in the relative numbers of neurons in corresponding layers. Although the average cell density was nearly constant, the extreme monocular periphery that has reciprocal connections with only the three contralateral layers of the lateral geniculate nucleus was 20-30% thinner than central binocular cortex. This reduction was seen in all layers but was greatest in layers 5 and 6 (layers that are the source of subcortical projections to the superior colliculus and lateral geniculate nucleus), and consequently, the fraction of neurons present in these layers was reduced from 30% in probes of central representation to less than 20% in peripheral representation. There was a relatively gradual reduction in the thickness of cortex moving from more central binocular sites toward the binocular periphery, a finding that may correlate with the two-fold reduction in the width of ocular dominace stripes in the binoucular periphery (LeVay et al., '85, J. Neurosci. 5:486.) The most dramatic reduction in overall thickness, to under 40% of the central average in one case, was noted in a region that is likely to correspond to the blind spot. The structural heterogeneity of area 17 was obvious in each cardinal plane of section and was not due to differences in patterns of cortical folding or angle of cutting. (Qualitatively similar differences were also noted in the striate cortex of an adult human.) We conclude that [1] the striate cortex of rhesus monkeys contains about twice as many neurons as previously estimated, and that [2] there are consistent diffferences in the thickness and cellular demography of parts of this cortex that subserve binocular and monocular vision, differences that are most marked in the infragranular layers. A preliminary developmental analysis indicates that this structural heterogeneity is present at birth. As we show in our companion study (Rakic & Williams, this vol.), these differences in neuronal demography and cortical thickness are dependent on normal retinal input and are regulated in part by binocular interactions.

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405.9 THALAMIC REGULATION OF CORTICAL PARCELLATION: AN EXPERIMENTAL PERTURBATION OF THE STRIATE CORTEX IN RHESUS MONKEYS. P. Rakic and R.W. Williams, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The cerebral cortex is composed of morphologically and functionally distinct cytoarchitectonic areas that can be further subdivided into radial columnar modules having layer-specific characteristic reciprocal connections with thalamic nuclei. We reasoned that the possible regulatory developmental role of the periphery via thalamic afferents in the specification of areal, radial, and laminar differentiation of the cortex could be tested if we were able to experimentally alter the size of individual thalamic nuclei at early embryonic stages, leaving the rest of the thalamus intact. For this purpose, binocular enucleation was performed on 4 monkey embryos between E59 and E65, after all neurons of the lateral geniculate nucleus (LGd) have been generated (Rakic, '77, J. Comp. Neurol. 176:23), but well before their axons have penetrated the developing cortical plate (Rakic, '76, Nature 261:467). After enucleation, embryos were returned to the uterus, delivered at full term (E165), and sacrificed between 3 months and 3 years of age. The LGd in enucleated animals was smaller, devoid of layers, and contained 400,000-500,000 neurons compared to 1.3 million in controls. The occipital lobe displayed dramatic changes in its pattern of folding, including the appearance of new gyri and deep fissures. Anterograde and retrograde transport of HRP showed topographically well defined reciprocal connections between the LGd and the occipital lobe. Despite severe abnormalities of the LGd and the absence of any retinal input, striate cortex could be readily identified and developed the normal complement of layers and sublayers. Although the number of neurons per radial unit remained the same as in controls, unexpectedly, layers IVC and VI, which have major reciprocal connections with the LGd, were relatively wider than those layers not receiving thalamic input. The most dramatic change was a significant reduction in the area of striate cortex. The reduction was roughly proportional to the loss in the number of LGd neurons: from 500-700 mm^2 in age matched controls (Williams and Rakic, this vol.) to 200-280 mm^2 in experimental cases.

We conclude that: [1] the number and pattern of cerebral convolutions depends in part on normal development of thalamic afferents; [2] the size of cytoarchitectonic fields may be regulated by the number of these afferents; [3] although the number of radial cortical units is reduced by a decrease in thalamic afferents, the number of neurons within units remains constant; and [4] the basic topography of geniculocortical and corticogeniculate connections can be established in the absence of signals from the retina. Supported by EY02593 and NS22807.

405.10 CORTICAL NEURON RESPONSES AND PERCEPTUAL ORGANIZATION. E. Peterhans* and R. von der Heydt* (SPON: H.R. Lüscher). Department of Neurology, University Hospital Zürich, CH-8091 Zürich, Switzerland.

A dotted line can be as conspicuous in perception as a solid line. A few isolated spots moving in synchrony can mimic a solid object. To learn more about the cortical principles of visual coding, we have studied neuronal responses to such stimuli in rhesus monkeys during behaviorally induced fixation.

In V1 we found that continuity across the receptive field was important; rows of dots regularly evoked weaker responses than bars of equal light flux, and tiny gaps in bars or edges often caused dramatic decrements. In a simple cell, a gap as small as 4 min arc abolished the response.

In V2, some cells responded to a row of fine dots (size 1, and spacing 24 min arc, typically) equally well as to a solid bar of much higher flux. When three spots were moved as if fixed on a rod (eg 6 min arc squares spaced 60-120 min arc), some cells in V2 responded, and were selective for the orientation of the 'rod' as for the orientation of a solid bar; when each spot was presented alone, or the three were moved asynchronously, the cells responded much less, or not at all. (With asynchronous motion, the spots are no longer perceived as a single object.) Often the same neurons also signalled contours of illusory figures, similarly as described earlier (R. von der Heydt, E. Peterhans and G. Baumgartner, Science, 224:1260, 1984). In this case, the spatial alignment of local features (line ends and corners) seemed to be important.

Thus, compared to V1, the representation of visual information in V2 depends less on local stimulus features such as luminance, pattern of line, and connectedness, but appears to be related more to object qualities such as spatial coherence. Similar as in perception, this quality may be derived from connectedness as well as from spatial alignment or synchrony of movement.

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405.11 SPATIAL PERIODICITY DETECTION IN MONKEY VISUAL CORTEX. R. von der Heydt*, E. Peterhans* and M.R. Dursteler. Dept. of Neurology, University Hospital Zurich, CH-8091 Zurich, Switzerland

Responses of visual cortical neurons to spatially periodic and aperiodic stimuli were studied in rhesus monkeys during behaviorally induced fixation. Cells were found that responded to gratings of certain periodicity and orientation, but not to aperiodic stimuli, even at high contrast. In 23 of 475 units studied (5%) in V1, and 7 of 465 (1.5%) in V2, single bars evoked less than 1/10 of the grating responses, or had even inhibitory effects. Most of these units gave unmodulated reponses to drifting gratings, and were unselective for direction of drift. Those in V1 were often monocular, and were nearly always found in the supragranular layers (mostly 2+3). They preferred high spatial frequencies (peaks at 3.6-19 c/deg, median 8.5, in V1), and were often sharply tuned (bandwidths at half amplitude 0.43-1.4 oct, median 1.0, in V1). With optimum frequency gratings, spatial summation was found over 4-14 grating cycles (median 7.5).

In spite of their preference for high-frequency gratings, however, they failed to signal high-frequency Fourier components in crucial tests: Square-wave gratings of 1/3 the optimum frequency produced no responses, although their third harmonic components presented alone were quite effective. Bars and edges evoked no responses, although from their Fourier spectra and the band-pass tuning of the units, one would have predicted significant responses on the linear spatial filter assumption. These results could not be reconciled with a linear-filter plus threshold model either; in order to suppress responses to higher harmonics one would have to assume high output thresholds, but the cells had low contrast thresholds for gratings. A non-linear model was proposed instead.

Since high-frequency components are essential for the perception of bars and square-wave gratings, and since our cells did not signal these components, we concluded that they are not involved in any spectral analysis of the stimulus. They seem to be specialized for detecting periodicity, and may be used for perception of texture. It is possible that some of the studies designed to investigate spatial-frequency channels in human vision have tested in fact the performance of such periodicity detectors.

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405.12 OPTICAL IMAGING OF CORTICAL ACTIVITY WITHOUT VOLTAGE-SENSITIVE DYES. A. Grinvald, E. Lieke*, R.D. Frostig, C.D. Gilbert and T.N. Wiesel. Lab. of Neurobiology, The Rockefeller Univ., New York, NY 10021, IBM T.J. Watson Res. Ctr., Yorktown Heights, NY 10598 and The Weizmann Institute of Science, Rehovot, Israel.

Optical mapping of the cortical surface offers several advantages over conventional electrophysiological and anatomical techniques: One can map a relatively large region at high spatial resolution in minutes, one can obtain successive maps to different stimuli in the same cortical area and one can follow variations in response over time. Voltage sensitive dyes have opened the possibility for using this approach in the intact brain. However, presently available dyes may produce irreversible photodynamic damage or pharmacological side effects. Thus optical imaging of the activity from unstained cortex based on intrinsic signals would be particularly valuable.

In experiments on the cortices of rats, cats and monkeys we discovered that the intensity of reflected light from unstained cortex decreased over regions of active cortex. The optical signal, which was 6 to 10 seconds in duration, had a risetime of 1 to 2 seconds and often included a large undershoot. The amplitude of the activity-dependent optical signal changed by 0.05% to 2% depending on the wavelengths, the stimulus parameters and the animal. It is not yet clear if this in vivo signal is related to the light scattering signals that were detected in other preparations in vitro. Since the present signals were larger in proximity to the arteries, their origin may be related to a local activity-dependent metabolic changes.

We used a 10 x 10 photodiode array to determine if the intrinsic optical signal can be used for the mapping of cortical activity. We first mapped individual barrels in the rat somatosensory cortex by stimulating individual whiskers. The map obtained without dyes was confirmed by optical mapping with fast voltage-sensitive dyes. In the cat and monkey visual cortex we obtained signals in response to stimulation by horizontal or vertical drifting gratings. The pattern of activity recorded by the photodiode array was not uniform, but showed clear peaks and valleys. Though the largest signals were associated with arteries, regardless of stimulus orientation, taking the ratio of the maps, on a pixel-by-pixel basis, yielded a striped pattern of activity independent of the positions of arteries. We confirmed that the optical maps corresponded to orientation columns by recording single units in multiple (30 to 60) perpendicular electrode penetrations. The correspondence between the optical and electrophysiological maps was very close ($p < 0.05$).

The above approach therefore offers promise for use in a number of experimental and clinical situations.

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406.1 **DISTRIBUTION OF SYNAPSES AND DENDRITIC SPINES ON THE SURFACES OF RUBROSPINAL NEURONS. A STUDY EMPLOYING HIGH VOLTAGE ELECTRON MICROSCOPY OF INTRACELLULARLY STAINED CELLS.**

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Studies of rubrospinal neurons have demonstrated that synapses formed by two major excitatory inputs to these neurons, one from the cerebral cortex and one from the interpositus nucleus of the cerebellum, are differentially distributed on the surface of the postsynaptic cells. This differential distribution, with cortical afferents ending distally and cerebellar afferents ending proximally, has been shown to be associated with dramatic differences in the time courses of postsynaptic potentials evoked in rubrospinal neurons by activity in individual axons of these pathways. In addition, both the synaptic potential shapes and the synaptic locations have been shown to be modified by a variety of treatments. Interpretation of the relationship between synaptic function and location on the cell requires precise information on the morphological features of the rubrospinal neurons, and especially on the structure of dendritic and somatic appendages involved directly in the synapses. Rubrospinal neurons were identified in anesthetized cats by antidromic activation from the contralateral rubrospinal tract, and injected with horseradish peroxidase. Tissue sections containing the injected cells were postfixed in osmium tetroxide and embedded in plastic. These sections were subsequently sectioned at thicknesses ranging from 2 to 5 µm, and these sections were examined in the light microscope. Certain of these sections were selected for high voltage electron microscopic study (using the Hitachi H-1250M at the Japanese National Institute of Physiological Sciences). Others of these sections were reembedded and thin sections cut from them for examination at conventional accellerating voltage.

The surface of the rubrospinal neuron exhibited two clearly distinguishable zones. The proximal region of the cell included the soma and the proximal 200-300 µm of the dendrites. There the cell is covered with an extraordinary number of extremely fine straight appendages. They surround and invaginate the many axon terminals that cover the surface of the rubrospinal neuron in this region. Synaptic contacts were made longitudinally on these hair-like spines from both of the two major axon terminal types that contact this part of the cell. These are: (1) the boutons with round vesicles and asymmetrical contacts that are known to arise from the cerebello-rubral pathway and (2) the boutons with pleomorphic vesicles and symmetrical junctions that have been previously shown to contain glutamate decarboxylase and that arise from neurons intrinsic to red nucleus. These spines probably function primarily to increase the synaptic surface area of the proximal part of the rubrospinal neuron. The more distal portion of the dendritic tree of the rubrospinal neuron had a very different appearance, and the transition between the two zones on the surface of the rubrospinal neuron was very abrupt. The dendrites in the distal region were more branched, and exhibited many dendritic appendages. The latter were of three major types. One was a large spine with a cup-shaped head that formed the principal postsynaptic component of a glomerulus that also involved an axon terminal and often a presynaptic dendrite. The number of these was estimated to be at least 1000/cell, and usually was higher. The second type of appendage was a long thin filiform appendage that usually occurred around the spines. These appendages could form complicated lacy networks in the area around the dendrites. Although they were occasionally postsynaptic, a large portion of the surface area of these appendages was free of synaptic contacts. The third type of appendage was a spherical one containing many residual bodies. Synapses were seen on the shafts of distal dendrites, but they were very rare in comparison to the arrangement seen on the proximal dendrites. Examination of boutons degenerating after cortical lesions demonstrated that cortico-rubral axons end both onto dendritic shafts and in the glomeruli formed by the spines.

406.2 A PLANT LECTIN, PEANUT AGGLUTININ, BINDS SPECIFICALLY AT THE NEUROMUSCULAR JUNCTION IN THE FROG. C.E. Smith* and C.-P. Ko. Department of Biological Sciences, Neurobiology Section, University of Southern California, Los Angeles, CA 90089-0371.

Molecules in the extracellular matrix (ECM) influence the differentiation of neural tissue. In an attempt to identify molecules concentrated at synapses with potential synaptogenic functions, we used peanut agglutinin (PNA) to discover a glycoconjugate that is restricted to the ECM of the frog neuromuscular junction. To the best of our knowledge, this is the first report of the unique localization of PNA receptors in the neuromuscular junction.

Fluorescent derivatives of PNA bind avidly and specifically to the neuromuscular region, outlining the periphery of the nerve terminal arborization in the frog cutaneous pectoris muscle. Garter snake costo-cutaneous neuromuscular junctions also stain intensely with derivatized PNA, while mouse diaphragm staining is faint. Double-staining with either fluorescently-tagged alpha-bungarotoxin or Karnovsky's cholinesterase stain confirms that PNA binding occurs specifically at the neuromuscular junction. That fluorescent staining represents binding of the lectin to a junction-specific carbohydrate was demonstrated by inhibition with both asialofetuin, a glycoprotein containing the recognition sequence, and native lectin.

To further localize the PNA-receptor site we performed several experiments. First, protease and collagenase treatments, known to digest the ECM in the frog neuromuscular junction, completely remove PNA binding sites. Conversely, a second procedure which removes junctional cytoplasm and plasma membrane while sparing ECM constituents has no effect on PNA binding. In this procedure we denervated, froze and surgically removed all but a thin bridge of muscle containing endplate regions. Ten days post operation, fibers completely degenerate while PNA receptors are retained at synaptic sites in the ECM as confirmed by cholinesterase staining. These results indicate that the PNA receptor is closely associated with the ECM in the neuromuscular junction. Additionally, electron microscopy of peroxidase-PNA stained junctions also assigns the lectin receptor to the ECM surrounding the nerve terminal with reaction product absent from the synaptic cleft.

Flourescent derivatives of PNA applied in situ on the frog cutaneous pectoris persist in vivo for at least three weeks. Additionally, the lectin does not block spontaneous miniature nor evoked endplate potentials indicating that fluorescently-tagged PNA has potential as a vital stain for neuromuscular junctions. Both the persistent nature of in vivo PNA binding and its physiological compatibility make the lectin an attractive probe for investigating synaptogenic functions of a receptor specifically concentrated in junctional ECM.

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406.3 PURIFICATION OF A NERVE TERMINAL ANCHORAGE PROTEIN FROM ELECTRIC ORGAN. S.S. Carlson#, E. Ertel#, P. Caroni*^, and R.B. Kelly+. #Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195. and +Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143. (^Present address: Hirforschungs-Institut, Universitat Zurich, August Forel Strasse 1, Zurich, Switzerland.)

The electric organ nerve terminal is attached to the synaptic basal lamina presumably by integral membrane proteins of the presynaptic plasma membrane. We have purified an extracellular matrix protein whose physical properties suggest that it is one of these anchorage proteins. The protein was identified because it shares an antigenic site with a proteoglycan component of synaptic vesicles. It too behaves like a proteoglycan, but is much larger than the synaptic vesicle component. It is solubilized from extracellular matrix fractions only under denaturing conditions in the presence of detergent; it elutes from DEAE sephacel at pH 4.9 only at high ionic strength; and it binds to laminin affinity columns from which it can be eluted by heparin. Upon gel filtration under reducing and denaturing conditions, it behaves as a proteoglycan with a molecular weight of about 1 million. This large highly charged molecule can be readily reconstituted into liposomes, which indicates that it posseses the hydrophobic domain of an integral membrane protein. By immunoelectron microscopy the antigen is found both in synaptic vesicles and on the plasma membrane of the nerve terminal. We have purified this molecule from an electric organ extracellular matrix fraction approximately 700 fold using a combination of gel filtration, sedimentation velocity, and ion exchange chromatography. Further, we have identified a molecule of similar charge and size in the mammalian spinal cord. Since this is the first protein described which links the nerve terminal to the extracellular matrix, we propose calling it terminal anchorage protein one (TAP-1).

406.4 A SYNAPTIC VESICLE PROTEIN WITH A SELECTIVE DISTRIBUTION IN SPINAL CORD AND BRAIN. T.C. Ritchie, M.A. Thomas*, C.L. Olson*, S.S. Kanekar*, and J.D. Coulter. Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

A monoclonal antibody generated against rat brain synaptosomes localizes to a highly selective population of nerve terminals in the spinal cord and brain (see other abstracts this volume). The S-7B8 antibody, when employed for immunocytochemistry, produces punctate staining of nerve terminals in the superficial laminae of the spinal dorsal horn. In the brain, the antibody produces intense staining of nerve terminals associated with primary sensory systems, and other selected areas of high nerve terminal density, including the cerebellar cortical molecular layer, and certain synaptic layers of the hippocampus. Immunocytochemical staining is absent in neuronal cell bodies and axons, as well as in non-neuronal endocrine tissues including the adrenal medulla, thyroid, pancreas and anterior pituitary.

The S-7B8 antibody appears to be directed against a synaptic vesicle membrane protein. Electron microscopy of the spinal dorsal horn shows that the antibody stains a subpopulation of nerve terminals and that the staining is associated with synaptic vesicles. Analyses of synaptosomal fractions on a solid phase immunobinding assay indicate that binding of the antibody is abolished by treatment of synaptosomes with proteolytic enzymes (pronase, trypsin). Extraction of lipids from synaptosomes with chloroform/methanol does not affect S-7B8 antibody binding to the insoluble residue. The S-7B8 antigen is likely to be an integral membrane protein since relatively high concentrations of detergents (Triton X-100, Zwittergents) are required to solubilize the protein from synaptosomal membranes, and it is not solubilized with high salt (1.0 M NaCl). These results are consistent with the suggestion that the S-7B8 antibody binds to an integral membrane protein of synaptic vesicles in a subpopulation of CNS nerve terminals.

Synaptic vesicle membrane components with functions common to all secretory vesicle membranes would be expected to have a ubiquitous distribution in the nervous system. In contrast, those vesicle membrane elements involved in the synthesis, storage or release of a specific neurotransmitter or with some other specialized function, such as maintenance of specific synaptic contacts, would be selectively distributed. In view of the highly specific pattern of immunocytochemical staining in the brain and spinal cord revealed by the S-7B8 antibody, the vesicle membrane protein recognized by the antibody seems likely to have a unique role in synaptic function.

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406.5 PROTEIN P38 IS SELECTIVELY ASSOCIATED WITH SMALL SYNAPTIC VESICLES IN NEURONS AND WITH VESICLES DISTINCT FORM PEPTIDE-CONTAINING SECRETORY GRANULES IN ENDOCRINE CELLS. F. Navone*, R. Jahn+, G. Di Gioia*, P. Greengard+ and P. De Camilli*. *CNR Center for Cytopharmac. and Dept. of Med. Pharmac., Univ. of Milano, Italy, +Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY, 10021.

P38 is a major intrinsic glycoprotein of the membrane of synaptic vesicles which has recently been purified and partially characterized (Jahn et al., PNAS 82:4137-4142, 1985; Wiedenmann and Franke, Cell 41:1017-1028, 1985, Rehm et. al., EMBO J. 5:535-541, 1986). We have now carried out a systematic analysis of the distribution of P38 in the nervous system and we have compared this distribution with that of synapsin I, an extrinsic membrane protein present at the cytoplasmic surface of all small synaptic vesicles. Light microscopy immunocytochemistry revealed an almost identical distribution of the two proteins at virtually all nerve terminal-containing regions of the central and peripheral nervous system. In addition, the two proteins were found by quantitative immunochemistry to be present at a roughly constant stoichiometric ratio (close to 1) in all of several major areas of the brain examined. Within nerve terminals, P38, like synapsin I, was found by EM immunogold to be localized on all small synaptic vesicles but not on large dense-core vesicles, i.e. the secretory organelles which contain peptide neurotransmitters. In spite of this very similar distribution in the nervous system, P38 immunoreactivity, but not synapsin I immunoreactivity, was also found in a variety of endocrine cells specialized for vesicular secretion, including chromaffin cells of the adrenal, cells of the diffuse neuroendocrine system of the the gut, endocrine cells of the pancreas and pituicytes. EM immunogold revealed that in these endocrine cells P38 was localized on smooth surfaced vesicles of variable size and with clear content which were scattered throughout the cytoplasm and were concentrated in the Golgi area. Peptide-containing secretory granules were unlabeled. P38 immunoreactivity in endocrine cells was found to be accounted for by a protein with electrophoretic mobility slightly different from that of brain P38, but such difference was eliminated by deglycosylation of the molecule.

Our findings indicate that: a) in neurons the biochemical characteristic of the membrane of small synaptic vesicles are distinct from those of the membrane of large dense-core vesicles; b) virtually all small synaptic vesicles of neurons share at least certain biochemical features (presence of P38 and synapsin I on their membrane in a roughly constant stoichiometric ratio); c) endocrine cells contain vesicles which are biochemically related to small synaptic vesicles of neurons and are distinct from classical secretory granules.

406.6 A Rapid Isolation of Synapsin I: A Neuron Specific Spectrin Binding Protein. K.E. Krebs*, I.S. Zagon and S.R. Goodman* (SPON: T.C. Pritchard). Depts. of Anatomy and Physiology, Div. of Cell and Mol. Biology, The M.S. Hershey Med. Ctr., The Pennsylvania State University, Hershey, PA 17033

We have developed a one chromatographic step isolation protocol for the neuron specific protein synapsin I. Synapsin I was extracted from demyelinated brain membranes with 2M KCl, 2% Triton X-100, 5 mM $NaPO_4$, 0.4mM DFP, pH 7.2 at 37°C for 30 min. The synapsin I extract was stirred with 3g carboxymethyl cellulose (5 mM $NaPO_4$, 20 mM KCl, 0.05% Tween 20, pH 8.0), poured into a column (1 x 5 cm), and washed with 20 ml of the above buffer. The synapsin I was eluted from the column with a 100 ml linear gradient of 0.02 M KCl to 0.10 M KCl in 5 mM $NaPO_4$, pH 8.0, 0.05% Tween 20. The synapsin I eluted between 50-70 mM KCl (peak fraction at 60 mM KCl). The final yield was 0.48 mg synapsin I from 6.17 g mouse brain, or 0.08 mg/g brain. This yield is ten fold higher than the greatest yield yet reported for this protein. The authenticity of the synapsin I isolated by this procedure is demonstrated by: 1) comigration with authentic synapsin Ia and Ib at 76 and 70 kDal respectively on SDS-polyacrylamide gels; 2) crossreactivity with antibody specific against synapsin I; 3) nearly identical (95% peptide homology) two dimensional chymotryptic iodopeptide maps of authentic synapsin I and the protein purified by this protocol. Synapsin I isolated by this procedure retains its functional properties, demonstrated by the ability of synapsin I to stimulate the formation of a brain spectrin(240/235)/synapsin I/F-actin ternary complex, as determined by a low shear falling ball viscometry assay. Incubation of spectrin (0.0135 mg/ml) and F-actin (0.50 mg/ml) with increasing [synapsin I] (0.006-0.025 mg/ml) resulted in a 25% increase in viscosity over that for synapsin I (0.025 mg/ml) and F-actin (0.50 mg/ml), or spectrin (0.0135 mg/ml) and F-actin (0.50 mg/ml) alone. This novel protocol therefore has the advantage of being a rapid, high yield procedure that retains the functional properties of synapsin I. This work was supported by the National Institutes of Health Grants NS-19357 and HL-26059 awarded S.R.G. and NS-21246 to I.S.Z. and S.R.G. S.R.G is an Established Investigator of the American Heart Association.

406.7 IN VIVO TURNOVER OF BRAIN SPECTRIN: STIMULATION OF DEGRADATION BY DEPOLARIZATION. W.Bodsch*, M.Baudry, and G.Lynch (SPON:H.Koopowitz). Center for the Neurobiology of Learning and Memory. UC Irvine, CA 92717, U.S.A.

Brain spectrin is a major cytoskeletal protein which has been postulated to play roles analogous to its erythrocyte counterpart, such as regulation of cell surface receptor distribution and cell shape. It is an excellent substrate for calcium-dependent proteases (calpain) and it has been proposed that calpain-induced degradation of brain spectrin as a result of high frequency electrical stimulation of various hippocampal pathways leads to long-term changes in synaptic efficacy (Lynch and Baudry, Science, 1984, 224: 1057-1062). The present study was directed at testing some aspects of this hypothesis by investigating the in vivo synthesis and degradation of brain spectrin.

The time-course of brain spectrin synthesis was determined by measuring the amount of labeled spectrin following intraventricular infusion of ^{35}S-methionine for various periods of time. Brain spectrin is synthesized rapidly, with a ratio of α/β subunits of about 2/1 in the soluble compartment; it is rapidly incorporated into the cytoskeletal compartment of synaptosomal fractions with equimolar amounts of α and β-subunits. This suggests that the attachment of the β-subunit to cytoskeletal elements regulates the incorporation of spectrin into the cytoskeleton. Five to six hours after this incorporation, a major breakdown product of the α-subunit with a M_r of 140,000, and previously identified as a product generated by calpain-induced proteolysis of brain spectrin, appears in both the soluble and cytoskeletal compartments. This result suggests that calpain-mediated degradation of brain spectrin in the cytoskeleton is a dynamic process and that spectrin is turning over relatively rapidly (estimated half-life of about 4-5 hours).

We next investigated the effect of prolonged depolarization on the formation of this major breakdown product in hippocampus of rats which had received a continuous infusion of ^{35}methionine for 4-5 hours, i.e., at a time when the amount of labeled breakdown product is very low in control animals. Induction of spreading depression by local applications of KCl in hippocampus resulted in a 2-3 fold increase in the amount of labeled breakdown product. This result suggests that the rate of spectrin degradation can be be greatly accelerated by depolarization and strengthens the hypothesis that calpain-induced spectrin degradation is a plausible triggering mechanism for activity-dependent changes in synaptic efficacy.

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406.8 BIOCHEMICAL AND MORPHOLOGICAL CHARACTERIZATION OF FREE AND TUBULIN-ASSOCIATED FORMS OF THE SOLUBLE NEURONAL CALMODULIN-DEPENDENT PROTEIN KINASE II H. LeVine, III*, and N.E. Sahyoun* (SPON: S.K.Burgess Department of Molecular Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The soluble form of the calmodulin-dependent protein kinase type II from rat fore- and midbrain regions is resolved into two species by ion exchange chromatography on DEAE-Fractogel following calmodulin-Sepharose affinity chromatography. The enzyme eluting at a low ionic strength corresponds to the kinase purified by several labs. The later eluting enzyme is obtained in complex with tubulin and several high Mr proteins. The unassociated form of the kinase does not exchange into or coprecipitate with the tubulin-containing form and both forms rechromatograph on ion exchange in their respective positions.

Subunits of the kinase in this complex have been identified by their characteristic increase in apparent Mr upon autophosphorylation, calmodulin binding, two-dimensional IEF/SDS-PAGE, and by two-dimensional iodopeptide mapping. The ratio of enzyme subunits to tubulin and the high Mr proteins remains constant through gel permeation chromatography and sedimentation velocity centrifugation. Hydrodynamic parameters derived from these studies demonstrate that the tubulin-associated enzyme is larger than the unassociated enzyme and displays heterodisperse behavior as well. The same complex of proteins coprecipitates with a monoclonal antibody to the kinase. Electron microscopy of negatively stained complex reveals an interesting ultrastructure distinct from that of the unassociated enzyme. These two species of enzyme thus represent distinguishable forms of the calmodulin-dependent protein kinase, with the tubulin-associated form possibly representing a putative precursor of the cytoskeletal form of the kinase since the complex SDS-PAGE protein pattern is contained in that of the postsynaptic density.

406.9 AUTOPHOSPHORYLATION REVERSIBLY REGULATES THE Ca^{2+}/CALMODULIN-DEPENDENCE OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II. Y. Lai*, A. C. Nairn, and P. Greengard (SPON: Mike Browning). Dept. Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York 10021.

Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (Ca/CaM Kinase II), purified from rat forebrain, is known to contain two types of subunit, α (M_r 50,000) and β/β' (M_r 60,000/58,000), both of which undergo Ca^{2+}/CaM-dependent autophosphorylation through an intramolecular mechanism. In the present study, we have studied the effect of this autophosphorylation reaction on the activity of the enzyme. Autophosphorylation of Ca/CaM kinase II converted the enzyme to one that was almost fully independent of Ca^{2+}/CaM for its activity. No change in the total activity, measured in the presence of Ca^{2+}/CaM, was observed. Moreover, dephosphorylation of autophosphorylated Ca/CaM kinase II with protein phosphatase 2A resulted in an enzyme that was again totally dependent on Ca^{2+}/CaM for its activity. Autophosphorylation of Ca/CaM kinase II also resulted in the enzyme becoming more labile to heat. For example, if autophosphorylation was carried out at 30°C, an apparently irreversible loss of activity was observed. This loss of activity could not be reversed by protein phosphatase 2A. Thus, autophosphorylation and dephosphorylation regulate the Ca^{2+}/CaM requirement of Ca/CaM kinase II.

In order to determine whether one or both subunits of the enzyme are responsible for the generation of Ca^{2+}/CaM-independence, we examined the effect of autophosphorylation on isozymes of Ca/CaM kinase II that contain different ratios of the α and β subunits. Forebrain Ca/CaM kinase II (described above) contains predominantly the α subunit. Cerebellar Ca/CaM kinase II and skeletal muscle Ca^{2+}/CaM-dependent glycogen synthase kinase contain predominantly the β subunit. Autophosphorylation also converted the cerebellar and skeletal muscle isozymes to species that were partially independent of Ca^{2+}/CaM for activity. These results suggest that autophosphorylation of both α and β subunits may be responsible for the generation of Ca^{2+}/CaM-independence.

Forebrain Ca/CaM kinase II was found to have 2 autophosphorylation sites on the α subunits and 3 on the β subunits. Phosphorylation of only 2-4 of the estimated 27 available sites in the holoenzyme appeared to be sufficient for Ca^{2+}/CaM-independent activity to occur. For both subunits, threonine residues were initially autophosphorylated, with serine residues being phosphorylated at a much slower rate.

406.10 ATYPICAL SYNAPTIC VESICLES IN HIPPOCAMPAL MOSSY FIBER BOUTONS FOLLOWING RECURRENT LIMBIC SEIZURES. R.M. Pico, J. Athanikar*, C.E. Ribak and C. Gall. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

A unilateral electrolytic lesion restricted to the hilar region of the dentate gyrus initiates a series of bilateral hippocampal seizures which end 8-12hr post-lesion. Light microscopic immunocytochemical investigation has shown that met-enkephalin immunoreactivity (ENK-I) in the mossy fiber projections to CA3 is affected by the seizure activity (Gall, in preparation). The principal changes are seen at 12hr postlesion when ENK-I is virtually depleted in the mossy fibers, and at 4 days postlesion when ENK-I is dramatically increased bilaterally in the axonal and terminal fields. Control levels of ENK-I return 14 days postlesion. The present analysis was made to determine the ultrastructural features of mossy fibers at these experimental time points and to compare them with the normal morphology of mossy fibers.

Seven adult male Sprague Dawley rats received a unilateral hilus lesion while under ketamine/xylazine anesthesia. These subjects were then observed over the next 10hr for class 5 motor seizures, and were found to display 5-10 per rat. At 11hr postlesion 4 experimental rats were anesthetized with sodium pentobarbital and perfused with aldehyde solutions. At 4 days postlesion 3 experimental rats were also prepared in this manner. A series of control rats were used for normal morphology. Vibratome sections were collected from the hippocampus contralateral and posterior to the lesion, and a portion of CA3b was microscopically dissected from each tissue section. These sections were postfixed in osmium, dehydrated and embedded in Epon/Araldite. Silver thin sections were collected on copper mesh grids, stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

A characteristic feature of the mossy fiber terminal boutons is the orderly accumulation of small agranular round vesicles 35-45nm in diameter (Amaral & Dent, JCN, 195, 1981). In all 11hr postlesion hippocampi, a large percentage of the mossy fiber terminal profiles contained a heterogeneous population of agranular vesicles with many enlarged clear vesicles distributed among the central vesicular array. Moreover, these terminals contained fewer dense core vesicles than is typically found in control samples. At 4 days postlesion the mossy fiber profiles contained a homogeneous collection of small agranular vesicles but lacked the large vesicles seen at 11hr postlesion. A more prominent feature of the 4 day postlesion mossy fiber boutons was an increase in the number of dense core vesicles and the intensity of their staining. In addition, an unusual organelle, electron-dense multivesicular bodies, were found in many mossy fiber axons and boutons suggestive of a precursor to the dense core vesicles.

Previous reports indicate that an increase in exocytotic profiles involving dense core vesicles occurs in the mossy fiber boutons following chemically-induced hippocampal seizures (e.g., Nitsch & Rinne, J. Neurocytol., 10, 1981). Our findings suggest that the dense core vesicles undergo additional seizure-related changes, and these events coincide with the reported fluctuations in mossy fiber ENK-I localization. Thus, the possibility exists that enkephalin may be stored in the dense core vesicles of the mossy fiber system. Supported by NIH MH093555 (RMP), NSF BNS8417098 and RCDA NS00915-10 (CG).

406.11 THE CENTRAL IMPULSE ACTIVITY OF A CRAYFISH MOTONEURON REGULATES ITS NEUROMUSCULAR SYNAPTIC PROPERTIES. G.A. Lnenicka and H.L. Atwood, Dept. of Physiology, University of Toronto, Toronto, Canada M5S 1A8

Increasing the impulse activity of a phasic motoneuron (the fast excitor to the crayfish claw) through in vivo stimulation has been previously shown to alter the neuromuscular synapses so that they resemble those of a tonic motoneuron. These changes in neuromuscular synaptic properties include: less initial transmitter release; greater resistance to synaptic fatigue; and an increase in the volume of mitochondria in the motor terminals (J. Neurosci. 5: 459; J. Physiol. 364: 26P).

We have previously shown that increasing the subthreshold synaptic input to the fast closer excitor (FCE) produces a smaller, though qualitatively similar change in neuromuscular synaptic physiology (Neurosci. Abstr. 11: 1288). These experiments suggested that increased impulse activity in the FCE may act to produce transformation of neuromuscular synapses through an increase in the central activity of the motoneuron. In order to test this directly, we developed a procedure to antidromically stimulate the FCE without increasing the activity of its neuromuscular synapses.

Electrodes were implanted at the base of the meropodite to stimulate the FCE and over the closer muscle to record the FCE myogram as previously described (J. Neurosci. 5: 459). Prior to in vivo stimulation, a cube of gelatin containing 10uM TTX was placed against the nerve distal to the stimulation electrodes. This procedure normally blocked orthodromic FCE impulses within 10 minutes. The FCE was then electrically stimulated antidromically for two hours at 12 Hz in 1 sec trains delivered every 2 sec. At the end of stimulation the TTX block was removed and impulse conduction was normally restored within 30 minutes.

The conditioning regimen was delivered daily to one of the paired claws for 3 consecutive days. One day after the final conditioning period the FCE EPSPs were measured in closer muscle fibers of the conditioned and contralateral control claws. The conditioning procedure was found to decrease initial FCE EPSP amplitude by 48% and reduce synaptic fatigue by 90%. Thus, the adaptation of the neuromuscular synapses to increased activity is triggered by increased central electrical activity of the motoneuron.

Preliminary evidence indicates that local application of colchicine (a blocker of fast axonal transport) to the nerve during chronic stimulation prevents the decrease in initial EPSP amplitude. Thus, some factor(s) whose production is regulated by central electrical activity may be transported to the terminals to control initial transmitter release.

406.12 RELATIONSHIP BETWEEN SYNAPTIC SIZE AND TARGET CELL SIZE IN THE PARASYMPATHETIC CARDIAC GANGLION OF *XENOPUS LAEVIS*. Peter B. Sargent. Division of Biomedical Sciences, University of California, Riverside, CA 92521.

The parasympathetic ganglion cells in the heart of the frog *Xenopus laevis* are innervated by preganglionic axons which terminate on the cell body and its axon in a number of synaptic boutons. Electrophysiological analysis of e.p.s.p.'s elicited from the unipolar ganglion cells by preganglionic nerve stimulation in adult frogs indicates that ganglion cells are innervated by 1-4 axons and that the average number of inputs per cell is 1.6 (Heathcote and Sargent, unpublished results). In adult frogs there is also a direct relationship between the number of boutons on the ganglion cell body and its size: larger cell bodies have more synaptic boutons in proportion to their increased surface area [Sargent, P.B., J. Physiol. 343(1983)85-104]. In the present study, I sought to determine whether this relationship is due primarily to differences in convergence or to differences in synaptic size. Do larger cell bodies have more boutons because they tend to be innervated by more preganglionic axons or because each innervating preganglionic axon tends to supply them with more boutons? To address this question I investigated whether the cell bodies of ganglion cells are multiply innervated, and, if so, whether the extent of convergence can account for the known correlation between somatic size and bouton number. The experimental rationale was to stain single preganglionic axons innervating individual cell bodies and then to determine whether there are any boutons on the cell body which cannot be accounted for by the stained axon. Preganglionic axons innervating the cardiac ganglion were filled with HRP and stained brown by using diaminobenzidine as a substrate. In order to visualize all the synaptic boutons in the ganglion, I used immunocytochemical techniques to locate the binding sites for a monoclonal antibody which binds to synaptic vesicles and which quantitatively stains synaptic boutons in the cardiac ganglion (no. 48, a generous gift of Dr. Louis Reichardt, University of California, San Francisco). Bouton staining was done using the ABC technique, and boutons were rendered blue-purple by using chloronaphthol as a substrate. Following this procedure boutons supplied by the filled preganglionic axons remained brown while all other boutons were blue-purple. Ganglion cells whose somata were innervated by only a single *filled* preganglionic axon were then analyzed. All the boutons on such cells were invariably brown and arose from the stained axon (121 cells examined in 10 ganglia). Blue-purple boutons were found on cells whose somata were not supplied by *filled* axons as well as on the axons of many cells. This suggests that all ganglion cell bodies are supplied by a single preganglionic axon, and that additional inputs to the cell, when present, must be located on their axons. Furthermore, this result indicates that larger ganglion cell bodies have more boutons because the single axon that innervates them supplies them with more boutons. Thus, there is a direct correlation between target cell size and synapse size in the cardiac ganglion. (Supported by the NSF and the AHA.)

407.1 THE DEVELOPMENT OF PROPRIOSPINAL PATHWAYS IN CHICK EMBRYO SPINAL CORD. A. Shneiderman, N. Okado, R. W. Oppenheim and R. Shiba* (SPON: P. B. Smith). Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103 and Department of Anatomy, University of Tsukuba, Tsukuba, Japan.

Recent studies have shown that most supraspinal projections to cervical and lumbar cord of the chick develop between embryonic day (E)5 (stage 26) and E10 (stage 36) (Okado, N. and Oppenheim, R., J. Comp. Neurol., 232: 143-161, 1985). The onset of propriospinal projections very likely occurs earlier than this although there is only one report in the literature that has addressed this problem in the chick (Nornes, H., et al., J. Comp. Neurol., 192: 119-132, 1980). By the use of in ovo and in vitro preparations we have begun to study the ontogeny of propriospinal pathways and projections between E3 (stage 19) and E10. Small amounts of recrystallized HRP or WGA-HRP on the tips of Minuten insect pins or glass micropipettes were inserted into various regions of spinal cord (cervical, brachial, thoracic, lumbar). Embryos were allowed to survive for 5-24 hrs after which they were processed for HRP histochemistry using TMB, DAB or DAB with heavy metal intensification procedures on either frozen (50-80 μ) or Vibratome (80-100 μ) sections. At present, only cases in which injections were made into lumbar segments 2-5 have been analyzed in detail. At stage 21 (E3.5) labelled interneurons can be found 2-4 segments rostral to the injection. Prior, to this (stages 19-20), labelling is only found within and approximately one segment rostral to the injected segment. The earliest labelled cells are found in various parts of what will become lamina VII, especially adjacent to the prospective lateral funiculus, and many of these are commisural neurons whose labelled axons can often be followed through the ventral commisure. By stages 22-23 (E3.5-4.0) increased numbers of labelled cells appear in lamina VII extending as far as six segments rostral to the injection site (i.e., in thoracic segments). From stage 24 (E4.5), lumbar injection results in labelled cells in brachial segments. By stage 27 (E5.5) labelled cells are found in lamina VI in brachial segments and between E6 (stage 29) and E10 labelled cells progressively appear in laminae I, II, IV, V, VIII and X, similar to the adult condition. Preliminary analysis of labelled neurons in lumbar segments following thoracic or brachial injections indicates a similar spatio-temporal pattern of labelling as just described. Thus, by E4 (stage 24) there are neuroanatomical interconnections between lumbar, thoracic and brachial regions that could be the basis for previously described intersegmentally organized motility and electrophysiological patterns (Provine, R., Brain Res., 29:155-158, 1971).

407.2 ROLE OF THE SUBVENTRICULAR ZONE IN GROWTH AND GUIDANCE OF CALLOSAL AXONS.
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During fetal development the subventricular zone comprises an area of proliferating cells which forms between the ventricular germinal cells and the intermediate layer, and which appears to consist of precursor cells for neurons and macroglia. A previous study in our laboratory suggested that subventricular cells may be a preferred substrate for growth of callosal axons. In the present study we have perturbed the subventricular zone in fetal mice, and have observed the subsequent growth of callosal axons.

Perturbations of the subventricular zone were accomplished in two different ways: 1) using a needle, which was passed through the cortex and into a lateral ventricle on embryonic day 16; 2) using whole body x-irradiation (125 R) of pregnant mice on E14. Brains were taken from treated and control animals between E17 and postnatal day 2 for light and electron microscopic examination.

After x-irradiation or needle-lesioning a similar pattern of disrupted axonal growth was observed. Both treatments produced breaks in the subventricular zone or eliminated patches of subventricular cells. In the irradiated animals the remainder of the cortex was thinner than usual, but was intact. In lesioned animals the cortical tissue, through which the needle passed, appeared to mend itself readily.

When callosal axons, growing towards the midline, encountered areas with abnormal subventricular tissue, they either followed a path which kept them in contact with subventricular cells - even if this described a dead-end route into the ventricle - or, where patches of subventricular cells were apparantly eliminated, they failed to continue normal growth toward the midline, and instead formed a neuroma-like structure. In the irradiated animals few axons reached the midline, and thus they were either acallosal or had much smaller than normal corpus callosi. In lesioned animals the growth of axons appeared normal in areas where the subventricular zone was not disrupted.

The results of this study are consistent with the hypothesis that subventricular cells are a preferred substrate for growth of callosal axons.

407.3 AXONAL GUIDANCE IN AVIAN SYMPATHETIC PREGANGLIONIC NEURONS. J. W. Yip. Department of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

The patterns of preganglionic projections in the avian sympathetic system are highly specific. Preganglionic axons arising from the T1 spinal segment, for example, project predominantly in the rostral direction whereas T4 preganglionic axons project predominantly in the caudal direction. This pattern of preganglionic projections, moreover, is correct from the outset, suggesting that there are specific cues for the guidance of these axons.

In a recent study, I have investigated whether target cues are required for the guidance of preganglionic axons by examining the preganglionic projections in embryos deprived of their cervical and thoracic sympathetic ganglia through removal of the neural crest. My results indicate that target cues are not required for the establishment of the correct patterns of preganglionic projections, since preganglionic axons deprived of their target ganglia still project in the proper directions.

To examine if the rostral or caudal projections of preganglionic neurons are determined strictly by their segmental origins in the spinal cord, segments of the thoracic spinal cord were translocated. In one set of experiments, the thoracic segments T1 to T4 were rotated 180° along the rostro-caudal axis, and in another, segments T1 to T4 were transplanted to the cervical level where preganglionic neurons are normally absent. Following rotation at the thoracic level, preganglionic axons project according to their new segmental locations, i.e., T4 preganglionic neurons (which are now located rostrally) project rostrally, and T1 preganglionic neurons (which are now located caudally) project caudally. By contrast, T1 and T4 preganglionic neurons translocated to cervical levels no longer project predominantly in either a rostral or caudal direction, but project more or less equally in both directions. These results indicate that the directions of preganglionic projections are not strictly determined by their initial segmental origins in the spinal cord but appear to depend on factors in the tissue environment outside of it.

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407.4 ANATOMICAL AND PHYSIOLOGICAL DISTORTION OF THE XENOPUS RETINOTECTAL PROJECTION BY ANTIBODIES TO N-CAM.
S. E. Fraser, S. Carhart, B. A. Murray*, C.-M. Chuong, and G. M. Edelman. Dept of Physiology & Biophysics, University of California, Irvine, CA 92717, and The Rockefeller University, New York, NY 10021.

The neural cell adhesion molecule (N-CAM) mediates a dominant neuron-neuron adhesion, is ubiquitous in the nervous system of developing and mature vertebrates, and undergoes major alterations in both distribution and amount during development. To determine the role of this defined intercellular adhesion in the formation of neuronal maps, agarose implants containing antibodies specific for Xenopus N-CAM were inserted into the tectum and their effects on the projection followed with anatomical and physiological techniques.

Both monoclonal and polyclonal antibodies against N-CAM altered the retinotectal projection as determined by horseradish peroxidase (HRP) tracing. Following labelling of the entire optic nerve with HRP and processing of the brains with diaminobenzidine & cobalt, the region of the tectum near the implant showed a decreased density of optic nerve innervation. The antibodies also distorted the pattern of the retinotectal projection and decreased the precision of the projection as determined by electrophysiological techniques. The size of the receptive fields recorded in the tectum were significantly ($p<.005$) enlarged.

The specificity of the immune reagents was shown by their ability to immunoprecipitate Xenopus N-CAM and to inhibit membrane vesicle aggregation. The inhibition of aggregation was neutralized by preincubation of the antibodies with purified N-CAM. Rabbit antibodies against the brain membrane, depleted in anti-N-CAM activity, were used as the control reagent. These antibodies bound to the tectal membranes, but had no effect on either the aggregation of brain membrane vesicles or on the density or the pattern of the projection.

The distortion of the retinotectal projection by anti-N-CAM became detectable after three days and was maximal by eight to ten days. The antibody spread from the implant within hours to fill most of one tectal lobe, where it remained at detectable levels for five to six days. The delayed effect of the antibody and the lack of any blind regions of the retina argue that the effects do not result from damage of the retina or optic nerve fibers. The time-course is consistent with the antibody disrupting an on-going dynamic process of neurite sprouting and retraction which appears to underlay the outwardly static retinotectal projection.

This work extends our previous work by demonstrating an anatomical correlate of the physiological distortion of the projection, by establishing the specificity of the reagents for Xenopus N-CAM, by documenting the spread of the perturbing antibodies from the implant, and by including a more relevant control reagent. The results further support the conclusion that N-CAM mediated cell adhesion plays an important role in the formation and maintenance of topographic neural projections.

Supported by NSF (BNS11224), McKnight Scholars Award, and NIH (Jacob Javits Center of Excellence and HD09635).

407.5 INVOLVEMENT OF CREST MIGRATION IN CREATING AXON BOUNDARIES AT THE TECTAL MIDLINE. Michael R. Poston* and Jerry Silver (SPON: M.J. Katz). Dept. Develop. Genetics and Anatomy, Case Western Reserve Univ., Sch. of Med., Cleveland, OH 44106.

In the vertebrate embryo, optic fibers from the retinal ganglion cells innervate the entire surface of the optic tectum. However, the fibers which have arrived at one tectal lobe rarely grow across the midline to innervate the other even though a potential avenue of growth, the future roof of the Sylvian aqueduct, is present. We have studied the development of the tectal midline in timed chicken embryos in hopes of gaining insight into the question of why this region is refractory to the passage of axons. It has been suggested that the marginal endfeet of the neuroepithelium provide a preferred surface for the growth of optic axons (Silver, J.C.N., 223, 1984; Silver and Rutishauser, Dev. Biol., 106, 1984). A question that remained was whether some naturally occurring disruption of the endfoot zone might create a boundary where optic axons would not grow, similar to the "glial knot", a structure that may play a role in partitioning fibers of the optic and olfactory systems at the chiasm of chick embryos (Poston, Rutishauser and Silver, Neurosci. Abstr., 15, 1984). In order to address this question in E6-8 chick embryos, TEM and immunohistochemical techniques using antibodies to NCAM, 8D9 and HNK-1 antigens were used to stain neurons, axons and support cells. As the optic fibers arrived at the tectum on E6, we observed an unexpected phenomenon: a number of axons approaching the tectal midline projected out of the brain and into the mesenchyme immediately dorsal to the midline. The process of axon misguidance continued for at least two more days (E7-8). The axons exited the neuroepithelium through gaps in the endfeet and the glial limitans. During this same time period we observed that a population of very large cells migrated across the glial limitans exclusively at the tectal midline. The migratory cells were positive for the HNK-1 antigen (a marker expressed by migrating neural crest) but negative for the NCAM antigen, while other cells at the tectal midline were negative for both HNK-1 and NCAM. At such late stages (E6-8) the HNK-1 positive cells may be a population of neural crest that contributes to the formation of the mesencephalic nucleus of the trigeminal nerve by migrating back into the brain. We hypothesize that the large HNK-1 positive cells which migrate across the glial limitans disrupt the endfeet of the midline tectal neuroepithelium. Nearby axons that might otherwise have crossed the midline are instead misrouted through discontinuities in the glial limitans and out into the mesenchyme. This combination of novel phenomena may constitute a mechanism for creating an axon-refractory boundary between the two lobes of the optic tectum.

Supported by NIH EY05952.

407.6 REGENERATING OPTIC AXONS OF GOLDFISH DO NOT GROW ON THE BASAL LAMINA. S.S. Easter, Jr. and C. Malinoski*. University of Michigan, Ann Arbor, MI 48109.

New ganglion cells are added to the goldfish retina at its edge, the ora terminalis, throughout life. Their new axons grow on the innermost surface of the retina, in contact with the basal lamina of the retinal inner limiting membrane and, more centrally, in contact with the basal lamina of the pia. In the peripheral nervous system, the basal lamina around Schwann tubes is an important substrate for peripheral axonal regeneration; and in vitro, a component of the basal lamina, laminin, is an effective substrate for axonal growth. These several observations suggest that axonal regeneration in the goldfish optic nerve might recapitulate de novo development. Accordingly, we examined regenerating optic axons electron microscopically to see if regenerating axons grow along the basal lamina.

Normal and crushed optic nerves were prepared with standard methods for transmission electron microscopy. The normal optic nerve is divided into interleaving fascicles surrounded by astrocytic cytoplasm and a basal lamina. In the crushed nerve, the axons degenerate downstream from the lesion but the fascicular organization is maintained; that is, fascicles are surrounded by astrocytic cytoplasm and basal lamina, as normally. At 5 and 10 days post crush, all regenerating axons were found in the centers of the fascicles, separated by degenerating debris and astrocytic cytoplasm from the basal lamina. We conclude that the regenerating axons do not retrace the original path.

We have not yet identified the substrate followed by the pioneering axons. However, one series of 435 sections, which included hundreds of bundles of axons, yielded many growth cones. All contacted other axons extensively. We conclude that other axons are the preferred substrate when they are present. (Supported by EY-00168 to SSE.)

407.7 GUIDANCE OF REGENERATING BULLFROG MOTOR AXONS. M.T. Lee and P.B. Farel. Dept. Physiol., UNC Sch. of Med., Chapel Hill, NC 27514.

Numerous studies provide indirect support that axons regenerating in the peripheral nervous system are guided by empty sheaths contained within the distal nerve stump. According to this idea, regenerating axons non-specifically enter denervated endoneurial sheaths at the site of nerve transection. Once having entered a sheath, an axon is confined and forced to follow the pathway laid down by the sheath. We have been testing this idea by examining the regeneration of hindlimb motoneurons in tadpoles of the bullfrog, Rana catesbeiana.

Ventral roots (VRs) 8, 9, & 10, which supply motor axons to the hindlimb, were transected on one side of tadpoles at stages XI-XVIII. After 35 - 124 days, the segmental innervation of 17 hindlimb nerves was determined on both sides, by recording the antidromic activity elicited in VRs 8, 9, & 10 in response to shocking each limb nerve. For these experiments, the CNS was removed to exclude synaptically evoked potentials from the VRs. Such recordings reveal that the distribution of axons from each transected VR is nearly identical to normal. For example, normal and regenerating axons from VR 10 are found in only 1 of the 3 branches of the crural nerve, and in 4 of the 6 branches of the profundus posterior nerve, even though they grow in close proximity to the origins of all of these branches. It is unlikely that this results from an active selection of particular pathways by the regenerating axons, since retrograde labeling studies (Farel & Bemelmans, in press) indicate that motoneurons transected at these stages reinnervate targets non-specifically. Instead, the reestablishment of a normal pattern of segmental innervation suggests that the regenerating axons are somehow constrained from entering nerves outside the normal territory of their VR. This constraint may be provided by the denervated sheaths through which the axons elongate.

Regenerating motor axons can divide and send branches to more than one muscle, as demonstrated by severing a muscle nerve, stimulating its proximal stump, and observing the contraction of other muscles (CNS removed). As in other systems, much of this branching occurs at the transection site. However, additional branches form within the crural and sciatic plexuses at the base of the limb, since inter-nerve communication remains after the spinal nerves are transected just proximal to the plexuses, and since HRP applied to one limb nerve labels branches in other nerves in such reduced preparations. We are now in the process of comparing the branching patterns of regenerating axons with those of normal axons, in order to determine whether the pathway constraints on regenerating axons might be provided by endoneurial sheaths, which should branch only at sites of branching of the axon originally surrounded by each sheath.

407.8 **HYPOTHALAMIC NEURONS DISPLAY MORPHOLOGICALLY IDENTIFIABLE AXONS AND DENDRITES THAT GROW AND DIFFERENTIATE RAPIDLY IN A THREE-DIMENSIONAL EXTRACELLULAR MATRIX.** Penelope W. Coates, Kerri Ming-Hsin Meyer* and Suzette Stubblefield*. Dept. Cell Biol. & Anat., Texas Tech Univ. HSC, Sch. Med. Lubbock, TX 79430.

We have previously shown that single, unconnected cerebral or spinal neurons rapidly exhibit characteristic differentiated features including two morphologically distinct types of processes - axons and dendrites - when cultured in a three-dimensional (3-D) extracellular matrix (ECM) consisting of a hydrated collagen lattice (Cochran & Coates, Soc.Neurosci.Abs.11:762, 1985; Coates, Dev. Brain Res.25:11-20, 1986). We now demonstrate that single hypothalamic neurons also have the capacity to express similar characteristics. Hypothalami from embryonic d.8-9 chicks were dissociated by gentle trituration and enzymatic treatment. Cells were plated at 10^5 cells/ml which assured that neurons would be widely separated and not in physical contact with other cells or processes. Within 24 hours, many neurons initiated axon and dendritic outgrowth into the matrix as evidenced by phase microscopy of living cultures and scanning electron microscopy of fixed cultures. Axons grew relatively straight. Nissl and silver stained whole mounts revealed well differentiated monopolar, bipolar and multipolar neurons. Varicosities were present on some axons. Growth cones were prominent. A quantitative analysis of growth and differentiation was performed using an image analysis system coupled to a phase contrast microscope and microcomputer. Data from three independent experiments were compared using an ANOVA multiple range test. After 1 d. in culture, mean axon length was 192 (±14) um/neuron and reached 395 (±25) um/neuron by d. 3. The mean length of the entire dendritic tree/neuron increased from 54 (±11) um to 113 (±19) um over the same time period. The combined total process length that a neuron was capable of generating in 3 d. averaged 508 (±34) um. Rate of growth for axons and dendrites was 169 um/d., which is within the range of rates for slow transport of cytoskeletal components and suggests that growth is linked to this. Differences in the number of primary processes, branch points, segments and terminals were not statistically significant, although differences between individual hypothalamic neurons were obvious visually. These data demonstrate that hypothalamic neurons have an inherent capacity to develop axons and dendrites when cultured in a 3-D ECM and that this is not dependent on contact with other neurons or non-neuronal cells. Thus, the culture system promises to provide a convenient vehicle with which to study inherent properties of single hypothalamic neurons and the influence of extrinsic factors including other cells and factors, on individual neurons. (Supported in part from TTUHSC BSRG, the APA and SITRF)

407.9 **NICOTINIC CHOLINERGIC BLOCKING AGENTS ENHANCE PROCESS OUTGROWTH BY SOLITARY RAT RETINAL GANGLION CELLS IN CULTURE.** M.P. Frosch, M.D. Phillips*, E. Aizenman, D.L. Tauck and S.A. Lipton, Dept. of Neuroscience, The Children's Hospital and Harvard Medical School, Boston, MA 02115.

Rat retinal ganglion cells from postnatal animals (7 to 12 days) were identified with fluorescent labels, dissociated and cultured as previously described (Leifer et al., *Science* 224;303, 1984). During patch-clamp recordings, these central neurons displayed nicotinic responses to acetylcholine (ACh) similar to autonomic ganglia and Renshaw cells (Lipton et al., *Soc. Neurosci. Abstr.,* 1986). One day after plating cultures in d-tubocurarine or mecamylamine (10 μM), the proportion of solitary retinal ganglion cells that had regenerated processes longer than the diameter of the cell body increased relative to control by 78 and 89%, respectively ($p < .001$). The total length of processes per cell also increased by 40-45% with these drugs compared to control. In contrast, atropine (10 μM) did not significantly increase process outgrowth; this concentration of atropine did not alter cholinergic currents measured in whole-cell patch clamp. Moreover, bicuculline methiodide and picrotoxinin (10 μM) which block the large currents induced by GABA (1-100 μM) in these cells (Tauck et al., *ibid,* 1986) did not affect process outgrowth. These results suggest a degree of specificity to the effect of nicotinic antagonists on the plasticity of process outgrowth.

The enhancement of process outgrowth by nicotinic blockers suggested that ACh might be present in the culture medium. To measure endogenous ACh, cultures were incubated with ^{3}H-choline followed by high energy electrophoresis of the culture fluid to separate radiolabelled ACh from precursor; ^{3}H-ACh was found in the culture medium in nM concentrations. Thus, the growth enhancing effect of nicotinic antagonists may be due to removal of inhibition of growth by tonic levels of ACh. Since several workers have shown that there is a spontaneous 'leak' of ACh in the intact retina, the effects of nicotinic cholinergic drugs on process outgrowth in culture may reflect a control mechanism for growth of retinal ganglion cell processes *in vivo.*

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407.10 NEURONAL CELL ADHESION MOLECULES AND CYTOTACTIN ARE COLOCALIZED AT THE NODE OF RANVIER Francois Rieger, Joanne K. Daniloff, Martine Pincon-Raymond*, Kathryn L. Crossin*, Martin Grumet, and Gerald M. Edelman. Laboratory of Developmental and Molecular Biology, The Rockefeller University, New York, New York, 11021, and Groupe de Biologie et Pathologie Neuromusculaires, Institut National de la Sante et de la Recherche Medicale, Paris, France

Two neuronal cell adhesion molecules (CAMs), neural CAM, N-CAM, and neuron-glia CAM, Ng-CAM, mediate binding between neurons and between neurons and glia. An extracellular matrix protein, cytotactin, has also been identified that is involved in glia-neuron interactions. Immunocytochemical and ultrastructural gold methods have been used to show that Ng-CAM, N-CAM, and cytotactin are highly concentrated at nodes of Ranvier of the adult chicken and mouse. In contrast, unmyelinated axonal fibers were uniformly stained by antibodies to both CAMs. Both N-CAM and Ng-CAM were enriched in the nodal axoplasm and axolemma of myelinated fibers as well as within the nodal regions of the myelinating Schwann cell. In chick embryos at embryonic day 14, before myelination had occurred, small-caliber fibers showed periodic coincidental accumulations of the two CAMs, with faint labeling in the axonal regions between accumulations. On the other hand, cytotactin was found on Schwann cells and in connective tissue. By embryonic day 18, nodal accumulations of CAMs were first observed in a few medium- and large-caliber fibers. Immunoblot analyses indicated that E to A conversion of N-CAM occurred while nodes were forming with a progressive decrease in the amount of Ng-CAM and N-CAM. Sciatic nerves of mouse mutants with defects in cell interactions showed abnormalities in the distribution patterns and amount of Ng-CAM, N-CAM and cytotactin, that were consistent with the known morphological nodal disorders. In trembler (+/Tr), intense staining for both CAMs appeared all along the fibers and the amounts of N-CAM in the sciatic nerve were found to be increased. In mice with motor endplate disease (med/med), Schwann cells stained intensely for cytotactin, in contrast to normal Schwann cells which stained only slightly. All of these findings are consistent with the hypothesis that surface modulation of neuronal CAMs mediated by signals shared between neurons and glia may be necessary for establishing and maintaining the nodes of Ranvier. Supported by grants from INSERM, CNRS, and Fondation pour la Recherche Medicale (FR), a Senator Jacob Javits Center of Excellence in Neuroscience Grant (NS-22789), USPHS grants AM-04256, HD-16550, HD-09635 (GME) and Postdoctoral Fellowship NS-07636 (JKD).

407.11 IDENTIFICATION OF A PERIPHERAL NERVE ANTIGEN THAT PROMOTES AXONAL REGENERATION. A.W. Sandrock* and W.D. Matthew. Dept. of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115

In mammals, severed neuronal axons of peripheral nerves regenerate readily, whereas axons of the central nervous system fail to regrow effectively. Ramon y Cajal was the first to postulate that the rapid regeneration of peripheral axons was made possible by the presence of "neurotrophic substances" in the environment of elongating growth cones, and that the relative inability of central axons to regenerate was due to the absence of these substances in CNS tissue rather than an inherent defect of these neurons. This hypothesis has received furthur confirmation by the recent experiments of Albert Aguayo et al., who have demonstrated that many types of CNS neurons possess the ability to elongate axons for considerable distances when provided with a supportive environment, e.g., a peripheral nerve graft.

In an effort to identify the "neurotrophic substances" which may underlie these phenomena, we raised a panel of monoclonal antibodies against antigens specific to the PNS, by repeatedly tolerizing Balb/c mice to adult rat spinal cord (by concurrent injections with cyclophosphamide) and immunizing with the distal stump of previously transected adult rat sciatic nerve. All of the 15 antibodies that were judged to be "PNS-specific" by immunocytochemistry were tested for their binding to peripheral nerve neurite-promoting activities in an in vitro bioassay which we have previously described (Sandrock and Matthew, Soc. Neurosci. Abstr. 11: 592, 1985). This assay relies on the ability of monoclonal antibodies against putative neurite-promoting molecules to interfere with the outgrowth of sympathetic fibers from rat superior cervical ganglia that have been explanted onto fresh-frozen cryostat sections of sciatic nerve. Of the antibodies tested, only 3 significantly reduced the rate of axonal regeneration in the assay. The inhibition of neurite outgrowth by two of these antibodies, called PC1C5 and PC2A3, was virtually complete.

Both PC1C5 and PC2A3 appear to stain the basal lamina of Schwann cells in peripheral nerve and of myocytes in skeletal muscle. Since the distribution of these antigens was similar to that of laminin, we examined the binding of the antibodies to purified mouse EHS sarcoma laminin, by RIA and ELISA. There was no detectable binding. This experiment, coupled with the finding that these antibodies do not stain any structures in kidney, lead us to conclusion that the antigens are not laminin. The antigens are present in medium conditioned by dissociated cell cultures of mouse sciatic nerve non-neuronal cells, and appears to stain a subset of these cells intracellularly. We are currently characterizing these antigens biochemically and examining their role in axonal regeneration in vivo.

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407.12 ULTRASTRUCTURAL IDENTIFICATION OF SYMPATHOHIPPOCAMPAL AXONS USING ANTEROGRADE TRANSPORT OF WGA-HRP. K.A. Crutcher and C. Marfurt. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132 and Dept. of Anatomy, NW Center for Med. Ed., Indiana Univ. Sch. of Med., Gary, IN 46408.

Loss of septohippocampal neurons results in collateral sprouting of perivascular sympathetic axons into the dentate gyrus and CA3 region of the hippocampus. The only published ultrastructural data on this sprouting response has been limited to the sprouts associated with penetrating blood vessels. Fascicles of sympathetic axons were found to be separated from both the blood vessel and the adjacent neuropil by basal lamina (Crutcher and Chandler, Brain Res., 308:177, '84). In order to identify sympathohippocampal axons not isolated by basal lamina an ultrastructural marker is required.

We used wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) as an anterograde tracer to label sympathetic axons for ultrastructural identification. Sprague-Dawley rats received either a medial septal lesion or a control lesion of the tissue overlying the septum but not resulting in septohippocampal denervation. A 2% solution of WGA-HRP was injected into the superior cervical ganglion 4-8 weeks following the lesion and, in some cases, the ganglion was reinjected with WGA-HRP 24 hours later. Three days following the initial injection the animal was perfused with aldehydes. If successful labeling of sympathetic axons in the iris was obtained, transverse vibratome sections through the dorsal hippocampal formation were reacted for HRP using a modification of the TMB procedure. Following the reaction some hippocampal sections were mounted for light microscopic analysis and others were osmicated, dehydrated and embedded in Spurrs resin for thin sectioning.

HRP-filled fibers were clearly visible at the LM level within the dentate gyrus from the septal-lesioned animals but not in control-lesioned rats. The vast majority of labeled fibers appeared to be associated with the parenchymal vasculature. Electron microscopic examination of unstained thin sections revealed HRP reaction product within axonal and vesicle-filled profiles, all of which, so far, have been found closely associated with blood vessels. Since the majority of HRP-labeled fibers were not separated from the neuropil by basal lamina, the perivascular environment may be more favorable for axonal growth than the surrounding neuropil. Axonal elongation may occur more readily in the perivascular environment due to the presence of basal lamina or to the perivascular accumulation of the hippocampal NGF-like growth-promoting factor (Collins and Crutcher, this meeting). (Supported by NIH grant #NS-17131 and NSF grant #BNS-8501269.)

408.1 COMPARTMENTALIZATION OF ANTEROGRADELY AND RETROGRADELY TRANSPORTED ORGANELLES IN AXONS AND GROWTH CONES FROM CHICK OPTIC TECTUM. T.P.O. Cheng and T.S. Reese, Lab. of Neurobiology, NINCDS, NIH, at the Marine Biological Lab., Woods Hole, MA 02543.

The distribution of various organelles along microtubules in axons and growth cones has been examined in detail on the assumption (Cell, 40:455,'85) that organelles contacting microtubules are engaged in fast axonal transport. Optic tecta from 6½-6½ day old chicken embryos were quick-frozen and then freeze-substituted in acetone-osmium tetroxide (5%). Five axon segments, each ten um long, and four entire growth cones were reconstructed from aligned serial electron micrographs. Approximately 90% of microtubule segments in reconstructed axons, as well as two-thirds of those in growth cones, were contacted by organelles. Most large vacuoles contacted several microtubules in axons, but usually only one microtubule in a growth cone. The presence of microtubule segments which did not contact organelles prompted us to ask whether there is a class of microtubules which never contacts organelles. Analysis of 38 microtubule segments extending through the entire 10 um long axonal segments indicated that the observed distribution of organelles fits better with the Poisson distribution generated by assuming that 90-100% of the microtubules contact organelles than with the distribution generated by assuming that only 75% do. Thus, there are, at the most, only a small number of microtubules in axons which do not contact organelles.

Microtubules typically form fascicles in axons which enclose small vesicles. Large vacuoles lie outside of but also frequently contact these fascicles. Most of the vesicles and vacuoles in axons contact microtubules but most of those in growth cones do not. Microtubule-contacting organelles in axons are evenly distributed along microtubules; in contrast, they are prevalent only in the most proximal domains of growth cones where the fascicular configuration of microtubules is still evident. On the assumption that contact with a microtubule is associated with transport, and that small vesicles mediate anterograde transport while larger vacuoles mediate retrograde transport, it appears that the vesicles in anterograde transport are confined to the inside of microtubule fascicles whereas those in retrograde transport lie outside the fascicles. The vesicles in anterograde transport also appear to be unloaded in a specific domain of the tectal growth cone which is slightly proximal to the mid-segment of the growth cone where vacuoles destined for retrograde transport are loaded onto microtubules. We conclude that there are separate zones along growth cone microtubules where organelles are introduced or removed from fast axonal transport; we propose that organelle binding to microtubules is inhibited by proteins (or other factors) in the distal segment of the growth cone.

408.2 REGENERATION-ASSOCIATED CHANGES IN PHOSPHORYLATION OF OPTIC NERVE PROTEINS IN VIVO. Denis C. Larrivee and Bernice Grafstein. Dept. of Physiology, Cornell University Medical College, New York, NY 10021

Following axotomy, the retinal ganglion cells of the goldfish show greatly increased production of RNA and protein, including a substantial increase in the synthesis of proteins that are conveyed by axonal transport and that are presumably essential for reconstitution of the optic axons. In an investigation of the role of protein phosphorylation in regeneration, normal goldfish and goldfish in which the optic tracts had been sectioned 3 weeks earlier were injected intraocularly with inorganic ^{32}P. Six or 17 hours after the injection the animals were sacrificed, the optic nerves excised, and the nerve proteins separated by 2-dimensional gel electrophoresis. More than 20 proteins, ranging from 19 to 180 kD and pI 4.4-6.8, were seen to be phosphorylated, and many of them increased their incorporation of ^{32}P during regeneration. Five proteins displayed particularly robust increases in labelling. Among these was an acidic (pI 4.5) 45 kD protein, which undergoes a dramatic increase in synthesis during goldfish optic nerve regeneration (Benowitz et al., J. Neurosci. 1: 300, 1981). Three proteins showed decreased uptake of radiophosphate during regeneration, despite significant increases in their incorporation of ^{3}H-proline.

In order to identify proteins that could become phosphorylated along the course of the optic nerve, excised nerves were incubated in ^{32}P-containing medium. Under these conditions, there was little if any labelling of the 5 proteins that had shown a large regeneration-associated increase in ^{32}P incorporation following intraocular injection. Thus these proteins are phosphorylated in the retinal ganglion cell bodies prior to their entry into the axons.

Both regenerating and normal nerves incubated with ^{32}P showed strong labelling of intermediate filament proteins and a number of other proteins, ranging from 50 to 58 kD and pI 4.9-6.4. If the axons were caused to degenerate by removing the retina one week before the incubation, labelling of these proteins was abolished, although active protein synthesis in the glial cells still continued. This suggests that phosphorylation of these proteins is dependent upon the integrity of the optic axons.

This research was supported by NIH Research Grant NS-14967 and Training Grant NS-07138.

408.3 FAST AXONAL TRANSPORT IN EXPERIMENTAL DIABETIC POLINEUROPATHY. ANALYSIS OF INDIVIDUAL POLYPEPTIDES. R. Medori*, L. Autilio Gambetti, H. Jenich* and P. Gambetti. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106.

In previous studies we observed that slow transport is not uniformly impaired in the BB rat, a model of genetically determined diabetes. Transport of major polypeptides migrating with the component b of the slow transport (SCb) is slowed down about 40%, whereas that of polypeptides migrating with the component a (SCa) is slowed down 20%.

We now examined transport of major polypeptides migrating with the fast component of axonal transport. BB rats were injected in the lumbar spinal cord with 35S-methionine two months after onset of diabetes. Animals were sacrificed six hours later. The sciatic system was cut into 5 mm segments which were processed individually for 1 and 2 dimension gel electrophoresis and fluorography. Polypeptides of approximately 147, 107, 58, 53, 43 and 21 Kd were examined. The distribution of radioactivity associated with these polypeptides along the sciatic system was determined by densitometry of the fluorograms. In controls the peak of radioactivity related to these polypeptides was at approximately 55 mm from the spinal cord while in diabetic animals the peak was at 45 mm from the cord.

It is concluded that fast transport is delayed in the BB rat and that the impairment of the polypeptides migrating with the fast transport is uniform affecting different polypeptides to the same extent.

Our studies show that all types of anterograde transport are affected in this model of genetically determined diabetes. The SCb is the most severely affected whereas the fast component and the SCa are less impaired. It is therefore likely that the impairment of SCb plays a major role in the degeneration of the axons which in this animal model takes place at advanced stages of diabetes. (Supported by grants from the Diabetes Association of Greater Cleveland and NS #14509)

408.4 ACRYLAMIDE INHIBITS RETROGRADE TRANSPORT IN HEN SCIATIC NERVE. M.I. Sabri, A. Moretto* and Spencer, P.S. Institute of Neurotoxicology, Albert Einstein College of Medicine, Bronx, NY 10461

Repeated exposure of animals to the neurotoxin acrylamide results in hindlimb paralysis associated with distal axonal degeneration of affected nerves. The biochemical mechanism underlying axonal degeneration is unknown. Recent studies from our laboratory have shown that single injections of acrylamide result in a dose-dependent impairment of retrograde transport (RT) in the sciatic nerve of rodents within hours of dosing. Repeated daily doses of acrylamide led to a progressive reduction in transported material and distal axonal degeneration. We have extended this research in an avian species to determine whether acrylamide produces RT deficits prior to axonal degeneration. Adult white Leghorn hens (1.5 -1.8 kg) were given a single injection of acrylamide (100 mg/kg; i.p.) and RT of ^{125}I-tetanus toxin was studied in sensory and motor neurons following the injection of ^{125}I-tetanus toxin (10 uCi) in the gastrocnemius muscle. Acrylamide (100 mg/kg) did not reduce RT as measured by the accumulation of ^{125}I-tetanus toxin in dorsal root ganglia and ventral spinal cord 35 h after dosing. This suggested either that acrylamide has no effect on RT in chicken neurons or that the effect of acrylamide lasts only a few hours after dosing. To investigate the latter, we studied the action of acrylamide on RT at 3, 6.5 and 48 hours after dosing. One hour before the injection of acrylamide (100 mg/kg i.p.) or saline, ^{125}I-tetanus toxin was injected into the gastrocnemius muscle and the rate of RT was determined by the position of the leading edge of ^{125}I in the sciatic nerve measured at 4 h and 7.5 h after radiolabel injection. Acrylamide virtually blocked RT without altering the uptake of radiolabel in the nerve. However, when the radiolabel was injected 48 hours after dosing, the position of the leading edge of retrogradely transported ^{125}I-tetanus toxin was unaltered compared to saline treated animals. These data show that a single injection of acrylamide impairs the RT initially and that RT returns to normal velocity within 48 hours. Repeated disruption of RT appears to be associated with the induction of axonal degeneration.

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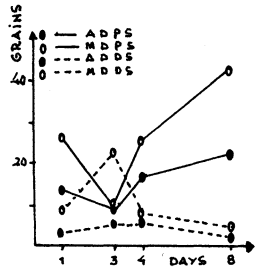
408.5 LOCAL NERVE PROTEIN SYNTHESIS DURING EARLY NEUROMA DEVELOPMENT AND WALLERIAN DEGENERATION. J.R.Sotelo Jr., R. Correa-Luna* and C. Benech*. Biophysics Division. Inst. I. Biol. Clemente Estable. Montevideo-Uruguay.

Local Nerve Protein Synthesis (LNPS) was studied in the Proximal Stump (PS) and Distal Stump (DS) of sectioned rat sciatic nerves. Proteins were labeled with a tritiated aminoacid pool (3H-glycine, 3H-leucine, 3H-phenylalanine, 3H-aspartic acid and 3H-lysine) 50 µCi in each nerve. Aminoacid pool was supplied simultaneously to both PS and DS of the nerves -which were crushed and ligated previously 4 cm away from the DRG - as a solution (50µCi/50µl) filling a trough of polyethilene placed around each nerve and sealed in both ends with silicone grease. Labeling was made for 30 min and after 23h and 30 min, 3 days, 4 days and 8 days after crushing. When labeling was accomplished both stumps were excized and processed for light microscope autoradiography as it has been described elsewhere (Exp. Neurol. 76:72-82, 1982), but having the special care that both stumps were not separated until washing and dehydration was not accomplished. Autoradiographies has been quantitized using a closed TV circuit attached to the microscope and an X-Y plain digitizer connected to DEC PDP-11 computer. Grain density per 100 µm2 has been calculated in myelin and axoplasm of both stumps and promediated. Mean grain density of Myelin (MD) and axoplasm (AD) of DS were always lower than in PS, but allways significant different from background. In both stumps MD was greater than AD. As it is shown in the figure, evolution of protein synthesis in PS is quite different than in DS; since 3rd day, protein synthesis increases (beginning of outgrowth), while in DS slowly disminish. Activity is not due to soluble contaminants since PS and DS were washed together. Grain density diminution in the fibers of DS suggests that LNPS depends on the connection to the neuronal soma. Supported by OAS & MEyC



408.6 ABSOLUTE SPECIFICITY FOR RETROGRADE FAST AXONAL TRANSPORT DISPLAYED BY LIPID DROPLETS ORIGINATING IN THE AXON OF AN IDENTIFIED APLYSIA NEURON IN VITRO. M.J. Savage*, D.J. Goldberg and S. Schacher. (SPON:E. Shapiro). Department of Pharmacology and Center for Neurobiology and Behavior, Columbia University College of P&S, New York City, NY 10032.

An organelle moving in an axon by fast axonal transport travels essentially in one direction. How the organelle comes to acquire this directional specificity is not understood. Because organelles originating in the cell body travel anterogradely and those originating, or modified, in the axon ending travel retrogradely, it is attractive to think, and has been suggested, that directionality is conferred on organelles at these sites. It would be interesting to see if an organelle originating neither in the cell body nor the axon ending, but in the axon, would demonstrate a directional specificity. Using video-enhanced contrast-differential interference contrast microscopy to study organelle movement and development in the giant cerebral neuron (GCN) of Aplysia californica in culture, we have identified such an organelle and have studied its transport characteristics. Spherical, membranated organelles up to 3um in diameter developed in large numbers simultaneously throughout the axon of the GCN growing in culture. These were lipid droplets, since they fluoresced in the presence of nile red and appeared dark in electron micrographs after fixation with glutaraldehyde and osmium tetroxide. Eighty instances of retrograde fast axonal transport were observed for lipid droplets whose diameter ranged from 0.3-1.4um. These droplets exhibited an absolute directional specificity for retrograde transport since not one instance of anterograde movement was observed. They are the largest spherical organelles reported to move by fast axonal transport.

Clearly, an organelle need neither originate nor be modified in the axon ending to be specified for retrograde transport. It is possible that the cell body is the only site important in conferring directionality, with organelles there being coded for anterograde transport. Retrograde transport may occur simply because of the absence of this coding. Alternatively, the site of origin could be insignificant in determining directionality, with organelle size being the critical factor, small organelles moving anterogradely and large organelles retrogradely.

DEVELOPMENT AND PLASTICITY: AGING III

409.1 AGE-RELATED CHANGES IN LH PULSE AMPLITUDE AND FREQUENCY AFTER CASTRATION: EFFECT OF LONG-TERM OVARIECTOMY, BREEDING AND NALOXONE. P.M. Wise, E.M. Dükerx, W. Wuttkex. Dept. of Physiol., U. of MD, School of Medicine, Baltimore, MD 21201 and Frauenklinik, Abteilung Klin. u. Exptl. Endokrinol., U. Göttingen, D3400 Göttingen, FRG

Long-term ovariectomy and multiple pregnancies delay age-related deterioration of reproductive potential in rodents. Long-term ovariectomy extends the period during which older rats can respond positively to estradiol. Rats that have been breeders exhibit estrous cycles and are fertile longer than virgins. We tested whether long-term ovariectomy and multiple pregnancies decelerated age-related decreases in the frequency and amplitude of LH pulses observed after ovariectomy.

We determined LH pulse amplitude and frequency in 4 groups of ovariectomized (OVX) rats: (1) young (3-4 mo.) virgin short-term OVX (ST-OVX), (2) old (18-22 mo.) virgin LT-OVX, (3) old virgin long-term OVX (LT-OVX) and (4) old retired breeder ST-OVX rats. ST-OVX rats were OVX 2 wks. prior to use; LT-OVX rats were OVX at 4 mo. of age, approximately 14-18 mo. prior to the experiment. Retired breeders were used as breeders by the commercial supplier from 2 to 10 mo. of age and then were maintained in our animal facility until they were used. Rats were implanted with right atrial cannulae and the next day were bled at 10 min. intervals for 3 h. To test whether pulsatility of LH could be restored by suppressing the inhibitory effects of opiates, rats were bled for 1 h and then treated with naloxone (2 or 10 mg/kg, i.v.) and bled for an additional 3 h.

A pulsatile pattern of LH secretion was observed in all young ST-OVX rats: mean interpulse interval was 40.8±3.46 min and mean pulse amplitude was 2.91±0.29 ng/ml. In contrast, old virgin ST-OVX rats exhibited lower basal LH concentrations and 61% exhibited no pulses during the entire 3 h interval. In those old virgin ST-OVX that did exhibit LH pulses, the interpulse interval increased 4-fold and pulse amplitude decreased 55% compared to young rats. LT-OVX and breeding restored basal LH concentrations to those observed in young rats, significantly decreased interpulse interval and increased pulse amplitude compared to old virgin ST-OVX rats. Although naloxone stimulated LH transiently in all groups of rats, it did not restore a pulsatile pattern of secretion in old virgin ST-OVX nor further improve the LH secretion pattern in old LT-OVX or old ST-OVX retired breeders.

These data indicate that age-related changes in the pattern of castration-induced LH secretion can be attenuated by long-term ovariectomy and breeding, and that these changes are not regulated by an opiate mechanism. The results suggest that long-term exposure to cyclic increases in estradiol may deleteriously affect the neural "pulse generator".

409.2 AGING CHANGES IN INNERVATION OF LHRH NEURONS IN MALE RAT PREOPTIC AREA. J.W. Witkin. Dept. of Anatomy & Cell Biology, Col. Univ. Coll. P & S. New York, NY 10032.

The number and distribution of and synaptic input to immunocytochemically identified luteinizing hormone-releasing hormone (LHRH) neurons and their processes in the male rat brain preoptic area were compared in young adult (2-4 mos) and middle aged (12-14 mos) animals. At the light microscopic level there were no age dependent differences in numbers of neurons nor in their distribution in the brain. At the electron microscopic level the distribution of reaction product within the neurons was similar in all respects. The synaptic input to LHRH neurons and nonidentified neurons in the same tissue blocks was measured and reported as percent of membrane that showed synaptic modification. Dendrites were more densely innervated than perikarya of both LHRH and nonidentified neurons. Synaptic input to LHRH perikaryal and dendritic membrane was one fifth that to nonidentified elements. Synaptic input to LHRH perikaryal membrane was three times as dense in the middle aged as young adult sample, while synaptic input to LHRH dendritic membrane did not change with age. There were no aging changes in percentage of membrane with synaptic modification in nonidentified elements. Synapses were also classified on the basis of their synaptic vesicle content. There were proportionately more synaptic boutons containing round clear than pleomorphic vesicles in the young sample. The proportion of synapses with pleomorphic vesicles increased with age onto both LHRH and nonidentified perikarya. The proportion of synapses containing electron dense-core vesicles decreased slightly with age onto both LHRH and nonidentified neurons.
Supported by USPHS AG 05366

409.3 NORADRENERGIC MARKERS, NEUROPEPTIDE-Y AND ENKEPHALINS IN AGING HUMAN SYMPATHETIC GANGLION. A.L.J. Hervonen, R. Järvi*, Jari Koistinaho* and Pauli Helen. Laboratory of Gerontology, University of Tampere, P.O. Box 607, SF 33101 Tampere, Finland.

The sympathetic ganglia provide a useful model for studying aging in the nervous system. The homogenous population of adrenergic neurons shows well defined age-related changes: Decline of cytoplasmic catecholamine stores, compensatory increase of tyrosine hydroxylase activity, accumulation of lipopigments and neuromelanin in the cytoplasm. Aging in the sympathetic ganglia is associated with increasing heterogeneity: Some neurons accumulate signs of oxidative damage and loose the noradrenergic markers sooner than others.

The purpose of the present study was to correlate the lipopigment accumulation and tyrosine hydroxylase immunoreactivity with the Neuropeptide-Y-like- and Enkephalin-like-(NPYL and ENKL) immunoreactivities. The stellate ganglia were obtained from both operations and from cadavers. The age range was from 16 to 92 years. The peroxidase antiperoxidase double staining was used.

Number of neurons innervated by enkephalin containing nerves declined with age. The neurons in contact with ENKL-nerves were larger and accumulated less lipopigments. The ENKL-innervated neurons were devoid of tyrosine hydroxylase immunoreactivity. NPYL immunoreactivity was restricted only to cells also showing tyrosine hydroxylase immunoreactivity.

ENKL-immunoreactivity seems to be associated with a subpopulation of neurons which loose the tyrosine hydroxylase activity early but show wery little age pigment accumulation. On the other hand, neurons with both NPYL- and tyrosine hydroxylase immunoreactivity showed heavy loading with lipopigments. This pigment shares the histochemical properties of neuromelanin.

Two main conclusions were drawn: First, higher level of metabolic activity, as shown by tyrosine hydroxylase and NPYL-immunoreactivity leads to enhanced accumulation of neuromelanin type lipopigments. Second, ENKL-innervated neurons show less degenerative changes, which may be due to the enkephalin mediated inhibition of cellular metabolism or trophic effects of the peptide.

The neuromelanin type of lipopigment derives from catecholamines. It is possible that the toxic effects of some intermediates of melanin have caused a compensatory burst of tyrosine hydroxylase activity which ends up with loss of compensatory reserves and neuronal death.

409.4 BIOCHEMICAL FUNCTIONAL EXPRESSION OF DOPAMINE D_2 RECEPTORS IS NOT MODIFIED BY AGING. A.Valerio*, M.Memo, C.Missale, M.Pizzi*, L.Castelletti*, L.Trivelli*, S.Govoni, M.Trabucchi, P.F.Spano. Inst Pharmacol., Sch. of Med., Univ. of Brescia, Italy and Inst. of Pharmacol. Univ. of Milano and Roma, Italy.

A large body of data have shown age-related decrease in various dopaminergic (DA) features. Radioreceptor binding studies in vitro in both animal and human tissue have revealed a decline in D_2 receptor concentration with age, with no change in the dissociation constant. Quite recently, the significance of these data has been enphasized by studies showing that a decrease in the number of DA receptors with age can be demonstrated in the living human brain through positron emission tomography after i.v. injection of ^{11}C-3, N-methyl-spiperone. However, the drowback of this approach is that in vitro or in vivo receptor measurement does not reflect functioning of the receptor itself. In our laboratory, we first found that D_1 DA receptors, as measured by stimulation of adenylate cyclase (AC) activity, are markedly reduced in various DA brain regions of old animals. In this study we evaluated a direct index of functional expression of D_2 DA receptors in young (3-4 months) and old (20 24 months) rats. D_2 DA receptors were studied by measuring the inhibitory action of DA on basal AC activity in neostriatum and the inhibitory activity of DA on VIP-stimulated AC activity in the pituitary. The cyclic AMP generating system in striatal homogenates is stimulated by DA and other DA agonists, giving an index of D_1 receptor function. However, when SCH 23390, a specific D_1 antagonist is present in the incubation medium, DA and other DA agonists dose-dependently inhibit AC activity. This action has been pharmacologically characterized as a stimulation of D_2 DA receptors. We found that DA and bromocriptine posses the same potency in inhibiting cyclic AMP formation in striatal homogenates of young and old rats. The IC_{50} and V_{max} values of DA for this effect were virtually superimposable for young and old animals. On the contrary, DA inhibited VIP-stimulated AC activity to a greater extent in pituitary membranes from old rats than in those from young animals. These results indicate that D_2 DA receptors in the pituitary became supersensitive. The finding that D_2 DA receptor function is unmodified during aging has to be recounciled with the data showing decreased number of D_2 binding sites.

409.5 PROTEIN PHOSPHORYLATION DURING AGING IN DOPAMINERGIC RAT BRAIN AREAS. S. Govoni, R.A. Rius,* F. Battaini, P.F. Spano and M. Trabucchi . Institute of Pharmacological Sciences, University of Milan, Department of Pharmacology and Therapeutics, University of Brescia and Chair of Toxicology, 2nd University of Rome, Roma Tor Vergata, Italy.

The present study investigates the effect of aging on cyclic AMP dependent phosphorylation of phosphoproteins specifically associated with dopaminergic nerve terminals.

Male Sprague Dawley rats (Crl:COBS CD (SD) BR) aged 3 and 24 months were obtained from Charles River (Calco, Italy). Particulate and cytosolic fractions of striatum (S) and nucleus accumbens (N.A.) were prepared and the phosphorylation of endogenous proteins measured according to Walaas et al. (J. Neurosci. 4:84, 1984). SDS-polyacrylamide gel electrophoresis was carried out as detailed by Krueger et al. (J. Biol. Chem. 252:2764, 1977). In the particulate fractions of both S and N.A. of aged rats cAMP stimulated ^{32}P incorporation was less in the bands corresponding to a MW of 80 (S: -37%, N.A.: -26%), 60 (S: -24%, N.A.: -26%) and 55 (S: -23%, N.A.: -25%) K daltons.

A reduced cAMP-stimulated ^{32}P incorporation was observed also in several protein bands of the cytosolic fractions prepared from S and N.A. of aged rats. In particular a significant decrease was observed in the protein bands corresponding to the following molecular weights: 130 K, 98 K, 81 K, 55 K, 50 K, 45 K, 32 K.

Complexively the results indicate that the cAMP dependent phosphorylation of several protein bands is reduced in S and N.A. of aged rats.

The decreased phosphorylation of the 80 K band in the particulate fraction may be relevant to the age related changes in neurotransmission since this protein band represents synapsin 1 which is associated and regulates synaptic vesicles traffic.

The 32 K phosphoprotein in the soluble fraction is DARPP-32, which is specific for dopaminoceptive neurons and is linked to dopamine D_1 receptors. The decreased phosphorylation of this protein band further stress the concept that the effector systems linked to dopamine D_1 receptors present an age-related deficit.

409.6 AGING OF THE CHOLINERGIC SYSTEM: COMPARATIVE AUTORADIOGRAPHY OF MUSCARINIC RECEPTORS IN YOUNG AND AGED RAT BRAINS. R. Duvdevani*, V. Greenberger*, M. Segal* and A. Biegon (SPON: A. Mayevsky). Weizmann Institute of Science, Rehovot, Israel.

Cognitive deficits associated with aging may be the result of changes in cholinergic transmission in the brain. One approach to testing this hypothesis is to correlate cholinergic markers in young and old animals with cognitive function. Cholinergic muscarinic receptor binding in 66 discrete brain regions and nuclei was examined in two groups of young and aged rats, by quantitative autoradiography of tritiated quinuclidinyl benzilate (^{3}H-QNB). The subjects were 7 young (8 months) and 7 old (24-33 months) hooded rats, and 8 young (4 months) and 8 old (31 months) Wistar rats. Animals of both strains were first run in an open field and a water maze, then sacrificed and their brains processed for quantitative autoradiography of muscarinic receptors (Rainbow et al., J. Neurosci. Methods 5: 127, 1982). Cryostat sections were labeled with lnM ^{3}H-QNB and non specific binding was defined in the presence of excess atropin. Tiritum microscales (Amersham) were used to convert film density to relevant concentration units, and the autoradiograms were analyzed by a video-camera based computerized image analysis system. Cholinergic muscarinic binding was reduced by up to 60% in specific regions of the aged rat brain. The most significant reductions were found in the medial septum, substantia innominata, nucleus basalis magnocellularis, ventral pallidum, horizontal limb of the diagonal band and caudate nucleus. Significant reductions were also found in the anterior cingulate cortex, frontoparietal-motor cortex and the locus coeruleus. These reductions are found in both strains. No changes were found in other areas sampled, e.g. thalamic and hypothalamic nuclei, parts of the hippocampal formation, striate cortex, amygdaloid nuclei, substantia nigra and cerebellum. These findings are corelated with the results of acetylcholinesterase histochemistry and behavioral tests performed on the same animals.

409.7 DEFICITS IN TRANSMITTER PHENOTYPIC PLASTICITY IN AGED SYMPATHETIC NEURONS. J.E. Adler and I.B. Black. Division of Devel. Neurology, Cornell Univ. Med. Coll., N.Y., NY 10021.

We have previously reported that striking transmitter plasticity occurs in adult rat sympathetic ganglia, but fails during senescence: the 10-fold increase in substance P (SP) consequent to explantation of adult ganglia to culture is absent in aged ganglia. To ascertain whether deficits are intrinsic to senescent ganglia, and whether other functions are affected, we examined the scope of the deficiency. To determine whether aged neurons have lost normal responsiveness to trans-synaptic signals, aged (2 yr.) and adult (6 mo.) rats were subjected to unilateral decentralization (denervation) of the superior cervical ganglion (SCG). Surgery elicited the normal, 2-fold rise of SP in adult ganglia, but failed to elevate peptide in aged ganglia, suggesting that deficits are a) intrinsic to aged neurons in vivo, and b) not peculiarities of culture. Further, the deficiency in vitro did not simply reflect delayed responsiveness, since prolonged culture for 3 wks. did not evoke a rise in aged ganglion SP, while control, adult ganglia maintained high levels of peptide.

To determine whether faulty plasticity affected other transmitter characters, we examined ganglion somatostatin (SS), tyrosine hydroxylase (TH) and choline acetyltransferase (CAT). SS, another SCG peptide is also regulated by trans-synaptic stimuli. Upon explantation, SS in adult ganglia behaved similarly to SP, increasing markedly during the first week before attaining plateau levels. In aged ganglia, however, the SS response was only 50% of control, suggesting that deficient regulation extends to other neuropeptides. In marked contrast, catalytic activity of TH and CAT responded identically in aged and adult ganglia. Further, phase microscopy demonstrated that morphologic characteristics, such as neurite extension were identical in cultures of adult and aged ganglia. Our studies suggest that aged neurons are specifically deficient in peptidergic but not adrenergic or cholinergic transmitter regulation. We are currently defining the defective molecular mechanisms underlying the loss of peptidergic plasticity. (Supported by NIH Grant B.R.S.G. S07 RR05396 and Grants from the Hereditary Disease Foundation, American Federation for Aging Research and Alzheimer's Disease and Related Disorders Assoc.).

409.8 LOCUS COERULEUS: COMPUTERIZED 3-DIMENSIONAL ANALYSIS OF DEGENERATION IN ALZHEIMER'S AND DOWN'S DISEASES. V.M.Ingram, H.M.Moore*, J.H.Koenig*, D.E.Parry* and C.H.Miller*, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

The locus coeruleus is one of several areas of the brain suffering very severe neuronal degeneration in advanced Alzheimer's disease (AD). The beginning and the progress of the degenerative changes in different parts of the locus coeruleus and in the other affected areas are unknown. Every Down's syndrome (DS) individual over the age of 35 years shows similar and very severe degenerative changes; the changes are also in the locus coeruleus and the other affected areas. We have begun a longitudinal study of the degenerating locus coeruleus in Down's brains of different ages, using this condition as a putative model for the development of Alzheimer's disease. The locus coeruleus was chosen because its functional complexity and widespread efferent fields, as well as its clear involvement in the disease process, leads one to expect this group of neurons to play key roles in AD etiology.

Autopsy material, fixed in formalin or paraformaldehyde, was blocked to contain the part of the brainstem which includes the locus coeruleus. Serial frozen coronal sections were mounted and stained with a monoclonal antibody to tyrosine hydroxylase or with cresyl violet or with hematoxylin/eosin. The monoclonal antibody revealed very clearly the noradrenergic neurons of the locus coeruleus and the subcoeruleus; in addition noradrenergic neurons of the lateral tegmentum are identified. In their distinct and characteristic locations, the dopaminergic neurons of the substantia nigra and of the ventral tegmentum are also seen with the same antibody. Reconstruction of loci coerulei from different specimens is from outlines of individual cells and of nuclei using the three-dimensional software programs of the Zeiss Videoplan II or the R & M Biometrics Bioquant. In either system pseudorotation of the structures allows one to localize and compare the degenerated portions of the loci coerulei dramatically and clearly. For example, an initial comparison of the locus coeruleus of a 64-year old DS brain with that of a 73-year old normal brain shows striking [>90 %] loss of neurons in certain parts of the structure. The analysis of these and other specimens continues.
[Supported by grants MOD 1-909 and USPHS AG04792.]

409.9 EFFECT OF AGE AND GENDER ON RESTING AND VISUALLY ACTIVATED CEREBRAL GLUCOSE METABOLISM. F. Yoshii*, W.W. Barker*, J.Y. Chang*, A. Apicella*, M. Ginsberg, A. Emran* and R. Duara. Mt. Sinai Medical Ctr., Miami Beach, FL 33141 and Univ of Miami School of Medicine, Miami, FL 33101.

Resting and visually activated cerebral glucose metabolic rates (rCMRglc) in mg/100g/min were measured by PET using [F-18] fluorodeoxyglucose (FDG). Following the injection of FDG, subjects remained awake with eyes covered for 30 min. and were then scanned. Overall, CMRglc was 6.22 ± 1.53 in 16 resting males and 8.08 ± 1.98 in 18 resting females ($p = 0.01$). CMRglc tended to decrease with age in females ($r = -0.46$, $p = 0.09$) but not in males ($r = -0.27$, $p = 0.30$). Metabolic rates normalized to the sensori-motor cortices (which were used as reference regions in these studies) showed reduction with age in the right and left prefrontal regions in males and females ($r = -0.48$ $p = 0.004$; $r = -0.41$, $p = 0.015$, respectively) and increases with age in the right and left occipital regions ($r = 0.42$ $p = 0.013$; $r = 0.37$ $p = 0.032$, respectively).

In 14 females and 5 males FDG/PET studies were performed in visually activated states while viewing colored pictures. Normalized values were used to compare young and elderly resting and visually activated subjects. In 12 young visually activated subjects (age: 46.8 ± 8.7 yrs.) compared to 16 young resting subjects (age 38.6 ± 12.5 yrs.) significant ($p < 0.05$) increases were found in the right and left occipital lobes (10% and 9%), the right superior temporal region (10%) and the left medial temporal region (13%). In 7 elderly visually activated subjects (age: 67.4 ± 2.9 yrs.) compared to 18 elderly resting subjects (age 69.7 ± 5.8 yrs.) no significant increases were found in any brain region but a trend ($p < 0.10$) to increase was found in right and left prefrontal (6% and 7%), right orbito-frontal (11%), left occipital (10%), left superior parietal (6%) and right inferior parietal (8%) regions. In the entire group of visually activated subjects increases with age in the left superior parietal ($r = 0.51$, $p = 0.02$) and in the left inferior parietal ($r = 0.51$, $p = 0.02$) regions were found.

Our findings show that females have 25-30% higher CMRglc values than males. Overall, in the resting state the prefrontal regions show reduction in normalized values with increasing age and this is accompanied by increases in the occipital regions. Visual activation produces increases in normalized values in the occipital and temporal regions in young subjects but no significant effect in elderly subjects who show a trend towards a more diffuse pattern of activation. The absence of prefrontal reduction in the elderly in the visually activated state is also notable.

409.10 BLOOD BRAIN BARRIER (BBB) CHOLINE TRANSPORT IN AGED RATS. A.D. Mooradian*, (SPON: Jerome Siegel), GRECC, VA Medical Center, Sepulveda, CA 91343

In vivo choline is transported from plasma to the brain mostly via a carrier mediated transport mechanism. Since the brain cholinergic system has been implicated in the pathophysiology of diseases commonly affecting the elderly, we were interested in determining the effect of aging on the kinetics of BBB choline transport. The carotid artery single injection technique of Oldendorf was used to measure the brain uptake index (BUI) of choline in the forebrain of Fischer 344 rats at different choline concentrations. ^{3}H tryptamine was used as the internal reference substance. In the young (2 month old) rats anesthetized with pentobarbital (50 mg/kg), the cerebral blood flow as determined from the efflux rate of $^{3}H_2O$ was found to be 0.67 ml/min/gm. The saturable uptake of choline in this model was characterized with a single kinetic system with a mean Km of 69 uM and Vmax of 1.4 nmol/min/gm. The Kd was 5 ul/min/gm.

Young rats were compared to old (24 month old) for choline BUI at three different concentrations of choline. BUI (%) results are expressed as mean ± SEM.

Choline conc. (mM)		Young	Old		P
0.028	(n=6)	24.3 ± 2.4	27.3 ± 4.6	(n=3)	N.S.
0.128	(n=6)	21.9 ± 2.9	8.1 ± 2.5	(n=3)	<0.025
0.528	(n=5)	15.4 ± 2.4	4.6 ± 2.9	(n=3)	<0.05

These results suggest that: (1) the kinetics of BBB choline transport is substantially altered in the aged rat; and (2) the high degree of variability in choline BUI of aged rats may reflect the variability in the effect of aging on BBB transport kinetics.

409.PO SPARING OF FUNCTION AFTER BILATERAL HEMISECTIONS OF THE MID-THORACIC SPINAL CORD IN NEONATAL RATS IS NOT DUE TO DESCENDING CONNECTIONS. J.M. Cullen* and D.J. Stelzner (SPON: M.J. Lyon) Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

Developing axons from various regions of the mammalian CNS often grow around a lesion placed in a normal pathway by taking an aberrant route through intact neural tissue to reach normal target areas. Such anomalous growth sometimes underlies the sparing of function seen after injury to the developing CNS. In the present study, opposite-sided spinal hemisections were made in the mid-thoracic spinal cord of rats (N=36) less than 24 hrs. old. Hemi-sections overlapped slightly at the midline and were separated by one cord segment. When mature, all rats exhibited supportive, locomotor and placing reactions, behaviors not observed in rats given opposite-sided hemisections at one month of age (Anat. Rec. 214: 27A, 1986). To see whether the behavior of the neonatal operates might be related to anomalous growth around the lesions, 8 animals received a complete spinal transection two segments rostral to the neonatal injury at one month of age. Immediately after the transection, supportive, placing and locomotor responses were seen in the hindlimbs of all rats. Function returned to pretransection levels over the 5 day postoperative testing period in 6 of 8 cases. The re-emergence of these behaviors strongly suggests that axonal growth or regrowth around the neonatal injury did not contribute to the sparing of function seen in the neonatal operates. In addition, Fink-Heimer staining of degenerating axons 5 days after transection gave little evidence of descending axonal growth past the caudal neonatal injury site, even though viable spinal cord remained between lesions and degenerating axons filled this zone. Controls for degeneration staining showed no degeneration in the cord of 35 day old neonatal operates (N=4) who did not receive the spinal transection, while exuberant degeneration appeared distal to a spinal transection in a second group of controls (N=4) who did not have neonatal lesions. These findings are presently being confirmed in the remaining neonatal operates using the tracers HRP and tritiated proline. The sparing of function in newborn operates is likely to be associated with intrinsic properties of the spinal cord caudal to the neonatal lesion sites, comparable to the sparing seen after complete spinal cord transection in the neonatal rat. This work was supported by NINCDS grants NS07539 (JMC), NS14096 (DJS) and by a grant from the Eastern Paralyzed Veterans (JMC).

409.12 NEUROFILAMENTOUS SWELLINGS IN PROXIMAL AXONS OF PURKINJE CELLS INDUCED BY A THIOL-PROTEINASE INHIBITOR. G.O. Ivy, J.T. Do*, M. Baudry and G. Lynch. Center for the Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92717.

We have previously shown that infusion of high concentrations of leupeptin, a thiol proteinase inhibitor into brains of young rats, induces the accumulation of intracellular dense bodies that morphologically and histochemically resemble lipofuscin, a hallmark of aging and certain disease states (Ivy et al., Science, 226:985, 1984). Since changes in neurofilaments (NF) are another prominent feature of aging and of various pathological conditions, we decided to investigate the possibility that leupeptin can elicit changes in NF.

Young adult rats were implanted with leupeptin (n=7) or saline (n=5) filled osmotic minipumps connected to the lateral cerebral ventricle. The rats were sacrificed by decapitation after 2 to 10 weeks of infusion and their brains removed and quickly frozen. Sections were processed for immunocytochemistry using antibodies to NF 68kd or NF 200kd (Boehringer-Mannheim). Additional sections were stained with toluidine blue or the PAS method.

In cerebellum, both antibodies decorated primarily basket cell terminals around Purkinje (Pk) perikarya and various axons of the inner molecular layer, granule cell zone and white matter. However, in leupeptin treated rats, the proximal axons of many Pk neurons contained large varicosities that were recognized by both antigens. These axonal swellings were often elongated and contained one or more bulbous protrusions; they strongly resembled those that form in Amyotrophic Lateral Sclerosis. The perikarya of these affected cells, as well as the basket terminals around them, also stained more densely with both antibodies than did those of Pk cells that did not have swollen axons or Pk cells in control rats. In rats treated with leupeptin for the longest periods tested both the dendrites of Pk cells and axons of the inner molecular layer were more prominently labelled by the NF antibodies than were those of control rats. Also, while some folia were denuded of Pk cells, as determined by Nissl stains, the basket cell terminals in these areas were still anti-NF positive.

There are a number of possible explanatons for the effects described here. Calcium activated thiol proteinases (calpains) are among the targets of leupeptin and these enzymes are known to degrade NF proteins. Conceivably then, inhibition of calpain activity could disturb the turnover equation for the NFs, leading eventually to an accumulation too great to be processed by the axonal transport system. Possibly related to this is the recent finding that ventricular infusion of leupeptin causes a selective increase in spectrin (also a substrate for calpain) and the NF triplet (Siman et al, submitted). In any event, these results reinforce our previous suggestion that perturbations in proteinase activity result in some of the most prominent hallmarks of aging and certain disease states. Supported by NIA grant #AG00538.

ACTION POTENTIALS AND ION CHANNELS XI

410.1 WHAT REALLY DETERMINES THE RESTING POTENTIAL OF THE NERVE CELL? D. C. Chang. Dept. of Physiol. of Mol. Biophysl, Baylor Coll. of Med., Houston, Tx 77030.

It is generally assumed that the resting potential of a nerve cell is determined by a mixture of chemical potentials of K+ and Na+, as described by the Goldman - Hodgkin - Katz (GHK) equation. Using the squid giant axon as a biological model, we studied how the resting potential (V) varied as a function of internal and external ionic concentrations. We found that, although the [K]o - dependence of V under high external Na could be fitted with the GHK equation, this fitting was fortuitous. When one reduced the external Na+, the GHK equation could no longer describe the [K]o - dependence of the resting potential. Furthermore, the GHK equation failed to describe the effects of [K]i and [Na]o on V under the condition of low external K+ concentration.

One possible explanation for the failure of the GHK equation is that the assumption of equilibrium between the K+ activities at the outer surface of the membrane and in the external solution may be violated. By modifying this assumption, we are able to derive a new electrodiffusion equation which varies slightly from the GHK equation. This modified equation appears to describe the effects of [K]o, [K]i, [Na]o and [Na]i on V satisfactorily.

Our study suggests that the nerve cell has a mechanism of regulating its resting potential. The membrane acts as a potential buffer, which automatically limits the membrane potential to a pre-set value. The K+ gradient is required mainly to charge the membrane capacitor. Once the chemical potential of K+ exceeds the pre-set membrane potential, the resting potential becomes self-regulated and is no longer sensitive to the variation of internal or external concentrations of K+.

(Work supported partially by ONR contract N00014-85-K-0424 and NSF grant BNS-84-06932)

410.2 INFERRED INTERNAL STRUCTURE OF THE ACETYLCHOLINE RECEPTOR PORE John A. Dani. Section of Molecular Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Functional nicotinic acetylcholine receptors (AChRs) are composed of five glycosylated polypeptide subunits in a molar stoichiometry of $\alpha_2\beta\gamma\delta$. All of the AChR subunits have been cloned and the amino acid sequences used to model the subunits' organization into a transmembrane structure (Guy, Biophys. J. 45:249-261, 1884; Finer-Moore & Stroud, PNAS USA 81:155-159, 1984). The models propose that transmembrane helices serve as staves of a barrel to form a hydrophilic channel lined with polar or charged amino acid side chains. The receptor has been estimated to be about 14 nm long, with 7 nm extending extracellularly and 4.5 nm extending into the cytoplasm (Brisson & Unwin, Nature 315:474-477, 1985). The external entrance of the pore is funnel shaped with a 3 nm opening that narrows to about 0.8 nm deeper in the pore, and evidence suggests that the subunits have different lengths that do not match up at the cytoplasmic end. The primary goal of this work is to provide information about the internal structure of the pore by estimating the length of its narrowest cross section.

The length of the narrow region of the channel can be determined indirectly by estimating the number of water molecules that occupy this region. The estimate can be made by measuring streaming potentials, which directly indicate the number of water molecules coupled to the transport of a cation. If the permeant cation completely fills the narrow cross section, then, as it passes through the channel, all the water molecules in the narrow region will be pushed along in front of the cation. A pore with a long narrow region will give a large streaming potential because many water molecules would have to be coupled to the transport of the largest permeant. The streaming potential associated with a permeant (triethanolamine) that is expected to fill the narrow cross section of the AChR is small, indicating that the narrow region is short.

Another indirect way to estimate the length of the narrow region is to determine the number of cations it can hold. If the narrow region is very short, then, due to ion-ion repulsion, it will only be able to hold one cation at a time. One of the strongest pieces of evidence for single occupancy of the narrow region is if the permeability ratio of two permeants remains constant over a wide concentration range. In the AChR, P_{Li}/P_{NH4} was found to remain constant when the concentration was changed over a 15 fold range. Along with other transport data, this result also indicates that the narrow region is short. Supported by NIRA NS 21229.

410.3 TOPOLOGICAL LOCALIZATION OF A C-TERMINAL REGION OF THE VOLTAGE-DEPENDENT SODIUM CHANNEL USING ANTIBODIES RAISED AGAINST SYNTHETIC PEPTIDES. R.D. Gordon*, W.E. Fields*, D.L. Schotland*, R. Hogue-Angeletti and R.L. Barchi. Univ. of Penn. Sch. of Medicine, Philadelphia PA 19104.

A series of oligopeptides have been synthesized corresponding to defined regions of the primary sequence of the voltage dependent sodium channel from Electrophorus electricus. The purity of each peptide was confirmed by amino acid analysis and peptide sequencing. Antisera have been raised to each peptide and these antisera are being used to probe the topology of the sodium channel within electroplax membranes. One antiserum (Jenny) raised against a region near the C-terminus (amino acid residues 1781-1792) has been most fully characterized. This antiserum specifically immuno-reacts with the immobilized synthetic peptide in solid phase radioimmunoassay. The specificity of this antiserum was shown by the specific binding of ^{125}I antibodies to a 280 kDa protein in western blots of an enriched fraction of solubilized electroplax vesicles. Examination of electroplax membranes at the light microscopic level by immunofluorescent labelling showed that only the innervated membrane fluorescently labelled with this antiserum. No detectable labelling of the non-innervated membrane was observed, consistant with the known distribution of the sodium channel within this tissue. The binding of antibodies to the sodium channel in western blots and in cryostat sections of electroplax could be blocked by the synthetic peptide (at concentrations = 50uM). When electroplax membranes were examined at the EM level with colloidal gold labelled antibodies, extensive labelling of the membrane was confirmed, with no significant labelling of the non innervated membrane, cytoplasm, organelles or extracellular space. Inspection of the labelled innervated membrane revealed the presence of gold particles associated with the cytoplasmic surface. In typical fields more than 90% of the gold particles were on the intracellular side. We conclude that the C-terminal region of the sodium channel is exposed on the cytoplasmic side of the electroplax membrane, this finding is consistent with current models of the tertiary structure of the sodium channel. Topological mapping of additional peptides is in progress.

410.4 **CATION-SELECTIVE, TTX-SENSITIVE INFLUX BY RECONSTITUTED VOLTAGE-SENSITIVE Na CHANNELS CHEMICALLY ACTIVATED WITH N-BROMOACETAMIDE.** E.C. Cooper* and W.S. Agnew (SPON: W. James). Yale University School of Medicine, New Haven, CT 06510

Brief treatment of the internal surface of nerve and muscle cell membranes with proteases or group-specific peptide-cleaving reagents, such as N-bromoacetamide (NBA), abolishes inactivation of the Na currents of the action potential, but has little effect on activation or ion selectivity (Oxford, G.S., et al., (1978) J. Gen. Physiol. 71:227; Patlak, J. & Horn, R. (1982) J. Gen. Physiol. 79:333). It has not been possible, however, in native membrane preparations to identify sites of proteolysis which may be associated with inactivation gating. Reconstituted Na channels purified from Electrophorus electricus (Rosenberg et al. (1984) Proc. Natl. Acad. Sci., U.S.A. 81:5594; Tomiko, S.A., et al. (1986) Biochem 25:2164) provide a convenient preparation in which to identify the sites of functionally specific chemical modifications. The channels are reincorporated in random orientation, permitting access to either surface of the molecule, and the channels are amenable to either biochemical screening or electrical recording.

In ion-tracer influx studies with reconstituted vesicles, treatment with 2.5 uM batrachotoxin (BTX), enhanced $^{22}Na^+$ influx 5-12 fold over controls (1% ethanol vehicle). Influx was blocked 50-70% by 1 uM external TTX and ~100% by internal plus external TTX, suggesting that protein orientation is nearly random and external TTX blocks right-side out facing channels. Conversely, the membrane impermeant anesthetic QX-314 preferentially blocked inside-out oriented channels. Dual label radio-tracer influx assays were conducted with combinations of $^{22}Na^+$, $^{42}K^+$, $^{86}Rb^+$ and $^{137}Cs^+$. Control incubations (without BTX) revealed low, non-selective tracer influx. BTX-activated influx, however, was distinctly ion selective. Permeability ratios, based on relative initial BTX-activated uptake, were $Na^+(1.0)$: $K^+(0.14)$: $Rb^+(0.045)$: $Cs^+(0.03)$.

Vesicles treated with external NBA (500uM) exhibited a 2-3 fold increase in $^{22}Na^+$ uptake over NBA-free controls, with little or no increase in $^{86}Rb^+$ uptake. This ion-selective NBA-activated influx was insensitive to 1 uM external TTX but was completely blocked by internal plux external TTX, and by external QX-314 (K_i~0.5 mM). NBA-activated flux was generally ~50% of that BTX-activated influx which was not blocked by external 1uM TTX. These results suggest that NBA acts upon inside-out facing Na channels to activate an ion-selective conductance, as might be expected if inactivation gating had been eliminated.

410.5 SELECTIVITY SEQUENCE OF METAL AND ORGANIC CATIONS FOR BATRACHOTOXIN-MODIFIED RECONSTITUTED Na CHANNELS PURIFIED FROM ELECTROPHORUS ELECTRICUS. S.A. Tomiko* & W.S. Agnew Dep't of Physiology, Yale University School of Medicine, New Haven, CT 06510

A voltage-sensitive cyanine dye (diS-C_3-(5)) was used to measure the hyperpolarization generated by reconstituted voltage-dependent sodium channels in the presence of different Na^+ gradients. The voltage gradient generated across the vesicle membranes was collapsed by various monovalent cations in proportion to their permeabilities through the batrachotoxin (BTX)-activated channels. Purified Na channel protein from eel electroplax was reconstituted as before (Tomiko, et al., Biochem., 1986) after dialysis in 84 mM Na_2SO_4, 10 mM HEPES, 1 mM EGTA, pH 7.4. Reconstituted vesicles were enlarged by freeze-thaw-sonication (0.5 sec), and diluted 10 fold. Vesicles were incubated 40 min at 30°C + BTX (2.5 uM, in ethanol at 1% final). Samples were eluted through small columns of Dowex 50X8-100 with a 6 fold excess of isosmolar ethanolamine, 10 mM imidazole solution (pH 7.4 with H_2SO_4), to remove all external Na^+. They were mixed 1 to 1 with isosmolar ethanolamine, 10 mM imidazole solutions (pH 7.4 with H_2SO_4) that contained 0.05 or 5.07 mM Na_2SO_4. Fluorescence ($6.1x10^{-7}$ M diS-C_3-(5), in ethanol at 0.5% final) was measured in a spectrofluorometer (excitation 645 nm). The difference between reference fluorescence (same [dye] in 3 ml ethanol) and sample fluorescence 5.9 min after dye addition was set = 100%. Monovalent cation as the sulfate (5 mM), nitrate (5 mM) or chloride (10 mM) salt was then added. The difference between this signal 100 sec after salt addition and the reference was expressed relative to 100%. The fraction >100% for Na^+ was set =1, and signals for the other cations were compared with that value. Vesicles incubated in ethanol vehicle alone did not hyperpolarize, nor depolarize on addition of Na^+ salt. BTX-activated vesicles hyperpolarized in proportion to external [Na^+], and depolarized to about the same final level upon addition of excess external Na^+. Hyperpolarization was blocked by adding BTX together with dibucaine (0.1 mM) or TTX (1 uM) + QX-222 (3 mM). The rank order of cation permeability through BTX-modified channels was Tl(1.4)> Na(1.0)~ Li(1.0)> NH_4(0.6)> formamidine(0.5)> guanidine(0.4)> K(0.2)> Rb(0.1)> Cs(0.02)~ methylamine(0.02). Both the high Tl^+ permeability relative to Na^+, and the difference in permeability between NH_4^+ and guanidine are diagnostic of the permeation selectivity changes induced in voltage-dependent Na channels by BTX (c.f. Khodorov et al., Neurosci., 1978). Thus it seems likely that BTX does not simply act as an allosteric modulator which increases the probability that channels are open.

410.6 EFFECT OF LOCAL ANESTHETICS ON THE DISSOCIATION RATE OF THE BATRACHOTOXIN-SODIUM CHANNEL COMPLEX. C. R. Creveling*, G. A. Lewandowsky, and J. W. Daly*. Laboratory of Bioorganic Chemistry, NIDDK, NIH, Bethesda, Maryland 20892.

[^{3}H]Batrachotoxin-B ([^{3}H]BTX-B), a steroidal alkaloid, binds specifically with high affinity to a single site associated with the voltage-sensitive component of sodium channels. α-Scorpion toxin binds specifically to another site, thereby shifting the channel toward the open or conducting state, and enhances the specific binding of [^{3}H]BTX-B. Local anesthetics bind specifically to a site or sites associated with the channel, thereby shifting the channel towards a closed or nonconducting state and markedly decreasing the specific binding of [^{3}H]BTX-B. A wide variety of local anesthetics and compounds with local anesthetic activity have been shown to competitively inhibit the binding of [^{3}H]BTX-B. Recent studies (Postma and Catterall, Mol. Pharm. 25: 219, 1983) suggest that the competitive inhibition of binding of [^{3}H]BTX-B is only apparent and is due to an allosteric increase in the dissociation rate of [^{3}H]BTX-B.

We report the effect of local anesthetics on the dissociation rate of [^{3}H]BTX-B from sodium channels in a synaptoneurosome preparation from guinea pig cerebral cortex (Hollingsworth et al., J. Neuroscience 5:2240, 1985) in the presence and absence of specific ligands for the BTX-binding site. The dissociation rate for [^{3}H]BTX-B in the presence of excess BTX-B expressed as $T_{1/2}$ was 31.3 ± 3.2 min. True competitive ligands, such as BTX, aconitine and veratridine, did not affect the dissociation rate. A linear, concentration-dependent increase in the dissociation rate of the [^{3}H]BTX-B complex was demonstrated for 22 compounds with local anesthetic activity in the presence and absence of veratridine. The concentration dependence of this effect was determined for 15 compounds ranging in inhibition potency from dibucaine (K_i = 0.6 μM) to procaine (K_i = 49 μM). The increase in dissociation of [^{3}H]BTX-B was determined at 3-5 concentrations of local anesthetic. Double reciprocal plots of the increase in rate relative to the dissociation rate of BTX-B against the concentration of local anesthetic was a linear function. The horizontal intercept yielded the EC_{50} value for each local anesthetic. Comparison of the inhibition constants, K_i, with the EC_{50} for the local anesthetic-induced increase in the dissociation rate yielded a 1:1 relationship (r = 0.94). Thus, the inhibition of binding is a direct consequence of the increase in the dissociation rate constant for [^{3}H]BTX-B.

410.7 TWO SODIUM CURRENTS IN DISSOCIATED BULLFROG SYMPATHETIC NEURONS. Stephen W. Jones. Dept. Neurobiology and Behavior, SUNY at Stony Brook, NY 11794, and (present address) Dept. Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106.

Cell bodies of bullfrog sympathetic neurons have two kinetically and pharmacologically distinct sodium currents. One is relatively large and fast, and is sensitive to tetrodotoxin (TTX) and saxitoxin (STX); the other is blocked by cadmium and STX, but not TTX.

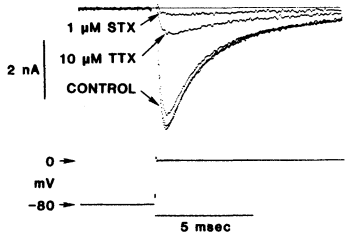
1 μM TTX blocked 76 ± 3% (n=15) of the peak inward current at +10 mV; 10 μM TTX had little or no additional effect. 10 nM TTX blocked 38 ± 3% (n=6) of the current, consistent with a $K_d \sim 10$ nM for the TTX-sensitive current.

The current remaining in 0.1-10 μM TTX inactivated more slowly than the total current (see figure below). Furthermore, the TTX-resistant current was blocked by 200 μM cadmium (88 ± 2%, n=4), whereas the total current was blocked only 25 ± 1% (n=5) [see also Bowers, Brain Res. 340:143 (1985)].

The TTX-resistant, cadmium sensitive current appears to be a sodium current, as (1) it is recorded in Mn^{2+}, which is poorly permeant in most calcium channels, (2) the reversal potential of the TTX-resistant current depends on the external sodium concentration (shifting 48 ± 2 mV/10-fold change in sodium, for 1/2 or 1/3 normal sodium, n=4), and (3) the TTX-resistant current is largely blocked by 1 μM STX (see figure).

Even in a single cell, sodium currents can be heterogeneous. This diversity, like that of potassium and calcium currents, may prove to be functionally important.

Supported by NIH grants NS 20751 to SWJ and NS 18579 to Dr. Paul R. Adams. I also thank Jim Cummings for computer programming and Claire Adams for preparing the cultures.



Methods: Bullfrog sympathetic neurons were dissociated by the method of Kuffler & Sejnowski [J. Physiol. 341: 257 (1983)], cultured at 4°C for 0-40 days, and voltage clamped with 0.5-2 MΩ patch pipets in the whole-cell configuration, using a discontinuous single electrode voltage clamp. The pipets contained (mM) CsCl 103, NaCl 9, TEA·Cl 5, CsHEPES 2.5, CsEGTA 1, pH 7.3; the extracellular solution was NaCl 97.5, TEA·Cl 20, $MnCl_2$ 4, Tris base 2.5, titrated to pH 7.3 with HCl. Records shown are averaged, leak subtracted, digitized at 25 kHz, and filtered (3 kHz current, 10 kHz voltage). Control records include recovery from each drug.

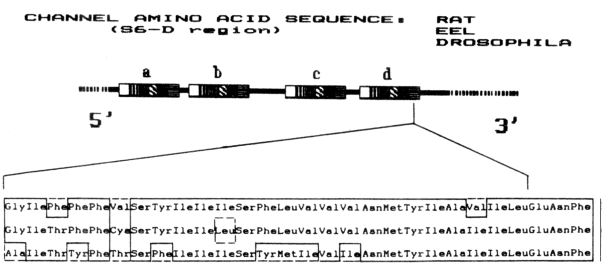
410.8 A CADMIUM- AND TETRODOTOXIN-RESISTANT VOLTAGE-DEPENDENT TRANSIENT INWARD CURRENT IS PRESENT IN CULTURED RAT AND MOUSE SPINAL NEURONES *D.G. Owen and J.L. Barker. (Spon: C. Bourque) MRC Neuropharmacology Unit, The School of Pharmacy, 29/39 Brunswick Sq., London WC1N 1AX, UK. and LNP, NINCDS, NIH, Bethesda, MD 20892, USA.

Under conditions of Cs^+-loading we have observed a transient inward current activated at the level of the resting membrane potential following hyperpolarizing voltage steps. This appears to be distinct from $I_{Cl(Ca)}$ (Owen et al, 1984) and other Ca^{2+} -dependent currents activated by depolarization. Recordings were made from rat spinal cord neurones and FACS-sorted mouse motoneurones (Schaffner et al, 1983) after about 4 weeks in culture. Electrodes were filled with either CsCl or Cs_2SO_4 and the bath solution contained TEA and 1 μM TTX. In a proportion of cells (ca 10%) a depolarizing overshoot or 'bounce' occurred following electrotonic hyperpolarizing responses to negative current injection, sometimes triggering Ca^{2+} action potentials or Ca^{2+}-activated Cl^- depolarizing potentials. Under voltage-clamp it was apparent that the 'bounce' was due to a transient inward current (I_b) activated following hyperpolarizing steps. I_b turned-on slowly (time to peak about 80 ms) and inactivated exponentially with time (tau=190 ms at -60 mV). The activation and inactivation curves did not overlap and lay quite hyperpolarized (threshold for activation was ca -75 mV). I_b was substantially resistant to Cd^{2+} (200 μM) and was only slightly reduced in a bathing solution nominally free of Ca^{2+}. In some cells depolarizing voltage steps activated a transient and relatively sustained inward current (thresholds -35 mV to -40 mV and -25 mV to -30 mV respectively), both of which were abolished in Cd^{2+} or zero Ca^{2+}, in contrast to I_b. Similarly $I_{Cl(Ca)}$, which is dependent on Ca^{2+} entry for activation, was abolished by Cd^{2+}. Furthermore, whereas Ba^{2+} inhibits activation of $I_{Cl(Ca)}$(Owen et al, 1984) and enhanced Ca-dependent currents activated at depolarized potentials, I_b was unaffected. The estimated reversal potential for I_b from paired voltage step experiments was ca 0 mV in the presence and absence of Cd^{2+} which together with the above suggests that Ca^{2+} is not a major charge carrier. Neither Cl^- or Na^+ are likely to be involved since in cells recorded with Cs_2SO_4 electrodes I_b was still clearly inward at potentials more depolarized than E_{Cl} and substitution of 90% of Na^+ had little effect upon I_b currents. Further experiments are required to determine the precise ionic dependence of this current and under what circumstances I_b may perform a physiologically relevant role.

Owen, D.G., Segal, M. and Barker, J.L. (1984) Nature, 311, 567-570.
Schaffner, A.E., St John, P.A. and Barker, J.L. (1983) Soc. Neurosci. Abstr., 9, p7.

410.9 **A DROSOPHILA GENE WITH HOMOLOGY TO THE VERTEBRATE Na^+ CHANNEL** L. Salkoff, A. Butler*, M. Hiken*, A. Wei, K. Giffen*, C. Ifune* and G. Mandel*, Dept. Anat. & Neurobiol., Washington University Sch. of Med., St. Louis, MO 63110, and *Dept. Med., Tufts New England Med. Ctr., Boston, MA 02111.

We have isolated a Drosophila genomic clone containing a gene sequence with close homology to the vertebrate sodium channel (Noda, et al., Nature 320:188). The coding region of the Drosophila gene segment is homologous to the vertebrate gene membrane spanning regions S3,S4,S5, and S6 of the 4th internally repeated homology unit. We have currently sequenced an open reading frame of 588 bp coding for 196 amino acids; 107 are identical to the eel sodium channel. Most differences are conservative and total conservation is greater than 80%. By in situ hybridization to polytene chromosomes the clone was mapped to the cytogenetic locus 60 D-E on the right arm of the 2nd chromosome. This position is close to but not coincident with the reported position of the seizure locus (60 B-C). Mutations at this locus confer temperature-induced seizure and paralysis (Jackson, et al., Nature 308:189). One mutant allele alters the affinity of the saxitoxin binding component for the ligand. The diagram below shows the S6D membrane spanning region and compares the sequences of Rat (top), Eel (middle) and Drosophila (bottom). Of 28 amino acids shown, 20 in Drosophila are identical to Rat or Eel. There are no major nonconservative differences in this region. In many positions the precise hydrophobic side chains of the amino acids appear to be vital.



Supported by NSF Grant BNS 8311024 and a grant from the Esther A. and Joseph Klingenstein Fund

410.10 CLONING OF DROSOPHILA MELANOGASTER SEQUENCES HOMOLOGOUS TO THE VOLTAGE-SENSITIVE SODIUM CHANNEL GENE FROM ELECTROPHORUS ELECTRICUS. D.W. Gil*, J.K. Keen* and L.M. Hall. Department of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

We are cloning the gene for the alpha subunit of the voltage-sensitive sodium channel from Drosophila melanogaster as an approach to studying the molecular basis of sodium channel function and regulation. A number of different mutants have been identified in Drosophila which effect propagation of action potentials and/or the properties of ^3H-saxitoxin binding to sodium channels. After cloning the gene for sodium channels from Drosophila, we will use in situ hybridization to salivary gland chromosomes to determine which, if any, of these mutants identify the structural gene for this channel. In addition, sequencing of the Drosophila sodium channel gene will provide further information concerning highly conserved regions of the channel and thus aid identification of functionally important amino acid sequences of vertebrate and invertebrate channels.

Two oligonucleotide probes complementary to the S4 and S5 transmembrane regions of the Electrophorus electricus sodium channel gene have been synthesized using deoxyinosine in the third position for amino acids with degenerate codons. The sequences chosen are highly conserved in the cDNA from electric eel and rat brain (Noda et al., Nature, 320:188, 1986). A recombinant lambda phage library of Drosophila genomic DNA was screened with the labeled probes at high stringency using the tetramethylammonium chloride wash procedure in which the melting temperature of the hybridizing DNA strands is independent of the base composition and dependent only on the length of matching sequence (Wood et al., Proc. Natl. Acad. Sci. USA, 82:1585, 1985). Under wash conditions that require at least 20 base pairings for hybridization to occur, nine positive clones were identified from approximately 250,000 plaques. These clones are being characterized by restriction enzyme digestion and in situ hybridization to Drosophila salivary gland polytene chromosomes to determine how many distinct genes are represented by the clones. Two criteria are being used to determine which of the distinct clone(s) encode regions of a Drosophila sodium channel alpha subunit. Because Drosophila sodium channels are only found in neuronal tissue and are large proteins, sodium channel clones should preferentially hybridize to head-specific mRNA that is approximately 8 kb long. In addition, the distinct clone(s) will be sequenced by standard dideoxy nucleotide methodology. The sequences will be compared with the electric eel and rat brain cDNA sequences to identify the clones with strongest homology in an open reading frame.

410.11 NEUROPHYSIOLOGICAL DEFECTS IN SEIZURE AND ENHANCER OF SEIZURE MUTANTS IN THE GIANT FIBER PATHWAY OF ADULT DROSOPHILA. J.C. Nelson*, D.P. Kasbekar*, and L.M. Hall (SPON: R.J. Wyman), Dept. of Biol., Yale U., New Haven, CT 06511.

We used the Giant Fiber (GF) pathway to look for temperature induced defects in Drosophila mutations seizure and enhancer of seizure [e(sei)]. No neurophysiological defects have been reported in seizure (isolated by Ganetsky), and e(sei) is a new mutation isolated by its interaction with seizure. The sodium channel blocker, saxitoxin, has altered binding in seizure flies compared to wild-type flies (Jackson et al, Nature 308:189, 1984). Thus, the seizure gene product is thought to be involved in the production and/or structure of the voltage gated sodium channel.

Brain stimulation excites the GFs, which descend into the thoracic neuropile and make two electrical synapses. These two synapses excite the Tergotrochanteral motoneuron which drives the jump muscle (TTM), and the Peripherally Synapsing Interneuron which chemically synapses onto the motoneurons that excite the wing depressor muscles (DLMs) (King and Wyman, J. Neurocytology 9:753, 1980). We recorded spontaneous activity (SA) and GF stimulated responses from the DLMs, TTMs, and wing elevator muscles (DVMs). The DVMs respond to GF stimulation by an unknown pathway.

We have found that hyperactivity and rigid paralysis in e(sei) and seizure at high temperatures is due to spontaneous activity (SA). The normal flight motor pattern excites the DLMs and DVMs at ~6 Hz (Harcombe and Wyman, J. Comp. Phys. 123:271, 1978). Heat pulses to 44°C for 4 minutes never induced SA in wild-type flies (n=7). Mutant flies were heat pulsed to 42.5°C for 2-5 minutes and returned to 22°C. seizure flies had continuous SA from 10 to 50 Hz (DLMS, n=16). The SA induced behavioral hyperactivity, and more induced rigid paralysis in seizure (~37-41°C). The SA in e(sei) flies varied on a continuum: from no DLM activity to DLM activity at 40 Hz (n=26). High temperature does not cause paralysis in e(sei) flies, but the SA causes hyperactivity. Double mutants of seizure, e(sei) have higher rates of activity (40 to >80 Hz), and lower temperatures at which SA and behavioral paralysis start (<35-37°C). We found no defects in axon conduction or spike initiation when stimulating the GFs directly. Different initiation sites of SA exist for each mutation within GF pathway neurons.

nap, para, and tip-E are additional mutants thought to affect sodium channels. They differ from seizure and e(sei) in that they block neuronal conduction or spike initiation in neurons, instead of increasing the activity in neurons. In addition, double mutants of nap, para, and tip-E enhance each other, while they all suppress seizure (Ganetzky, J. Neurogen. 3:19, 1986; Jackson et al., J. Neurogen. 3:1, 1986). Thus, if seizure and e(sei) affect the sodium channel, they probably act to keep the channel open.

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410.12 ELIMINATION OF RAPID ADAPTATION FROM AN INSECT SENSORY NEURON BY CHLORAMINE-T. A.S. French. Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7.

The cockroach femoral tactile spine is a mechanoreceptor in which the response to a step deformation is a burst of action potentials ceasing completely after about 1 s. This rapid adaptation has similar dynamic properties to a range of other vertebrate and invertebrate sensory receptors. The adaptation can also be demonstrated by direct electrical stimulation of the single sensory neuron in the base of the spine, showing that it occurs during action potential encoding, not transduction. One possible mechanism for rapid sensory adaptation is a slow inactivation of sodium current. Oxidizing agents such as Chloramine-T (CT) selectively eliminate sodium inactivation in a range of neural tissues, so we have examined the effects of CT on the tactile spine neuron.

Experiments were performed on isolated legs from adult cockroaches. Tactile spines were amputated above the sensory neuron and microelectrodes (0.5 M NaCl, 5 MΩ) were advanced into the lumen adjacent to the axon-soma junction. Stimulating current was driven by a constant voltage to current converter and monitored by a virtual ground circuit. Action potentials were detected extracellularly from nerve 5. CT was applied through the spine lumen dissolved in cockroach saline. After CT application a suprathreshold pulse was applied at 10 s intervals until double spikes appeared. CT was then removed and the oxidation stopped with sodium metabisulfite. The tissue was rinsed several times in saline. The threshold properties of treated and untreated neurons were measured by determining the strength-duration relationship for current pulses by an automated procedure.

CT had dramatic effects on the dynamic properties of the tactile spine neuron, converting it from a rapidly adapting to a tonically firing receptor. Measuring the rheobasic current as a function of background current showed a large shift in the threshold of the neuron which depended on the length of treatment and the membrane potential. Preliminary experiments with other oxidizing agents indicate that *N*-chlorosuccinimide, with similar chemical reactivity to CT, has almost identical effects on rapid adaptation, while *N*-bromoacetamide, a stronger oxidizing agent, destroys excitability without first affecting adaptation. These results suggest that rapid adaptation in this neuron is caused by a sodium current whose inactivation, while different in temporal behavior to the more familiar sodium current, has a similar chemical basis.

Supported by The Canadian Medical Research Council and The Alberta Heritage Foundation for Medical Research.

DOPAMINE NEURONS: ELECTROPHYSIOLOGICAL STUDIES

411.1 ELECTROPHYSIOLOGICAL EFFECTS OF THE PSYCHOMOTOR STIMULANT, FENCAMFAMINE, ON MIDBRAIN DOPAMINE NEURONS. R.T. Matthews. Dept. Anatomy, Texas A&M University, College Station, Texas 77843.

Fencamfamine is a psychomotor stimulant that has appeared on the U.S. illicit drug market as a substitute for cocaine. Behavioral studies on fencamfamine in rodents have shown that it causes increased locomotor activity and stereotyped behavior and its actions are blocked by the dopamine receptor antagonist, haloperidol (Hotovy, et al., Arzneim.-Forsch, 11:20, 1961; DeLucia, et al., Gen. Pharm., 15:407, 1984). Biochemical studies suggest that fencamfamine does not directly stimulate dopamine (DA) receptors or block DA catabolism by monoamine oxidase, but it does increase DA metabolite levels and block catecholamine uptake into cerebral nerve terminals (Seyfried, Biochem. Pharm., 32:2329, 1983). Reserpine plus α-methyltyrosine, but neither drug alone, attenuated the behavioral effects of fencamfamine suggesting that fencamfamine may be an indirect DA agonist of the non-amphetamine type. In the present study, single unit recordings were performed in chloral hydrate anesthetized male rats and the effects of systemic or microiontophoresed fencamfamine on the spontaneous activity of midbrain DA neurons were measured. Standard single unit extracellular recording technics were used with either tungsten or 5-barrel micropipette electrodes. Mesotelencephalic DA neurons were identified by their long duration (≥ 2 msec) action potentials, burst firing patterns, triphasic wave form and location within the A9/A10 cell group area of the midbrain. Fencamfamine (0.25-16.0 mg/kg, i.v.) dose-dependently decreased the firing rates of both A9 and A10 DA neurons and this effect was reversed by haloperidol (0.1 mg/kg, i.v.). Some neurons were not completely inhibited by the highest doses of fencamfamine although low doses of apomorphine (10-70 µg/kg, i.v.) could further inhibit firing rates of these neurons. Reserpine plus α-methyltyrosine, but not α-methyltyrosine alone, prevented the firing rate inhibition by fencamfamine administration. Fencamfamine (0.2 mM, pH 4.0) microiontophoresed from 5-barrel pipettes onto neurons inhibited firing of A9 DA neurons. These data are in general agreement with previous studies which suggest that fencamfamine is an indirectly acting DA agonist whose pharmacological profile resembles that of non-amphetamine psychomotor stimulants. In addition, fencamfamine at high doses may have additional unidentified effects on some DA neurons which limits its ability to decrease spontaneous firing rates. (Supported by NIMH Grant MH40774).

411.2 ELECTROPHYSIOLOGICAL EFFECTS OF APOMORPHINE AND CIS-FLUPENTIXOL ON MEDIAL PREFRONTAL CORTICOMESENCEPHALIC NEURONS IN THE RAT. S.L. Peterson, J.S. St. Mary* and N.R. Harding*. Dept. of Med. Pharmacology and Toxicology, Texas A&M University, College Station, TX 77843.

The deep layers of the medial prefrontal cortex (PFC) receive dopaminergic innervation from the ventral tegmental area (VTA) and contain neurons which project back to the VTA. The synaptic transmission in the cortical component of this reciprocal innervation is believed to be mediated in part by dopamine (Ferron et al., Brain Res., 302:257, 1984) and this study was designed to test that hypothesis.

The spontaneous activity of neurons in the PFC of chloral hydrate anesthetized rats was studied using single unit extracellular recording techniques. Only neurons with large amplitude (0.5-2.0 mV), positive first deflection action potentials that were antidromically stimulated from the VTA were studied. Electrical stimulation of the VTA (1.0 msec. pulse, 200-400 µA, 1 pps) induced a 40-400 msec ($\bar{x}$ = 140) inhibition of the spontaneous activity of the PFC neurons that occurred 10-30 msec ($\bar{x}$ = 19) after the stimulus, presumably a result of the activation of VTA projections to the PFC.

Cis-flupentixol (1.0 mg/kg, iv) significantly reduced the VTA mediated inhibition of PFC neuronal activity in 12 of 19 (63%) neurons. Cis-flupentixol also decreased the average spontaneous firing rate of the neurons as compared to vehicle treatment (saline, iv, n=8). The vehicle treatment had no effect on the VTA mediated inhibition of the spontaneous firing rate.

Apomorphine (25 µg/kg, iv) reduced the spontaneous activity of the PFC neurons (n=7) to an average of 37% of the preinjection rate. Cis-flupentixol (1.0 mg/kg, iv) blocked the response of the same neurons to a subsequent injection of the same apomorphine dose.

The results indicate that the activity of the PFC efferent neurons can be influenced by endogenous dopamine or dopamine agonists. The inhibitory effect induced by VTA stimulation is probably mediated in part by dopamine as the inhibition was blocked by cis-flupentixol in 63% of the neurons. Apomorphine produced a cis-flupentixol sensitive depression of the spontaneous firing rate in all cells tested suggesting a more profound response of PFC neurons to exogenous dopamine agonists. Although it has been proposed that VTA mesocortical neurons do not respond to DA agonists or antagonists, neurons in the cortical area they innervate are responsive and should be considered a site of action of such drugs.

(Supported by a PMA Foundation Research Starter Grant)

411.3 ELECTROPHYSIOLOGICAL EVALUATION OF A PARTIAL AGONIST-ANTAGONIST OF DOPAMINE RECEPTORS. M.F.Piercey and W.E.Hoffmann*. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Biochemical experiments have suggested that the ergot derivative trans-dihydrolisuride (THDL) is a partial agonist-antagonist of central dopamine (DA) receptors (Eur. J. Pharmacol. 97:111, 1984). We have now tested TDHL for its effects on firing rates of DA neurons located in the substantia nigra pars compacta (SNPC) of chloral hydrate anesthetized rats. Using extracellular single-barrelled microelectrodes, DA neurons were identified by their long duration, positive-negative action potentials, and their slow bursting pattern of spontaneous firing, as well as by the location of recording sites through histological recoveries of dye deposits (JPET 185:560, 1973). Like haloperidol and clozapine which are full DA receptor antagonists, TDHL antagonized the depression in DA neuron firing induced by systemic amphetamine. However, where full antagonists completely reversed the amphetamine effect, TDHL could do so only partially, the maximal effect being around half. Like DA agonists, but unlike DA antagonists, TDHL also depressed the spontaneous firing rates of DA neurons. But, whereas the full agonist apomorphine completely inhibited firing of DA neurons, TDHL only depressed firing rates by about half, even at high doses. These data confirm the original characterization of TDHL as a partial DA agonist-antagonist.

411.4 IN VIVO EFFECTS OF SELECTIVE D1 AND D2 DOPAMINE (DA) RECEPTOR AGONISTS ON A9 DA NEURONAL ACTIVITY IN RATS. M.D. Kelland, D. Clark*, and L.A. Chiodo, Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235

Behavioral and biochemical studies have shown that selective D1 DA receptor agonists, while exhibiting few effects of their own, can influence the actions of selective D2 DA receptor agonists. For example, the selective D1 agonist SKF38393 has been shown to reduce the inhibitory effects of the selective D2 agonists pergolide and LY171555 on striatal DA turnover. Since this effect may be mediated by alterations in DA nerve impulse flow, we are currently examining the actions of SKF38393 and LY171555, either alone or in combination, on A9 DA cell firing.

Standard extracellular single-unit recording techniques were used to monitor the activity of A9 DA neurons in vivo in anesthetized, adult male rats. Intravenous injections of LY171555 dose-dependently inhibited the firing of A9 DA neurons (ED_{50} = 5.7 ± 1.5 µg/kg, N=10). This effect was reversed by the selective D2 antagonists 1-sulpiride and haloperidol, but not by the selective D1 antagonist SCH23390. SKF38393 did not inhibit the firing of these neurons at doses up to 20.5 mg/kg (N=5). Following a 4 minute pretreatment with SKF38393 (1.0 mg/kg i.v.) there was no significant change in the LY171555-induced inhibition of A9 DA neuronal firing rate (ED_{50} = 5.7 ± 1.2 µg/kg, N=9).

Arnt (1985) showed that SKF38393, which has only minimal behavioral effects in normal animals, produces locomotor stimulation in rats administered chronic reserpine, suggesting a sensitization of D1 receptors following this treatment. In rats administered reserpine for 4 days (2.0 mg/kg s.c. daily) intravenous SKF38393 still failed to inhibit the firing of A9 DA neurons at doses up to 20.5 mg/kg (N=4). Observation of awake rats injected with SKF38393 (15.0 mg/kg s.c.) following the same reserpine pretreatment confirmed Arnt's findings.

Under the present conditions, we have demonstrated that while stimulation of D2 receptors produces an inhibition of DA cell firing, activation of either normal or sensitized D1 receptors is without effect. In addition, D1 receptor stimulation did not influence the effects of a D2 receptor agonist. Current investigations with these compounds are proceeding in the paralyzed rat preparation. (Supported by grants from the American Parkinson Disease Assoc. and Tourette Syndrome Assoc.).

411.5 CHOLECYSTOKININ OCTAPEPTIDE (CCK-8) MODULATES THE ACTIVITY OF NIGROSTRIATAL DOPAMINE (DA) NEURONS. A.S. Freeman and L.A. Chiodo. Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

DA/CCK co-localization in the substantia nigra zona compacta (A9) is confined to its medial and extreme lateral (pars lateralis) aspects. A9 cells excited by i.v. and microiontophoretic sulfated CCK-7 have been found only in these areas (Skirboll et al., Neurosci 6:2111, 1981) and we have observed similar responses to the biologically prevalent CCK-8. Although it is tempting to conclude that CCK-8 actions in the midbrain are restricted to regions of extensive DA/CCK coexistence, our preliminary electrophysiological data suggest that CCK-8 exerts widespread modulatory effects on midbrain DA cells. This work focuses on identified (antidromically activated from the caudate nuc.) rat nigrostriatal DA neurons located in an area of A9 (middle third) which is devoid of CCK- or DA/CCK-containing cell bodies. After a stable firing rate was obtained, sulfated CCK-8 was injected i.v. at a dose (8 µg/kg) known to transiently stimulate DA/CCK neuronal firing and to modulate the responsiveness of those cells to the inhibitory action of apomorphine (APO); unit activity was monitored for an additional 10 min prior to i.v. APO administration. Sulfated CCK-8 had no effect on the firing rates of 10 nigrostriatal DA cells but stimulated the firing rates of 6 others (mean rate increase of 37%). These excitations lasted between 2 and 5 min. The responsiveness of these neurons to subsequent doses of APO was correlated with the initial effect of CCK-8. Cells whose firing rates were unaffected by CCK-8 displayed enhanced sensitivity to the inhibitory effect of APO compared to controls; diminished sensitivity to APO was observed in cells initially excited by CCK-8.

These preliminary results show, for the first time, the effects of CCK-8 on the activity and subsequent APO sensitivity of a population of DA neurons identified by antidromic activation. Our results suggest that CCK-8 can modulate the effect of a DA agonist despite having no discernible effect of its own and also show that a small percentage of nigrostriatal DA cells originating in an area of A9 devoid of DA/CCK co-localization are excited by sulfated CCK-8. Finally, i.v. injection of sulfated CCK-8 (but not unsulfated CCK-8) resulted in an increase in the number of spontaneously active DA neurons encountered in A9 and A10. Together, these findings support our hypothesis that CCK-8 has widespread modulatory effects on midbrain DA systems independent of DA/CCK co-localization and suggest that the complexity of CCK-8 actions on midbrain DA neuronal populations is greater than once thought. (Supported by American Parkinson Disease Assoc. and Tourette Syndrome Assoc.)

411.6 ELECTROPHYSIOLOGICAL AND PHARMALOGICAL CHARACTERIZATION OF IDENTIFIED NIGROSTRIATAL AND MESOACCUMBENS DOPAMINE NEURONS. D. Clark* and L.A. Chiodo (SPON: R.B. Holman). Laboratory of Neurophysiology, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235

Recent evidence from electrophysiological studies has suggested that brain dopamine (DA) neurons innervating different forebrain areas are not homogeneous and are regulated in a different manner. For example, DA neurons projecting to the prefrontal cortex (PFC) exhibit a higher firing rate and greater bursting activity than nigrostriatal DA neurons (NS-DA) which project to the caudate nucleus. While the latter neurons possess somatodendritic DA autoreceptors responsive to DA agonists, PFC-DA cells appear devoid of these regulatory sites. In the present study, we have used extracellular single-unit recording techniques to compare the basal activity and pharmacological sensitivity of antidromically activated NS-DA neurons, and mesoaccumbens DA cells (MA-DA) which innervate the nucleus accumbens. In addition, we decided to determine whether the antidromic activation itself can alter basal DA neuronal activity and pharmacological sensitivity. Antidromic activation (100-500 µsec, 0.5-3.0 mA) of DA neurons produced only transitory alterations in firing rate and pattern. The inhibitory actions of DA agonists were identical in cells whose projection area was determined either before or after drug treatment, indicating that antidromic activation did not alter pharmacological sensitivity. The mean basal firing rate and the percentage of discharges occurring within bursts did not differ between NS-DA (3.6 ± 0.3/sec; 5.3 ± 2.7%) and MA-DA (4.0 ± 0.3/sec; 10.1 ± 4.0%) neurons. In addition, the dose-dependent inhibitory effects of the direct-acting DA agonists apomorphine (ED_{50}: 12.0 ± 1.8, 7.9 ± 1.5 µg/kg for NS-DA and MA-DA, respectively) and B-HT 920 (ED_{50}: 2.3 ± 0.5, 2.8 ± 1.1 µg/kg) on the two DA cell populations were not statistically different. As observed previously, differences in the spontaneous recovery of cell firing following inhibition by these agonists were observed; NS-DA neurons exhibited a significantly faster recovery in the 10 minutes following complete inhibition than did MA-DA neurons. The two cell populations were equally responsive to inhibition by intravenous d-amphetamine (1.2 ± 0.3, 1.1 ± 0.2 mg/kg); the population differences in spontaneous recovery were less pronounced than observed with the direct-acting DA agonists. These findings suggest that the basal activity and pharmacological sensitivity (to intravenous DA agonist administration) are similar for DA neurons innervating the caudate or the nucleus accumbens. However, differences in recovery have been observed and a further characterization is ongoing. (Supported by grants from the American Parkinson Disease Assoc. and Tourette Syndrome Assoc.)

411.7 D2 DOPAMINERGIC 'GATING' IN THE NUCLEUS ACCUMBENS OF SIGNAL TRANSMISSION FROM THE HIPPOCAMPUS TO THE PEDUNCULOPONTINE NUCLEUS.
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The nucleus accumbens (NAC) receives converging afferent inputs from the hippocampus (HIPP) and the mesolimbic dopaminergic neurones of the ventral tegmental area (VTA). In turn, HIPP signals are relayed via the NAC to the subpallidal region (SP) which includes the substantia innominata and the lateral hypothalamus. A descending projection from the SP to the pedunculopontine nucleus (PPN), a part of the mesencephalic locomotor region (MLR), has also been demonstrated. Since HIPP signals reach the SP area via the NAC and activation of the VTA dopaminergic neurones modulates the excitatory input to the NAC, it is of interest to investigate whether HIPP signals will reach SP-PPN neurones and whether these signals are influenced by a dopaminergic 'gating' mechanism in the NAC.

Extracellular single unit recordings were obtained in the SP region of urethane-anaesthetized rats. SP-PPN neurones were identified by their antidromic responses to electrical stimulation (200-800 µA, 0.15 ms, 1.0 Hz) of the PPN and the adjacent lateral portion of the central gray. 156 SP neurones were antidromically activated by PPN stimulation and 76% (118 neurones) of them had no spontaneous discharge activity. However, 30 (19%) spontaneously discharging SP-PPN neurones, antidromically activated by PPN stimulation, were inhibited orthodromically (mean onset latency = 19 ms) by HIPP stimulation. The inhibitory responses of 8 of these neurones were selectively attenuated by LY171555 (2.5 µg/0.2 µl), a dopamine D2 agonist, but not by SKF38393 (2.0 µg/0.2 µl), a dopamine D1 agonist, microinjected sequentially into the medial NAC via a stainless steel double-cannulae ($p<0.02$). In 2 groups of 10 SP neurones, not antidromically activated by PPN stimulation, their inhibitory responses to HIPP stimulation were separately tested with the D1 and D2 agonists injected into the NAC. LY171555 (D2) injected into the NAC produced a significantly greater attenuation [$F(3,29)=8.4$; $p<0.05$; n=10] of the inhibitory response than SKF38393(D1) [$F(3,31)=2.87$; $p<0.05$; n=10] during the 15 min post-injection period. Taken together these results suggest that SP neurones projecting directly to the PPN, a part of the MLR, convey HIPP signals which are modified by a D2-mediated 'gating' mechanism in the NAC. This HIPP-NAC-SP-PPN pathway appears to convey HIPP signals to the brainstem-spinal cord movement generating site (PPN) and these signals can be 'gated' by a D2 mechanism in the NAC.
(Supported by Medical Research Council of Canada).

411.8 ELECTROPHYSIOLOGICAL EVIDENCE FOR AUTORECEPTORS ON DOPAMINERGIC MESOCORTICAL NEURONS.
R.F. Gariano*, J.M. Tepper, S.F. Sawyer*, S.J. Young*, & P.M. Groves (SPON: R.B. Livingston). Dept. Psychiatry, Univ. California, San Diego, La Jolla CA 92093.

Dopaminergic nigrostriatal neurons possess autoreceptors on the cell body and terminal regions which regulate spontaneous firing rate and dopamine (DA) release. In nigrostriatal DA neurons, local or systemic administration of DA agonists reversibly reduces the excitability of striatal DA terminals to direct electrical stimulation, effects that are mediated by autoreceptor activation (1). It has been proposed on biochemical and electrophysiological grounds that DA neurons in the adjacent ventral tegmental area (VTA) projecting to frontal and cingulate cortices lack such autoreceptors (2). This view has been recently challenged (3,4). We have applied the method of terminal excitability testing to the mesocortical DA system to test for the presence of terminal autoreceptors.

Extracellular recordings from the area of the VTA and medial substantia nigra were obtained from urethane-anesthetized male albino rats (200-350 gm) with stimulating electrodes implanted in the frontal cortex. A total of 56 cells was identified as DAergic mesocortical neurons based on extracellularly recorded waveform, antidromic activation from frontal cortex, and later histological verification of recording site. The average firing rate of these cells was 2.9 ± 0.2 Hz. Local (10 µM) or intravenous (0.5 mg/kg) administration of amphetamine decreased terminal excitability in 6 of 11 neurons tested. The decreases were comparable in magnitude to those observed in nigrostriatal neurons. Two additional cells with variable spontaneous firing rates exhibited a significant inverse correlation ($r = -0.72$, $p < 0.001$) between firing rate and terminal excitability over time, suggesting that these autoreceptors are responsive to moment-to-moment fluctuations in endogenous DA release. The lack of excitability changes found in five cells may indicate an absence of autoreceptors on these cells or that the site of electrical stimulation was in non-terminal axonal regions within frontal cortex. Our results provide electrophysiological evidence for physiologically functional terminal autoreceptors on at least some DAergic mesocortical neurons. Further work is needed to determine what proportion of these cells possesses autoreceptors.

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411.9 ELECTROPHYSIOLOGICAL EFFECTS OF NICOTINE ON DOPAMINE MID-BRAIN NEURONS.
K-W.P. Yoon*, G.L. Gessa*, V. Boi*, L. Naes*, G. Mereu and T.C. Westfall (SPON: K. Smith). Depts. of Pharmacology and Neurosurgery, St. Louis Univ. School of Medicine, St. Louis, MO 63104 and Inst. of Pharmacol., Univ. of Cagliari, 09100, Italy.

Nicotine, the active ingredient in tobacco smoke, is well known to produce multiple psychopharmacological, behavioral and neurochemical effects in the central nervous system. Like several other drugs of abuse, the rewarding and stimulant effects of nicotine appear to be linked to its activation of some central dopamine neurons. Nicotinic receptors have been observed in dopaminergic areas such as the substantia nigra pars compacta (A9), ventral tegmental area (A10), striatum and nucleus accumbens. Moreover, nicotine has been shown to stimulate motor behavior in rats, as well as to increase dopamine synthesis and release from the striatum and nucleus accumbens.

It has been previously reported that the systematic administration of nicotine increases the firing rate of A9 cells (Clarke, P.B.S. et al., Br. J. Pharmac. (1985) 85:827-835). However, there have been no reports describing the effect of the drug on A10 neurons which may be more important in the mediation of the rewarding and reinforcing properties of psychostimulant drugs. We have administered nicotine intravenously while recording single unit activity from A9 or A10 cells. Since it has been observed that general anesthetics may disrupt the pharmacological responses of these neurons, experiments were carried out using chloral hydrate (400 mg/kg) anesthesia as well as locally anesthetized animals paralyzed with d-tubocurarine (5 mg/kg). It was observed that nicotine, in a range of doses between 50 and 200 µg/kg, enhanced the activity of A9 cells up to some 25% above the baseline in both general and local anesthetized preparations. However, the same range of doses was more effective in stimulating A10 cells in local anesthetized rats increasing up to 70% above baseline their firing rate. In contrast, the majority of these cells were temporarily depressed in deeply anesthetized animals. All of the above effects were reversed and prevented by mecamylamine suggesting the involvement of nicotinic-cholinergic receptors. Moreover after nicotine-induced stimulation, low doses of apomorphine (10-50 µg/kg) inhibited the firing rate as in control rats suggesting that dopamine receptors are not directly involved in the nicotine action.

Our results support the idea that nicotine enhances dopamine transmission and demonstrate its greater effect on A10 meso-limbic and meso-cortical than on nigro-striatal dopaminergic neurons.

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411.10 DOPAMINE HYPERPOLARISES NEURONES IN THE RAT SUBSTANTIA NIGRA ZONA COMPACTA IN VITRO BY INCREASING POTASSIUM CONDUCTANCE.
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The firing rate of dopamine (DA)-containing neurones in the substantia nigra zona compacta (SNC) has been shown to be reduced by DA in vivo. The ionic mechanism underlying this effect was determined in the present experiments.

Intracellular recordings were made from neurones in the SNC identified under visual control in coronal slices (300 µm) of rat mesencephalon. The slices were totally submerged in and continuously superfused by oxygenated, bicarbonate-buffered artificial cerebrospinal fluid containing 2.5 mM K at 36°C. The neurones of the SNC generally fired action potentials spontaneously at rates of 1-5 Hz. The firing rate was reduced by DA (3-100 µM) in a concentration-dependent manner. Hyperpolarising the neurones to -60 mV by current injection abolished cell firing; under these conditions 95% of neurones showed a hyperpolarisation accompanied by a decrease in input resistance in response to DA. In the presence of 10 µM cocaine the amplitude of the hyperpolarisations induced by DA (0.3-30 µM) was increased, reaching a maximum value of 15 mV. Reversible and reproducible hyperpolarising responses were evoked by brief applications of DA from the tip of a micropipette; these were reduced in amplitude when the membrane was hyperpolarised by current injection and the response reversed polarity at a membrane potential negative to -100 mV. A single electrode voltage clamp amplifier (Dagan 8100) was used to measure membrane currents. Brief applications of DA caused transient outward currents at holding potentials of -60 mV, which became smaller at more negative potentials and reversed polarity negative to -100 mV. Comparison of steady-state I/V plots (obtained with slow voltage ramps) in the presence and absence of DA was also used to measure DA currents.

The mean reversal potential for DA in 2.5 mM K obtained with these three methods was -109.5 ± 2.3 mV (n=7 cells). In 10.5 mM K the reversal potential of the DA hyperpolarisation was -67 mV. The correspondence of reversal potential of the DA response to the expected equilibrium potential for K ions, together with the close to Nernstian shift in 10.5 mM K, indicates that DA increases potassium conductance in these neurones.

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411.11 **LOCALLY APPLIED DOPAMINE ALTERS ACTIVITY OF VENTRAL PALLIDAL/SUBSTANTIA INNOMINATA CELLS.** T.C. Napier and G.R. Breese. Bio. Sci. Res. Ctr., Univ. North Carolina School of Medicine, Chapel Hill, NC 27514

Neurons of the ventral pallidum/substantia innominata (VP/SI) are often excited, in a dose-related fashion, following administration of apomorphine. These excitations are readily antagonized by subsequent haloperidol treatments[1]. VP/SI responses to systemically administered dopamine (DA) agonists may be mediated indirectly through alterations of a DA sensitive afferent system (eg. ventral striatum or nucleus accumbens). However, recent reports provide evidence that DA agonists may also directly influence VP/SI cells. For example, DA, DOPAC and HVA are present in VP/SI tissue and are greatly reduced following 6OHDA lesions of the substantia nigra[2]. The present study electrophysiologically characterizes the effects of locally applied DA on firing of VP/SI cells. Extracellularly recorded single units (n=37) were sampled from VP and SI regions in chloral hydrate anesthetized rats. VP/SI neurons were predominately biphasic, with a firing frequency of 15±4 Hz and demonstrated regular as well as irregular firing patterns. Approximately half of the cells tested to date were sensitive to microiontophoretically applied DA (0.2 M, 20-120 nA) with a suppression of firing frequency occurring more often than rate potentiations (75% vs 25%). Local application of the D_1 agonist SKF38393 (0.02 M, 20-140 nA) elicited neuronal responses in 42% of the cells tested and a decrease in firing was most often observed. Systemic administration of the D_1 antagonist, SCH23390 (0.01-0.5 gm/kg i.v.), slightly attenuated effects of locally applied DA but a complete blockade has never been observed. Haloperidol (0.1-0.5 g/kg i.v.), a D_2 antagonist, did not cause a robust antagonism of DA-induced rate changes. However, in the majority of cells tested, simultaneous administration of both antagonists did block responses to iontophoretically ejected DA. These experiments provide physiological evidence for a dopaminergic component to neuronal integration of VP/SI cells which likely involves both D_1 and D_2 DA receptor subtypes.

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411.12 INTERACTIONS BETWEEN D-1 AND D-2 RECEPTORS WITHIN THE RAT MESOACCUMBENS DOPAMINE SYSTEM: ELECTROPHYSIOLOGICAL STUDIES. S.R. Wachtel and F.J. White (SPON: D.P. O'Brien). Neuropsychopharmacol. Lab., Univ. of Illinois, Champaign, IL 61820.

The existence of two subtypes of dopamine (DA) receptors within the CNS is now well established. The D-1 receptor is defined as that receptor at which DA stimulates adenylate cyclase, whereas the D-2 receptor is either unlinked to the cyclase or mediates an inhibition of its activity. In previous electrophysiological studies (White and Wang, J. Neurosci. 6, 1986), we have demonstrated the existence of both receptor subtypes within the rat nucleus accumbens (NAc), each of which is associated with an inhibition of NAc neuronal activity. In contrast, the impulse-regulating DA autoreceptor on A10 DA neurons within the ventral tegmental area (VTA) which innervate the NAc appear to be exclusively of the D-2 subtype (White and Wang, JPET, 231, 1986). However, in spite of the lack of inhibitory effect of intravenous (i.v.) or iontophoretic SKF-38393 (a D-1 selective agonist) on A10 DA neuronal firing, the D-1 selective antagonist SCH-23390, when administered i.v., caused an increase (15-46%) in the firing of 4 out of 6 A10 DA neurons. These findings suggest possible interactions between D-1 and D-2 receptors within the mesoaccumbens DA system. Such interactions have been further investigated in the present experiments.

In the first experiments, we tested the possibility that the D-1 agonist SKF-38393 might alter the rate suppressant effect of the D-2 selective agonist quinpirole (LY-171555). SKF-38393 (0.1-3.1 mg/kg, i.v.) failed to alter either the firing rates of A10 DA neurons or the potency of quipirole (1-31 µg/kg, i.v.) to inhibit these cells. However, SCH-23390 (50-150 µg/kg, i.v.) increased the firing of 3 out of 10 A10 DA cells that had been inhibited by quinpirole. We have obtained similar results when SCH-23390 was administered following apomorphine-induced inhibition.

In experiments designed to determine how i.v. SCH-23390 might increase A10 DA activity when there are no D-1 receptors on the A10 DA cells, we used antidromic stimulation techniques to explore the possibility that NAc-VTA feedback pathways might be involved in this effect. We have antidromically identified 8 NAc neurons which project to the VTA. Two of these cells were inhibited by iontophoretic SKF-38393, an effect which was blocked by SCH-23390. Thus, the rate-enhancing effects of SCH-23390 on a subpopulation of A10 DA cells may be indirectly mediated by neuronal feedback pathways. Apparently, only a portion of NAc-VTA feedback fibers emanate from NAc neurons possessing D-1 receptors. Therefore, interactions between D-1 and D-2 receptors within the meso-accumbens DA pathway may occur primarily postsynaptically rather than directly on A10 DA cells. (Supported by a PMAF Research Starter Grant, the Univ. of Ill. Research Board, and DA-04093).

411.13 ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE IN THE MESOACCUMBENS DOPAMINE SYSTEM: STUDIES IN THE NUCLEUS ACCUMBENS. F.J. White. Neuropsychopharmacol. Lab., Univ. Illinois, Champaign, IL 61820.

The nucleus accumbens (NAc) is known to be involved in the reinforcing effects of cocaine. Our previous studies have indicated that, in chloral hydrate anesthetized rats, iontophoretic (ionto) administration of cocaine potentiates the inhibitory effects of dopamine (DA) on NAc neurons (White, Soc Neurosci Abst, 11, 1985). These results suggest that cocaine increases DA neurotransmission within the NAc by blocking the DA reuptake mechanism. Similar effects of cocaine occur in somato-dendritic regions of mesoaccumbens A10 DA neurons within the ventral tegmental area which, along with feedback inhibition, causes a suppression of their activity (Einhorn and White, this volume). When the effects of ionto-cocaine on NAc neurons and A10 DA neurons were directly compared in the present studies, cocaine (0.01M) was significantly more potent at inhibiting NAc cells. This was surprising since A10 DA autoreceptors are more sensitive to DA than postsynaptic DA receptors in the NAc. In addition, cocaine was a more potent inhibitor of NAc neurons than was DA. These results suggest that both DA and non-DA mechanisms are involved in cocaine's effects within the NAc. Since cocaine also inhibits serotonin (5-HT) reuptake and since the NAc receives 5-HT inputs from the dorsal raphe nucleus, we have studied a possible 5-HT involvement in the effects of cocaine on NAc cells.

As previously reported (White, Pharmacol Biochem Behav, 24, 1986), ionto-5-HT (0.3M) produced two effects on NAc cells. Most cells, whether spontaneously active or driven to fire by ionto-glutamate (0.1M), were inhibited by low 5-HT ejection currents (5-20nA). However, 5-HT increased the activity of a subset of spontaneously active NAc cells. In contrast, DA inhibited the activity of both types of NAc neurons. Cocaine was more potent at inhibiting those cells on which both DA and 5-HT were inhibitory as compared to those on which 5-HT was excitatory. In addition, cocaine was more potent than either 5-HT or DA alone. Low ejection currents (2-4nA) of cocaine potentiated the inhibitory effects of both DA and 5-HT on NAc cells. The selective DA uptake inhibitor GBR-12909 (0.01M) potentiated the effects of DA but not those of 5-HT and, by itself, produced an inhibition of activity which was significantly less than that produced by cocaine. The D-2 DA antagonist l-sulpiride (0.05M) completely blocked the effects of DA, partially blocked the effects of cocaine, but exerted no effect on those of 5-HT. The local anesthetic procaine (0.01M) did not alter the firing rates of NAc neruons. These results indicate that cocaine is a potent inhibitor of most NAc cells because it blocks the reuptake of both DA and 5-HT, each of which is inhibitory on most NAc cells. (Supported by a PMAF Research Starter Grant, BRSG NIH RR-7030 and NIDA Grant DA-04093).

411.14 ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE IN THE MESOACCUMBENS DOPAMINE SYSTEM: STUDIES IN THE VENTRAL TEGMENTAL AREA. L.C. Einhorn* and F.J. White (SPON: W.G. Wood). Neuropsychopharmacol. Lab., Univ. of Illinois, Champaign, IL 61820.

Behavioral evidence has demonstrated that the reinforcing properties of cocaine are mediated, at least in part, by the mesoaccumbens dopamine (DA) system projecting from the A10 DA cells within the ventral tegmental area (VTA) to the nucleus accumbens (NAc). We have recently reported that intravenous (iv) administration of cocaine to chloral hydrate anesthetized rats causes a dose-dependent partial inhibition (50-60%) of the activity of identified mesoaccumbens A10 DA neurons (White, Soc Neurosci Abst, 11, 1985). Although this effect appeared to be due to cocaine's ability to block the DA neuronal uptake mechanism, other effects of cocaine were not ruled out. Because cocaine also inhibits the reuptake of norepinephrine (NE) and serotonin (5-HT) and exerts local anesthetic effects, we explored the possible roles of these various mechanisms in the inhibitory effects of cocaine on A10 DA cells. Administration of the catecholamine uptake inhibitor nomifensine and the selective DA uptake inhibitor GBR-12909 resulted in a dose-dependent suppression of A10 unit activity comparable to that observed with cocaine (0.1-3.1 mg/kg, iv). In contrast, neither the selective NE uptake inhibitor, desipramine, nor the selective 5-HT uptake inhibitor, fluoxetine, caused any significant alteration of A10 DA neuronal activity. The local anesthetic procaine caused a transient increase in the activity of most cells tested. In microiontophoretic experiments, GBR-12909 (0.01M, 20nA) caused a partial suppresion of A10 DA unit activity similar to that previously found with cocaine while procaine (0.01M 40nA) failed to alter neuronal activity.

In addition, because we have previously shown that microiontophoretic application of cocaine onto A10 DA cells caused only a weak (15-20%) inhibition of A10 DA cells while similar administration in the NAc resulted in a significantly greater inhibition (White, this volume), we sought to determine whether NAc-VTA neuronal feedback pathways might be involved in the effects of iv cocaine. Rats received unilateral ibotenic acid (5-6 µg) injections into the NAc to destroy neuronal somata. Following 5-7 days of postoperative recovery, the inhibitory effects of iv cocaine on A10 DA cells were significantly reduced as compared to those observed in sham operated controls.

These results indicate that the effects of cocaine on A10 DA neurons are due to alterations in DA activity rather than 5-HT, NE or local anesthetic effects. In addition, both impulse-regulating somatodentritic DA autoreceptors and inhibitory NAc-VTA feedback processes appear to be involved in the effects of iv cocaine on A10 DA neurons. (Supported by a PMAF Research Starter Grant, BRSG NIH RR-7030 and NIDA Grant DA-04093).

411.15 NEURONAL RESPONSIVENESS TO BIOGENIC AMINES AND GABA IN RAT CINGULATE VS PARIETAL CORTEX: COMPARATIVE MICROIONTOPHORETIC STUDY. M. Beauregard* and A. Ferron (SPON: R.A. Benoit). Centre de recherche en sciences neurologiques (Dép. physiologie), Université de Montréal, Montréal (Qué.), Canada H3C 3J7.

The cingulate and the parietal cortex of rat both receive a triple monoamine innervation, by dopamine (DA), noradrenaline (NA) and serotonin (5-HT) afferents. Whereas NA and 5-HT axon terminals are relatively abundant and distributed to every layer in these two cortical regions, the DA terminals are also found in great number and in every layer of the cingulate cortex, but they are only scarce and strictly confined to layer VI in the parietal cortex (Descarries et al., 1986). In contrast, the density of GABAergic nerve cell bodies and axon terminals appears to be fairly uniform throughout both cortical regions (Mugnaini et Oertel, 1985). The effect of these transmitters on spontaneously active neurons of the cingulate and the parietal cortex was compared by microiontophoresis in urethane-anaesthetized rats. The responsiveness to microiontophoretically applied DA (0.5 M, pH 4), NA (0.5 M, pH 4), 5-HT (0.1 M, pH 4) and GABA (1.0 M, pH 4) was assessed with the IT50 method, i.e. by measurement of the current (in nA) X time (in sec) required to obtain a 50% depression of firing rate. Both cortical regions were examined in the same rats, most of the data for the parietal cortex being obtained from deep layer neurons.

A vast majority of spontaneously active neurons in both the cingulate and the parietal cortex responded by depression of their firing rate to all or either one of the biogenic amines and to GABA. There were no significant differences in IT50 values for NA (632 ± 72 vs 711 ± 177 nC) and for 5-HT (667 ± 70 vs 616 ± 143 nC) between these two regions. However, responsiveness to DA was nearly twice lower in cingulate than in parietal cortex (1732 ± 336 vs 1016 ± 154 nC). In the case of GABA, a reverse observation was made, cingulate cells showing four times higher responsiveness than the parietal neurons (56 ± 9 vs 209 ± 30 nC).

These data suggest that the presence of DA terminals exerts a profound influence on the responsiveness of cortical neurons to this biogenic amine and to GABA, but not to NA or 5-HT. The lower DA responsiveness in more densely DA-innervated cortex (cingulate) could reflect a greater capacity for reuptake and/or a regional difference in sensitivity of D1 and/or D2 postsynaptic receptors. The weaker responsiveness to GABA in the less densely DA-innervated cortex (parietal) suggests a modulatory interaction between these two transmitters, unless the intrinsic properties of the GABA system differ between cortical regions.

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411.16 ELECTROPHYSIOLOGICAL PROPERTIES OF DOPAMINE-SENSITIVE NEURONS IN SUBSTANTIA NIGRA TISSUE SLICES. N.L. Silva and B.S. Bunney Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

Identified dopamine (DA) neurons have been observed in vivo to fire in two types of patterns: 1) a slow irregular spiking mode and 2) a burst firing mode. Intracellular studies have shown that DA neurons have a slow depolarization (SD) which results in an action potential (AP) which is followed by an afterhyperpolarization (AHP). It is believed that this is an intrinsic pacemaker modulated by afferents to produce the irregular and burst firing patterns of the DA neuron. Recently, DA-sensitive neurons in the nigral tissue slice were reported to exhibit characteristics which are similar to those found in vivo, except that they have a very regular firing pattern. We further examined the firing pattern of DA-sensitive neurons in vitro, utilizing intracellular recording techniques and investigated the effect of DA on these cells.

Midbrain tissue slices (300-400μm) were prepared from 200-250gm male Sprague Dawley rats. Slices were placed into an air interface recording chamber which was continuously perfused with an oxygenated nutrient medium and maintained at 36.5°C. The perfusate could be rapidly switched between normal and DA containing media (1-100 μM). Intracellular electrodes (50-70 Megohms) were filled with 3M KCl.

Intracellular recordings of DA-sensitive neurons revealed that they possess an input resistance ranging between 100-200 Megohms and a time constant of 37.5 ± 5 ms. Hyperpolarizing current pulses demonstrated a nonlinear current-voltage relationship. The AP's were of long duration (>2mS) and firing rates were slow (0.5-5 spk/s). All cells exhibited a regular firing pattern. As was described in vivo, a SD (11± 3mV, 210 ± 60 ms) initiated each AP (50-55mV) which was followed by an AHP. The SD, firing rate and firing threshold were voltage sensitive. The introduction of DA was associated with a dose-dependent hyperpolarization and decrease in firing rate, along with a decrease in input resistance which was maintained when the membrane potential was manually clamped to resting levels. These effects were antagonized by sulpiride.

These results, obtained from slices in which most if not all afferents are cut, support the in vivo studies suggesting that DA cells have intrinsic pacemaker activity which is modified by inputs external to the substantia nigra to produce the different firing patterns observed in vivo.

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411.17 EXCITABILITY PROPERTIES OF MEDIAL FOREBRAIN BUNDLE AXONS OF PUTATIVE A10 DOPAMINE CELLS. J.S.Yeomans, N.T. Maidment* and B.S.Bunney. Dept. Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06510.

Putative ventral tegmental dopamine cells were recorded extracellularly and antidromically activated by electrical stimulation of the medial forebrain bundle at the level of the preoptic area in rats. The cells were identified by location, waveform, spontaneous firing rate, antidromic latency and collision tests. The absolute refractory periods, measured with test pulses 1.5 to 3 times current threshold, ranged from 1.2 to 2.5 msec. Minimum latency varied from 5.0 to 11 msec, as measured at the peak of the initial segment spike. Collision intervals were near the antidromic latency, and considerably less than the latency plus the absolute refractory period.

The latency of antidromic spikes in a given cell varied by as much 1.5 msec, depending on when the stimulus was delivered relative to spontaneous spikes. When two pulses were delivered, the latency following the second pulse was more reliable, always being longest at interpulse intervals slightly longer than the absolute refractory period and shortest at interpulse intervals from 20 to 200 msec (the supernormal period). The peak of supernormal period conduction was similar to the interspike interval of bursts observed spontaneously, suggesting that bursting favors fast conduction through axons.

In several cells MFB stimulation below antidromic threshold resulted in a burst of firings. The latency of the first action potential was 30-100 msec and was followed by 1-4 spikes within a few hundred msec, then a relatively inactive period followed by a return to normal spontaneous firing. This suggests that MFB stimulation with single, high-current stimulating pulses (800 uA and above, 0.5 msec duration pulses) can synaptically activate DA cells to produce a single burst.

The current-distance relationship, tested in 4 cells by moving the stimulating electrode past the axon, was approximately parabolic (I = K d exp 2), with K ranging from 1000 to 2000 uA/mm exp 2 for 0.5 msec pulses. This allows calculation of the radius of the field of dopamine axon excitation at any current.

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411.18 EFFECTS OF DOPAMINE AGONISTS ON SLOW AND FAST FIRING DOPAMINE NEURONS. L.T. Meltzer and C.L. Christoffersen*. Warner-Lambert/ Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105

There is recent evidence that the baseline firing rate of dopamine (DA) neurons can influence the percent inhibition produced by different DA agonists. In the present study, we have extended these observations by evaluating the cumulative IV dose-response curves for EMD 23 448, pergolide (PER), lisuride (LIS), apomorphine (APO), and (-)3PPP (-3P) on slow (<4 Hz) and fast (>4 Hz) firing DA neurons. Extracellular single-unit recordings were obtained from chloral hydrate anesthetized male S-D rats. DA neurons were identified by standard criteria. Unless stated, doses are cumulative.

Slow Firing Neurons. APO (32 ug/kg), PER (10 ug/kg), and EMD (200 ug/kg) produced complete inhibition (100%) of slow firing DA neurons. LIS (40 ug/kg) completely inhibited 5 of 6 DA neurons. -3P (640 ug/kg) produced near-complete (93%) inhibition of slow firing DA neurons. Thus, all these DA agonists show full or near full agonist activity on slow firing DA neurons.

Fast Firing Neurons. APO (128 ug/kg) and PER (40 ug/kg) produced complete inhibition of fast firing DA neurons. In contrast, EMD (4.0 mg/kg), -3P (5.1 mg/kg), and LIS (1.28 mg/kg) produced only partial inhibition (60%) of fast firing DA neurons. Thus, APO and PER exhibit full agonist activity while EMD, -3P, and LIS exhibit partial agonist activity on fast firing DA neurons when a cumulative dose-response is determined. However, when doses of EMD (0.5 mg/kg) and LIS (.64 mg/kg) which produced partial inhibition (60%) in the cumulative dose-response curve were injected as bolus doses, they produced near complete inhibition (>90%) of fast firing DA neurons. Thus, LIS and EMD can display full DA agonist activity similar to APO and PER. However, it appears that a dose of LIS or EMD in some way effects a subsequent dose's ability to produce inhibition. This is not observed with APO and PER. In contrast, a large bolus dose of -3P (2.5 mg/kg) still only produced a partial inhibition (46%) of fast firing DA neurons. Thus, as reported previously, -3P appears to be a partial DA agonist.

For all compounds, the percent inhibition produced by the same dose was greater in slow compared to fast firing DA neurons. However, the magnitude of the inhibition in actual HZ was always equal or greater in fast compared to slow firing cells. This suggests that there are no intrinsic differences between these arbitrarily defined cell populations in their response to DA agonists.

411.19 MIDBRAIN DOPAMINE NEURONS IN GENETIC MOUSE STRAINS: NEURONAL RESPONSE PROPERTIES. G.L. Bernardini*, S.G.Speciale, and D.C.German (SPON: E. Orr). Depts. of Physiol. and Psychiat., Univ. of Texas Health Sci. Cntr., Dallas, TX 75235.

There are strain-dependent differences in midbrain dopamine (DA) neurons. The BALB/c mouse; (a) exhibits more DA-related behavior (Fink & Reis, Brain Res. 222:335,1981); (b) has more DA type-2 receptors in the striatum (Helmeste & Seeman, Psychiat.Res. 7:351,1982); and (c) has more midbrain DA neurons (Fink & Reis, 1981;German et al.Neurosci. Abstr. 9:1150, 1983) than the CBA strain. In order to determine how single DA neurons function in nuclei of different sizes we have made in vivo recordings from the midbrain DA neurons in BALB/c and CBA mouse strains. We found that the midbrain DA neurons fired significantly slower in the BALB/c than in the CBA strain (Sanghera & German, Neurosci. Abstr. 10:950, 1984), but the BALB/c neurons were significantly more sensitive to autoreceptor stimulation than those in the CBA (Walker et al. Neurosci. Abstr. 11:890, 1985).

The purpose of the present study was to determine whether the strain difference in midbrain DA neuronal firing rate was due to a difference in afferent drive on these neurons or a difference intrinsic to the midbrain. Single cell recording techniques were used in the in vitro slice preparation. Coronal midbrain sections were cut at 400 µm thickness. Just as we observed in vivo, the in vitro firing rates of DA neurons in the BALB/c mice were significantly slower than the firing rates in the CBA mice (2.85 $\pm$ 0.19 vs. 3.84 $\pm$ 0.22 impulses/sec., n=15/group, $p < 0.001$). We also observed that the cells in both strains uniformly fired with an irregular pattern (vs. burst firing pattern). These data are consistent with the hypothesis that (a) basal DA neuronal firing rates are inversely related to the number of neurons in the nucleus, and (b) burst firing patterns, exhibited by some DA neurons, are dependent upon afferent inputs to these cells. Research supported by NIMH grant MH-30546 and the Dallas Area Parkinsonism Society.

411.20 Iontophoretically applied exogenous dopamine produced different electrophysiological response in accumbens neurons from that of endogenous dopamine released by VTA stimulation. C.Y. Yim and G.J. Mogenson, Department of Physiology, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

We have previously reported that pulse-train stimulation of the ventral tegmental area (VTA) produced consistent membrane depolarization of neurons in the nucleus accumbens (NAcc) and attenuated EPSPs evoked from amygdala (AMY) or hippocampal (HIPP) stimulation (Yim and Mogenson, Brain Res. 369:347-352,1986). The effects of VTA stimulation were attenuated by dopamine (DA) antagonists such as haloperidol, sulpiride or clozapine, suggesting that the response was mediated by DA released by activation of the mesolimbic DA pathway. We recently compared these responses to those produced by iontophoretically applied DA and found similar as well as different effects.

Intracellular recordings were made from neurons in the nucleus accumbens of urethane-anesthetized rats in situ using multibarrel microelectrodes which permit simultaneous intracellular recording and extracellular iontophoretic application of putative transmitters. Evoked postsynaptic potentials (PSPs) following stimulation of the AMY or HIPP were recorded and the effects of VTA stimulation and iontophoretically applied DA on these PSPs were compared. From a preliminary series of 11 experiments, it was found that both VTA stimulation and iontophoretically applied DA produced consistent membrane depolarization accompanied by an increase in membrane conductance and an attenuation of EPSP evoked from amygdala or hippocampal stimulation. Membrane depolarization produced by DA (10-15 mV) was greater than that produced by VTA stimulation (5-10 mV) but attenuation of the EPSP was quantitatively similar in both cases. However, VTA stimulation produced a selective attenuation of the evoked EPSP leaving a late IPSP unaffected whereas iontophoretically applied dopamine attenuated both. Moreover, in neurons that receive convergent input from both the AMY and the HIPP, VTA stimulation usually produced preferential attenuation of the evoked response from AMY stimulation. Iontophoretically applied DA, on the other hand, did not show such selectivity and always produced similar attenuation of responses from AMY and HIPP stimulation. We conclude from these experiments that responses produced by iontophoretically applied exogenous DA may not be all physiological and that the action of DA in the striatum may depend on specific sites of action and perhaps on the co-release of other transmitter substnaces. (supported by MRC of Canada.)

411.21 INCREASED EXCITABILITY OF NON-DOPAMINE CELLS IN VENTRAL MIDBRAIN OF RAT TO DA AGONISTS IS CORRELATED WITH HYPERMOTILITY AND ORAL STEREOTYPY. M. E. Olds, Division of Biology, California Institute of Technology, Pasadena, California 91125.

The responses of identified non-DA cells in ventral tegmental area (VTA) and substantia nigra (SN) to dopamine (DA) agonists were investigated with the extracellular recording technique in behaving rats. In each subject, multi- and single-unit activity was recorded simultaneously from 7 electrodes, shaped into 3 bundles of 3 wires each, of differing lengths. The cells were identified by their biphasic, short duration action potentials (<2.0 ms) and high rates of firing (>20 spikes/s). Motor activity was recorded with the open-ended wire technique in which movements of locomotion generated high counts and movements of the head and mouth, low counts. Systemic d-amphetamine (5.0 mg/kg) and apomorphine (3.0 mg/kg) induced hypermotility/stereotypy and large increases in unit discharge. These effects were dose-responsive with doses of 1.0 and 2.0 mg d-amphetamine resulting in increases of lower magnitude, shorter duration, and more variability. Systemic apomorphine (3.0 mg/kg) also induced large increases in movement and unit counts, but of shorter duration than those seen with amphetamine. Lower doses of apomorphine (0.75 mg and 1.5 mg) induced responses of less duration and lower magnitude.

Analysis of the increases induced by d-amphetamine showed that 86% of the rats showed 100% increase in movement counts, 65% showed 200% increase, and 46% showed 300% increase. For units, 64% showed 100% increase, 19% showed 200% increase, and 11% showed 300% increase (criterion: increase had to be sustained, without interruption, during the 15-130 min interval of the 150 min post-injection period. Pretreatment with haloperidol (1.5 mg/kg) severely attenuated the increases in movement and unit counts to d-amphetamine (5.0 mg/kg).

Pretreatment with a subanesthetic dose of urethane (600 mg/kg) to prevent the expression of locomotion but not head and mouth movements led to an attenuated response in VTA but not in SN to d-amphetamine (5.0 mg/kg). D-amphetamine (5.0 mg/kg) given to rats with bilateral electrolytic lesions in n. accumbens induced an attenuated motor response but normal neuronal responses.

These data are interpreted to mean that (1) a subpopulation of non-DA neurons in VTA and SN transmit signals related to the DA motor function, (2) the response of these cells to DA agonists depends upon the stimulation of DA receptors, (3) the non-DA cells in VTA may transmit to premotor neurons signals related to locomotor movements, the non-DA cells in SN, signals related to oral stereotypy, and (4) the response of these cells to DA agonists may also depend on the release of DA in SN-VTA, since lesions of n. accumbens and ventral striatum reduced the motor but not the neuronal response to amphetamine.

412.1 EFFECT OF DOPAMINERGIC AGONISTS AND ANTAGONISTS ON α-MSH RELEASE FROM RAT PITUITARY. W.Fratta, L.Lai* and G.L.Gessa*. Institute of Pharmacology, University of Cagliari, Cagliari, Italy.

It is khown that α-MSH release from the intermediate lobe of pituitary is under inhibitory dopaminergic control. "In vitro" studies had shown that such inhibitory control is specifically mediated by Dopamine D2 type receptor. (K.Tsuruta et al., Nature, 292; 463; 1980). We have tested the effect of selective Dopamine D1 and D2 agonists and antagonists on α-MSH levels in rat plasma, with the purpose to verify whether the "in vivo" α-MSH release is essentially regulated as shown by "in vitro" studies. Our results indicate that (-) Sulpiride, a specific D2 receptor antagonist , is extremely potent in increasing α-MSH levels in rat plasma. In fact, 30' after a single dose of 100 μg/Kg of (-) Sulpiride, α-MSH levels in rat plasma increase of about 200% and return to control values 60' after treatment. The high potency of (-) Sulpiride may reasonably suggest that its effect on α-MSH release is directly mediated at pituitary level, since this dose is far lower than those active at SNC level. The D2 agonist LY 171555 causes a dose dependent decrease of α-MSH levels in rat plasma in a dose range between 10 and 100 μg/Kg. These doses are the same known as fully effective for the central effects mediated by LY 171555. On the contrary, the new compound SCH 23390, wich has been characterized as a potent and selective central D1 antagonist, does not show any effect on plasma α-MSH levels with doses up to 500 μg/Kg. Only higher doses significantly increase the levels of rat plasma α-MSH. However, at these doses the specificity of SCH 23390 as D1 antagonist is very questionable. Thus, these data confirm the role of the Dopamine D2 receptor as specific regulator of α-MSH release from pituitary intermediate lobe. Furthemore, the selective response of α-MSH release to the specific D1 and D2 ligands might offer an interesting model of functional activity to study and to differentiate dopaminergic agents on the basis or their specificity for the two types of Dopamine receptors "in vivo".

412.2 EFFECTS OF HYPOTHALAMIC ELECTRICAL STIMULATION ON THE ACTIVITIES OF DOPAMINERGIC AND 5-HYDROXTRYPTAMINERGIC NEURONS IN THE MEDIAN EMINENCE. J.W. Gunnet* and K.E. Moore. (Spon: J. Thornburg) Dept. Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

The median eminence (ME) is innervated by tuberoinfundibular dopaminergic (TIDA) neurons and 5-hydroxytryptaminergic (5-HT) neurons. The cell bodies of TIDA neurons are located in the arcuate nucleus (ARN). 5-HT cell bodies are located in the midbrain but the dorsomedial nucleus (DMN) of the hypothalamus is also believed to contain cell bodies of intrahypothalamic 5-HT neurons. In the present study electrical stimulation was applied to regions in the medial hypothalamus in an effort to activate these intrahypothalamic 5-HT neurons and to locate possible afferents to TIDA neurons.

Ovariectomized Long Evans rats were anesthesized with Equithesin (3 ml/kg) and given bilateral electrical stimulation (biphasic 1 msec pulses, 10 Hz, 200 uA peak-to-peak) of the ARN, DMN or ventromedial nucleus (VMN). The activities of DA and 5-HT neurons were determined by measuring the rates of precursor accumulation (DOPA or 5-HTP) in the ME following injection of the aromatic L-amino acid decarboxylase inhibitor, NSD 1015. In the initial experiment NSD 1015 (100 mg/kg, i.p.) was given at the start of the 30 min stimulation period. Stimulation of the ARN and the DMN but not the VMN increased DOPA levels in the ME. There were no changes in 5-HTP accumulation in the ME regardless of the region stimulated. When the frequency of DMN stimulation was varied (0, 5, 10, 20 and 50 Hz) only stimulation at 5 and 10 Hz increased DOPA accumulation (15 min after NSD 1015 25 mg/kg, i.v.) in the ME.

DA released from TIDA neurons in the ME acts at the anterior pituitary to inhibit the secretion of prolactin. To determine if DMN stimulation increases DA release from TIDA terminals, serial measurements of plasma prolactin concentrations were made during DMN stimulation. There was a significant decline in prolactin levels after 15 min of DMN stimulation at 5 and 10 Hz. DMN stimulation (10 Hz, 30 min) in male rats causes an increase in DOPA accumulation in the ME similar to that seen in female rats. The effect of DMN stimulation was also determined when basal TIDA neuronal activity was elevated by haloperidol (1 mg/kg,i.p., 24 hr) or depressed by bromocriptine (3 mg/kg,i.p., 24 hr). Regardless of the initial rate of DOPA accumulation, stimulation of the DMN (10 Hz, 30 min) increased DOPA levels in the ME. These results are not consistent with the presence of putative 5-HT neurons originating in the DMN and projecting to the ME, but do suggest the existence of afferent neurons, projecting from or passing through the DMN, which stimulate TIDA neuronal activity. (Supported by USPHS grant NS9174.)

412.3 ATRIOPEPTIN III INCREASES THE NEUROSECRETORY ACTIVITY OF TUBEROHYPOPHYSEAL DOPAMINE NEURONS. K.T. Demarest and L.I. Fedoronko*. Depts of Physiology and Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824.

Hemorrhage and dehydration have been demonstrated to increase the neurosecretory activity of tuberohypophyseal dopamine neurons. These two physiological states are also associated with an increased secretion of vasopressin from the neurointermediate lobe of the pituitary. Recent studies have demonstrated that the hemorrhage- and dehydration-induced increase in vasopressin can be inhibited by the systemic administration of atriopeptin III. It has also been suggested that vasopressin release may be inhibited by tuberohypophyseal dopamine neurons. The objective of the present study was to determine if tuberohypophyseal dopamine neurons are stimulated by atriopeptin III. Atriopeptin III was administered via intravenous or intracerebral ventricular infusions at doses of 0.5 ug/ul/30 min or 10 ug/10 ul, respectively. Dopamine neuronal activity was estimated by measuring the rate of DA synthesis and turnover in the neurointermediate lobe, median eminence, and the striatum which are the terminal regions of the tuberohypophyseal, tuberoinfundibular, and nigrostriatal dopamine neuronal systems, respectively. Dopamjne synthesis was estimated by measuring the rate of dihydroxyphenylalanine (DOPA) accumulation following NSD 1015 administration. Dopamine turnover was determined by the rate alpha-methyltyrosine induced decline of dopamine concentrations after 0, 30, and 60 minutes. Dopamine and DOPA concentrations were analyzed by a radioenzymatic assay. A significant increase in dopamine synthesis and turnover was demonstrated in the neurointermediate lobe following both the intravenous (vehicle, 0.66±0.04; atriopeptin III, 1.22±0.19) or intracerebral ventricular (vehicle, 0.81±0.07; atriopeptin III, 1.92±0.21) infusions of atriopeptin III compared to vehicle treated control rats. No change in dopamine synthesis or turnover was observed in the median eminence or striatum. These experiments suggest that atriopeptin III selectively stimulates the tuberohypophyseal dopamine neurons, therefore at least partially supporting the hypothesis that the tuberohypophyseal dopamine neurons may mediate the atriopeptin III-induced inhibition of vasopressin secretion. (Supported by a Biomedical Research Support Grant from the College of Osteopathic Medicine, Michigan State University).

412.4 HALOPERIDOL EFFECT ON HUMAN PLASMA BETA ENDORPHIN LEVELS: DOSE RESPONSE AND EFFECT OF DEXAMETHASONE. M.M. Murburg, R.C. Veith, G. McDonald and D.M. Dorsa* (SPON: C.W. WILKINSON). Seattle VA Medical Center and Departments of Psychiatry, Medicine and Pharmacology, University of Washington School of Medicine, Seattle, WA 98195.

Dopamine (DA) plays an important role in regulating release of beta endorphin (βE) and related peptides from the neurointermediate lobe (NIL) in a number of species, while corticosteroids exert feedback-inhibition over release of βE from the anterior lobe (AL). We have previously reported that intravenous (IV) administration of the DA antagonist haloperidol to normal young men increases plasma levels of beta endorphin-like immunoreactivity (βE-LI) and cortisol. Here, we attempt to define dose response characteristics of this effect, and also to determine whether corticosteroids inhibit the response. Thirty-three normal-weight male nonsmokers, aged 20 to 37, without psychiatric or medical disorders or substance abuse, were studied after one week of abstinence from alcohol or drugs of any kind. Fourteen of these subjects were randomly assigned to receive pretreatment with placebo capsules (N=7) or dexamethasone 1.5 mg p.o. (N=7) at 11:00 p.m. the night before the study. The remaining 19 subjects received no pretreatment. All subjects fasted except for water from midnight the night before the study.

On the morning of the study, IV catheters were inserted, and after a 30-minute rest period, three baseline blood samples were drawn. Haloperidol 0.05 mg/kg IV was then given to subjects taking dexamethasone or placebo the night before; the remainder of subjects were randomly assigned to receive IV saline placebo (N=6), or IV haloperidol 0.015 mg/kg (N=5) or 0.030 mg/kg (N=8). All medications were given on a double-blind basis. Following injection, blood samples were taken at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180 and 210 minutes.

We found a dose-related increase in βE-LI following IV haloperidol ($F=3.82$; DF 3,22; $p < 0.025$, ANOVA). Peak increases from baseline βE-LI were 10.9 ± 2.0, 23.0 ± 6.8, 38.2 ± 4.3 and 46.3 ± 11.7 pg/ml ($\bar{x}$ ± SEM) for subjects given placebo, 0.015 mg/kg and 0.03 mg/kg and 0.05 mg/kg, respectively. Dexamethasone, compared to placebo, suppressed both baseline βE-LI levels (16.1 ± 2.1 vs 23.4 ± 2.1 pg/ml, $p < 0.01$, t test) and haloperidol-induced peak increases in βE-LI (7.2 ± 1.4 vs 46.3 ± 12.6 pg/ml, $p < 0.01$, t test). We conclude that neuroregulatory mechanisms which are specific to the NIL (inhibition of βE release by DA) or the AL (inhibition of βE release by corticosteroids) in other species, interact to regulate βE release into plasma in humans.

412.5 A COMPARTMENTALIZED CHAMBER FOR STUDYING THE RELEASE OF DOPAMINE (DA) FROM THE ISOLATED HYPOTHALAMO-NEUROHYPOPHYSIAL COMPLEX. Paul A. Garris* and Nira Ben-Jonathan, (SPON: Joseph DiMicco). Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, Indiana 46223.

We have recently shown that DA from the rat posterior pituitary (PP) participates in the regulation of anterior pituitary prolactin secretion. Given that the short portal vessels linking the posterior and anterior lobes are inaccessible to cannulation, in vitro studies must be employed to determine the regulation of DA release from the PP. The objectives of this study were to: 1) develop a technique for measuring DA release from an isolated hypothalamo-PP complex, and 2) compare DA release under different endocrine states. Tissue explants from male rats containing the medial basal hypothalamus (MBH), pituitary stalk and PP were placed in a specially designed chamber. The MBH and PP compartments were isolated by a sliding partition with a notch through which the intact pituitary stalk traversed. Leakage of fluid between the two compartments was 0.05%/h. The PP was incubated with tritiated DOPA (2 uCi) for 1 h, followed by 2h of chase. DA release was elicited by electrical stimulation via platinum electrodes placed alongside the PP or MBH. Tritiated DA (^{3}H-DA) was determined by HPLC with a radioactive flow detector. Three identical 1 min trains of pulses (10 Hz, 0.5 msec, 10 V) were delivered to the PP 20 min apart. ^{3}H-DA release was similar for each train, comprising about 2.5% of the total tissue ^{3}H-DA content which averaged about 50,000 dpm. There was negligible release of ^{3}H-DA before and after stimulation. Frequency-dependent release was examined in male, ovariectomized (OVEX) and OVEX rats implanted with estradiol pellets (OVEX+E_2). Male PP, stimulated with 5, 10 and 20 Hz, released 1, 2 and 3% of total ^{3}H-DA, respectively. In contrast to males, the same frequencies caused a significantly higher release of ^{3}H-DA from PP of OVEX and OVEX+E_2. Electrical stimulation of male MBH also evoked a frequency-dependent release of ^{3}H-DA from the PP. However, this required higher frequencies (10, 20 and 40 Hz) and resulted in a lower percent of released ^{3}H-DA.

Conclusions: 1) The compartmentalized chamber is well suited for studying hormonal control of DA release from the hypothalamo-neurohypophysial complex. The advantage of this method is that the tuberoinfundibular DA neurons remain intact, with cell bodies in the MBH and nerve terminals in both the MBH and PP. 2) There are marked differences in the amount of DA released from the PP under different endocrine states, which could be correlated with the postulated role of DA from the PP in suppressing prolactin secretion. Supported by NIH grant NS 13234.

412.6 ANTERIOR PITUITARY DOPAMINE IS INFLUENCED BY ESTRADIOL AND PROGESTERONE, BUT NOT BY 5-ALPHA-DIHYDROTESTOSTERONE. N.Telford and C.E.Finch. Department of Biological Sciences and Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

Estradiol (E2) induces up to 5-fold increases in anterior pituitary dopamine (DA) in C57BL/6J female mice (Telford, et al., Neuroendocrinology, 1986, in press). To assess the effects of other gonadal steroids on pituitary DA, ovariectomized (OVX) mice were treated with progesterone (P) or 5-α-dihydrotestosterone (DHT) in the absence or presence of E2 (ca. 20 pg E2/ml serum). The anterior pituitary was assayed for DA by reversed-phase HPLC with electrochemical detection.

Progesterone (ca. 35 ng P/ml serum) alone had no effect on pituitary DA, yet inhibited by 50% the increase in pituitary DA produced by exogenous E2. DHT alone had no effect on pituitary DA, and did not affect the E2-induced increase in pituitary DA.

As female mice approach midlife, estrous cycles lengthen and eventually cease. This cessation of cycles, which occurs in about 50% of the mice in our colony by 12-13 months of age, is accompanied by tonic secretion from the polyfollicular ovaries of moderate levels of E2 relatively unopposed by P. We previously demonstrated that these altered ovarian secretions are responsible for the age-related 4-5 fold increase in pituitary DA in female mice, and that removal of the ovaries from aging mice causes pituitary DA to decline to levels comparable to those seen in young cycling mice (Telford, et al., ibid.). To further confirm that ovarian E2 unopposed by P is responsible for the age-related increase in anterior pituitary DA, OVX and E2-replaced or intact mice were treated with P. Progesterone antagonized the E2-induced increase in pituitary DA in aging (12 month) mice, as was seen in the young (6 month) mice. Progesterone had no effect on pituitary DA in intact young mice, but decreased pituitary DA in intact aging mice to levels indistinguishable from those seen in young intact mice, indicating that decreasing the E2/P ratio can attenuate the age-related increase in pituitary DA. The antagonism of both the E2-induced and the age-related increases in pituitary DA by P is further evidence that the age-related increase in pituitary DA is caused by tonic E2 secretion from the polyfollicular ovaries.

Estradiol is the only gonadal steroid investigated which increases pituitary DA. DHT alone or in combination with E2 has no effect on pituitary DA. Progesterone alone has no effect on pituitary DA, but can antagonize the effect of exogenous or endogenous E2.

This work was supported by NIA grant AG00443 (CEF) and NIA training grant AG00093 (NT).

412.7 PULSATILE ACTH SECRETION IN RHESUS MONKEYS: VARIATION WITH TIME OF DAY AND RELATIONSHIP TO CORTISOL. M. Carnes, N.H. Kalin*, S.J.Lent*, C.M. Barksdale*, and M.S.Brownfield. William S. Middleton Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705, and Univ. of Wis. School of Vet. Med., 2015 Linden Dr., Madison, WI 53706.

The circadian rhythm of pituitary ACTH secretion has been well characterized. Recently, using narrow sampling intervals, very frequent "micropulses" of ACTH have been described in humans, dogs, horses and rats.

To assess the variation in ACTH "micropulse" parameters which contribute to the overall circadian rhythm of the pituitary-adrenal axis, four chair-restrained rhesus monkeys were continuously bled and samples were collected at one-minute intervals for up to 70 minutes at three different times of day: 530, 1100, and 1730 hours. Significant pulses were identified by a threshold criterion method. Pulse amplitude (amp), frequency (freq), interpeak interval (IPI), and mean levels were computed for ACTH and cortisol.

ACTH

time (hr)	mean freq (#/hr ± SE)	mean amp (pg/ml ± SE)	mean IPI (min ± SE)	mean level (pg/ml ± SE)
530	2.4 ± 0.7	25.3 ± 5.0	18.0 ± 4.3	55.2 ± 7.5
1100	2.3 ± 0.6	15.0 ± 1.8	16.3 ±.2.8	47.2 ± 8.8
1730	2.3 ± 0.9	11.4 ± 1.0	13.3 ± 3.4	42.4 ± 4.6

CORTISOL

time (hr)	mean freq (#/hr ±SE)	mean amp (ug/dl ± SE)	mean IPI (min ± SE)	mean level (ug/dl ± SE)
530	4.1 ± 1.5	16.2 ± 2.4	12.7 ± 3.5	39.6 ± 3.7
1100	3.1 ± 1.0	13.3 ± 2.1	16.2 ± 6.1	32.5 ± 6.3
1730	2.3 ± 0.7	12.6 ± 3.1	17.6 ± 6.5	18.9 ± 1.6

ACTH pulse amplitude and mean levels of both ACTH and cortisol showed significant correlations with time of day and were significantly higher ($p<.05$) at 530hr than at 1730hr. No significant correlation was found between time of day and the other pulse parameters or within pulse parameters at different times of day for either ACTH or cortisol. Levels of ACTH and cortisol were significantly correlated as were their pulse frequencies.

In conclusion, 1) pulses of ACTH and cortisol occur in non-human primates; 2) differences in pulse amplitude but not frequency or interpulse interval may contribute to the circadian variation in mean ACTH levels; and 3) a relationship appears to exist between the frequencies of ACTH and cortisol pulses.

412.8 DIFFERENTIAL PLASMA CORTICOSTERONE RESPONSES TO ELECTRICAL STIMULATION OF THE MEDIAL SEPTAL-DIAGONAL BAND COMPLEX. J. Dunn and D. Miller*. Dept. of Anat., Sch. Med., Oral Roberts Univ., Tulsa, OK 74171.

Previous studies reported from this laboratory have shown that electrical stimulation of cytoarchitecturally distinctive sites within several limbic forebrain areas produces differential changes in plasma corticosterone levels. To further pursue the question of differential limbic influences on pituitary-adrenal function blood samples obtained prior to and following electrical stimulation of the medial septal-diagonal band complex of urethane ($1.3g \cdot Kg^{-1}$) anesthetized female rats were assayed (RIA) for plasma corticosterone (Cpd B) levels.

Hippocampal EEG, ECG, heart rate, mean arterial pressure and respiration were monitored and timed blood samples (0.2ml) were obtained from a catheterized tail artery. Samples were taken at 0.5 min. prior to and at 5, 10, 15 and 30 min. after initiation of stimulation (monophasic square waves, 100uA, 50HZ, 0.5 msec, 1 sec on/1 sec off for 30 min.). For purposes of plotting Cpd B responses to stimulation a change in plasma Cpd B was considered different from no change when the average of the 5, 10, 15 and 30 min. samples deviated by more than 10% from the pre-stimulus level. However, statistics were derived from Cpd B responses grouped according to stimulation site.

Whereas no change in Cpd B levels were observed following sham stimulation or stimulation of several forebrain fiber systems, stimulation of the medial septal-diagonal band complex produced varied changes in plasma Cpd B levels. Stimulation of the medial septal nucleus (MS) and the nucleus of the dorsal division of the vertical limb of the diagonal band (VDBD) produced marked decreases in plasma Cpd B levels. In contrast stimulation of the nucleus of the ventral division of the diagonal band (VDBD) produced an increase in plasma Cpd B levels. The overall change in plasma Cpd B following MS, VDBD and VDBV stimulation was -20%, -14% and +22% respectively. In all cases the greatest change in Cpd B levels occurred at 15 min (-23%, -18% and +28%). Plasma Cpd B responses to stimulation of the nucleus of the horizontal limb of the diagonal band (HDB) produced varying and inconsistent results.

These data are consistent with our previous observations and offer additional support for the suggestion that differential control mechanisms reside within as well as between limbic forebrain areas.

412.9 CHANGES IN CONCENTRATIONS OF SERUM PROLACTIN, CORTICOSTERONE, THYROXINE, PITUITARY PROLACTIN AND HYPOTHALAMIC CATECHOLAMINES IN RESPONSE TO THE STRESS OF LOW AMBIENT TEMPERATURE. D.L. Palazzolo* and S.K. Quadri, Kansas State University, Manhattan, KS 66506.

Concentrations of serum prolactin, corticosterone, thyroxine, pituitary prolactin and hypothalamic catecholamines were determined in adult male Sprague-Dawley rats during exposures to low ambient temperatures. Separate groups of rats were decapitated after a 4.5 hr exposure to 22 (control), -5, -10 and -20C. Hormones were determined by radioimmunoassays and catecholamines by high performance liquid chromatography with electrochemical detection. Serum prolactin concentrations decreased significantly ($P < 0.05$) from 10.0 ± 1.3 ng/ml at 22C to 6.0 ± 0.8, 4.4 ± 0.4 and 4.3 ± 0.3 ng/ml after 4.5 hrs of exposures to -5, -10 and -20C, respectively. Pituitary prolactin levels increased significantly ($P < 0.05$) from 1.4 ± 0.2 µg/mg at 22C to 2.3 ± 0.1 and 2.0 ± 0.1 µg/mg after exposures to -10 and -20C, respectively. The increase in pituitary prolactin concentration at -5C (1.8 ± 0.1 µg/mg) was not significant. This inverse relationship between serum prolactin and pituitary prolactin concentrations indicated that cold stress caused a decrease in the release of prolactin. Corticosterone concentrations increased significantly ($P < 0.05$) from the 31.0 ± 12.2 ng/ml at 22C to 218.4 ± 29.7, 303.7 ± 62.2 and 465.8 ± 78.7 ng/ml after 4.5 hrs of exposures to -5, -10 and -20C, respectively. Serum thyroxine showed an increase from 8.1 ± 1.0 µg/dl at 22C to 12.5 ± 1.4 µg/dl after exposure to -20C ($P < 0.05$). Although serum thyroxine concentrations were elevated after exposures to -5C (9.9 ± 0.9 µg/dl) and -10C (9.5 ± 0.9 µg/dl), these elevations were not significant ($P < 0.4$). Hypothalamic dopamine and norepinephrine levels remained unchanged during exposure to -5C, but exposure to -10C produced a decrease from 0.27 ± 0.05 at 22C to 0.03 ± 0.03 ng/mg in dopamine and from 1.9 ± 0.2 at 22C to 0.4 ± 0.1 ng/mg in norepinephrine ($P < 0.05$) indicating an increased release of dopamine which inturn led to a decrease in the release of prolactin. Interestingly, after exposure to -20C, hypothalamic concentrations of both dopamine and norepinephrine increased back to the control level, indicating the possibility of an increase in both the synthesis and release of catecholamines at this temperature.

412.10 BRAIN AND PITUITARY GLUCOCORTICOID FEEDBACK IN CHRONIC STRESS. E.A. Young*, M.F. Dallman and H. Akil. (SPON. G. Quarton), Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720 and Department of Physiology, University of California, San Francisco, CA 94143.

We have previously demonstrated that acute stress in a naive rat leads to a blunted response of the anterior pituitary in vitro to stimulation with oCRF. In contrast, in an animal which has been exposed to footshock stress one half hour daily for 14 days, acute stress produces a potentiation of the anterior pituitary's response to oCRF in vitro. We suggested that a change in the anterior pituitary's sensitivity to glucocorticoid negative feedback might be involved in this potentiation seen in acute stress combined with chronic stress. When anterior pituitary cell suspensions from animals which have been chronically stressed (one half hour of intermittent footshock daily for 14 days) are incubated with oCRF, glucocorticoid (dexamethasone or corticosterone), or a combination of glucocorticoid and oCRF, we no longer see the steroid induced antagonism of CRF stimulated release that occurs in pituitary cell suspensions from normal rats. In fact, we see a 50% increase in CRF stimulated release in these suspensions from chronically stressed rats while we see a 50% decrease in CRF stimulated release in anterior lobe suspensions from control unhandled rats. Since in vitro we see positive feedback at the pituitary, while in vivo the levels of ACTH, B-Endorphin and corticosterone at the end of the 30 minute stress session are identical following the first stress session (acute stress) or the 15th stress session (chronic stress/acute stress), we examined another steroid feedback paradigm, fast feedback, to assess changes in steroid sensitivity in vivo.

Fast feedback is believed to reflect primarily hypothalamic inhibition of CRF release during the course of stress, and thus to be involved in the "turning off" of the stress response. Rats were given a 5 minute swim stress immediately following an injection of either corticosterone or saline. Blood was collected at the end of the stress and 25 minutes post stress. There was no difference in plasma levels of ACTH or B-Endorphin following 5 minutes of stress between saline injected or corticosterone injected rats. Neither was there a difference between acute stress and chronic stress/acute stress groups in their response to saline or corticosterone at this 5 minute point. Twenty-five minutes after the end of stress, the corticosterone injected rats showed significantly lower plasma ACTH and B-Endorphin levels than saline injected rats. There was no differences in plasma ACTH levels between corticosterone injected groups 25 minutes post stress. We conclude that despite the demonstration of glucocorticoid positive feedback at the pituitary in vitro, these chronically stressed animals show normal fast feedback.

412.11 THE ACTH SECRETORY RESPONSE TO ARGININE VASOPRESSIN IN MAJOR DEPRESSION. R.S. Jaeckle, J.F. Lopez*, R.G. Kathol and W.H. Meller*. Neuroscience Program, Department of Psychiatry, and Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

Arginine Vasopressin (AVP) is a brain peptide that potentiates corticotropin releasing hormone in stimulating ACTH secretion from the pituitary. We have used AVP to assess the HPA axis function in both healthy controls and patients with major depression (MD) (DSM-III). Twenty control (C) subjects and 24 MD patients received 0.18 pressor-units/kg body weight of AVP intramuscularly. Plasma ACTH was measured immediately prior to injection and every 15 minutes after injection for 1-1/2 hrs. Six of the MD patients were dexamethasone non-suppressors (NS) and 17 were suppressors (S). There were no significant age or sex differences between depressed patients and control subjects. There were no significant differences in baseline or peak ACTH levels or in the maximal increase (Δmax) ACTH levels between C, NS and S. When the groups were divided by gender, female controls had a significantly higher peak ACTH (55.0±30.2 pg/ml) than male controls (34.6±15.6 pg/ml), males with MD (30.8±18.1 pg/ml) and females with MD (33.6±14.2 pg/ml) (ANOVA DUNCANS MULTIPLE RANGE TEST, $p<0.04$). No sex differences were found in baseline ACTH levels. No correlations were found between the ACTH response to AVP and age in any of the groups..

A stepwise regression analysis in the MD group revealed no significant correlations between the ACTH response to AVP and severity of depression, weight, age of onset, length of illness, number of depressive episodes or dexamethasone suppression test status. Since most of the MD patients were on psychotropic medications, we divided the MD groups on those taking different classes of medications (TCA, MAOI, Lithium and antipsychotic). No significant differences were found in baseline, Δmax and peak ACTH levels between groups.

Our findings suggest that: 1) the ACTH response to AVP is not impaired in depressed patients, unlike the ACTH response to CRF; 2) females may have higher ACTH response to AVP than males; 3) there seems to be no correlation between age and the ACTH response to vasopressin in healthy controls and in MD patients; 4) chronic psychotropic administration does not seem to significantly impair the ACTH response to AVP in humans.

412.12 ACTH RESPONSE TO HYPOGLYCEMIC STRESS IN MAJOR DEPRESSION. J.F. Lopez*, R.S. Jaeckle and R.G. Kathol Department of Psychiatry, Neuroscience Program and Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242

Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis in major depression (MD) can be examined by studying the secretion of ACTH following insulin-induced hypoglycemia (IH). In this study we analyze the relationship of the ACTH response to age, sex, severity of depression, duration of the depressive episode, duration of the illness, age of onset (or age at the time of the first episode), and number of depressive episodes. We administered 0.15 units/kg IV of regular insulin to 20 healthy controls and 21 inpatients with MD (DSM-III). Plasma ACTH levels were assayed at regular intervals for 3 hrs. after insulin infusion. Twelve of the MD patients were dexamethasone suppressors (S) and five were dexamethasone non-suppressors (NS). Four of the MD patients did not have a DST. There were no age or sex differences between the MD and control groups.

MD patients had significantly lower ACTH peaks ($p<0.004$) and Δmax ACTH ($p<0.004$) than controls. There were no differences between S and NS MD patients. No baseline ACTH differences were found between the three groups. There were significant positive correlations between age and peak ACTH levels as well as age and Δmax ACTH levels for all groups. The age effect was greater for MD patients ($R=0.66$, $p<0.004$ for peak ACTH; $R=0.61$, $p<0.008$ for Δmax) than for controls ($R=0.54$, $p=0.014$ for peak ACTH; $R=0.54$, $p=0.13$ for Δmax). Female controls had higher peak and Δmax ACTH levels than male controls or male or female MD patients (ANOVA, Duncans Multiple Range Test, $p<0.001$).

A stepwise linear regression analysis in MD patients revealed that Δmax ACTH was positively correlated with the age of onset of MD ($p<0.004$) and negatively correlated with duration of the current MD episode ($p<0.03$). Peak ACTH levels were also negatively correlated with duration of the MD episode ($p<0.002$), and positively correlated with the age of onset ($p<0.005$). These findings suggest: 1) In MD patients, abnormal ACTH secretory responses to IH may not be reflected by the DST; 2) Age and sex are confounding variables in studies of ACTH responses to IH; 3) Age of onset of MD and length of the current MD episode are also correlated with ACTH responses to IH.

412.13 **REDUCED SOMATOSTATIN mRNA LEVELS IN HYPOPHYSECTOMIZED RATS DETERMINED BY IN SITU HYBRIDIZATION.** K.V. Rogers*, L. Vician*, R.A. Steiner and D.K. Clifton*. Depts of Obstetrics and Gynecology, Physiology and Biophysics, and Psychology, University of Washington, Seattle, Washington 98195.

Physiological evidence suggests that hypothalamic somatostatin (SRIF) inhibits pituitary GH release and that GH acts via shortloop feedback to stimulate SRIF secretion. Hypophysectomy (hypox) reduces hypothalamic SRIF content in rats, an effect which may be attributable to a change in SRIF synthesis, storage or release. We tested the hypothesis that hypox would reduce hypothalamic SRIF synthetic capacity, as reflected by a reduction in SRIF mRNA levels. Using in situ hybridization and a computerized image analysis system, we measured SRIF mRNA signal within individual cells in the periventricular nucleus of male rats. We compared intracellular SRIF mRNA signal in hypox (n=3) vs. sham hypox (n=3) rats. Four days following surgery animals were perfused with 4% paraformaldehyde containing 10% sucrose and their brains were frozen and sectioned at 10 um. Sections were pretreated with proteinase K and acetic anhydride, then incubated overnight at 45°C with either a 35-S-labeled ribonucleotide probe complementary to proSRIF mRNA or a noncomplementary, control, probe. Following incubation sections were treated with RNAse, washed, dipped in photographic emulsion and exposed for 7 days. Developed slides were counterstained with cresyl violet, randomized and read blindly under darkfield illumination. SRIF mRNA signal levels in periventricular cells were significantly reduced from 91.5 ± 8.5 in control animals to 56.4 ± 3.4 in hypox animals, representing a 38.4% reduction ($p<0.02$). These results demonstrate that SRIF synthetic capacity is influenced by the presence of the pituitary. We infer that GH may feed back to regulate the synthesis of somatostatin in the periventricular nucleus of the hypothalamus. This system represents an example of the regulation of CNS neuropeptide gene expression by a circulating pituitary hormone. (Supported by NIH Grant HD-12629)

412.14 GROWTH HORMONE-RELEASING FACTOR AND TYROSINE HYDROXYLASE IMMUNOREACTIVE NEURONS IN TRANSPLANTED FETAL MEDIOBASAL HYPOTHALAMUS. T.O.Bruhn, P.A.Tresco*, E.L.P.Anthony*, and I.M.D.Jackson. Division of Endocrinology, Brown University/Rhode Island Hospital, Providence, R.I. 02902.

Previous studies have shown that treatment of neonatal rats with monosodium glutamate (MSG) results in a destruction of growth hormone-releasing factor-immunoreactive (GRF-i) neurons in the arcuate nucleus and a dramatic reduction of growth hormone secretion from the pituitary gland. We undertook experiments to examine whether GRF-i neurons could be replaced in MSG-lesioned rats by grafting fetal mediobasal hypothalamus into the third ventricle.

Fetal (18 days gestation) mediobasal hypothalamus or cerebral cortex was dissected from normal donors and grafted under sterile conditions into the third ventricle of MSG-treated rats via a 20g stainless steel cannula. 30-40 days after transplantation, rats were injected with 150 µg colchicine into the lateral ventricle and perfused with heparinized saline and 2.5% acrolein 24 h after the colchicine treatment. GRF-i and tyrosine hydroxylase-immunoreactive (TH-i) neurons were visualized with the ABC or PAP technique. Viable grafts were found in approximately 80-90% of all treated rats. Grafts were located in the third ventricle with variable ventral/dorsal position and a variable degree of integration with the host hypothalamic tissue. Numerous (20->200) GRF-i and TH-i neurons were found in hypothalamic transplants but not in cortical grafts. Median eminence innervation by grafted GRF-i neurons was achieved in some animals with transplants in a ventral position. When grafts were located dorsally in the third ventricle, median emminence-like structures developed within the grafted tissue.

In conclusion, our studies demonstrate that GRF-i and TH-i neurons develop in transplanted fetal mediobasal hypothalamus. The grafted neurons may serve as a replacement of those cells which had been previously destroyed by MSG treatment.

412.15 MEASUREMENT OF HYPOTHALAMIC GROWTH HORMONE RELEASING FACTOR (rGRF) IN MALE AND FEMALE RATS. S.M. Gabriel, W.J. Millard, T.M. Badger, J.B. Martin, Departments of Neurology and Gynecology, Massachusetts General Hospital, Boston MA 02114 and College of Pharmacy, University of Florida, Gainesville FL 32610.

Rats exhibit sexually dimorphic patterns of growth hormone secretion. While male rats have high amplitude growth hormone pulses which are low in frequency (3-4 hours), pulsatile growth hormone secretion in the female is lower in amplitude, higher in frequency, and does not vary appreciably across the estrous cycle. Furthermore, our laboratory has shown that these patterns do not depend solely on the gonadal steroid environment during early development, but rather display remarkable plasticity to changes in the ongoing gonadal steroid milieu. These differences cannot be fully explained by changes in pituitary growth hormone responses to gonadal steroids, growth hormone releasing factor (rGRF), or somatostatin.

To determine the involvement of hypothalamic rGRF in these sexually dimorphic patterns of growth hormone secretion, we have developed a radioimmunoassay for rGRF. Antibodies to rGRF(1-43) were generated in rabbits and ^{125}I-rGRF(1-43) was radiolabeled using chloramine-T and purified with a sephadex G-50 column. Under equilibrium conditions, the assay has an ED_{50} of 50 fmoles, while under disequilibrium conditions, the ED_{50} is 12 fmoles with a sensitivity of approximately 2 fmoles. On a molar basis, the assay displays 10% crossreactivity with [His^1, Nle^{27}] hGRF(1-32) amide, 1% crossreactivity with hGRF (1-44) amide and hGRF(1-40), and no significant crossreactivity with glucagon, secretin, vasoactive intestinal polypeptide or peptide-histidine-isoleucine. Acid extracts of rat hypothalamus coelute with rGRF standard using HPLC. Concentrations of rGRF in whole male rat hypothalamus were 4.79 ± 0.24 ng. More than 90% of rGRF immunoreactivity was located in the medial basal hypothalamus, with detectable amounts also in the anterior hypothalamus-preoptic area. Preliminary studies suggest that there are no differences in hypothalamic rGRF levels in castrated male and female rats. Studies are currently in progress to determine whether changes in hypothalamic rGRF occur in response to gonadal steroid treatments. Supported by AM26252 to JBM and AM07561 to SMG.

412.16 SYMPATHETIC INNERVATION TO THE RAT PITUITARY GLAND. J. Franzer*, P. Beaston-Wimmer*, and A.J. Smolen (SPON: W.H. Bridger). Dept. of Anatomy, The Medical College of Pa., Philadelphia, PA 19129.

The superior cervical ganglion (SCG) has been implicated in the regulation of various endocrine functions because it is known to provide innervation to several endocrine structures, including the pineal and thyroid glands. Cardinali et al. (J. Neural Transmission, 52:1-21, 1981) have proposed that the SCG functions as a peripheral neuroendocrine center, providing innervation directly to the pituitary gland and to the basal hypothalamus. To date, the presence of sympathetic axons in the pituitary has been examined by biochemical techniques (measurement of catecholamine levels after ganglionectomy) or by interpretation of catecholamine histofluorescence (presence of coarse fibers). The conclusions from these earlier studies were that sympathetic axons provided some innervation to the posterior pituitary and the median eminence, but that this innervation was restricted to blood vessels. In the present study, we reexamined the question of sympathetic innervation to the pituitary using the highly sensitive and specific technique of anterograde transport of a conjugate of horseradish peroxidase and wheat germ agglutinin (HRP-WGA). Approximately 10 ul of a 3% solution of HRP-WGA was injected by pressure into the SCG of adult Sprague-Dawley rats. After a 1-2 day survival period, the animals were perfused with a mixture of aldehydes, and 50 um frozen sections of the pituitary and hypothalamus were reacted with tetramethylbenzidine and hydrogen peroxide to demonstrate sites of fast anterograde transport (axon terminals). The posterior pituitary receives a fairly sparse sympathetic innervation, with varicosities distributed throughout this region of the gland. The innervation does not appear to be restricted to the vasculature, but rather seems to invade the parenchyma. The intermediate lobe of the pituitary receives little, if any sympathetic innervation. The anterior lobe of the pituitary is innervated by sympathetic fibers to an even greater extent than the posterior lobe, and the innervation appears to be concentrated around particular cell clusters. In addition, there is a distinct projection to the median eminence region of the hypothalamus. In these locations, as well, the sympathetic innervation does not appear to be restricted to the vasculature. Electron microscopic studies are in progress to elucidate the synaptic associations of the sympathetic innervation of the pituitary gland and median eminence.
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412.17 RECORDINGS FROM KITTEN MAGNOCELLULAR NEUROENDOCRINE CELLS USING THE HYPOTHALAMIC SLICE PREPARATION. M. Fagan* and R. D. Andrew, Anatomy Dept., Queen's University, Kingston, Ont. Canada, K7L 3N6.

Rat hypothalamo-neurohypophysial neurons are a model for vertebrate neuroendocrine systems. These magnocellular neuroendocrine cells (MNCs) in supraoptic and paraventricular nuclei (SON and PVN) release vasopressin or oxytocin from axon terminals in the neurohypophysis. Electrophysiological studies using the coronal hypothalamic slice preparation have supported and extended in vivo recordings from rat MNCs. In particular, this preparation has facilitated intracellular studies of endogenous bursting and osmosensitivity by these cells. However, it is unclear to what extent the electrophysiology of the rodent magnocellular neuroendocrine system is representative of higher mammals. We have examined this system in kitten at the level of the single MNC to determine similarities and differences with the rat counterpart.

Kittens (0.3 - 1.0 kg) were anesthetized with Ketamine (0.35 ml/kg) and quickly decapitated. The hypothalamus was blocked and 500 µm slices were perfused with oxygenated saline in a Haas-type chamber. An array of three bipolar concentric electrodes was placed 0.5 mm dorsal to the SON and an extracellular micropipette (2 M NaCl or 3 M K^+ acetate; R= 15-25 MΩ) positioned in SON.

A total of 54 cells were recorded extracellularly (46 in SON; 8 in PVN). Of these, 3 were silent, 2 fired intermittently, 4 fired slowly (< 1 Hz) and 45 were continuous (1 - 11 Hz). In contrast to rat where repetitive bursting is associated with increased vasopressin release, spontaneous phasic firing by kitten MNCs was rarely seen, as previously reported in the intact cat (Koizumi and Yamashita, 1978). Although commonly observed in slices from rat, steady K^+ iontophoresis from the recording electrode did not promote phasic firing in 9 of 9 MNCs tested in kitten. These cells simply increased their firing rate. Also unlike the rat, brief excitatory orthodromic input to kitten MNCs only rarely triggered a burst of action potentials (3 of 22 cells).

These findings suggest that the intrinsic mechanism promoting bursting in the rat (Andrew and Dudek, 1984) is less developed in the cat. This was further supported by intracellular recordings from kitten SON. In 4 of 4 MNCs, the ability to generate a plateau potential, (the slow depolarization driving spontaneous or evoked bursting in rat MNCs), was absent or reduced. Therefore, both extracellular and intracellular recordings indicate that the differences in MNC firing patterns between rat and cat results to a large extent from the reduced ability of cat MNCs to support endogenous bursting.

Koizumi, K. and Yamishita, H. J. Physiol. 285, 341 (1978)
Andrew, R.D. and Dudek, F.E. J. Neurophysiol. 51, 552 (1984)

412.18 PITUITARY-ADRENAL RESPONSE IN YOUNG CONGENITALLY HYPOTHYROID (hyt/hyt) MICE. L.M. Juárez*, M.T. Stark*, and L.A. Meserve* (SPON: R.M. Kantner). Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

Appropriate development of the pituitary-adrenal response to stressful stimuli, as mediated by the hypothalamus, is important for the homeostatic well-being of mammals. Young rodents experience what has been termed a relative stress non-responsive period during the first two weeks of life, only after which time does a general stressor cause a pronounced elevation in circulating levels of the adrenal glucocorticoid, corticosterone. Previous studies in our lab have demonstrated that induction of hypothyroidism in young rats and mice by feeding the mother the chemical goitrogen thiouracil prolongs the stress non-responsive period (Meserve and Leathem, J. Endocrinol., 90:403, 1981). At least in 15 day old thiouracil-exposed rats, the failure to respond to stress appears to be the result of severely depressed hypothalamic content of bioactive (Meserve and Pearlmutter, Hormone Metab. Res., 10:488, 1983) and immunoactive (Meserve, Hormone Metab. Res., 12:674, 1974) corticotropin-releasing factor (CRF). The relationship between hypothyroidism and delayed development of stress response in the above studies is weakened by the possibility that thiouracil is directly modifying hypothalamic CRF synthesis.

The development at Jackson Labs (Bar Harbor, ME) of the C·RF/J-hyt strain of mouse which is congenitally hypothyroid in the homozygous, hyt/hyt, condition has made it possible to re-examine the development of pituitary-adrenal response without the confound of thiouracil. The present study was done to compare hypothalamic CRF levels and increase in circulating corticosterone 15 min after ether stress in hyt/hyt mice and their phenotypically normal littermates at 15, 20, 25 and 30 days of age. Extracts of hypothalami from unstressed mice were assayed for CRF content by radioimmunoassay (RIA) using kits purchased from Peninsula Labs (Belmont, CA). Circulating corticosterone levels were determined before stress or 15 min after exposure to ether fumes using RIA kits purchased from Cambridge Medical Diagnostics (Billerica, MA).

Hypothalamic CRF content was approximately 50 pg less in hyt/hyt mice than in normal littermates at 15, 20, and 25 days of age, but the difference was not statistically significant. However, 30 day old hypothyroid pups had significantly less hypothalamic CRF than did controls (169.5 vs 238.9 pg/hypothalamus). Hypothyroidism did not alter circulating corticosterone levels but stress response was significantly attenuated in hyt/hyt pups, at least at 20 days of age. Since the hypothalamus appears to contain sufficient CRF to elicit a normal response at this age, it is possible that the attenuation is the result of a subnormal release of CRF as recently suggested in relatively stress non-responsive rats (Walker et al., Endocrinology, 118:1445, 1986).

412.19 NEONATAL INJECTIONS OF MSG INHIBIT THE DEVELOPMENT OF NEUROPEPTIDE Y IN THE RAT HYPOTHALAMUS AND POSTERIOR PITUITARY. J. K. McDonald, P. Collins* and C.A. Reich*. Dept. of Anatomy & Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The arcuate nucleus (AR) of the hypothalamus contains numerous neuropeptide Y (NPY) immunoreactive perikarya which project axons to other hypothalamic nuclei such as the medial preoptic nucleus (Chronwall, Peptides 1985). NPY is also located in brainstem catecholaminergic neurons which project fibers to the hypothalamus (Sawchenko et al., J. Comp. Neurol. 1985). NPY-labeled fibers are found in the infundibular stem and in the posterior pituitary of the rhesus, orangutan, and human (Tigges et al., Soc. Neurosci. Abstr. 1986; Pelletier et al., Cell Tiss. Res. 1984). Furthermore, NPY exerts direct and indirect effects on the secretion of luteinizing hormone (LH) and growth hormone (GH) from the anterior pituitary (McDonald et al., Soc. Neurosci. Abstr. 1984; PNAS 1985).

Injections of MSG destroy neurons in the AR and alter normal growth and development. In this study we have analyzed the effects of neonatal treatment with MSG on the development of NPY-immunoreactivity in the hypothalamus and posterior pituitary. Sprague-Dawley rats were injected i.p. with MSG (4 mg/g B.W. in 0.9% saline) on postnatal days 2, 4, 6 and 8 while littermate controls received 10% saline. Rats were sacrificed by decapitation on days 9, 14, 21 and 65, the brains removed, posterior pituitaries collected and hypothalamic tissue dissected from the preoptic-suprachiasmatic region to the antetior aspect of the mammillary bodies. The posterior pituitaries were pooled from each group. Tissues were homogenized in cold 0.2 N HCl, centrifuged and the supernatant removed for measurement of NPY using an antiserum and RIA developed in our lab. The body weights and naso-anal lengths of MSG treated animals were significantly less than controls on 8, 9, 21 and 65 days of age. On day 65, the weights of the anterior pituitaries, adrenals and ovaries of MSG-treated rats were significantly less than those of controls. MSG treatment caused a rapid and significant reduction (50%) in the hypothalamic content of NPY by day 9 which was sustained at days 14, 21 and 65 when compared to controls (all $P<0.001$, Student's t-test). The concentrations of NPY in the pooled posterior pituitaries from MSG-treated rats were less than controls at all times examined. These results demonstrate that neonatal injections of MSG inhibit the normal development of hypothalamic NPY, probably by destroying arcuate neurons which synthesize NPY. These effects of MSG on hypothalamic levels of NPY should be considered when interpreting the results of MSG treatment on normal development especially in view of previous reports which have described direct and indirect effects of NPY on LH and GH secretion. Supported by the March of Dimes Birth Defects Foundation and NIH HD-19731.

412.20 RAPID TRANSPORT OF HORSERADISH PEROXIDASE TO NEURAL PARENCHYMA AND PITUITARY FOLLOWING INFUSION INTO THE LATERAL VENTRICLE. S. Saporta and C.P. Phelps, Dept. Anat., Coll. Med., Univ. S. Fla., Tampa, FL 33612.

The accessibility of substances released into the cerebral spinal fluid (CSF) to various areas of the central nervous system has been of interest for a number of years. We have recently begun to study the transport of substances in the CSF by examining the distribution of horseradish peroxidase (HRP) in the central nervous system of male and female Sprague-Dawley rats (200-300g) at various times after infusion of 10µl of 50% HRP over a 4-5 min interval into the lateral ventricle. Following survival times from 4 min to 24 hrs, the animals were perfused with mixed aldehydes and 60µm frozen sections of the brain, brainstem and pituitary were reacted for HRP with diaminobenzidine dihydorchloride or tetramethylbenzidine as the chromagen.

Within 4 min after the start of HRP infusion, reaction product was present within neural parenchyma along ventricular and pial surfaces and was present in deeper areas of parenchyma along large penetrating arteries. Diffuse HRP label appeared in progressively greater amounts in the posterior vs. the anterior pituitary lobe during the first 10 min after injection. The intermediate lobe of the pituitary consistently remained unlabeled. Penetrating arteries in various areas of the brain, especially in the cerebral cortex and the hypothalamus, exhibited cuff-like densities of HRP reaction product throughout their length. In some areas of brain and brainstem, homogeneously labeled neurons which presumably sent at least one process into the paravascular HRP cuff, or into HRP filled parenchyma along ventricular or pial surfaces were seen. When animals were allowed to survive for 24 hr after infusion of HRP, reaction product was found in perivascular cells and in a large number of labeled neurons which were predominantly labeled with granular HRP reaction product. No HRP was present in cuff-like arrangements. Colchicine (75-200µg) given 24 hrs before HRP infusion into the lateral ventricle failed to significantly decrease the number of cuff-like HRP blushes around penetrating arteries or the number of homogeneously labeled neurons 10 min after infusion. Five minutes after an intravenous bolus of 50mg of HRP in mice, a number of homogeneously labeled neurons were seen in areas which do not have an intact blood-brain barrier. The results of these studies suggest that CSF circulation and paravascular circulation (see Rennels, M.L. et al., Brain Res. 326:47, 1985) is a rapid, efficient route for the transport of a variety of substances to neurons, as well as the adeno- and neurohypophysis.

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412.21 SUSTAINED-BRAIN SPECIFIC DELIVERY OF ESTRADIOL DERIVATIVES USING A NOVEL CHEMICAL REDOX SYSTEM. K.S. Estes*, M.E. Brewster*, E. Shek*, and N. Bodor* (SPON: W. Stern). Pharmatec, Inc., Alachua, FL 32615 and College of Pharmacy, University of Florida, Gainesville, FL 32610.

A brain specific chemical drug delivery carrier, based on interconversion of a lipophilic dihydropyridine and hydrophilic pyridinium salt drug carrier and analogous to the NADH $\rightleftarrows$ NAD^+ redox system, was shown capable of sustained and specific delivery of dopamine to brain tissues (Science 221:65, 1983). When this chemical delivery system (CDS) was applied to estradiol (E2), castrated rats treated with 17β-[1-methyl-(1,4-dihydropyridinyl) carbonyl]oxy estra-1,2,5(10)-trien-3-ol (CDS-E2) showed suppressed serum LH for more than 24 days in contrast to the 4 day LH decrease in animals treated with equimolar E2. This activity could not be attributed to peripheral E2 or CDS-E2 levels. Three new CDS's were synthesized with E2 derivatives to optimize CDS penetration and "lock in" by the blood brain barrier (BBB). A CDS for the 3-benzoate ester of E2 (CDS-3Bz-E2) and for 3,17-bis(1,4-dihydrotrigonellinate)E2 (CDS-2-E2) were prepared by reacting estradiol-3-benzoate and E2, respectively, with nicotinoyl chloride followed by quaternization with methyl iodide and reduction. An ethinyl estradiol (EE) CDS was synthesized by reacting EE with nicotinic anhydride followed by preferential phenolic hydrolysis, quaternization and reduction to yield CDS-EE. Serum LH was determined by radioimmunoassay from samples collected 12 and 18 days post-treatment in rats ovariectomized 14 days prior to treatment with CDS-E2 (0.5 mg/Kg), or equimolar controls of estradiol valerate (EV) or EE. On day 12, serum LH was suppressed 76%, 72%, 53% and 80% in CDS-E2, CDS-2-E2, CDS-3Bz-E2 and CDS-EE treated rats, respectively, compared to controls. In contrast LH was not different from controls on day 18 post-treatment with CDS-2-E2 or CDS-3Bz-E2, but remained more than 80% decreased compared to controls in CDS-E2 and CDS-EE treated rats. Uterine and pituitary weights were not different between rats showing suppressed and non-suppressed LH values on day 18. It appears that the 17-ester linkage is important in determining the ability of the CDS to maintain a slow and sustained release of estrogen. This application of CDS to steroids which normally penetrate the BBB can be extensively used to specifically deliver drugs centrally in a sustained-manner, the duration of which can be controlled by chemical design as well as dose.

LIMBIC SYSTEM AND HYPOTHALAMUS III

413.1 **BILATERAL MICROINJECTION OF GABA SYNTHESIS INHIBITORS INTO THE POSTERIOR HYPOTHALAMUS (PH) ELICITS AVERSIVE REACTIONS.** **A. Shekhar,* J.A. DiMicco & J.N. Hingtgen, Depts. of Pharmacology & Toxicology and Psychiatry, Indiana University School of Medicine, Indianapolis, Indiana 46223.**

Previous studies have shown that blockade of GABA in the PH elicits cardio-respiratory stimulation similar to that seen during a defense reaction in anesthetized rats, and locomotor activity suggesting a "flight" response in conscious rats. This flight response was quantified as number of crossings and rearings. These responses were also seen when the GABA blockade was induced with the GABA synthesis inhibitor isoniazid (INH), but the time to peak effect was much longer (24±1 min.) as compared to GABA antagonists (6 - 10 min.). Even though this gross locomotor activation suggests an aversive effect judging from the well co-ordinated "escape" reactions, a non-specific activation of locomotion could not be excluded. Therefore, male rats were trained in an aversive paradigm of avoiding foot shocks by pressing a lever (Sidman; RS 20: SS 10). Guide cannulae were stereotaxically placed bilaterally in the PH where microinjection of the GABA antagonist bicuculline methiodide (BMI) 25 ng increased heart rate under anesthesia. After a recovery period of 4 - 5 days, 70 ug of INH was microinjected bilaterally into the PH and the rats were placed in the aversive paradigm. INH increased the avoidance responses gradually, attaining peak effects by 30 min. in contrast to BMI which increased avoidance behavior immediately (time to peak effects 5 - 10 min.). However, GABA blockade failed to increase lever pressing in an approach paradigm, where the rats obtained sweetened milk by pressing a lever (VI 1). These results suggest that GABA synthesis inhibitors, like the antagonists, may specifically stimulate an aversive drive when injected bilaterally into the PH, and that the time course of this effect corresponds to those of their cardio-respiratory and locomotor effects. (Supported by grants USPHS NS 19883 and PHS MH 17107, NIMH.)

413.2 FIRING OF SINGLE CELLS IN ENTORHINAL CORTEX IS LOCATION SPECIFIC AND PHASE LOCKED TO HIPPOCAMPAL THETA RHYTHM. G.J. Quirk* and J.B. Ranck, Jr., Dept. of Physiology, Downstate Medical Ctr., SUNY, Brooklyn, N.Y., 11203.

Single neurons in the hippocampus have been shown to fire when the rat is in a particular location. The entorhinal cortex supplies the hippocampus with processed sensory information through the perforant path, which arises from layers II and III. Employing techniques similar to those used in hippocampus, entorhinal cells were examined for spatial and other firing correlates.

Rats were trained to retrieve food pellets in simple geometric enviroments until they would run almost continuosly, at which time they were implanted with movable and fixed electrodes in entorhinal cortex and hippocampus, respectively. The firing rate of an isolated entorhinal cell was then correlated with the rat's position using an automatic system developed by Muller and Kubie. After simultaneous monitoring of the action potential rate and the animal's position, a color coded rate map was produced for each cell comparing rates of firing and location of the rat throughout a 16 minute session. The majority of cells analyzed in this way yielded maps that showed, upon visual inspection, a reproducable spatial pattern. Both spatial and non spatial cells were seen in several cortical laminae, including layers II and III.

Previous work from this laboratory has shown a generator of theta rhythm in entorhinal cortex. In order to study firing correlates, EEG was recorded from both hippocampus (CA1) and entorhinal cortex and was stored on video tape along with the unit for later analysis. Theta rhythm was recorded from CA1 and used as a trigger for examining the spike train. A pronounced rythmicity was observed with the majority of cells firing 30 degrees after the peak negativity of CA1 theta ($p=.002$, Rayleigh's test). This rythmicity was found in both place and non-place cells, and in cells exhibiting high rates and low rates. Fox, et. al., observed, in walking rats, a majority of cells in Ammon's horn and dentate gyrus firing at a similar time in the theta cycle (Exp. Brain Res., in press).

In addition to its projection to hippocampus from superficial layers, the entorhinal cortex receives a projection from hippocampus in deep layers. Place correlates in the entorhinal cortex, therefore, could represent a spatial input to the hippocamus, a spatial output of the hipocampus, or some interaction between the two. The phasic firing observed supports a recent claim that connections through entorhinal cortex are critical for hippocampal theta in walking rats (Vanderwolf, et. al., Brain Res., 347:58, 1985).

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413.3 **Rhythmically Bursting Neurons of the Medial Septum/Nucleus of the Diagonal Band Are Excited by Bicuculline.** B.S. Givens and G.R. Breese. Curriculum in Neurobiology and Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27514

A population of neurons of the medial septum and the vertical limb of the nucleus of the diagonal band (MS/DB) is known to have a rhythmically bursting pattern of activity. These cells are believed to receive a GABAergic inhibitory input from neurons of the lateral septum. To test whether ongoing GABA afferent activity to MS/DB cells underlies their rhythmic bursting pattern, the response of single bursting neurons to locally administered bicuculline was assessed in urethane anesthetized rats.

Single units were recorded extracellularly through a glass micropipette which was attached to a five-barrel pipette for drug administration. MS/DB neurons encountered in this study fired at a mean burst frequency of 4.1 bursts per second (range = 3.0-5.4) with an average of 7 action potentials per burst (range = 2-16). In all units tested, unilateral fornix stimulation led to a period of inhibition (mean duration = 245 ms) in the spontaneous activity of MS/DB neurons. In 83% of the cells, this period of inhibition was followed by a resetting of the bursting pattern which was time-locked to the stimulus. Iontophoretic application of bicuculline (methiodide; 5mM; 10-50 nA) to these MS/DB cells consistently increased spontaneous firing rate in a dose related manner. Similar responses were elicited by pressure ejection (5-50 psi x sec) of bicuculline. Maximal increases occurred within 20 seconds of drug application and lasted 30-180 seconds after the ejection current or pressure was terminated. Bicuculline-induced rate increases reflected the appearance of interburst spikes in addition to an increase in the number of spikes per burst. The burst frequency (4.1 bursts/ sec) remained unchanged. Every unit tested was inhibited by iontophoretically applied GABA and this response was reversible by bicuculline ejected with either pressure or an iontophoretic current. These data support the hypothesis that there is a tonically active GABAergic input to rhythmically bursting neurons of the MS/DB which acts to suppress cell firing both between as well as within bursts.

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413.4 RAT MEDIAL FRONTAL CORTEX SINGLE UNIT RESPONSES TO ELECTRICAL STIMULATION OF HIPPOCAMPUS AND AMYGDALA. K.G. Ruit* and E.J. Neafsey. Department of Anatomy, Loyola University Medical Center, 2160 S. First Avenue, Maywood, IL 60153.

Our laboratory has previously reported a direct projection from the infralimbic and prelimbic regions of rat medial frontal cortex (MFC) to the vagal solitary nucleus (Terreberry and Neafsey, BR 278, 1983), and we have also found that stimulation of the MFC evoked changes in heart rate, blood pressure, gastric motility and respiration, prompting us to label this region the "visceral" motor cortex (Hurley-Gius and Neafsey, BR 365, 1986). Major afferent projections to this portion of the MFC arise in the basolateral nucleus (BL) of the amygdala (Krettek and Price, JCN 172, 1977) and the CA1 and subicular regions of the hippocampal formation (H) (Swanson, BR 217, 1981). The present study examined the effect of electrical stimulation of these limbic structures on single unit activity in the MFC.

Rats were anesthetized with chloral hydrate (400 mg/kg, IP) and placed in a stereotaxic frame. The cortex on one side was exposed, and concentric bipolar stimulating electrodes positioned stereotaxically in the BL and H. Stimulation parameters were single or dual pulses of 0.15 msec duration, current intensity between 200-500 uamps. A glass insulated tungsten microelectrode was used to record unit responses in the MFC between 3 and 4 mm rostral to bregma and from the surface to 6 mm below it. Unit responses were evaluated from PST histograms, cumsum graphs and dot raster oscilloscope displays. Marking lesions were made along each electrode track to facilitate histological reconstruction.

Within the MFC, 60-70% of the neurons responded to either BL or H stimulation, with most responsive cells located between 2 and 6 mm from the cortical surface. Most of these cells (80%) were effected by both BL and H stimulation. The initial response to BL stimulation was either an decrease (48%) or increase (20%) in firing rate; the initial response to H stimulation was a decrease in firing rate. The latency of the earliest change in activity to either BL or H stimulation was about 15 msec. The predominant effect of both amygdala and hippocampal stimulation appeared to be a decrease in the firing rate of MFC cells; these "inhibitory" periods could last for 300-400 msec. The zone of responsive cells overlaps the cortical origin of the direct projection to the solitary nucleus, indicating that the limbic system has ready access to central visceral control systems. The concept of the limbic system as the "visceral brain" (MacLean, EEG 4, 1952) still appears to have merit.

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413.5 EPILEPTIFORM ACTIVITY AND SPREADING DEPRESSION IN THE ABSENCE OF EXTRACELLULAR Mg^{2+} IN THE RAT HIPPOCAMPAL FORMATION. I. Mody, J.D.C. Lambert* and U. Heinemann*. Max Planck Institute for Psychiatry, 8033 Martinsried, F.R. Germany.

The effect of lowering Mg^{2+} on the neuronal activity and changes in free extracellular Ca^{2+} concentration($[Ca^{2+}]_o$) was studied in hippocampal slices. Twenty to forty minutes following the washout of Mg^{2+}, when its level declined to less than 0.1 mM, spontaneous field potentials developed in the CA1 and CA3 regions, but not in the dentate gyrus. Recorded extracellularly, these potentials occurred with a frequency of about 0.3-0.5 Hz and, in the pyramidal cell layer of CA3, consisted of 2-5 mV positive deflections with 2-10 superimposed population spikes, lasting 40-100 ms. In the str. pyramidale of CA1, the field potential transients were usually biphasic, with amplitudes generally less than 3 mV. Intracellular recordings from CA3 pyramidal cells revealed that the transients were associated with 20-30 mV paroxysmal depolarization shifts (PDS) with superimposed bursts of action potentials, but no significant changes in RMP or R_n of the cells. In contrast, in CA1 pyramidal neurons, it was EPSP/IPSP sequences that accompanied the transients, suggesting their synaptic origin. This hypothesis was also supported by the finding that surgical isolation of area CA3 from CA1 readily abolished the spontaneous activity in CA1, but not in CA3. Perfusion of the NMDA receptor antagonist 2-APV (30μM) or elevation of $[Ca^{2+}]_o$ to 3-4 mM also attenuated, or reversibly blocked, the spontaneous activity.

Decreases in $[Ca^{2+}]_o$, produced by orthodromic stimulation (20 Hz/10s) and measured with ion-selective microelectrodes, were also enhanced in the absence of Mg^{2+}. The increases in the Ca^{2+} responses were usually maximal near threshold stimulus intensities, and were blocked by raising $[Ca^{2+}]_o$ to 3.6 mM. In low Mg^{2+}, high intensity, suprathreshold repetitive stimulation also caused spreading depression (SD) to develop.

SDs could be evoked in all hippocampal areas studied, including the dentate gyrus, and were characterized by 30-40 mV depolarizations of the neurons together with negative field potential shifts of 10-15 mV and decreases in $[Ca^{2+}]_o$ by 1.2-1.4 mM, lasting for 1-2 min. Synaptic transmission remained impaired in excess of 2 min following the SDs. Perfusion of 30μM 2-APV reversibly blocked the tetanc stimulation-induced SDs, suggesting the involvement of NMDA type receptors.

The present findings show that in the absence of extracellular Mg^{2+} spontaneous epileptiform activity develops in the CA3 region of the hippocampus, which propagates to area CA1. The underlying mechanism for this type of activity and the induction of SD may be the reduction of surface charge screening together with the removal of the voltage-dependent blockade of NMDA receptors.

413.6 INDUCTION OF DELAYED SYNCHRONIZED BURSTS IN THE CA2/CA3 REGION OF RAT HIPPOCAMPAL SLICES IN THE ABSENCE OF EPILEPTOGENIC AGENTS. S.M. Bawin, A.R. Sheppard, I. Shahhal*, M.D. Mahoney* and W.R. Adey. Departments of Physiology and Neurosurgery, Loma Linda University and VAMC, Loma Linda, CA 92357.

It is well known that weak afferent stimulation of the CA2/CA3 area of disinhibited hippocampal slices results in delayed synchronized bursts (DSBs) which are synaptically transmitted to the CA1 region. DSBs are thought to reflect progressive recruitment of bursting neurons via a local network of recurrent excitatory processes in the CA2/CA3 area (Wong, RKS and Traub, RD, J. Neurophysiol., 49:442-471, 1983)

We studied the effects of weak repetitive stimulation of mossy fibers or Schaffer collaterals on field potentials evoked in the CA2/CA3 and CA1 cell layers of hippocampal slices perfused with control solution and with penicillin (PEN) at various concentrations (0.25 to 3.4 mM). Bipolar stimulating electrodes in either pathway were used for test pulse stimulation (0.2 Hz) or repetitive stimulation with sinusoidal currents (10 sec wave trains at 60 Hz, amplitude 10-50 uA). Much greater 60 Hz currents are used in kindling epilepsy. The test pulse intensities were sufficient to evoke short-latency population spikes (primary PSs) in control solution, or DSBs in the PEN containing solutions.

Although test pulses during perfusion with control solution induced only primary PSs prior to 60 Hz stimulation, DSBs were evoked regularly in both CA2/CA3 and CA1 regions during and following wave trains. DSBs occurred whether the sine wave was delivered in the mossy or Schaffer tracts, and lasted from a few sec to >10 min. Others have reported post-tetanic potentiation of primary responses and afterdischarges resembling DSBs following 10 sec, 13 Hz pulse trains in the lower and upper dentate blades (Yamamoto, C and Chujo, T, Exp. Neurol., 58:242-250, 1978). However, in our studies sinusoidal stimulation did not potentiate the primary PSs.

Adding PEN facilitated DSB initiation and transmission in a concentration dependent manner. During 60 Hz stimulation "spontaneous" DSBs occurred between test pulses. Aftereffects lasting up to 3 min varied from DSB potentiation to complete suppression as epileptiform activity increased in the tissue prior to sinusoidal stimulation. The suppression of DSBs may have been due to pathological conditions created by local intense bursting.

In summary, weak repetitive stimulation of CA2/CA3 afferents in the absence of disinhibiting agents can induce long-lasting potentiation of the synaptic elements responsible for DSBs. Weak interictal discharges reaching the hippocampus and reverberation between the CA1 and CA2/CA3 areas could prime DSB mechanisms and facilitate further epileptiform discharges.

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413.7 PROTECTION AGAINST HYPOXIA BY HIGH GLUCOSE - A STUDY USING THE IN VITRO HIPPOCAMPAL SLICE. C.A. West*, A. Schurr K.H. Reid and C.B. Shields. Laboratory for Cellular Neuroscience, ACCRU, Depts. of Anesthesiology, Physiology and Surgery, Univ. Louisville, Sch. of Med., Louisville, KY 40292

The effect of high (20 mM) and low (5 mM) glucose levels on synaptic activity and on the sensitivity to hypoxia in brain slices was examined. 400 μm rat hippocampal slices were maintained in a dual compartment interface chamber supplied with humidified 95% O_2/5% CO_2 and artificial CSF (ACSF) containing the standard level of 10 mM glucose. Evoked population spikes from the CA1 cell layer were continuously recorded (1/min) from one slice per compartment following orthodromic stimulation of Schaffer collaterals. After 15 min of baseline recordings, slices in one compartment were perfused with either 5 or 20 mM glucose/ACSF while control slices in the other compartment continued to be perfused with the standard (10 mM) ACSF. After 60 min, the slices were exposed to hypoxia (95% N_2/5% CO_2) for either 10 or 20 min, followed by recovery for 30 min (95% O_2/5% CO_2). Additional slices in the chamber were tested for their response to orthodomic stimulation; the presence of a population spike(>1mV) was considered recovery of synaptic function.

The high and low glucose levels had no apparent effect on the pre-hypoxia population spike but significantly affected recovery of synaptic function after hypoxia. Ninety-three percent (38/41) of the 20 mM glucose slices exposed to 10 min hypoxia recovered as compared to 41% (13/37) of the 10 mM glucose controls ($p<.0005$). None (0/25) of the 5 mM glucose slices recovered after this insult. Similarly, 73% (30/41) of the high glucose slices exposed to 20 min hypoxia recovered while non (0/39) of their control counterparts showed synaptic activity. The results show a dose-dependent relationship between glucose levels and the recovery from hypoxia of synaptic function in isolated brain tissue.

413.8 SENSITIVITY OF SYNAPTIC ACTIVITY IN THE CA1 REGION OF THE HIPPOCAMPAL SLICE PREPARATION TO HYPOXIA: EFFECTS OF TEMPERATURE AND ACSF POTASSIUM CONCENTRATION ON PROBABILITY OF RECOVERY. K. H. Reid, A. Schurr, C. A. West*, M. Tseng*, C. B. Shields and H. L. Edmonds, Jr.* Laboratory for Cellular Neuroscience, ACCRU, Depts. of Physiology, Anesthesiology, Anatomy and Surgery, School of Medicine, University of Louisville, Louisville KY 40292.

Hippocampal slices from 200-300 g Sprague-Dawley rats, cut 400μ thick, were maintained in an interface chamber for 2-3 hours before being subjected to hypoxia. The hypoxic insult was produced by changing the humidified gas flowing over the slices (1 liter/min) from 95% oxygen/5% carbon dioxide to 95% nitrogen/5% carbon dioxide for a period of 10 minutes, followed by a return to 95% oxygen/5% carbon dioxide. Synaptic responsiveness was evaluated by stimulating Schaffer collaterals once/minute at 2x threshold, and recording the CA1 population spike. Slices were considered unresponsive if stimulation at 2x threshold did not evoke a response > 1 mV in amplitude. The typical pre-hypoxic response at 2x threshold was 10-20 mV in amplitude.

At 34°C, with 5 mM K^+ in the medium, the hypoxic insult reliably rendered 30-50% of the slices exposed unresponsive after restoration of normal oxygenation. Changes in temperature had a marked effect on the percentage of slices retaining synaptic responsiveness (Table 1). Changes in ACSF K^+ level also had a marked effect, as shown in Table 2. In particular, K^+ levels in excess of 6.5 mM, which produced hyperexcitable slices, greatly reduced the ability of slices to recover electrical activity after a standard hypoxic insult.

TABLE 1

Temperature of incubation	Recovered/total slices tested	
40 °C	1/89	1%
37	12/74	16%
34	78/126	62%
31	81/83	98%
28	70/75	93%

TABLE 2

Concentration of potassium	Recovered/total slices tested	
2.5 mM	80/89	90%
5.0	78/126	62%
6.5	54/100	54%
7.5	11/72	15%

Preliminary data indicates that neither feedback inhibition (antidromic-orthodromic stimulation) nor feed-forward inhibition (orthodromic-orthodromic heterosynaptic stimulation) shows selective vulnerability to this form of hypoxic insult.

413.9 2-DEOXYGLUCOSE UPTAKE IN NORMOXIC AND HYPOXIC RAT HIPPOCAMPAL SLICES. M.T. Tseng*, A. Schurr, G.D. McClure Jr.*, K.H. Reid and B.M. Rigor. Lab. for Cellular Neuroscience, ACCRU, Depts. of Anatomy, Anesthesiology and Physiology, Univ. of Louisville School of Medicine, Louisville, KY 40292.

Demise of hypoxic brain tissue can partly be attributed to the accumulation of anaerobic glycolytic end products such as lactate. An increased glucose uptake and utilization after hypoxic insult has been documented. Among the various models for study of ischemia/hypoxia, the hippocampal slice preparation has become increasingly popular. While the electrophysiology of the hypoxic slice preparation is well documented, little is known about the time course of glucose uptake, its utilization, and the formation of lactic acid. In this study [^{3}H] 2-deoxyglucose (2DG) was used to determine the time course of glucose uptake under normoxic and hypoxic conditions in rat hippocampal slices. Slices, 400 um thick, were prepared from adult male rats immediately after decapitation and equilibrated for 1 hr in a linear flow dual chamber where they were superfused with ACSF (40 ml/hr) in a humidified atmosphere of 95%O_2/5%CO_2 at 34°C. Slices in one compartment were subjected to a 10 min hypoxic episode (95%N_2/5%CO_2) while those in the other remained under normoxic conditions. Ten min prior to hypoxic challenge, ACSF containing 50 μCi/ml 2DG was introduced. Slices were allowed to recover for an additional 60-90 min during which, at 10 min intervals, samples were removed from both compartments, dried, weighed and solubilized to determine 2DG incorporation by scintillation counting. Additional slices were fixed and embedded for histological and autoradiographic studies. Compared to that in normoxic slices, 2DG uptake after hypoxia was elevated. Sixty to 90 min after hypoxic challenge, 2DG uptake continued to rise while normoxic controls began to plateau. Morphological data revealed a differential preservation of CA1 pyramidal cells along the thickness of the slice between the air-liquid interface and the submerged surface. Moreover, pockets of pyramidal cells exhibited higher 2DG incorporation.

Our results showed that the onset of increased glucose uptake after hypoxic insult is rapid and that such enhanced uptake is sustained for at least 90 min. The present results are in accordance with other studies. The hippocampal slice preparation thus appears to be a suitable model for critical evaluation of post-hypoxic events.

413.10 PROTECTION AGAINST HYPOXIA BY TAURINE - A STUDY USING THE IN VITRO HIPPOCAMPAL SLICE. A. Schurr, M.T. Tseng* and B.M. Rigor. Lab. for Cellular Neuroscience, ACCRU, Depts. of Anesthesiology and Anatomy, Univ. of Louisville Sch. of Med., Louisville, KY 40292

The rat hippocampal slice preparation (Schurr, A. et al, Comp. Biochem. Physiol. 82A:701, 1985) has been used in the present study as an in vitro model for a study of cerebral hypoxia and possible pharmacological protection against it. Damage brought about by a standardized hypoxic insult was assessed electrophysiologically and histologically in the presence and absence of a protective agent. Hypoxia-induced Ca^{2+} influx has been implicated as having a major role in producing irreversible damage in neuronal tissue (Hansen A.J., Physiol. Rev. 65:101, 1985). Reoxygenation-derived free radicals have also been suggested to produce such damage (Demopoulos H.B. et al, Acta Physiol. Scand. Suppl. 492: 91, 1980).

Taurine, a sulfur-containing amino acid, is a putative modulator of Ca^{2+} fluxes across neuronal membranes. Its extracellular level increases significantly after ischemia in rabbit hippocampus (Hagberg, H. et al, J. Cereb. Blood Flow Metab. 5:413, 1985) and its decreased level in brain tissue postnatally coincides with a decrease in resistance to hypoxia.

Pretreatment of hippocampal slices with taurine almost doubled the recovery rate of synaptic function (orthodromically evoked population spike) from hypoxic insult in CA1 pyramidal neurons. Taurine's protective effect was dose-dependent and biphasic. Among the control slices, 49% (n=126) exhibited recovery of synaptic function after 10 min hypoxia. Of the experimental slices, 64% (n=22; n.s.), 88% (n=42; $P<.005$), 94% (n=19; $P<.005$) and 30% (n=20; n.s.) displayed recovery of synaptic function when treated with 0.5, 1.0, 2.0 and 5.0 mM taurine, respectively. A 15 min hypoxic insult resulted in only 20% (n=25) recovery of synaptic function in control slices while 1.0 mM taurine increased the recovery rate to 88% (n=26; $P<.005$). Histological assessment revealed fewer dark or edematous cells in taurine-treated slices after hypoxia as compared to control slices. When control slices were incubated in bathing medium containing no Ca^{2+}, the amplitude of the population spike fell 50% over a period of 40 min (slope = -0.57). Inclusion of 1.0 mM taurine in the above medium slowed the amplitude decline (12%; slope = -0.25) over the same period of time.

These results demonstrate taurine's antihypoxic potential. We hypothesize that the in vivo elevation of cerebral taurine after hypoxia is produced by oxidation of thiol compounds (cysteine, glutathione) by oxygen free radicals. Increased taurine slows down Ca^{2+} influx thus reducing irreversible neuronal damage.

413.11 **HYPOTHALAMIC TEMPERATURE-SENSITIVE NEURONS AND THEIR REGIONAL INTERACTIONS IN HORIZONTAL TISSUE SLICES.**
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Thermosensitive neurons in the preoptic region and anterior hypothalamus are known to be important in the regulation of body temperature. In intact animals, these same neurons have been shown to receive ascending inputs from the caudal hypothalamus and lower brain stem. Rostral hypothalamic neurons are also known to communicate with middle and caudal hypothalamic nuclei. Previously, we have used horizontal tissue slices to systematically map the locations of temperature-sensitive and insensitive neurons throughout the entire hypothalamus of the rat. The present study investigated regional interactions between hypothalamic nuclei by determining neuronal responses to temperature changes both locally and in remote areas of the slice. Horizontal tissue slices rested directly over three thermodes that allowed independent thermal stimulation of discrete rostral, middle, and caudal hypothalamic regions. Thermocouples measured tissue temperatures in these three locations. As in previous studies, locally warm-sensitive and cold-sensitive neurons were recorded in all major hypothalamic nuclei. The majority of neurons recorded in each area were classified as insensitive to local temperature changes. These temperature-insensitive neurons also did not respond to changes in remote temperatures. On the other hand, in the septum, anterior hypothalamus and lateral hypothalamus (adjacent to the fornix), neurons were recorded that responded to remote thermal stimulations, while local temperatures were held constant. This suggests that thermosensitive synaptic networks extend throughout the hypothalamus and may serve to explain the influence of temperature on a variety of homeostatic systems. In addition, most remote-responsive neurons were warm-sensitive to local temperatures but cold-sensitive to remote temperatures. This supports a previous contention that neuronal cold-sensitivity is strongly linked to synaptic input.
(Supported by NIH, NSF & American Heart grants.)

413.12 ANALYSIS OF HIPPOCAMPAL THETA RHYTHM SHOWS THAT CORRECT LOCATION OF CURRENT SOURCES AND SINKS GENERATING EGG PATTERNS CAN REQUIRE DC RECORDING. S.E. Fox and M. Stewart, Department of Physiology, Downstate Medical Center, SUNY, Brooklyn, NY 11203.

The DC component of the potential fields of hippocampal theta rhythm varies substantially with depth (Gerbrandt and Fowler, Prog in Brain Res 54:109-116, 1980). Current source density (CSD) analysis, a weighted second derivative of the voltage with respect to depth, indicates the magnitude and sign of the transmembrane current as a function of depth (Nicholson and Freeman, J Neurophysiol 38:356-368, 1975). CSD analyses including the DC components may differ from those derived from AC analyses. For the hippocampal theta rhythm, inclusion of the large DC components alters the magnitude and even the sign of the computed transmembrane current at some sites.

Anesthesia with urethane induces long bouts of 4-5 Hz theta rhythm in rats. Profiles of the peak to peak amplitude of this rhythm using AC recordings show one maximum on the basal dendritic side of CA1 and another near the hippocampal fissure, with a phase shift and null located between them.

Methods and equipment were optimized for stable DC monopolar recording from two sites. A stationary micropipet near the hippocampal fissure served as a phase-reference for recordings from a moveable electrode that was passed through the contralateral hippocampus. Changes in CSD evoked by stimulation of the ventral hippocampal commissure and entorhinal cortex were used to identify the laminar position of the moveable pipet.

The DC CSD analysis indicated three major sites for transmembrane currents: 1) a basal dendritic sink in CA1 having a maximum magnitude at the positive peak of the reference theta rhythm, 2) a sink near the level of the hippocampal fissure whose maximum occurred on the negative peak of the reference theta, and 3) an apical dendritic source in CA1 that retained a relatively constant magnitude throughout the theta cycle.

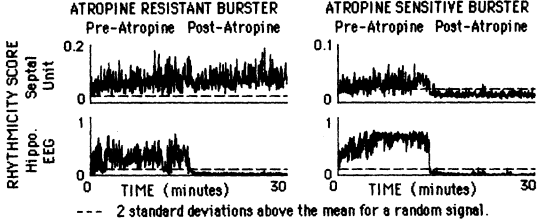
Whether a sink represents an "active" or an "electrotonic" transmembrane current depends on the direction, location and ionic basis of the underlying conductance changes. For example, an active sink can be produced by local EPSPs, while an electrotonic sink can be produced by distant IPSPs. The active vs. electrotonic decision about any source or sink must be based on information other than CSD. Increases in the amplitude of CA1 population spikes (Rudell and Fox, Brain Res 294:350-353, 1984) and in the probability of firing of CA1 pyramids (Fox, Wolfson and Ranck, Exp Brain Res, in press, 1986) on the positive phase of the dentate reference theta rhythm are consistent with the notion that there is a net excitation occurring at the basal CA1 current sink in this study, suggesting that it may be due to local EPSPs. (Supported by NIH NS17095.)

413.13 **TWO POPULATIONS OF RHYTHMICALLY BURSTING NEURONS IN THE SEPTAL NUCLEI ARE REVEALED BY ATROPINE.** M. Stewart and S. E. Fox, Dept. Physiology, Downstate Med. Ctr., SUNY, Brooklyn, NY 11203.

In rats, a large amplitude rhythmic EEG "theta" pattern can be recorded from the hippocampal formation during a set of well-defined behaviors (Vanderwolf, EEG Clin. Neurophys., 26: 407, 1969). A similar EEG pattern is seen in animals anesthetized with urethane. Petsche, Stumpf, and Gogolak (EEG Clin. Neurophys., 14: 202, 1962) identified cells in the medial septal nucleus and nucleus of the diagonal band of Broca (MSN-NDBB) that burst at the frequency of the hippocampal theta rhythm and suggested that they "paced" the rhythm. Many of these rhythmically bursting septal neurons project to the hippocampus. Lesions of MSN-NDBB abolish the hippocampal theta rhythm. The urethane-induced theta rhythm is also abolished by systemic administration of muscarinic cholinergic receptor blockers, so cholinergic synapses have been assumed to be involved in the generation of this theta rhythm. Since the excitability of hippocampal neurons is modulated by theta rhythm (Rudell and Fox, Brain Res., 294: 350, 1984), these septal "pacemaker" cells may be a major modulatory influence on information flow through the hippocampus.

Extracellular recordings were made from rhythmically bursting neurons in the septum of urethane anesthetized rats. Atropine sulfate (25mg/kg i.v.) was given to completely abolish the hippocampal theta rhythm. Some rhythmic neurons (7 of 12) continued to to burst at the theta frequency; others (5 of 12) lost their rhythmic firing pattern within seconds of atropine administration. This was comparable in time course to the elimination of the hippocampal theta rhythm (see figure). Bursting neurons that remained rhythmic after atropine were characterized by a significantly greater firing rate ($\bar{X}$=11.0 spikes/burst) than those bursting neurons that lost their rhythmic activity after atropine ($\bar{X}$=4.2 spikes/burst) [t=2.605, p<0.05].

These findings demonstrate the presence of two types of rhythmically bursting neurons in the rat septum. The elimination of the theta rhythm by atropine may be due to blockade of transmission between these two populations of rhythmically bursting septal neurons, disrupting the "pacemaker", or by blockade of septo-hippocampal transmission at the hippocampus. These alternatives are illustrated with a model of the septo-hippocampal projections.



--- 2 standard deviations above the mean for a random signal.

413.14 NEUROPHYSIOLOGICAL EVIDENCE FOR RECIPROCAL CONNECTIONS BETWEEN THE CINGULATE CORTEX AND THE BASAL FOREBRAIN IN THE RAT. D.M. Finch and A.M. Tan*. Reed Neurological Research Center and Brain Research Institute, University of California, Los Angeles, CA 90024.

A variety of studies have shown projections from the basal forebrain to the neocortex. Connections between the basal forebrain and the cingulate cortex, however, have not been well characterized. Here, we studied the neuronal responses of rat cingulate neurons to electrical stimulation of the basal forebrain, and vice versa. Adult male Sprague-Dawley rats were anesthetized with Chloral Hydrate (400 mg/kg, ip) or Urethan (1.5 g/kg), and were prepared for in vivo intracellular recording. Stimulation sites (using 0.2 msec duration pulses of 100-500 μA intensity) were located in the nucleus of the diagonal band of Broca, or in immediately adjacent tissue. Recording sites were in the posterior cingulate cortex (area 29). Stimulation of the basal forebrain evoked antidromic responses in cingulate neurons. These were characterized by an abrupt rise of the action potential from baseline in the absence of an EPSP. About 38% of the encountered cingulate neurons were activated antidromically. Most of the responsive cells were located in layer V. Response latencies varied from 0.4-1.4 msec. We estimated the distance between the stimulating and recording sites, and computed conduction velocities. The conduction velocities were fast, varying from 8.6-30 m/sec, with a mean of 20 m/sec. No clear evidence for orthodromic excitatory responses was seen, although a few cells showed evidence of hyperpolarizations in response to stimulation.

The positions of the stimulating and recording electrodes were reversed in order to examine the effects of electrical stimulation of the cingulate cortex on neurons within the nucleus of the diagonal band. No clear orthodromic responses were seen in this sample, but 71% of the encountered diagonal band neurons were activated antidromically at short latency (0.4-0.6 msec) by cingulate stimulation. These latencies corresponded to conduction velocities of 21.7-26 m/sec, with a mean of 24.9 m/sec. The present results suggest the presence of reciprocal, fast-conducting pathways between the basal forebrain and the cingulate cortex. Further studies are necessary to characterize the physiological action of the projections.

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413.15 LOCAL SYNCHRONOUS EPILEPTIFORM ACTIVITY IN CA1 RECORDED USING A 32 ELEMENT PLANAR MICROELECTRODE ARRAY

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Synchronized epileptiform bursts are initiated in the CA2/CA3 region and are propagated along the Schaffer collateral pathways, providing the orthodromic input to CA1. Although latency to bursting onset in CA1 increases with distance from CA3, the population spikes within a burst as recorded using pairs of conventional microelectrodes are not highly synchronized. (Schwartzkroin, P. A. and Prince, D. A., Brain Res., 147, 1978). Because of the presence of highly localized synchronizing mechanisms present in this preparation, it seems likely that there exist some regions of cells which fire in synchrony during the propagation of an epileptiform field burst.

In order to identify these locally-synchronized areas, we used a 32 element array of photoetched microelectrodes to make recordings on the surface of transverse hippocampal slices from male Sprague-Dawley rats. The 20 µm diameter platinum-plated electrodes are arranged in a 4 by 8 matrix on 200 µm centers. The glass array substrate served as the slice chamber floor, and was covered by gauze which was perfused with Earle's Balanced Salt Solution containing 100 µM picrotoxin. A hole was opened in the gauze over the electrode matrix and the brain slice was laid directly on the array. The CA1 s. pyramidale was positioned along the long axis of the array and a bipolar stainless steel electrode was used for mossy fiber stimulation (100 µs pulses, 100 - 500 µA). Sixteen amplified (gain = 5000 or 25000) signals were sampled simultaneously (5 kHz/channel), and the data were sent to an AT&T PC6300 computer for analysis and storage.

The simultaneous recordings revealed that the pattern of activity during a population burst varies significantly over the surface of CA1. The latency to the onset of bursting as recorded along the Schaffer collaterals in s. radiatum increased approximately linearly with distance from CA3. A maximum delay of 1.6 ms over a 600 µm distance was observed. In the s. pyramidale, the latency increased with distance in a much less regular fashion. Nearer CA3, some events were nearly synchronous (no delay between electrodes), but large delays (2.4 ms) were observed between other electrodes further from CA3.

These data suggest that columns of CA1 receive serial driving input via the Schaffer collaterals, and that the propagation of the burst response is modified by other synchronizing and propagation mechanisms.

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413.16 MONOSYNAPTIC COMMISSURAL FEED-FORWARD GABA-INHIBITION OF FASCIA DENTATA IN RAT HIPPOCAMPUS.

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Early studies of inhibition in the mammalian hippocampus described all inhibitory circuits as local, recurrent or intrinsic ("feed-back"), based on anatomical (Lorente de No J. fur Psychologie und Neurologie 1934, 46:113) and histochemical studies (Storm-Mathieson and Fonnum J. Neurochem. 1971, 18:1105) Recent physiological (Buzsaki & Eidelberg. Brain Res 1981, 230:346) and anatomical (Seress & Ribak J. Neurocytol. 1984, 13:215) studies have hypothesized a "feed-forward" inhibition from one dentate gyrus to contralateral dentate. The inhibitory circuit proposed was a projection through the hippocampal commissure by axons of granules that would monosynaptically excite inhibitory interneurons in the hilus of the contralateral dentate which would, in turn, inhibit granule cells.

The present study has demonstrated another type of "feed-forward" inhibition. We have demonstrated GAD+(GABA-releasing) neurons in the hilus of the dentate that project *monosynaptically* via the hippocampal commissure to end in the contralateral dentate gyrus. This "feed-forward" inhibitory circuit is more direct than the other proposed circuit, and it demonstrates that inhibitory interneurons may have long axons to inhibit neurons over 1-3 mms. distal and into different anatomical regions. Rats were injected with HRP in dorsal hippocampus (CA1 or fascia dentata), left for 24 hrs. to allow retrograde transport to cells of origin, and fixed for HRP histochemistry. Frozen sections (30µ) were processed first for HRP with cobalt intensification that allowed visualization of cells containing black granules. Immediately, alternate sections were processed for glutamic acid decarboxylase (GAD+) using a DAB procedure that made GAD+ cell bodies appear brown. All double-labelled cells (brown cytoplasm with black granules) were identified as inhibitory neurons projecting contralaterally. Double-labelled cells were found in the left fascia dentata (FD) only with HRP injected in the right FD, never when the HRP was in the right CA1. Also, HRP product was found in the hippocampal commissure with the FD-HRP injection.

GABAergic axons from the contralateral dorsal subiculum also projected into the fascia dentata; however there were no other double-labelled neurons in contralateral Ammon's horn. *Ipsilaterally*, inhibitory neurons projected into the fascia dentata. That is, with the HRP injection in the right FD, GAD+/ HRP double-labelled neurons were numerous in all fields of ipsilateral Ammon's horn and subiculum. This demonstrates a much greater divergence of basket cell inhibitory axons than previously thought. This distant inhibitory influence was reciprocal because double-labelled neurons with HRP in CA1 ipsilaterally : most in FD, several in all Ammon's horn fields and a few in ipsilateral dorsal subiculum. In conclusion, this study has demonstrated for the first time inhibitory axons projecting via both the hippocampal commissure and intrahippocampal pathways for several mms. from their cell bodies, presumably providing monosynaptic inhibition of distant hippocampal neurons. Supported by NS02808.

413.17 OPEN-FIELD GREGARIOUSNESS AND CHANGES IN EMOTIONALITY FOLLOWING ELECTROLYTIC, AF64A AND KAINIC ACID LESIONS OF THE SEPTUM.

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Septal lesions were produced in rats via chemical administration of AF64A or kainic acid to determine if selective destruction by chemical means would mimick the changes in emotionality and gregariousness seen after conventional electrolytic lesion. Infusion of kainic acid (.3ug in .3ul of vehicle) produced small lesions of the septum, involving only about 10% of the structure. Larger lesions were difficult to obtain as dosages larger than .3ug usually resulted in hippocampal and infratemporal damage or death of the animal. Infusion of AF64A (1nm in .5ul of vehicle) resulted in lesions which more closely approximated the damage produced by electrolytic means. The major difference between electrolytic and AF64A lesions was sparing of the fornix when AF64A was used. Infusion of vehicle only did not produce any noticeable lesion.

After a 1 month recovery period, the animals were observed and rated for emotionality (during handling) on each of 15 consecutive days using the King emotionality scale. On each day after being rated for emotionality the animals were placed by pairs in a Latane open-field and contact time was recorded for 5 min. Contact time was defined as cummulative number of seconds spent in any type of physical contact.

Analysis of variance of the emotionality data revealed that electrolytic septal and AF64A treated animals displayed significantly greater emotionality during the first few days of observation, but did not differ significantly from the other groups during the second half of our observations. By contrast, kainic acid treated animals were more similar to electrolytic lesioned animals in amount of open-field contact time. Both kainic acid treated and electrolytic lesioned groups displayed significantly higher contact times than AF64A or vehicle control groups. Because we found that kainic acid lesions were restricted to the medial septum, these results appear to support earlier work suggesting that medial septal damage which results from electrolytic lesion is the critical factor in postoperative increases in social gregariousness (c.f. Poplawsky & Johnson, Phys. & Beh., 11:845, 1973). While lesion site is critical to changes in gregariousness, our data also suggest that the amount of damage may be an important variable affecting emotionality, i.e. AF64A produced a greater amount of damage and higher subsequent emotionality scores. The present results support the notion that the effects of electrolytic septal lesions may be divisable through the use of chemical agents which selectively destroy specific neural content.

413.18 SUPRAMAMMILLARY INFLUENCES ON HIPPOCAMPAL PHYSIOLOGY: FACILITATION OF GRANULE CELL RESPONSIVITY TO PERFORANT PATH STIMULATION.

S.J.Y. Mizumori, C.A. Barnes, B.L. McNaughton and J. Keith*. Behavioral Neuroscience Program, Psychology Department, University of Colorado, Boulder, CO 80309.

Certain brainstem nuclei are thought to modulate the flow of information through the hippocampus. In particular, it has been suggested that the supramammillary complex (SuM) represents a relay station for median raphé and reticular formation modulatory influences on dentate granule cells (Winson, J., IBRO, Mon.Ser. 11: 131, 1984). The first experiment reported here assessed the influence of SuM stimulation on the dentate granule cell response to perforant path stimulation. The second experiment assessed whether the SuM spike facilitation observed in Experiment 1 might be mediated through septal neurons. The latter issue was of interest because of the known reciprocal connections between the SuM and septum, and the finding that stimulation of the medial septum also results in granule cell spike-facilitation (Swanson, L.W. and Cowan, W.M., J. Comp. Neurol., 186:621, 1979; Alvarez-Leefmans, F.S. and Gardner-Medwin, A.R., J. Physiol., 249:14P, 1975).

Male Fischer-344 rats were anesthetized with Nembutal. Two pulses (10-15 V, 2.5 msec apart) were delivered to the SuM 10 msec before perforant path stimulation. Compared to baseline evoked responses, SuM prestimulation significantly facilitated the population spike of the granule cell response without affecting the EPSP, a finding similar to that described by Winson (1984) and to the effects of medial septum prestimulation. In addition, it was found that the magnitude of spike facilitation was related to placement of the electrode within the SuM region. Prestimulation of lateral (ipsilateral) SuM resulted in a population spike that was 82 ± 18% (mean ± S.E.)+ larger than baseline, while prestimulation of the medial portion of SuM resulted in a 25 ± 7% increase. The regional specificity of the spike facilitation effect is in agreement with the transport study by Haglund, Swanson, and Köhler (J. Comp. Neurol., 229:171, 1984) which indicated lateral SuM to contain more cells that project to the dentate than medial SuM.

Stimulation of the medial septum resulted in an average increase in the granule cell population spike response to perforant path stimulation of 100%. No effect on the EPSP was observed. Electrolytic lesions of the septum eliminated the septal spike-facilitation effect without affecting SuM spike facilitation. This finding suggests that the SuM effect on granule cell excitability is not mediated through medial septum.

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+ Standard errors refer to comparisons across animals.

413.19 BEHAVIORAL MODULATION OF SEPTAL INFLUENCES ON HIPPOCAMPAL EXCITABILITY. E.J. Green, C.A. Barnes, and B.L. McNaughton, Dept. of Psychology University of Colorado, Boulder, CO 80309.

Electrical stimulation of the medial septal (ms) region prior to activation of the perforant path (pp) can result in a marked increase in the amplitude of the pp-evoked granule cell population spike (PS) in the fascia dentata (FD) (e.g., Alvarez-Leefmans and Gardner-Medwin, 1975; Fantie and Goddard, 1982; McNaughton and Miller, 1984). While this phenomenon has been extensively studied in anesthetized animals, there is little data which address the efficacy of ms modulation in behaving animals. We have compared the ability of ms pre-stimulation to modulate granule cell responses during various behavioral states.

Rats were implanted with electrodes for chronic stimulation of the ms and pp, and recording of evoked field potentials and EEG in FD. Ms modulation of granule cell excitability was assessed during four behavioral states: 1) walking on a treadmill, during which the EEG was dominated by rhythmic slow activity (theta); 2) sitting quietly in the same apparatus, characterized by irregular EEG activity; 3) while the animals were anesthetized with sodium pentobarbital; and 4) awake and unrestrained in their home cages 24 hr following recovery from anesthesia.

Pre-stimulation of ms resulted in a significant PS increase in each behavioral state, although the effect varied markedly among states. Rats tested in their home cages exhibited up to a 3-fold increase in PS area. By contrast, the same rats under anesthesia exhibited as much as a 12-fold increase. These increases returned to pre-anesthetic levels within 24 hr. Rats walking on the treadmill exhibited increases averaging only about one-third of those observed when the rats were sitting quietly in the same apparatus. The ms-induced increases in PS area were not accounted for by changes in baseline PS across behavioral states.

These results indicate that activation of hippocampal afferents originating in or passing through ms can have a substantial effect on granule cell excitability in conscious animals, and that the effect is dependent upon the behavioral state of the animal. Previous reports showed that sodium pentobarbital potentiates GABA-mediated inhibition in the FD (Douglas et al., 1983). The results of the anesthetic experiment, in conjunction with anatomical evidence for GABAergic projections from medial septal nucleus to hippocampus (Kohler et al., 1984), provide support for the idea that the septal pre-stimulation effect is mediated by inhibition of GABAergic inhibitory interneurons (Bilkey and Goddard, 1985).

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413.20 TEMPERATURE AND pH EFFECTS ON EVOKED RESPONSES FROM HAMSTER HIPPOCAMPAL SLICES. M.P. Thomas*, P.J. Eckerman*, J.M. Horowitz. Department of Animal Physiology, University of California, Davis, CA 95616.

Previous work has shown that hippocampal CA1 pyramidal neurons can be excited by Schaffer collateral stimulation at lower bath temperatures in slices from ground squirrels (10°C) than in slices from rats (14°C) (Hooper et al., J. Thermal Biol. 10:35-40, 1985). Moreover, in slices from hibernating hamsters, population spikes can be evoked at lower bath temperatures than in slices from nonhibernating hamsters. We are presently investigating the mechanisms responsible for this differing temperature sensitivity between hibernating and nonhibernating animals. Based on studies by Malan et al. (Respir. Physiol., 17:45-61, 1973) of acid-base states in hibernating animals, we investigated the effects of pH changes on evoked responses using the in vitro hippocampal slice preparation.

Hippocampal slices were prepared from hamsters as described previously (Hooper et al.). Extracellular evoked responses were recorded from area CA1 following Schaffer collateral stimulation. The pH of the bathing medium was altered by varying the concentration of CO_2 in the gas bubbled through the media reservoir (from 4% to 10%), with a buffer containing 26 mM bicarbonate ion. Slice temperature was varied using an external water bath.

We have observed that as slice temperature is lowered, the width of the population spike is consistently increased. In one set of experiments, population spike width was measured as pH was varied between 7.5 and 7.1, with slice temperature constant. These responses were compared with responses to identical stimulation parameters in the same slice, as temperature was varied between 25 and 20°C with CO_2 concentration held constant. Analysis of variance showed a significant difference in spike width between 25 and 20°C while no significant difference was seen as pH was altered and temperature held constant. Thus the lower threshold for evoking electrical activity in slices from hibernating vs. nonhibernating hamsters does not appear to be due to differential responses to the acid-base state of the bathing solution.

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413.21 AMYGDALOID NEUROPEPTIDES IN TEMPORAL LOBE EPILEPSY: A PRELIMINARY STUDY. M.L. Estes, H. Lüders* and C.H. Block. Departments of Pathology, Neurology and Brain and Vascular Research, Cleveland Clinic Foundation, Cleveland, OH 44106.

The amygdala is a complex anterior and mesial temporal nucleus. Although amygdala is easily kindled and has been studied as an animal model of temporal lobe epilepsy, the hippocampus has been the focus of the majority of research. In animal models of epilepsy, alterations of putative neurotransmitters, including neuropeptides, occur in hippocampus. In human hippocampus from epileptic patients, alterations in neurotransmitters have also been found. It is not known if there are changes in these transmitters in the amygdala of patients with epilepsy; however, pathologic abnormalities in amygdala have been demonstrated in temporal lobe epilepsy. These studies were undertaken to characterize and compare putative neurotransmitters in normal tissues with tissue obtained from patients undergoing temporal lobectomy for intractable epilepsy.

Brain tissue was processed for immunocytochemistry. Briefly, at the time of lobectomy, surgically removed mesial temporal tissue from three patients was oriented and placed in 10% buffered formalin. Frozen tissue was sectioned at 30 μm in a coronal plane using a sliding microtome. Serial sections were incubated overnight in antibodies generated against substance P (SP), somatostatin (SS), methionine enkephalin (ENK) and neurotensin (NT). The avidin-biotin technique with diaminobenzidine as the chromagen was used for immunocytochemical localization. Control tissue was obtained at postmortem within ten hours of death from brains of patients with no neurologic abnormalities. The tissue was oriented and processed in a similar manner to the surgical tissue.

Immunoreactive fibers for all substances were found in both the control and surgical tissue. Specifically, SP and SS immunoreactive perikarya were found within the amygdala. In addition, dense plexuses of SP fibers were present, while fewer fibers containing SS, ENK and NT were scattered in the amygdala. While we have demonstrated the existence of neuropeptides in both the control tissue and tissue from epileptic patients differences in their distributions remain to be determined. Quantitative studies are being undertaken for comparative analyses of these tissues.

413.22 GLYCOGEN PHOSPHORYLASE REACTIVITY IN THE ENTORHINAL PROJECTIONS TO THE HIPPOCAMPUS. C.W. Harley and J. O'Keefe* Psychology Dept., MUN, St. John's, Nfld. A1B 3X9 and Anatomy Dept., Univ. Coll. Lond. London, England WC1E 6BT

In the present study a histochemical method was used to examine glycogen phosphorylase 'A' distribution in the projections of entorhinal cortex to hippocampus in the rat brain. Glycogen phosphorylase A is detected by incubating tissue under conditions which promote glycogen formation. The newly formed glycogen can be visualized by reaction with iodine. Color differences in iodine staining reflect the presence of specific glycogen branching patterns.

The staining pattern obtained in the present study suggests the form of glycogen produced is common to both the origin and terminal areas of the entorhinal-hippocampal projection system. A distinct continuous color band, of orange or golden hue, characterizes layer 3 of both medial and lateral entorhinal cortex. A similarly well-defined band of the same color was observed in the hippocampal target area of the layer 3 projection, co-extensive with the molecular layer of CA1 adjacent to the hippocampal fissure. Layers 1 and 2 of entorhinal cortex were demarcated from layer 3 by a brown coloration which was sometimes more intense in layer 1 and often discontinuous in appearance. The same brown staining occurred in the middle and outer layers of the dentate gyrus molecular layer and in the adjacent molecular layer of CA3, projection targets of layer 2 entorhinal cells. Iodine staining was not seen in the dentate granule cells or pyramidal cells and the inner segment of the dentate molecular layer was typically unreactive.

A striking feature of glycogen distribution in the dentate molecular layer was the appearance of discrete reactive patches of 30-50 μM which formed a "leopard spot" pattern throughout the entorhinal projection area when sections were taken parallel to the cell body layer.

Microinjections of 0.2-0.3μL of 0.1M glutamate appeared to activate glycogen phosphorylase along the injection cannula. Intensification of staining was only seen in the projection targets of the entorhinal cells, i.e. the CA1 molecular layer and the dentate gyrus molecular layer, immediately adjacent sites were not activated.

413.23 HIPPOCAMPAL PLACE CELLS: CLASSIFICATIONS, CLARIFICATIONS AND MANIPULATIONS. L. T. Thompson* & P. J. Best, Neuroscience Program, University of Virginia, Charlottesville, VA 22903.

Past work from our lab has focused both on quantifying hippocampal place unit activity and its behavioral correlates, and upon defining sensory stimuli and neuronal circuitry necessary for the qualitative expression of place field activity. The present study reports that functional place cell classes defined both quantitatively and qualitatively are determined by environmental place stimuli, rather than by intrinsic properties of any given cell. Both plasticity and stability of the functional states of place cells were examined.

Single unit activity was recorded from the CA1 hippocampal cell field in 50 freely-behaving Long-Evans rats. The rats were tested in three different environments, under a variety of behavioral conditions. Hippocampal units were divided into complex-spike (CS) or theta (TH) cells, after quantitative analysis of their electrophysiological properties. The spatial firing correlates of over 200 place cells were then analyzed.

The spatial location of the rat within a particular environment was observed to be a sufficient condition to evoke reliable changes in the firing rates of CS place cells. The firing of CS cells was readily divided into six major classes after analysis of their place field topology within a given environment. This classification schema is only slightly modified from an earlier schema, which was based upon a smaller pool of place cell data (Olton et al., 1978). Classes included behaviorally silent cells, pure ON-cells, pure OFF-cells, and combinations of the latter two classes. The functional class to which a cell was assigned based upon its activity in one environment was not a predictor of its functional class in another environment. Some manipulations of sensory stimuli were found to produce reliable changes in place field topology, without altering the cell's functional class, while other manipulations altered the cell's class (e. g. from an ON-cell to an OFF-cell). We reliably held more than 10 cells for long periods (days) in stable environments without observing functional class changes in these cells. Place cell classes are therefore hypothesized to represent stabilized neuronal functional states, with plasticity modulated by spatial stimuli.

The firing of TH place cells was found to be less place specific than that of CS place cells, with less robust changes in firing rates delimiting a field. Although no attempt was made to distinguish behaviorally silent TH cells, both ON and OFF classifications could be applied to TH cell field topologies. TH cell place fields were less reliable than those of CS cells, since spatial location was not always sufficient to evoke changes in TH cell firing rate. Thus, the functional state of TH cells was less specifically determined by spatial stimuli.

413.24 WHEEL-RUNNING ACTIVITY AFTER THE IONTOPHORETIC INJECTION OF KAINIC ACID INTO THE VENTROLATERAL HYPOTHALAMUS OF RATS. W.E. Gladfelter and R.J. Millecchia, Dept. Physiology, West Virginia University Medical Center, Morgantown, WV 26506.

Bilateral electrolytic lesions placed in the ventrolateral hypothalamus of rats produces a decrease in wheel-running activity to 10% of preoperative levels. Since these lesions destroy both neurons intrinsic to the lateral hypothalamus and fibers of passage through this region, the identity of the neural substrate that must be destroyed to produce these results needs to be determined to know whether the hypothalamus plays a role in the control of wheel-running behavior. The neurotoxin kainic acid, when injected locally into the brain, has been reported to destroy neurons intrinsic to the site of injection but leave undamaged the fibers of passage through this site. When kainic acid is injected into the lateral hypothalamus of rats by means of an infusion pump, wheel-running activity is decreased, and the magnitude of the decrease in this behavior appears to be directly proportional to the magnitude of the hypothalamic damage produced by the kainic acid. In addition, wheel-running behavior after the injection of kainic acid is similar to that exhibited after the placement of electrolytic lesions, i.e., it is decreased both when the rats are left undisturbed and when they are being cared for. However, damage to structures outside of the hypothalamus is often produced when kainic acid is injected by infusion pump into the lateral hypothalamus. Tissue damage has been observed in the thalamus, the pyriform cortex, the amygdaloid complex, the hippocampus, the zona incerta, and the basal ganglia.

In an effort to produce damage to the lateral hypothalamus without concomitant damage to extra-hypothalamic structures, kainic acid was injected iontophoretically into the lateral hypothalamus of a small group of rats. Preliminary results from 8 rats indicate that extra-hypothalamic damage after the iontophoretic injection of kainic acid is much less than that observed after the injection of kainic acid by an infusion pump, but neuronal loss is sometimes observed in the region of the amygdaloid complex and the pyriform cortex. The magnitude of the decrease in wheel-running activity appears to be directly proportional to the magnitude of the tissue damage in the lateral hypothalamus. However, wheel-running activity is decreased only during the time the rats are left undisturbed. During the time the rats are being cared for, their wheel-running activity is at the same level it was preoperatively during this time. More data is needed before the role of the hypothalamus in the control of wheel-running behavior can be postulated with some degree of certainty. (Supported by WVU Med. Corp. and NIH Biomed. Res. Grant 5 S07 RR05433-18.)

413.25 EFFECTS OF TETRAHYDROCANNABINOL AND CANNABIDIOL ON HIPPOCAMPAL ACTIVITY IN THE INTACT, AWAKE RAT. D.M. Wilkison. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

Delta-9-tetrahydrocannabinol (THC) has been shown to have potent effects on the neurophysiology of the limbic system. Various authors have ascribed the behavioral changes induced by THC intoxication to changes in electrophysiological and neurochemical events in the septal-hippocampal system. The several electrophysiological studies have not led to a unified hypothesis as to the actions of THC in the hippocampal formation. We have previously reported the differential effects of THC on the perforant path/dentate response and the commissural CA1 response in urethane-anesthetized rats. This report details the actions of THC and the nonpsychoactive cannabinoid, cannabidiol (CBD) in the intact, awake rat.

Hooded Long-Evans rats were implanted stereotaxically under pentobarbital anesthesia. Pairs of electrodes were aimed at either the dentate/perforant path or the CA1/cCA3 sites. Commencing one week after surgery, each rat received a single dose, ip, of THC (2 to 16 mg/kg) or CBD (20 to 80 mg/kg) on a randomized weekly schedule. Full input/output curves (6 stimulus intensities) were established prior to drug administration and 15, 45 and 120 minutes after. Electrodes were in cell layers recording classical negative population spikes superimposed upon positive synaptic potentials. Each component was separately analyzed.

THC produced a dose-dependent decrease (20 to 50%) in the CA1 response to commissural stimulation in chronically implanted rats. This was most significant at the low threshold stimulus intensities and most consistent at 8 and 16 mg/kg. These effects were observed in the spike response and the synaptic potential. The data suggest the intrinsic coupling of spike generation and synaptic activity was not altered by THC. In contrast, THC did not significantly alter the dentate response to perforant path stimulation in conscious rats. CBD in doses 5 times those of THC was without effect on the hippocampal response to afferent stimulation.

These data substantiate earlier findings from this laboratory of the actions of THC in urethane-anesthetized rats. The finding that CBD did not have such effects is supportive of the hypothesis that the effects of THC on CA1 are likely related to the psychoactive properties of the drug. This study was supported by NIDA R01 DA 03785.

413.26 VAGAL NERVE-OLFACTORY BULB PATHWAY: AN EVOKED POTENTIALS STUDY. L. L. Jiménez-Montúfar*, D. E. García-Díaz*, R. Guevara-Aguilar and M. J. Wayner. (SPON: L. Cintra-McGlone). Depto. de Fisiología, Fac. de Medicina, UNAM, México 04510, D. F., and Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX. 78285.

We have already demonstrated the existence of a functional connection between visceral vagal afferents and cells of the periglomerular layer of the olfactory bulb (OB). Since the rostral portion of the nucleus of the tractus solitarius (NTS) receives afferents from the tongue and mouth associated with the V, VII, IX, and X cranial nerves, it is undoubtedly involved in taste and possibly ingestion. This portion of the NTS sends direct connections to hypothalamic and amygdaloid structures related to eating. The purpose of this study was to establish the connections between vagal afferents and the rostral portion of the NTS, lateral hypothalamus (LH) and the OB. Experiments were conducted on twelve Wistar rats anesthetized with cloral hydrate. Evoked potentials in the OB, LH and rostral portion of the NTS, were recorded after cervical vagus nerve stimulation. In the OB a 14 to 16 msec potential was recorded only at the periglomerular layer, though, a rapid component appeared deeper, probably at the granular layer. In the LH, an evoked potential could be observed with a latency of 3 to 5 msec. The two components observed in the rostral NTS occur with 2 to 3, and 5 to 7 msec delays. When the rostral NTS was stimulated and the evoked potentials were recorded in the OB and in the LH, the latency to the peak change in the OB was 7.5 to 8.5 msec and 3.5 to 4.5 in the LH. The early OB evoked responses by vagus nerve stimulation are still difficult to interpret, but the long latency 15 msec average response appears clearly in the surface of the OB and was eliminated when the rostral NTS was destroyed. Other data indicate a latency of 8 msec for the evoked potentials from the NTS to the OB. An examination of the evoked potentials in the NTS in response to vagal stimulation revealed two components; the longest latency component was 7 msec. Apparently this component represents the activity of a group of slow fibres which synapse in the NTS and then this is added to the 8 msec latency observed for evoked responses in the OB to vagal nerve stimulation resulting this in a 15 msec latency. After electrolytic lesion of NTS, the evoked potentials in LH by vagal nerve stimulation were not abolished. These results indicate that the pathway from the vagus nerve to olfactory bulb travels through the NTS but probably not through the LH.

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413.27 SUBFORNICAL ORGAN STIMULATION INFLUENCES THE EXCITABILITY OF SEPTAL NEURONS PROJECTING TO THE PARAVENTRICULAR NUCLEUS IN THE RAT. S.D. Donevan and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The subfornical organ (SFO) is a circumventricular structure that mediates drinking, and the increased plasma vasopressin concentrations observed in response to intravenous angiotensin II. Anatomical studies have revealed efferent projections from SFO to the paraventricular (PVN) and supraoptic (SON) nuclei. Although electrical stimulation of SFO enhances the excitability of the majority of oxytocin and vasopressin secreting neurons in the SON and PVN, inhibitory effects are also observed. This suggests that polysynaptic pathways may also be involved in connecting the SON and PVN with the SFO. Neurons in the medial septum and the adjacent diagonal band of Broca (MS-DBB) have anatomical connections with the SFO, PVN and SON. The SFO has been shown to influence the excitability of MS-DBB neurons that project to the SON. The present study examines the effect of electrical stimulation in the region of the SFO on the excitability of MS-DBB neurons that project to the PVN.

Male Sprague-Dawley rats (140-320 g) anesthetized with urethane (1.4 g/kg) were used in all experiments. A stimulating electrode was implanted, using stereotaxic coordinates, in the region of the SFO. The medial basal hypothalamus was exposed through a transpharyngeal approach, and a stimulating electrode lowered into the region of the PVN. Extracellular single unit recordings were obtained from MS-DBB neurons using 2.0 M NaCl-filled glass micropippetes. Neurons were antidromically identified according to standard criteria of an all or nothing constant latency response at threshold; the ability to follow high frequency (200 Hz) stimulation; and the presence of collision cancellation between antidromically evoked and spontaneous action potentials occurring within the critical period. Recordings were obtained from 214 MS-DBB neurons, 53 of which were antidromically identified as projecting to the region of the PVN. The effects of SFO stimulation were tested on 28 of these cells, of which 78% responded with short latency (20.78 $\pm$ 0.84 ms, SEM), short duration (10.58 + 1.06 ms) increases in excitability. The remaining neurons (22%) were unaffected by SFO stimulation.

These results indicate that the SFO has a predominantly excitatory effect on MS-DBB neurons antidromically identified as projecting to the PVN. It suggests that this may be a polysynaptic pathway through which the SFO influences the activity of oxytocin and vasopressin secreting neurons in the PVN.

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413.28 PERSEVERATIVE SPONTANEOUS TURNING: A NEW BEHAVIORAL MEASURE OF HIPPOCAMPAL DAMAGE. G.A. Mickley*, J.L. Ferguson*, C. Alderks, T.J. Nemeth and B.A. Dennison. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Exposure of the neonate to focused X rays produces discrete lesions of the dentate granular-cell layer of the hippocampus. This brain damage modifies the behavior of the rat by stimulating spontaneous locomotion and inhibiting the acquisition of passive avoidance (Bayer, S.A. et al., Nature New Biol. 242: 222-224, 1973). Here we report a newly discovered behavioral correlate of radiogenic hippocampal damage: perseverative spontaneous turning.

The cerebral hemispheres of neonatal rats were symmetrically exposed to x-rays using a procedure similar to that described by Bayer et al. (Brain Res. Bull. 2: 153-156, 1977). This protocol produces selective damage to the granular-cell layer of the hippocampal dentate gyrus. Other animals were sham irradiated. Ten- to 25-week-old subjects were placed in large bowls and spontaneous turning was recorded over at least 5, 30-minute sessions. We also measured several other behaviors: spontaneous locomotion, spontaneous alternation in a T-maze, and acquisition of a passive avoidance task.

Radiation-induced hippocampal damage caused rats to exhibit longer bouts of turns in the same direction. Once they began turning, irradiated rats perseverated in that turning to an extent significantly greater than the sham-irradiated subjects. Irradiated rats also had a more pronounced lateral bias. However, the radiogenic enhancement in bout length could not be attributed solely to the greater lateral bias of the irradiated animals. The irradiated rats also showed retarded acquisition of a passive avoidance task and some enhancement in measures of spontaneous locomotor activity. Rats with radiation-induced hippocampal damage did not exhibit perseveration in the spontaneous exploration of the arms of a T-maze.

These data confirm some of the previously reported behavioral correlates of hippocampal damage. Further the data suggest the usefulness of observing spontaneous turning as an indicator of discrete hippocampal damage produced by neonatal X irradiation.

413.29 AFTER DISCHARGES IN THE HIPPOCAMPUS FOLLOWING LOCAL TETANIZATION. K.A. Desborough* and L.S. Leung, Depts. of Clinical Neurological Sciences, Physiology and Psychology, Univ. Western Ontario, London, Canada N6A 5C5.

Following a short (1-10 sec) train of repetitive stimulation delivered to the hippocampal CA1 region, the following sequelae of afterdischarges (AD) was seen: (1) a silent period of 2-4 sec, (2) a large primary (1°) AD usually alvear-surface negative and deep positive, (3) a period of suppressed hippocampal EEG, (4) a secondary (2°) hippocampal AD, and at 3-6 min latency, (5) 15-25 min of enhanced (up to 10 times normal) fast (30-70 Hz) waves. The 2° hippocampal AD was preceded by or simultaneous with large AD at the amygdaloid electrodes. Large heat lesions of the amygdala (N=4) or electrolytic lesions of the medial septum (N=10) were not successful in abolishing the 2° hippocampal AD. However, 2 rats with stria terminalis lesions and 2 rats with bilateral aspiration lesion of the entorhinal cortex did not have the 2° hippocampal AD. Fast waves were reversed 180° across surface and deep CA1 electrodes. The fast wave increase was blocked by atropine sulfate (25-50 mg/kg i.p.), scopolamine hydrochloride (5 mg/kg i.p.) and medial septal lesions, but was enhanced by pilocarpine (25 mg/kg i.p.).

It was concluded that the 2° hippocampal AD may depend on reverberation of neural circuitry involving the ventral hippocampus (e.g. subiculum) and the entorhinal cortex. The 2° AD recorded from amygdala electrodes may partly reflect spreading of activities from the latter structures. On the other hand, the increase in fast waves after tetanization requires an intact septohippocampal, muscarinic cholinergic input, and may depend on an enhanced cholinergic input or an increased response. The long (15-25 min) duration of increased fast waves is similar to that of enhanced cholinergic supersensitivity to acetylcholine described in the hippocampal slice after tetanization (Burchfield et al. Science 204: 1096, 1979).

413.30 EFFECTS OF COOLING INFEROTEMPORAL CORTEX AND AMYGDALA ON MONKEY AMYGDALA AND LATERAL HYPOTHALAMIC NEURAL ACTIVITY DURING FEEDING BEHAVIOR. T. Ono, M. Fukuda*, K. Nakamura* and H. Nishijo*. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01, Japan.

It has been reported that monkey amygdala (AM) and lateral hypothalamic (LHA) neurons responded at the sight of food and nonfood during operant bar press feeding behavior (Fukuda et al., Brain Res. in press, 1986; Ono et al., Brain Res. Bull. 11: 515-518, 1983; Rolls et al., Brain Res. 130: 229-238, 1977). In the study reported here, functional and neuronal interaction between the inferotemporal cortex (ITCx) and the AM during operant bar press feeding by the monkey were investigated by inducing reversible deficits with cooling probes chronically implanted bilaterally over the dura of the anterior ITCx in one monkey. We similarly investigated relations between the AM and the LHA by bilateral cooling of the lateral part of the AM in two monkeys.

The activity of 43 AM neurons was analyzed. Before ITCx cooling, each AM neuron was classified into one of 4 groups based on its response pattern: 6 responded selectively to the sight of food (food related), 11 responded selectively to the sight of nonfood (nonfood related), 21 responded to the sight of both food and nonfood (arousal related), and 5 did not respond (no response). ITCx cooling changed the spontaneous firing rates of 15 AM neurons (2 increased, 13 decreased). Of 17 neurons that responded primarily to the sight of food or nonfood, the responsiveness of 8 (47 %) changed from selective to nonselective when the ITCx was cooled. The activity of 55 LHA neurons was analyzed. Of these, 22 were food related, 6 were ingestion related (responded during ingestion of food), 22 were arousal related, and 5 did not respond. AM cooling changed the spontaneous firing rate of 21 LHA neurons (12 increased, 9 decreased). Visual responses of 9 food related neurons and 2 arousal related neurons were depressed by AM cooling. Ingestion related responses of 3 of 6 food related AM neurons were not affected by ITCx cooling, but responses of 3 ingestion related LHA neurons (3/6) were depressed by AM cooling.

We concluded that visual information related to discrimination between food and nonfood flows sequentially through the ITCx and the AM to the LHA, and dynamic interaction among these structures is important in associating stimulus with reinforcement, i.e. reward or nonreward.

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413.31 THE STRIA TERMINALIS IS A ZINC CONTAINING PATHWAY. C.J. Frederickson, J. Perez-Clausell*, G. Danscher*, and J.G. Duesler Jr.*. (SPON: G. Moushegian) Lab. for Neurobiology, Univ. of Texas at Dallas, Richardson, TX 75080 and Institute of Anatomy B, Aarhus, Denmark.

The advent of new silver-amplification (Danscher In: Frederickson et al., The Neurobiology of Zinc, B., A.R. Liss (1984) pp. 237) and fluorescence (Frederickson et al., J. Neurosci. Meth. submitted) histochemical methods has recently made it possible to identify zinc-rich regions of the CNS with virtual certainty. The contemporary methods indicate that (a) CNS zinc staining is almost entirely restricted to regions of neuropil, and (b) in neuropil, the staining is limited to the vesicles of certain axon boutons (Perez-Clausell & Danscher Brain Res. (1985) 337:91)

We are currently searching for the cells of origin of some of the zinc-containing terminal plexuses in the CNS. One of these zinc-rich terminal fields encompasses the Bed Nucleus of the Stria Terminals (BNST) and subjacent perihypothalamic regions, including the outer capsule of the Ventro Medial Hypothalamus (VMH) and the Ventral Premammillary Nucleus (VPN). We have found that the zinc staining throughout the BNST, VMH, and VPN is completely abolished by prior ipsilateral interuption of the stria terminalis, accomplished by either simple transection of the stria or by amygdalar/subicular electrolytic lesion of the origins of the stria. Control lesions in the neocortex, hippocampus, and caudate/putamen have no apparent effect of the staining in the BNST/VHM/VPN zone. We conclude that zinc staining in the BNST/VMH/VPN region reflects the presence of zinc-rich boutons which derive from axons traveling through the stria terminalis.

Parallel studies now in progress suggest that both the cortico-striatal pathway and the hippocampo-septal projections may also be fiber systems which have zinc in the axon boutons. One interesting generalization emerging from these studies of the zinc-containing pathways is that the preponderance of the zinc-rich fiber systems are of limbic and/or cerebrocortical origin. (Supported by Danish Medical Council grants to CJF and GD and NIH-BSRG 807-RR07133 to CJF).

GABA PATHWAYS

414.1 IN VITRO AND IN VIVO MODELS OF IMINO ACID - NEUROTOXICITY
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Excitatory amino acids, naturally occurring in brain, such as glutamic acid may act as neurotoxins. This effect is believed to be mediated by a postsynaptic receptor mechanism. Other examples of neurotoxic amino acids are kainic, quisqualic, ibotenic, quinolinic and monocysteic acids. Recently, endogenous inhibitory imino acids such as pipecolic acid (PA) have also been found to be neurotoxic. Elevated blood and tissue concentrations of PA, a major metabolite of lysine in brain, are associated with a severe, destructive and demyelinating process in affected infants with hypotonicity and mental retardation. Mouse fetal cortical neurons were harvested and grown in modified Eagle's medium and treated by the addition of 3 and 5 nmole/ml PA. The cultures were grown, sequentially harvested and enzyme determinations were performed. Cultures predominantly affected by the presence of PA are those with cholineacetyltransferase (CAT) activity. Glutamic acid decarboxylase (GAD) activity is not affected in this model. The absolute levels of CAT/GAD are adversely affected by increasing levels of PA added to the culture medium particularly during days 7-10 of growth. Our study in mice in vivo shows that the offspring of mothers injected during pregnancy with 250 mg/kg PA demonstrate a significant decrease in both cortical and whole brain DNA content. Pups injected after birth demonstrated no adverse effects. Therefore, a high and steady level of PA comparable to cerebral levels in human hyperpipecolatemia can induce a neurotoxic effect in the fetal mouse brain. Both in vitro and in vivo results suggest that the critical period for neurotoxic effect is prenatal, probably due to a selective vulnerability of developing neurons to high PA. Damage to specific types of neurons may explain the mental subnormality evident in infants with hyperpipecolatemia. (Supported in part by SIU Central Research Grant)

414.2 GABA AND TAURINE SYSTEMS IN CONTROL AND ISCHEMIC RAT HIPPOCAMPAL FORMATION.
J.-Y. Wu[1], C.-T. Lin[1], J. W. Liu[1]* and F. F. Johanson[2] [1]Dept. Physiol., Milton S. Hershey Med. Ctr., Penn State Univ., Hershey, PA and [2]Institute of Neuropathol., Univ. Copenhagen, Denmark.

The changes of GABA and taurine neurons and their processes in the rat hippocampal formation after transient cerebral ischemia were investigated by immunocytochemical localization of their respective specific markers, namely, glutamate decarboxylase (GAD) and cysteine sulfinic acid decarboxylase (CSAD). Transient cerebral ischemia was induced in rats by ligation of both common carotid arteries for 20 min after electric cauterization of vertebral arteries. The control animals were sham operated but had no ischemia. All rats were intraventricularly injected with 20 µg of colchicine 2 days before they were sacrificed. In area CA_1 of sham operated animals, GAD-immunoreactivity (GAD-IR) was found in the somata of some interneurons and in many neuronal processes and terminals in stratum oriens (SO) and stratum radiatum (SR). Numerous punctate GAD-IR surrounding pyramidal neurons and some interneurons in SO and SR were seen. Very few basket pyramidal cells were stained. The pyramidal neurons were not stained. In ischemic hippocampus, most pyramidal neurons showed different degrees of degeneration, notably with shrinkage of cell body. Although punctate GAD-IR surrounding the pyramidal neurons were moderately reduced, no significant changes for GAD-positive interneurons in SO and SR were observed. Similar results were also found in areas CA_2 and CA_3. In the taurine system, CSAD-IR was seen in the somata of some pyramidal basket neurons and some interneurons in SO and SR in CA_1 area of sham operated animals. Some punctate CSAD-IR surrounding pyramidal neurons were also found. The pyramidal neurons were not stained. In ischemic condition, no significant changes of CSAD-IR in the somata of interneurons in SO and SR and in some pyramidal basket neurons in area CA_1 were observed. However, punctate CSAD-IR surrounding pyramidal neurons were markedly reduced. The same findings were also observed in areas CA_2 and CA_3. Hence, it is concluded that the GAD positive or CSAD-positive interneurons and pyramidal basket neurons appear to be less vulnerable to ischemic damage than pyramidal neurons which are not GABAergic nor taurinergic neurons. (Supported in part by NIH grants NS-20978, NS-20922 and EY-05385).

414.3 COMPARISON OF GABAERGIC AND TAURINERGIC NEURONS IN CONTROL AND ISCHEMIC RAT DENTATE GYRUS. C.-T. Lin[1] J. W. Liu[1]* F. F. Johansen[2]* and J.-Y. Wu[1]. [1]Dept. Physiol., Penn State Univ., Hershey, PA 17033 and [2]Institute of Neuropathology, Univ. Copenhagen, Denmark.

Glutamate decarboxylase (GAD) and cysteine sulfinic acid decarboxylase (CSAD), specific marker for GABAergic and taurinergic neurons, respectively, were identified in the sham operated (control) and ischemic rat dentate gyrus by immunohistochemistry. The ischemia was induced by ligation of both common carotid arteries for 20 min after electric cauterization of vertebral arteries. In the control dentate gyrus, GAD-immunoreactivity (GAD-IR) was seen in the somata of some interneurons (stellate cells) in molecular layer and of a few interneurons in the hilar region. Numerous punctate reaction products surrounding granule cells, interneurons in the hilar region, and the pyramidal neurons in area CA_4, as well as in the molecular layer were found. No pyramidal neurons nor granule cells were stained. In the ischemic dentate gyrus GAD containing interneurons were still visible in the molecular layer and occasionally in the hilar region. However, the number of GAD-positive processes and the staining intensity were markedly reduced. Some granule cells and pyramidal cells in area CA_4 showed degenerative change, notably with shrinkage of their somata. When sham operated rat dentate gyrus was stained with anti-CSAD, it was found that more dentate basket cells, interneurons in the hilar region and interneurons (stellate cells) in molecular layer were stained with anti-CSAD than anti-GAD. Some pyramidal basket neurons in the area CA_4 also contained CSAD-IR. The punctate reaction products surrounding granule cell bodies and pyramidal cells in area CA_4 were not as dense as GAD-IR containing terminals. No such punctate reaction products were seen surrounding interneurons in the hilar region and in the molecular layer. The granule cells and pyramidal cells in the area CA_4 contained no CSAD-IR. In the ischemic condition, CSAD-IR was still retained in some interneurons (stellate cells) and neuronal processes in both the molecular layer and in the hilar region. However, dentate basket cells and dentate pyramidal basket cells showed no or extremely weak immunostaining. Similarly almost no CSAD-IR containing terminals surrounding the dentate granule cells and pyramidal neurons in area CA_4 could be detected. These findings suggest that GAD containing interneurons appear to be less vulnerable than CSAD containing interneurons to the ischemic damage. (Supported in part by NIH grants NS-20978, NS-20922 and EY-05385).

414.4 DISTRIBUTION AND ULTRASTRUCTURE OF NEURONS IN PIRIFORM CORTEX DISPLAYING IMMUNOREACTIVITY TO GABA AND GAD AND HIGH AFFINITY ^{3}H-GABA UPTAKE. L.B. Haberly, S.L. Feig* and D.J. Hansen* (SPON: T. Duff), Dept. of Anatomy, Univ. Wisconsin, Madison, WI 53706.

GABAergic neurons in piriform cortex of the opossum have been identified with antisera to a GABA-BSA conjugate (Fex et al., Br. Res. 366:106) and glutamic acid decarboxylase (GAD; Oertel et al., Neurosci. 6:2689) and by the presence of high affinity ^{3}H-GABA uptake. Three distinctive populations have been identified on the basis of morphology, laminar distribution patterns and differences in anterior-posterior location: horizontal cells, spherical soma cells and multipolar cells.

Horizontal cells have large somata and are restricted to layer Ia of anterior piriform cortex. They are darkly stained by both GABA-BSA and GAD antisera but are labeled by ^{3}H-GABA uptake only at distances of 1 mm or more from injection sites. This later observation suggests a lack of local axonal arborizations. These cells clearly correspond to the spiny soma horizontal neurons previously described with Golgi and EM methods (Haberly, JCN 213:163; Haberly & Feig, JCN 216:69). They appear to be specialized for mediation of a rapid, feedforward inhibitory process.

Spherical soma cells are moderately small in size (10-13 μm) and distinctive by virtue of a thin rim of cytoplasm and thin dendrites that arise abruptly from the cell body. They are found in all layers of piriform cortex and in the underlying endopiriform nucleus, but a striking feature is a concentration in layers Ib and II in posterior piriform cortex in contrast to a relative absence in these layers anteriorly. These cells appear to correspond to the "neurogliaform" neurons observed in a Golgi study (Haberly, JCN 213:163) that have locally arborizing unmyelinated axons. Electron microscopy revealed a high density of symmetrical and asymmetrical synapses on dendrites but a low density on somata. It is postulated that these cells are responsible for the slow, K^+ mediated inhibitory process observed in piriform cortex (see Tseng & Haberly, this volume).

Multipolar cells are concentrated in the deep part of layer III and the subadjacent endopiriform nucleus in both anterior and posterior parts of piriform cortex. Variability in ultrastructure suggests the presence of at least two subpopulations. It is postulated that these cells are responsible for the well-known feedback, Cl^- mediated IPSP.

Preliminary results have also been obtained with a GAD antiserum in the rat. The same three neuron populations can be distinguished, although laminar patterns are less distinct and the superficial horizontal cells are more irregular in shape and confined to the immediate vicinity of the lateral olfactory tract. Supported by NSF grant BNS-8311118 to L.B.H.

414.5 GABA RELEASE IN THE GLOBUS PALLIDUS AFTER A DOPAMINERGIC DENERVATION CAUSED BY 6-OHDA. J.Segovia*, U. Tossman*, M. García-Munoz and U. Ungerstedt*. (SPON: A.M. Lopez-Colome) Institute of Cellular Physiology, U.N.A.M., P. O. Box 70-600, Mexico D.F.

There is evidence showing that the striato-pallidal projection contains GABA (Fonnum, F. et al, Brain Res., 143:125,1978). It has also been demonstrated using biochemical and electrophysiological techniques that this pathway is inhibitory upon pallidal cells (Lavine, M.S. et al, Exp. Neurol., 44:448,1974; Malliani, A. et al, Brain Res., 6:341,1967). Furthermore the GABAergic descending pathways from the striatum have been related to the motor activity induced by stimulation of dopamine receptors (Scheel-Kruger, J., Acta Neurol. Scand., 65, Suppl. 90:40, 1982).

The present experiments were designed to study the effect of striatal dopamine denervation in the "in vivo" overflow of GABA in the globus pallidus, using the intracerebral dialysis technique.

Male Sprague-Dawley rats (140-160 g) were anesthetized with a mixture of air and halothane and fixed to a stereotaxic frame. A unilateral microinjection of 6-hydroxidopamine (6-OHDA) (8ug/4 ul of saline with 0.2% ascorbic acid) was applied to the medial forebrain bundle. Two weeks after the operation the rats were tested with apomorphine (0.05 mg/kg, s.c.) to select the successfully denervated animals (Ungerstedt, U. et al, Chemical Transmission: 75 years. Academic Press, 326, 1981). Four weeks after the lesion the rats were tested for the release of GABA in the globus pallidus. The rats were anesthetized and placed on a stereotaxic frame, two dialysis probes (Carnegie Medicin) were bilaterally implanted in both nuclei. Samples were collected every 10 min. At the end of each experiment 100 mmoles of potassium chloride were added to the perfusing medium. The perfusates were determined for GABA using HPLC after o-phthaldehyde derivatization (Lindroth, P. et al, Anal. Chem., 51:1667, 1979).

In control unlesioned animals GABA release was similar on both hemispheres: 1.8±0.1 and 1.9±0.2 (pmoles/10 ul of perfusate ± SD), as well as the increase caused by the depolarization with potassium chloride (60.3±26; 62.9±11). On the lesioned side there was a small increase in GABA relaese either before or after potassium stimulation which was in contrast with a very marked decrease on the contralateral side.

The results suggest that although in control rats the overflow of GABA is similar on both hemispheres, the dopaminergic denervation in the striatum induces a slight increase in the release of GABA on the ipsilateral side and a very marked decrease on the contralateral nucleus. These effects were emphasized after potassium chloride. It is suggested that GABA release in globus pallidus is subject to control maybe by dopamine crossed fibres.

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414.6 GABA "A" RECEPTORS MODULATE THE RELEASE OF GABA IN NIGRA COMPACTA AND GABA "B" IN RETICULATA. B. Florán*, I. Silva* and J. Aceves*: (SPON.: Jorge Hernández). Dept. of Physiology, Biophysics and Neurosciences. Centro de Investigación del IPN. 07000 México, D.F. México.

In the last meeting, we reported the probable presence of GABAergic type "A" autoreceptors in nigra compacta but not in reticulata. This was derived from the bicuculline sensitive inhibition by muscimol of GABA release in compacta but not in reticulata. Here we have explored the possible presence of type "B" receptors in both nigra compacta and reticulata.

The experiments were done in slices of nigra compacta or reticulata. The tissues were loaded with tritiated GABA in the presence of β-alanine (1 μM), and the perfusion solution contained nipecotic acid (10 μM). Release was evoked by high (15 mM) K, which was maintained elevated throughout the experiment.

Baclofen (100 μM) inhibited GABA release from nigra reticulata but had no effect on the release from compacta. Bicuculline (10 μM) also inhibited release from reticulata but stimulated release from compacta.

The results suggest the presence of type "B" receptors, whose activation modulates either directly (autoreceptors) or indirectly (heteroreceptors) the release of GABA in nigra reticulata. Bicuculline, which by itself enhances (probably by blocking activation of autoreceptors) the release in nigra compacta, apparently activates GABA "B" receptors present in nigra reticulata, that is, it behaves as a GABA agonist for these receptors. (Supported by CONACyT of Mexico).

414.7 THE EFFECT OF NIPECOTIC ACID ON THE UPTAKE AND RELEASE OF ^{3}H-GABA AND ^{3}H-β-ALANINE FROM RAT HIPPOCAMPAL SLICES. L.J.Brahce* (Spon: J. French), R.D.Schwarz and T.A.Pugsley Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI 48105

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS, is released by depolarization of GABAergic neurons and removed from the synapse by high affinity uptake systems present in both neurons and glial cells. Biochemical and autoradiographic evidence indicates that in rat brain slice preparations, ^{3}H-GABA uptake is predominantly neuronal, while uptake of ^{3}H-β-alanine is relatively selective for glial uptake of GABA. Recently, nipecotic acid has been shown to promote the spontaneous release of GABA as well as to inhibit uptake, via the same carrier mechanism. However, the relative contribution of neuronal and glial uptake systems to the increased release of GABA by this agent has not been established. The purpose of this study was to investigate the effects of nipecotic acid on uptake and release of GABA in both neurons and glia, using ^{3}H-GABA and ^{3}H-β-alanine.

In the uptake experiments, hippocampal slices were incubated at 37° C with or without test compound for 15 min.; either ^{3}H-GABA or ^{3}H-β-alanine was added and the slices incubated for 2 or 15 min., respectively. The incubation was stopped by rapid filtration of the samples, followed by a wash with cold 0.9% saline. In the release experiments, the hippocampal slices were incubated at 37° C with either ^{3}H-GABA or ^{3}H-β-alanine for 15 min., washed three times and then incubated for 15 min. in the presence or absence of test compound. Tissue and medium were then separated by rapid centrifugation. High affinity uptake of both ^{3}H-GABA and ^{3}H-β-alanine was Na^+- and energy-dependent; but the rate of uptake of ^{3}H-β-alanine was much slower. Chemical specificity was also different as shown by the selective inhibition of neuronal uptake by 2,4-diaminobutyric acid (DABA) and glial uptake by 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THPO). Nipecotic acid inhibited uptake in both assays in a stereospecific manner, the (-)-isomer having IC_{50} values of 5.2 and 32.0 M in the ^{3}H-GABA and ^{3}H-β-alanine assays, respectively, and the (+)- isomer having IC_{50} values of 52.0 and 50.0 M, respectively. The spontaneous release of ^{3}H-GABA was shown to be both Ca^{++}-independent and Na^+-dependent, suggestive of a carrier- mediated process. Nipecotic acid markedly increased spontaneous release at concentrations that affected uptake, with the (-)-isomer showing the greatest effect (67% increase at 100μM). While spontaneous release of ^{3}H-β-alanine was also Ca^{++}- independent and Na^+-dependent, the effect of (-)-nipecotic acid was much less pronounced (26% increase at 100μM). In conclusion, this study indicates that nipecotic acid inhibits uptake of GABA into both neurons and glia, while also increasing spontaneous release from both types of cells.

414.8 GABA AND GAD-LIKE IMMUNOREACTIVITY IN THE PRIMATE RETINA. E. Agardh, B. Ehinger, Dept. of Ophthalmology, University of Lund, Sweden and J.-Y. Wu, Dept. of Physiology, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA.

GABA immunoreactivity was studied and compared with GAD immunoreactivity in the retinae of baboon, cynomolgus monkey and man. The central and perpheral parts of the retinae were investigated separately in cynomolgus monkey and man. The same kind of structures were stained with both antisera. Cells with a position corresponding to amacrine cells were stained, as well as the inner plexiform layer and some cells in the ganglion cell layer. The outer plexiform layer and some cells with the position and configuration of horizontal cells, also appeared immunoreactive. In some regions, more precisely the central parts of the retina, staining was observed in bipolar-like cells, in baboon with both antisera, in man with GABA antiserum. It is possible that horizontal cells as well as centrally localized bipolar-like cells may play a previously unsuspected role in GABAergic transmission in the primate retina.

414.9 LOW GLUCOSE PROMOTES THE EVOKED RELEASE OF ASPARTATE AND GLUTAMATE AND INCREASES THE RATIO OF ASPARTATE TO GLUTAMATE RELEASED AND RETAINED IN SLICES OF HIPPOCAMPUS. J.C. Szerb and P.A.O'Regan*.Dept.of Physiol.and Biophys. Dalhousie Univ. Halifax, N.S. B3H 4H7, Canada.

Glutamate (GLUT) is a transmitter and a precursor of GABA in the CNS, in addition to its metabolic function. Glucose (GLUC) and glutamine (GLN) are the two main precursors of GLUT. Changing the availability of GLN in 5 mM GLUC affects the Ca^{2+}-dependent evoked release of only GLUT but not of GABA (J.Neurochem.44,1724-1731,1985). This study was undertaken to see the effect of reducing the availability of GLUC, the other precursor of GLUT, on the evoked release of transmitter amino acids.

Longitudinal 0.4 mm thick slices of hippocampus were superfused with solutions containing 5, 1 or 0.2 mM GLUC without or with 0.5 mM GLN added. Six 10 min samples were collected and during samples 3 and 4 either electrical field stimulation or 50 mM K^+ was applied. Released and retained amino acids were analyzed by HPLC.

In 5 mM GLUC about 10 times more GLUT than aspartate (ASP) was released by field stimulation. GLN increased the evoked release of both, but the relative release of the two did not change. In 0.2 mM GLUC the evoked release of both GLUT and ASP was greatly potentiated, but the enhancement of ASP release was much greater, so that the ratio of released ASP to GLUT changed from 0.1 to 0.5 both in the absence or presence of added GLN. The ratio of ASP to GLUT retained by the slices also increased five fold in 0.2 mM GLUC. ~90 % of the enhanced release of ASP and GLUT in the presence of 0.2 mM GLUC and 0.5 mM GLN was Ca^{2+}-dependent and TTX-sensitive. Electrical field-stimulation did not evoke the release of GABA. 1 mM GLUC had only threshold effects on ASP and GLUT release and content.

The effect of different concentrations of GLUC on high K^+-induced release of ASP and GLUT was similar to that seen with electrically induced release. However, the high-K^+ evoked release of GABA decreased in 0.2 mM GLUC and 0.5 mM GLN increased only the depressed release of GABA in 0.2 mM GLUC.

Results suggest that reduced supply of GLUC increases the Ca^{2+}-dependent release of excitatory transmitters, by a mechanism which may involve decreased uptake or increased release. In addition, low GLUC increases the proportion of ASP in glutamatergic terminals, probably by causing the accumulation in the Krebs cycle of oxaloacetate, the precursor of ASP. Thus, glutamatergic terminals release ASP and GLUT in different proportions, depending on their metabolic state. In contrast, GABA release can be affected by GLN or GLUC only when the availability of both is severely limited, because normally the activity of GAD will be rate limiting.

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414.10 CORRELATION BETWEEN VIMENTIN AND GABA IMMUNOREACTIVITIES AND ^{3}H-GABA UPTAKE IN INTACT RABBIT RETINA AND RETINAL CELL CULTURES. H.Ghazi* D.W.Beaton*and N.N.Osborne*(SPON:C.D.Bridges).Nuffield Lab. of Ophthalmology, University of Oxford, Oxford OX2 6AW, U.K.

Autoradiography shows that intact rabbit retina exposed to ^{3}H-GABA for 30 mins is associated predominantly with the Müller cells. These same cells also "stain" positively for vimentin using a monoclonal antibody. However, in retinal cultures there is a lack of correlation between cells taking up ^{3}H-GABA and staining positively for vimentin immunoreactivity (1,2,3). The vimentin-positive cells in new cultures (4-6 hours) have a similar appearance both in size and shape with cells taking up ^{3}H-GABA. The vimentin-positive cells in older cultures (2-13 days) are very distinctive, being much larger. The vimentin-positive cells in older cultures also lack processes and from their morphology are identified as Müller cells (1,2). In contrast, the GABA-accumulating cells are "neurone-like" in shape and can also be stained for endogenous GABA using a polyclonal antiserum. Processing the intact retina for the localisation of GABA is consistent with this impression because GABA-immunoreactivity is found exclusively in neurones, particularly the amacrine cells (2). However, GABA-immunoreactivity is never found to be associated with the Müller cells in the intact retina, even if the retina was previously incubated in exogenous GABA.

The present results show that there is a lack of correlation between uptake and localisation of GABA in the intact retina but not in retinal cultures. It also appears that vimentin can be used as a marker for localising Müller cells both in intact retina and in retinal cultures.

(1) Osborne, N.N., Morgan, B., Ghazi, H. and Beaton, D.W. (in preparation).

(2) Osborne, N.N. in: Eric K. Fernström Symposium on Systems in normal and degenerating retina (edts. B. Ehinger and E. Agardh) Elsevier Press (in press).

(3) Osborne, N.N., Patel, S., Beaton, D.W. and Neuhoff, V. (1986) Cell and Tissue Research. 243: 117-123.

414.11 LOCALIZATION OF L-GLUTAMIC ACID DECARBOXYLASE (GAD) AND GABA TRANSAMINASE (GABA-T) IMMUNOREACTIVITY IN THE RAT SUPERIOR CERVICAL GANLION. O.Häppölä[1],H.Päivärinta[1],S.Soinila[2],J.-Y.Wu[3] and P.Panula[1]. [1]Department of Anatomy and [2]Neurobiological Research Unit, University of Helsinki, Siltavuorenpenger 20 A, SF-00170 Helsinki, Finland, [3]Department of Physiology, Pennsylvania State University, Milton S. Hershey Medical Center, Hershey, PA 170033.

GABA has been proposed to be one of the main inhibitory neurotransmitters in the central nervous system. In addition to the CNS, GABA has recently been identified in different peripheral mammalian tissues. There is biochemical evidence that GABA and the GABA-synthesizing enzyme, L-glutamic acid decarboxylase (GAD), are present in the rat superior cervical ganglion (SCG). Pharmacological studies have indicated that GABA receptors are present in the sympathetic ganglion and GABA has been shown to modulate ganglionic neurotransmission. The aim of the present study was to examine the location of the GABA-synthesizing enzyme, L-glutamic acid decarboxylase and the GABA-inactivating enzyme, GABA transaminase (GABA-T), in the rat SCG by indirect immunofluorescence technique and by immunoelectron microscopy with specific antisera raised against the corresponding enzymes.

Adult Sprague-Dawley rats were perfusion-fixed with 4% paraformaldehyde, and indirect immunofluorescence method was applied on 10 µm cryostat sections. For electron microscopy, fixation with 2% paraformaldehyde and 0.1% glutaraldehyde was used, and PAP method was applied on 40 µm vibratome sections. The sections were treated with 1% OsO_4 and embedded in a mixture of Epon and Araldite.

In the SCG, a large number of GAD- and GABA-T-immunoreactive principal nerve (PN) cells were detected. In addition, several small GAD- and GABA-T-immunoreactive cells (10-20 µm in diameter) were observed. The small cells occurred as solitary cells or in clusters and resembled small intensely fluorescent (SIF) cells in size and morphology. The small GAD-immunoreactive cells showed also tyrosine hydroxylase immunoreactivity in consecutive sections. In immunoelectron microscopy, solitary GAD- and several GABA-T-immunoreactive principal neurons were detected exhibiting evenly distributed cytoplasmic stainig. GABA-T, but not GAD, immunoreactivity was also localized in the satellite cells of the SCG.

The present immunocytochemical results provide evidence that the rat SCG contains an intrinsic neuronal system showing histochemical markers for GABA synthesis and inactivation, but its functional role in the modulation of ganglionic neurotransmission remains to be established.

414.PO PROPERTIES OF MONOCLONAL ANTIBODIES TO CHICKEN BRAIN GLUTAMIC ACID DECARBOXYLASE (GAD). D.I. Gottlieb, Y.-C. Chang* & J.E. Schwob. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110

Four hybridoma lines which secrete monoclonal antibodies to GAD were produced by immunizing mice with partially purified GAD preparations from chick brain, fusing their spleens and selecting hybridomas in an assay based on the ability to immunoprecipitate GAD activity. Four stable clones (GAD 1-4) were selected and established as permanent cell lines. Radioimmune assays of tissue extracts show chicken and rat brain are rich in GAD-1 antigen. Concentrations in muscle, gut, kidney and liver are at least 25-fold lower (the limits of the assay). GAD-1 also binds to antigen in the mouse, rat, rabbit, cat and monkey brain but fails to bind to enzyme in the goldfish brain. Thus, there is at least one conserved epitope on the GAD of higher vertebrates. In the chick and rat retina, GAD-1 and 2 stained the inner plexiform layer heavily. Staining of the outer plexiform layer is either very light or absent. In the chick and rat cerebellum the inhibitory neurons shown by others to be GABAergic stain, whereas known non-GABAergic elements are unstained.

GAD-1 was immobilized on Affi-Gel 10 to form an immunoaffinity column. Most of the GAD activity in crude chicken brain extracts is removed by passage over the column. Basic pH buffers were used to elute the enzyme resulting in increases in enzyme-specific activity of approximately 50-fold. The immunoaffinity enriched enzyme has a K_m for glutamic acid of 1.3 mM and is inhibited by the known GAD inhibitor AOAA. SDS-PAGE analysis of the column eluate reveals selective enrichment of proteins of 62 and 64 kd Mr. These proteins comigrate with GAD activity on subsequent rechromatography on DEAE-HPLC. Two further lines of evidence indicate that the 62 and 64 kd bands are GAD. These bands are enriched by immunoaffinity chromatography on GAD-3 columns even though GAD-1 and 3 recognize different epitopes on the GAD molecule. Finally, the affinity-purified 62 and 64 kd bands are recognized on Western blots by the anti-GAD antiserum prepared by Oertel et al. (J. Neurosci. 6: 2689, 1981).
Supported by grants from NINCDS and the McDonnell Center for Studies of Higher Brain Function.

INTERHEMISPHERIC RELATIONS II

415.1 CHANGES IN ROTATIONAL BEHAVIOR INDUCED BY FOOD DEPRIVATION IN THE LONG-EVANS RAT J. Carlson* and S.D. Glick. (SPON: B. Svare) Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208

Recent work in this laboratory has focused upon changes in rotational behavior induced by various stressors. Previously we have reported that uncontrollable footshock stress altered the intensity and direction of d-amphetamine induced rotation in unlesioned Sprague-Dawley rats in a manner that was dependent upon the animal's sex and pre-existing rotational bias (Carlson and Glick, Soc. Neurosci. Abstr. 11:870, 1985). These sex and bias dependent effects were similar to those previously found for the rotational effects of cocaine (Glick et.al., Science, 221:775, 1983).

In the Long-Evans rat, cocaine induces bias-dependent rotational effects (left > right) that are not sex-dependent and these effects are similar to those previously observed for uncontrollable footshock stress in this strain. The present studies sought to extend the generality of these stress effects using food-deprivation as a stressor.

Two days of food-deprivation selectively enhanced d-amphetamine-induced rotational behavior in left-rotating rats while decreasing this behavior in right-rotating rats. One day of food deprivation produced opposite effects, enhancing rotation in right-biased rats and decreasing rotation in left-biased rats. The opposing effects of different amounts of deprivation were comparable to those found for uncontrollable and controllable footshock stress, respectively. These data are discussed with regard to the differential effects of stress in its various forms on left and right sides of the brain (Supported by DA03817-02).

415.2 CLASSICAL CONDITIONING OF AMPHETAMINE-INDUCED ROTATION IN UNLESIONED RATS. K.L.Drew* and S.D.Glick. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208

Intact rats circle in response to d-amphetamine (d-A) and apomorphine (apo), and in most rats, the direction of the circling behavior is consistent across repeated testings. Carey (1986) classically conditioned d-A-induced and apo-induced circling behavior in rats with unilateral 6OHDA lesions of the substantia nigra. The direction of the conditioned circling responses were the same as the direction of the drug-induced responses. Elias et al., (1983) classically conditioned d-A-induced circling behavior in unlesioned rats but did not characterize the direction of the conditioned circling response. Presently, we have classically conditioned d-A-induced circling behavior in two strains of unlesioned rats and the direction of the conditioned circling is the same as the drug-induced circling.

Two groups of female Spraque Dawley rats received 1.25 mg/kg of d-A once per day for 2 days. In the classically conditioned (CC) group the rotometer apparatus was paired with the peak effect of the drug while in the pseudoconditioned (PC) control group the rotometer apparatus was never paired with the drug. On day 3 both groups were injected with saline prior to placement in the rotometer apparatus. Following saline the CC group made significantly more net turns than the PC group ($t=7.203$, $p<0.001$) and the direction of the conditioned circling response (CR) was the same as the drug-induced response. Significant sensitization of the d-A-induced turning behavior was observed on the second d-A trial ($t=3.29$, $p<0.02$). The contribution of the CR to the sensitization has not been determined. In two additional independent experiments 0.1 mg/kg of haloperidol blocked the conditioned circling behavior. Future experiments are planned to characterize more fully the neurochemical nature of the CR.

Similar results were obtained with female Long Evans rats except that the CR and the sensitization of the d-A-induced response were significant following 4 but not 2 d-A trials. Presently we are attempting to classically condition apo-induced circling in Long Evans rats. Preliminary results suggest that apo-induced circling (10 mg/kg) is not as easily conditioned as d-A-induced circling although the magnitudes of the drug-induced responses are similar. The study of conditioned circling behavior should further our understanding of the neural basis and plasticity of persistent directional preferences; it should also provide a novel paradigm for investigating the neurobiologic basis of learning as well as for studying the role of conditioning in altered drug responses and relapse in drug addicted individuals. (Supported by NIDA grant DA 03817).

415.3 INTERACTION BETWEEN SPONTANEOUS LATERALIZATION AND THE BEHAVIORAL AND ENDOCRINE EFFECTS OF ASYMMETRIC NEOCORTICAL ABLATIONS IN RATS. G. LaHoste*, V. Castagné*, P. Mormède*, P. Neveu*, M. Le Moal (SPON: J.J. Bouyer). Laboratoire de Psychobiologie des Comportements Adaptatifs, INSERM U.259, Université Bordeaux II, Domaine de Carreire, Rue Camille Saint-Saëns, 33077 Bordeaux Cedex. France.

Recent studies suggest that lateralization of function may be a basic property of brain organization with widespread evolutionary significance. However, nonhuman laterality can be distinguished from that in humans by the relative lack of relatedness between various behavioral asymmetries. To address this issue, we have sought to identify functional asymmetries which may be predictive of other behaviors under different conditions. Adult male rats were categorized as left-turning (LT) or right-turning (RT) on the basis of their spontaneous directional bias when given d-amphetamine (1.2 mg/kg i.p.) and observed for rotational behavior in standard plastic bowls. The effects of asymmetric neocortical ablations were then assessed with regard to d-amphetamine-induced rotation and open-field activity. As such lesions might induce neuroendocrine changes which in turn culd mediate behavioral effects, we also measured plasma prolactin (PRL), ACTH, and corticosteroids (CS) at sacrifice. Shallow cortical knife-cuts (<1 mm) extending from lambda 12.0 mm rostral and from 1.0 to 5.0 mm lateral from midline, were made on the right (n=10), left (n=10), or both (n=8) sides; a control group received sham surgery (n=10). These groups contained approximately equal number of LT and RT rats. Direction of amphetamine-induced rotation was not changed by the ablations. However, LT rats were more sensitive than RT rats to the postoperative amphetamine ($p<.01$) regardless of surgical treatment. Bilateral lesions decreased total activity in both LT ($p<.06$) and RT rats ($p<.001$) but the number of turns in the dominant direction was reduced only in RT rats ($p<.04$). Open field activity was highly elevated in all rats bearing bilateral ablations ($p<.001$) whereas right-sided lesions produced hyperactivity only in RT rats ($p<.001$). PRL levels were elevated compared to shams in LT rats bearing left-sided or bilateral lesions ($p<.05$). ACTH and CS were correlated with open field activity and various measures of d-amphetamine-induced rotation ($p<.05$) but only in RT rats.

We conclude that the d-amphetamine-induced rotation test is indicative of a more general property of brain lateralization since behavioral and endocrine changes following asymmetric neocortical lesions are strongly dependent on the preoperative direction of rotation.

415.4 EVIDENCE OF CORTICAL LATERALIZATION IN A VISUAL REACTION TIME TASK IN RATS. G. Mittleman,* I.Q. Whishaw, and T.W. Robbins*(SPON:E.S. Valenstein).Univ. of Cambridge,Dept. of Exp. Psych.,Cambridge, U.K.

Although clear anatomical and neurochemical asymmetries have been described in animals, which may be analogous to those observed in humans, evidence of functional asymmetry in the cognitive aspects of behavior has been more elusive. We report here evidence of striking functional asymmetries in intact rats in a "neglect" paradigm that predicts the effect of hemidecortication on performance. Rats were trained to hold their heads in the central hole of a specially designed chamber until a brief (200 msec), eccentric visual stimulus was presented to one or the other side of the head in the holes adjacent to the central one. Following presentation of the eccentric stimulus, rats were required to report the presence of the light by moving their head toward it and interrupting the photocell beam within the appropriate hole. Measures of performance included discrimination accuracy and reaction time to remove the head from the central hole. Following training, it was found that a majority of the rats consistently exhibited a significant asymmetry in reaction time to the visual stimuli. This difference in reaction time between right and left stimuli could be exaggerated by preventing unilateral visual input with an eye patch, and was correlated with rotational direction following amphetamine (1.2 mg/kg i.p.). Based on these asymmetries, the cerebral hemisphere contralateral to the more rapidly detected stimulus was designated as "dominant" for this task; the ipsilateral hemisphere was designated as "non-dominant". All rats then received a hemidecortication of either the "dominant" or "nondominant" cortex by aspiration under visual guidance. The effects of removing the "dominant" or "nondominant" cortex were strikingly different. Following removal of the "dominant" cortex rats responded exculusively to the side ipsilateral to the lesion regardless of which side the stimulus was presented. This deficit appeared to be permanent as there was no recovery over 4 postoperative months. These rats were also incapable of using compensatory strategies to mediate the task. In contrast, rats that had the "non-dominant" cortex removed showed relatively minor biases to respond ipsilaterally and were essentially unimpaired in discrimination. The behavioural effect of a "dominant" hemidecortication could not be reproduced by preoperative patching of either eye to prevent unilateral visual input, suggesting that the deficit was not sensory in nature. Nor were these deficits simply due to a motor incapacity to respond to contralateral stimuli as all rats tended to make premature responses to both ipsilateral and contralateral response holes before the stimuli were presented. These results indicate that hemidecortication impairs higher order processes of sensory-motor integration but this impairment depends upon pre-existing differences in functional lateralization.

415.5 DEVELOPMENTAL TRENDS OF CORPUS CALLOSUM PARAMETERS IN THE RAT. A.S. Berrebi, R.H. Fitch*, D.L. Ralphe*, V.L. Friedrich Jr., and V.H. Denenberg.

Previous work has demonstrated that, in adult rats, the cross-sectional area of the corpus callosum is larger in males than in females. Additionally, handling stimulation in infancy interacts with sex to further increase the area of the male callosum relative to that of the female.

This study was designed to further investigate the ontogeny of these differences. Litters were randomly assigned to either the handling or control treatments at birth. Handling was administered for three minutes daily throughout the pre-weaning period of 20 days. Male and female littermates were anesthetized and perfused at 21, 42, 70, and 110 days of age. Their brains were sectioned sagittally for light microscopic examination. Morphometric analyses yielded measures of callosal area, width, perimeter, and length. In addition, each of these primary measures was adjusted for brain weight differences. A unitless measure of callosal "form" was obtained by dividing the square of the perimeter by the area.

Between 21 and 110 days of age the increase in callosal area was 74%, in width 52%, in perimeter 20%, and in length 17%. In comparison, brain weight increased 29% and body weight 500% over this interval. A Sex effect for callosal length was found with the male callosum being longer at all ages. Sex main effects and Sex X Age interactions were found for callosal area and perimeter: the growth curves diverged over time with the males having the larger values. The effects of handling did not become significant until 110 days of age. At that time there were Sex X Handling interactions involving callosal area, width, and form. In all instances handling increased male-female differences relative to those of nonhandled controls. Therefore, handling and sex appear to affect different callosal parameters.

415.6 INTERHEMISPHERIC TRANSFER OF VISUAL LEARNING IN PIGEONS - Effects of unilateral rotundal lesion - S.Watanabe. Department of Psychology,Keio University Mita 2-15-45,Minato-ku,Tokyo,Japan.

Previously we reported that unilateral rotundal lesion after monocular discrimination caused deficits ininterocular transfer of this learning whereas unilateral OPT lesion did not (Shimizu et al.,1985). However,unilateral rotundal lesion either in the trained hemisphere or untrained hemisphere caused deficits in transfer and we suggested that the former deficits indicated memory-access deficit and the latter sensory deficit. In the present experiment unilateral rotundal lesion was made before and after the monocular discrimination to examine this point. If the lesion in the untrained hemisphere results in deficits in both before and after conditions and the lesion in the trained hemisphere causes deficit only when the lesion made after the original learning, separation of memory-access deficit and sensory deficit will be supported.

Pigeons were divided into four groups. Lesion was made in the trained hemisphere (contralateral to the eye for the original training) for the first two groups and in the untrained hemisphere (contralateral to the eye for transfer training) for the other two groups. The first group received the lesion before monocular discrimination then tested interocular transfer. The second group received the lesion after the subjects learned the monocular discrimination then tested its transfer. The third and fourth groups received lesion before and after the monocular learning respectively, but the lesion was made in the untrained hemisphere. The discrimination task was a horizontal-vertical line discrimination in an operant chamber with a single key.

The first group required longer training sessions to accomplish the original learning but showed clear interocular transfer. On the other hand, the second group showed deficits in transfer. These results suggest 1) the pigeons can learn monocular discrimination with a hemisphere to the viewing eye, but 2) the pigeons use contralateral hemisphere for monocular discrimination when both hemispheres are available.

415.7 VISUAL RECEPTIVE FIELD PROPERTIES OF CORPUS CALLOSUM FIBRES IN NORMAL AND SPLIT CHIASM CATS. L. Richer*, F. Lepore, M. Ptito and J.-P. Guillemot. Dép. de Psychologie, Univ. du Québec and Univ. de Montréal, C.P. 6128, Succ. A, Montréal, Québec, H3C 3J7

The corpus callosum (CC), the principal neocortical commissure, is involved in the inter-hemispheric transfer of sensory information. Previous electrophysiological and anatomical experiments have shown that neurons belonging to the principal visual cortical areas (17, 18, 19, lateral suprasylvian area-LSA) send information to the other hemisphere through axons crossing in the caudal portion of the CC. In order to determine the properties of this information, the present study examined the visual receptive field (RF) properties of callosal axons.

Acute recordings were carried out in the conventional manner using curarized and N2O:O2 anesthetized normal and split-chiasm cats. The recording site in the splenium of the CC was visually identified using an operating microscope and the electrodes consisted of glass micropipettes having an impedence of 6-10 MOhm. Fibre activity was amplified and displayed in the conventional manner. In some cases PSTH were computed in order to evaluate more quantitatively the properties of these axons.

The results in normal cats indicated that most of the cells were binocular and that their RFs were on or near the vertical meridian, on both sides of the horizontal meridian. RF areas were in general fairly large, along the range of those found in LSA. These RFs were also classified according to Hubel and Wiesel hierarchical model and the three types of units were found, although most were of the complex type. A seven point ocular dominance distribution was also computed (the different classes refer to left eye and right eye dominance and not to the classical ipsilateral-contralateral eye dominance). The distribution was similar to that found in the visual corteces, as were the orientation and directional properties of the RFs. The recording in the CC of split-chiasm cats indicated that the number of binocular cells was drastically reduced and only a small proportion remained binocular. The other RF properties of these axons were however similar to those of the normal cats.

These results suggest that callosal neurons essentially transmit sensory information and that they are probably involved in various functions, such as interhemispheric transfer of lateralized information and mid-line fusion.

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415.8 REGIONAL VOLUMETRIC ASYMMETRIES IN NEW AND OLD WORLD MONKEYS. A. Brown*, D. J. Woodward & M-C de Lacoste (SPON: C. E. Ginn, Jr.). Dept. of Cell Bio. & Anat. UTHSCD, Dallas, TX 75235.

This study was undertaken to examine regional volumetric asymmetries in ceboids and cercopithecoids. In previous studies we have delineated left/right regional volumetric asymmetries in a group of Lemuridae species and in hominoids, including humans. Results have suggested that although there may be species-specificity within the primate order for patterns of asymmetry, overall integrated prosencephalic indices of asymmetry do not correlate with phylogenetic status, brain weight, encephalization or degree of telencephalic fissuration; although in humans, at least, they may vary with the gestational age of the developing fetus (deLacoste & Horvath, 1985).

Complete anterior to posterior series of coronal sections from cerebra (cut at 10-20μm) of eight ceboid or cercopithecoid species were photographed and digitized. Sectional and regional volumes as well as regional and integrated indices of asymmetry were computed using the laboratory CARP system, SPSSx, data transformation programs to interface between CARP and SPSSx and a set of algorithms developed for these purposes. Anatomical delimiters varied with the species but included the coronal sulcus, genu of the corpus callosum, superior branch of the precentral sulcus, inferior branch of the post-central gyrus, Sylvian fissure, intraparietal sulcus, parallel sulcus, parieto-occipital sulcus, calcarine complex and lunate sulcus.

Species of new world monkeys confirmed our previous finding of a lack of correlation between the "complexity" or size of a primate brain and its integrated index of asymmetry: the nearly lissencephalic brains of Saguinus oedipus manifested indices of asymmetry comparable to those found in either the prosimians or subhuman hominoids. Both the new and old world monkeys exhibited the most significant asymmetry in retrocalcarine cortex, a pattern which appears to be characteristic of primates (deLacoste et al, 1986). Prefrontal cortex featured as the second most highly asymmetrical region. The other prosencephalic regions examined manifested variable but consistently lower regional indices of asymmetry. Our preliminary analysis further suggests that in some species of new world monkeys there may be a reversal of the patterns of asymmetry, depending on the sex of the case, with males exhibiting a right hemisphere bias across regions and, conversely, females showing a left hemisphere bias. At this point, we do not know if these trends are simply a reflection of small sample sizes. Futhermore, we have yet to determine if this same pattern is manifest in old world monkeys. Should a sex-related reversal in patterns of asymmetry of regional volumetric asymmetries prove to be a common occurence in primate cerebra, we hope to relate our findings to existing ethological observations.

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415.9 HEMINEGLECT AND LOCALIZATION OF FUNCTION IN THE BRAIN. R.K. Deuel. Depts. of Ped. and Neurol., Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Young adult cynomologus monkeys were studied with a battery of behavioral tests for symmetry of responses to visual and somatosensory stimuli delivered in the unilateral and bilateral simultaneous modes. Each upper extremity was tested as well as for strength manual dexterity and ability to use tactile exploration to obtain bait. Testing was carried out 5 to 7 days before a unilateral cortical excision from either the frontal periarcuate or posterior parietal association cortex.

Perfect symmetry was represented by a contralateral over ipsilateral ratio on behavior tasks of 1.00. Preoperatively the median frontal ratio was 1.03 and the median parietal 1.02. Animals were retested 3 to 6 days postoperatively. The median frontal symmetry ratio was then .37 and the parietal .32 indicating a marked impairment contralateral to the small cortical excision. Quantitative ^{14}C2deoxyglucose autoradiography (2DG) was used to study brains of half the animals, that were killed within 7 days of the cortical excision. The other half were studied with serial behavior batteries until asymmetries were largely recovered 4 to 10 weeks after operation (median frontal index =.85, parietal =.91) and then studied with 2DG. Throughout the study animals were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Distributed foci of decreased isotope density was found in the acute postoperative state. Foci after the frontal lesion included thalamic nMD, mf-pc, nVA, nVL, the caudate, putamen, Globus Pallidus and deep layers of the Superior Colliculus. After the parietal lesion thalamic nLP, pulvinar, nVL, nVPL and lateral geniculate, the Globus Pallidus, caudate, the deep and superficial layers of the Superior Colliculus and the striate cortex were affected. In recovered monkeys these foci were improved.

Findings suggest: (1) Distant structures are dysfunctional after association cortex lesions, and their dysfunction plays a role in the generation of the observed symptoms of the cortical lesion, and (2) more than one network of structures may control laterally directed attention.

415.10 ASYMMETRIES IN NEGLECT WITH UNILATERAL LESIONS OF RIGHT vs. LEFT MEDIAL PRECENTRAL PREFRONTAL CORTEX IN RATS J.V. CORWIN*, S. Kanter*, F. Caputo* and R. L. Reep. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148 and Depts. of Neurosurg., Psychol. and Physio. Sci., Univ. of Fla., Gainesville, FL 32610

Lateralization of function is recognized as one of the major characteristics of human brain organization, but reports of anatomical and behavioral lateralization in non humans are a relatively new phenomenon. We report on a behavioral asymmetry in response to lesions of medial precentral prefrontal cortex that produced severe neglect in rats.

The subjects were 46 Long-Evans hooded rats: 23 received a unilateral lesion of the left medial precentral prefrontal cortex, and 23 received a unilateral lesion of the right medial precentral prefrontal cortex. Behavioral testing for neglect consisted of rating the degree of orientation to the presentation of visual, auditory, somatosensory and nociceptive stimuli in a serial fashion to the left and right sides of the body. The degree of orientation was rated by an experimenter who was "blind" with respect to the hemisphere damaged.

The results of behavioral testing indicated that a unilateral lesion of medial precentral prefrontal cortex produces severe neglect across all modalities tested regardless of the hemisphere damaged, however, the nature of the behavioral findings depended on the hemisphere damaged. The 23 subjects with a left medial precentral prefrontal cortex lesion all had severe neglect on the contralateral side of the body. The 23 subjects with a right hemisphere lesion demonstrated a different pattern. Nine subjects demonstrated contralateral neglect, 9 demonstrated ipsilateral neglect, and 5 demonstrated a unique pattern of initial severe contralateral neglect followed by severe ipsilateral neglect.

The histological results indicated that the lesions in the left and right hemisphere groups were identical. Further, there were no differences among the lesions in the three behavioral subgroups resulting from damage to the right hemisphere.

The present results further support the contention that the rodent brain is asymmetrically organized with respect to some behaviors, and extends the findings of hemispheric laterally to the medial precentral prefrontal cortex.

416.1 CONNECTIONS OF THE SUPERIOR COLLICULUS IN THE MUSTACHE BAT, PTERONOTUS PARNELLII. E. Covey, W. C. Hall and J. Kobler, Depts. Surgery (Otolaryngology) and Anatomy, Duke University Medical Center, Durham, N. C. 27710

The mustache bat, Pteronotus parnellii, depends almost exclusively on echolocation to navigate and capture flying prey. This adaptation is reflected in the large and highly differentiated auditory structures of the central nervous system. In contrast, the eyes and central visual pathways appear rudimentary. Nevertheless, the superior colliculus, a structure usually associated with orienting the eyes, is large and well developed in this species.

The afferent and efferent connections of the superior colliculus in Pteronotus were studied using retrograde and anterograde transport of wheat germ agglutinin conjugated to HRP. The projection from the retina to the superior colliculus is very small and occupies a strip only about 50 μm thick on the outer surface. The remainder of the superior colliculus consists of layers which, in their cytoarchitecture and connections, appear to be equivalent to the intermediate and deep layers of other mammals.

These layers receive extensive afferent projections from motor structures, especially the substantia nigra pars reticulata and the deep cerebellar nuclei. Afferent projections from auditory structures are quite prominent, especially as compared to the retinal input. These auditory projections originate in the central and pericentral regions of the inferior colliculus (IC), the anterolateral periolivary nucleus (ALPO) and the rostral part of the dorsal nucleus of the lateral lemniscus (DNLL). The projections from IC and ALPO are mainly ipsilateral, but those from DNLL are bilateral. The superior colliculus also recieves projections from cortex, including a very rostral region which may be homologous to frontal cortex in other mammals.

The main efferent projections are to the contralateral medial reticular formation and inferior olive via the predorsal bundle, and to the ipsilateral lateral midbrain tegmentum and lateral pontine nuclei. The ascending projections to the diencephalon are relatively sparse.

Taken together, these results suggest that the superior colliculus in the echolocating bat is primarily involved in orienting the ears and/or body in response to auditory stimuli. (Supported by Grants BNS 82-17357, BNS 81-09794, NIH NS-21748 and PHS EY-04060).

416.2 THE INFLUENCE OF PASSIVE EYE ROTATION ON CAT SUPERIOR COLLICULUS NEURONS J.S. Nelson*, M. A. Meredith and B.E. Stein, (SPON: J.A. Astruc) Dept. of Physiol.& Biophys. and Dept. Anat., Med. Col. Va., Richmond, VA 23298.

Extraocular proprioceptive inputs to superior colliculus (SC) cells have been previously demonstrated in chloralose-anesthetized cats. The present experiments were initiated to determine the pattern of extraocular propriceptive convergence on visual, auditory and somatosensory SC cells and to examine the interactions that might occur as a result of combination of proprioceptive and visual or auditory stimuli.

Cats were anesthetized with chloralose (n=7), ketamine hydrochloride (n=3) or sodium pentobarbital (n=2) and were paralyzed and respired with 75% N2O and 25% O2. The ipsilateral eye was rotated 1-15° at 50-1000°/sec by a scleral suture attached to a moving-coil vibrator and monitored by a search coil. A total of 230 cells were studied in these different anesthetic conditions.

Eye rotation elicited responses in the majority (35/52; 67%) of cells in chloralose anesthetized animals. As in previous reports, eye rotation responses consisted of a brief burst of impulses of variable reliability, latency, number and frequency. In some cells, either visual or auditory stimuli could depress responses to eye rotation and the reverse was also observed. Surprisingly, however, most (77%) of the cells studied required eye rotation amplitudes (>10°) and velocities (>400°/sec) that were near or above the feline oculomotor range. They were neither sensitive to suprathreshold changes in amplitude and velocity, nor did they have amplitude thresholds that could be related to their anterior-posterior SC locations, as would be expected on the basis of the stimulus-evoked eye movement map within the SC. Often the responses to eye rotation were indistinguishable from that evoked by brisk taps to any body surface or loud sounds, even in cells located in laminae (superficial) that rarely receive non-visual inputs. These eye rotation responses appeared to depend on the presence of chloralose. No eye rotation responses were evoked in cells (n=178) studied when other anesthetics were used and eye rotation combined with other sensory stimuli produced no interactions in these cells. These data replicate previous reports demonstrating that passive eye rotation can evoke responses in SC cells under chloralose anesthesia. However, no evidence could be obtained here to indicate: (A) that the activity of these cells can code the parameters of an eye movement or (B) that, under non-chloralose anesthetic conditions, responses can either be initiated by passive eye rotation or that eye rotation can significantly affect the processing of other sensory stimuli.

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416.3 A TOPOLOGICAL INVESTIGATION OF THE MULTISENSORY ORGANIZATION OF THE TORUS SEMICIRCULARIS OF THE RAINBOW TROUT. N.A.M. Schellart*, L.J.A. Nederstigt* and M. Kamermans* (SPON: European Neuroscience Association), Lab. of Medical Physics, Univ. of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam-Zuidoost, The Netherlands.

In the torus semicircularis, a homologue of the mammalian colliculus inferior, acousticolateral (Acl) as well as visual (V) processing occurs in V sensitive, Acl sensitive and Acl-V sensitive units. The site of recording of 76 Acl, 95 V and 40 Acl-V units, all encountered at or sometimes just below the torus semicircularis, has been examined statistically. Details on the calculation of the position of the electrode tip, the sensory stimulation etc. have been given elsewhere (Nederstigt and Schellart, Pflügers Arch, 406, 151-157).

The three named modality types can be found all over the torus. With regard to the depth of recording and the rostro-caudal position, preference has been found neither for the modality nor for the response signs (ON and OFF behaviour to 0.7 s tone bursts or flashes); neither a combination of these characteristics. The Acl and Acl-V units are distinguished in L units (only sensitive below 125 Hz; 11%), A units (sensitive above 125 Hz; 34% and B (broadband; 50%) units. Since recordings from the lateral line nerve never showed a peak sensitivity above 100 Hz and always exhibited a strong high frequency attenuation, the A units are supposed to be fed exclusively by the otolith systems. The B units were especially encountered in the middle and caudal part of the torus and the A units more rostrally. AV cells, showing a preference for the rostral part, are relatively more numerous than BV cells. Superficially, in the laminated part of the torus, especially the B units and ventrally the A and L units are found. Along the mediolateral axis no topological preference was observed for either monomodal or bimodal cells.

In the torus, clusters (maybe columns or irregular slabs more or less perpendicular to the torus surface), 0.04 - 0.12 mm in width can be found dominated by either V or Acl units.

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416.4 **INPUTS TO SYRINGEAL MUSCLES IN THE ZEBRA FINCH** David Vicario, The Rockefeller University, New York, NY 10021

Previous work has shown that auditory responses can be obtained from the motor nucleus (nXIIts) and motor nerve that supply the muscles of the syrinx, the bird vocal organ (Williams & Nottebohm, 1985). The present study examines this system to determine 1) How individual syringeal muscles are represented in nXIIts; and 2) How sound stimuli affect activity in those muscles and the motorneurons that control them.

In anaesthetized adult male zebra finches, EMG activity was recorded simultaneously in 4 syringeal muscles. Low-current microstimulation was applied at many sites in nXIIts in order to map effective zones for the activation of the individual muscles. EMG responses to sound stimuli, including the bird's own song, song syllables and pure tones, were also recorded. Auditory responses were also recorded from single-units and multi-unit clusters in nXIIts of awake zebra finches with chronic implants.

Microstimulation revealed that NXIIts is organized into partially overlapping but separable zones which control single muscles, arranged along the rostro-caudal axis of the nucleus. The ventral and dorsal muscles have the largest domains and are most clearly separated; they are located at the rostral and caudal ends of the nucleus, respectively. Short-latency effects were limited to ipsilateral muscles, and the map was very similar from bird to bird. The mapping of nXIIts provides a foundation for further work on the detailed organization of the song control pathway, using physiological and anatomical techniques.

In anaesthetized birds, EMG responses to both natural and artificial sound stimuli were observed in the muscle groups studied. These responses typically consisted of phasic "on" responses with a latency of 50-100 ms, depending on the stimulus. While individual muscles sometimes showed larger responses to certain song syllables, in general, the muscles responded phasically to the onset of all the sounds presented.

Auditory responses were also present in the awake bird, but natural song and its constituent syllables were more effective than pure tones or white noise. Many neurons studied showed variable or labile responses, often influenced by their rhythmic activation during the expiratory phase of respiration. However, some individual neurons responded selectively to particular syllables in particular sound contexts; units simultaneously recorded at one site had similar response patterns. The possible role of these auditory responses in sound processing or song production is at present unknown. The overall spatial distribution of neurons with responses to stimuli with specific acoustic features, and the projection of those neurons to the different target muscles are currently being examined. (Supported by a Winston Fellowship)

416.5 CENTRAL GENERATION OF NATURAL ACTION SERIAL ORDER. K.C. Berridge and J.C. Fentress. Dept of Psychology, University of Michigan, Ann Arbor, MI 48104 and Dalhousie University, Halifax, Nova Scotia.

The behavioral stream of facial grooming or of taste-elicited ingestive/aversive consummatory actions of rats can be viewed as a long series of individual movements linked together to form functional sequences. In order to ascertain the sequencing rules that determine how these actions are linked together, many thousands of spontaneous grooming and elicited ingestive/aversive actions were videotaped and scored with a microcomputer. Information analyses of sequential stereotypy, tabulation of the sequential transitions between single actions and between action groups, and visual inspection for linear action chains, were employed to expose underlying rules of behavioral sequencing. Information analysis showed that Markov and higher-order sequential dependencies characterized both grooming and ingestive/aversive sequences. Transition analysis revealed three global rules: action perseveration, paired alternation, and alternating perseveration (alternation of bouts). Together these rules account for approximately 75% of all sequential transitions during grooming and taste-elicited ingestion/aversion. In addition, a specific fourth rule of linear chaining was found to apply only to facial grooming.

Peripheral deafferentation was used to ascertain the role of somatosensory cues and feedback in the generation of these serial patterns. Bilateral deafferentation of the mandibular and maxillary branches of the trigeminal nerve eliminated tactile sensation from the rostral face and mouth while preserving motor function. Neither the overall degree of sequential stereotypy (H) of grooming or ingestive sequences nor the generation of any of the natural sequencing rules were affected by trigeminal deafferentation. These natural rules thus appear to be specified by the brain without need of somatosensory feedback.

Previous observations have led us to suggest that somatosensory processing may be suppressed during the performance of grooming linear chain sequences. To test the hypothesis that somatosensory suppression is important to the successful completion of grooming linear chains, Kainic acid lesions of the corpus striatum were made (a structure reported to be involved in the modulation of trigeminal sensory responsiveness). Unlike deafferentation, striatal lesions specifically disrupted the performance of grooming linear chains. These data suggest the striatum may facilitate the central patterning of action serial order.

416.6 **MECHANICALLY EVOKED RESPONSES OF PERIORAL MUSCLES DURING FINE FORCE CONTROL.** S. M. Barlow and R. Netsell. Speech Physiology Laboratory, Boys Town National Institute, Omaha, NE 68131.

Recent evidence suggests that fine motor skills, such as manipulation and orofacial motor control, are more dependent on peripheral mechanosensory inputs than gross motor behaviors. A number of cutaneous mechanoreceptors located within skin and muscle exhibit their greatest sensitivity to small amplitude input signals. Furthermore, the linkage between these afferent systems and their target efferents of segmental and suprasegmental systems is greatest during the modulation of small forces.

The purpose of the present investigation was to characterize the mechanosensitivity of perioral muscle subgroups during fine force control in normal adults. A linear motor was used to deliver taps, with displacements ranging from 0.5 to 2.5 millimeters, to select areas of skin surrounding the mouth. Single ahd multiple motor unit activity was recorded from six sites in the lower face;Including medial and lateral placements within orbicularis oris inferior (OOI_m, OOI_l), orbicularis oris superior (OOS), and mentalis (MT). Subjects were required to generate fine levels of force in different vector orientations using select perioral muscles in the presence of a mechanical stimulus. Subjects monitored their ongoing force output in reference to a computer generated target signal simultaneously displayed on a digital oscilloscope. The magnitude of upper and lower lip bias forces ranged from 0.05 to 0.75 Newtons (N). Preliminary results suggest a high degree of response specificity among select muscles of the lower face. Individual components of the perioral response, with latencies ranging from 14 to 20 milliseconds, were found to be highly dependent upon the active, subject-generated, forces in select perioral structures. Further, evidence for muscle interdependence was found between OOI_l and MT, in that, systematic tradeoffs were observed in motor unit excitability between these two muscle groups within the range of force examined. The differential excitability of muscle subgroups in the lower face to mechanosensitive inputs reveals a level of fractionation and sculpturing of the motoneuron pool beyond current neuroanatomical specification.

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416.7 NEURONAL RELAXATION OSCILLATIONS IN THE HUMAN BRAIN: EVIDENCE FROM INTRA-HEMISPHERIC CHOICE REACTION TIME EXPERIMENTS. E. Pöppel and Th. Steinbach*, Inst. für Med. Psychol., Goethestr. 31, and Inst. für Rechtsmedizin, Frauenlobstr. 7a, 8 München 2, FRG

Histograms of choice reaction time are often multimodal (Pöppel, E., Naturwiss. 55: 449, 1968; Psychol. Forsch. 34: 1, 1970). The temporal interval separating the two, three or sometimes even four modes in such histograms is usually 30 to 40 ms. Multimodalities with similar intervals between modes have also been observed in latency distributions of stimulus-triggered eye movements like saccades (Frost, D., Pöppel, E., Biol. Cybern. 23: 39, 1976) and pursuit eye movements (Pöppel, E., Logothetis, N. Naturwiss. 73: in press, 1986). Why are multimodalities not reported more often? This is probably due a violation of stationarity. In case of instationarity, the variance of responses necessarily increases and thus, an intrinsic multimodal response characteristic might be masked. Using an intrahemispheric intermodal stimulus condition the question of multimodality in reaction time experiments was tested again. Visual or auditory stimuli were presented in random order, either to the left or to the right hemisphere, and the subject had to respond with a GO-response as fast as possible. In order to prevent instationarity due to learning, the subject was trained extensively. A plateau for mean reaction time was reached after several thousand single reactions. The intertrial interval varied randomly between 2 and 5 s. In order to prevent instationarity due to different stimulus probabilities within the intertrial intervals, the statistical probability of stimulus occurrence decayed exponentially; thus, the subjective probability of stimulus occurrence remained constant for successive bins within the intertrial interval. Furthermore, to prevent instationarities due to physiological changes dependent on circadian variations, all experimental sessions were scheduled for the same time of the day. With these conditions statistically significant multimodal histograms for visual or auditory choice reaction time were obtained quite often provided a large number of single trials was collected. Alcohol appears to affect the modal characteristics of the histograms. The multimodalities of histograms can be explained on the basis of neuronal relaxation oscillations. Every stimulus triggers after a constant transduction time a neuronal oscillation with a period of 30 to 40 ms. Responses to sensory stimuli are phase-locked to these oscillations. Such a neuronal oscillation may provide the temporal framework for a discrete sampling of stimuli. If sensory information would be processed continuously such multimodalities in histograms would not be possible. The same is true if one would assume a pendulum-like neuronal oscillation which would run continuously. A neuronal relaxation oscillation appears to define the formal structure in which information from different sense-modalities can be related to each other in an economical way. (Supported by DFG PO 121/8).

417.1 HRP FIBER LABELING IN THE BASAL GANGLIA: A POST MORTEM STUDY. S.N. Haber[1] and T. Eskin.[2] Dept. of Neurobiol. and Anat.[1] and Dept. of Pathology[2], Univ. of Rochester Sch. of Med., Rochester, NY 14642.

One of the primary goals of tracing pathways in the central nervous system of animals is to gain some understanding of the neuronal pathways in the human brain. Anterograde and retrograde methods generally depend upon active transport of molecules in the axon, and thus injection of tracer molecules are made in the living animal. However, axonal uptake of HRP has been reported during degeneration of axons (Olson and Malmgren, 1980, Neurosci. Lett. 20:135) and in vitro experiments in fish (Ekstron, 1985, Neurosci. Methods 15:21). We present here evidence of the ability to use HRP injections in post mortem brain material to study short fiber pathways.

Experiments used both human and monkey material. Brains were removed at autopsy and coronal blocks (1 cm thick) were cut through the basal ganglia. Pressure injections of WGA-HRP were made through a glass pipette into the internal segment of the globus pallidus or into the ventral pallidum. The blocks were incubated for 2, 24, or 48 hours in culture medium at 37°C. The blocks were then fixed in 4 paraformaldehyde for 2-4 days. Tissue was then sectioned serially at 50 and processed with the TMB (Mesulam, J. Histochem. Cytochem. 26:10 1978) proceedure or the DAB method.

The results showed fibers streaming out from the injection sites in the pallidal structures and forming bundles as they traveled medially towards the internal capsule and the striatum. These fiber bundles could be traced for at least 8 mm. Labeled cell bodies surrounded the injection site although none were detectable within the core of the injection site. Cells relatively close to the injection site were completely labeled giving the impression of a Golgi-impregnated cell. Cells at some distance from the injection site appeared less heavily labeled. These results show that axonal uptake of HRP occurs in post mortem material and that the material fills axons for a distance of at least 8 mm. Furthermore there were labeled neurons at least 4-5 mm from the injection site. Thus post mortem injections of WGA-HRP could be a useful method for studying short projections in human post mortem material.

417.2 NEWLY ESTABLISHED ENTOPEDUNCULO-STRIATAL PROJECTIONS EMERGE FROM ENTOPEDUNCULO-HABENULAR PROJECTION NEURONS. M. Takada* and T. Hattori. Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, Canada M5S 1A8.

Direct projections from the entopeduncular nucleus (Ep) to the striatum were examined in the rat using retrograde tracing techniques. After injecting WGA-HRP into the caudoputamen (CPU), labeled fibers could be traced medially to go through and terminate in both the globus pallidus (GP) and Ep ipsilateral to the injection. Surprisingly, HRP-positive neuronal perikarya were observed in the Ep as well as in the GP. These labeled Ep neurons were medium in size and restricted to the rostral 1/3 of the nucleus, in which they were distributed as overlapped with the terminal fields from the CPU.

Based on the data that the rostral 2/3 of the Ep (limbic portion of the Ep) projects to the lateral habenular nucleus (lHb) (1), the second experiment was designed to determine whether the Ep cells projecting to the CPU have axonal branching to the lHb. Following true blue (TB) injection into the CPU and diamidino yellow (DY) injection into the lHb, TB-positive neurons in the rostral 1/3 of the Ep never failed to be double labeled with DY. As might be expected, these TB-labeled Ep neurons have never been double labeled with DY injected into the centre median-parafascicular complex or ventroanterior-ventrolateral complex of the thalamus and nucleus tegmenti pedunculopontis pars compacta. In fact, these latter nuclei have been established to receive inputs only from the caudal 1/3 of the Ep (motor portion of the Ep) (1).

The present study clearly indicate that single Ep neurons of the rostral 1/3 of the nucleus innervate both the CPU and lHb, thus playing a critical role in integrating the motor and limbic functions in the basal ganglia, together with the GP (2) and substantia nigra pars compacta (3).

(Supported by the Medical Research Council of Canada)

References

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417.3 CORTICAL AND THALAMIC EXTRASTRIATE VISUAL AFFERENTS TO CAT STRIATUM: PUTATIVE VISUOMOTOR CIRCUITS. B.V. Updyke, Dept. Anat., LSU Med. Ctr., New Orleans, LA 70112.

Visual afferents to cat striatum were studied with injections of ^{3}H-proline in physiologically identified cortical visual areas, and with WGA-HRP injections of caudate and superior colliculus.

Cortical visual afferents to caudate terminate in a zone which adjoins the primary motor-recipient zone rostrally and shifts to the dorsomedial edge of caudate posteriorly. Within this zone, the upper field quadrant is represented dorsally, the lower field quadrant ventrally, and central gaze laterally. Cortical visual afferents also terminate in a small zone at the posterolateral margin of putamen; topographic organization of these projections resembles those to caudate. The patchy nature of both projections suggest that they may interdigitate with other afferents to the same striatal zone. Sparse contralateral projections are also evident.

Injections of WGA-HRP into the caudate visual-recipient zone result in dense retrograde labeling in visual areas ALLS, PLLS, DLS, VLS and PS, moderate labeling in areas 20a, 20b, 21a and 21b, and very sparse labeling within areas AMLS and PMLS. Dense retrograde labeling also occurs in the ectosylvian visual area, splenial visual area, area 7, cingulate gyrus, anterior ectosylvian gyrus, and the cat's presylvian and ventral cruciate eye fields (Guitton & Mandl, Brain Res., '78; Schlag & Schlag-Rey, Brain Res., '70).

The same WGA-HRP injections of caudate result in dense retrograde labeling within lateral posterior complex zones LPi, LPm, LPl-r and LPs-v (Updyke, JCN, '83) and suprageniculate nucleus. WGA-HRP injections in superior colliculus establish that each of these thalamic subdivisions also receive ascending projections from superficial or intermediate tectal layers.

Thus, thalamic zones which receive tecto-thalamic projections, and the extrastriate cortical areas with which they interconnect (Graybiel, Brain Behav. Evol., '72; Raczkowski and Rosenquist, J. Neurosci., '83; Symonds et al., Neurosci., '81), provide visual inputs which converge along with eye field inputs upon a common zone within striatum. In this context, strio-nigral (Royce & Laine, JCN, '84; Szabo, Exp. Neurol., '70) and nigro-tectal (Graybiel, Brain Res., '78; Jayaraman et al., Brain Res., '77) projections may be considered links in more extensive control loops. Collectively, these pathways appear to form a basic substrate for visuo-motor integration.

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417.4 EFFERENT CONNECTIONS OF THE NUCLEUS ACCUMBENS AND THE STRIATUM IN THE LIZARD GEKKO GECKO. F.T. Russchen, A.J. Jonker* and W.J.A.J. Smeets*, Dept. of Anatomy, Vrije Universiteit, Amsterdam, The Netherlands.

Immunohistochemical techniques were used to identify in the gekko possible homologues of structures which in mammals belong to the basal ganglia. Thus, the nucleus accumbens (Acc), striatum (Str), ventral pallidum (VP), globus pallidus (GP), entopeduncular nucleus (Ent), ventral tegmental area (VTA) and the pars reticulata of the substantia nigra (SNr) were identified on the basis of specific substance P- and/or enkephalin-like immunoreactive staining patterns. The VTA and the pars compacta of SN (SNc) were furthermore characterized by the presence of dopamine immunoreactive neurons. Injections of the tracers PHA-L and HRP were placed in various portions of Acc and Str in order to fill the striatofugal pathways. The major efferent projections from both Acc and Str are topographically organized. From Acc fibers distribute to VP, and from there course ventrally in the medial forebrain bundle. Caudally in the hypothalamus this bundle forms a superficially located sheet which continues into VTA. Varicosities are present throughout the entire rostrocaudal extent of this pathway. Additional projections were found to the preoptic area and to medial portions of the hypothalamus. The caudal portion of Acc also projects to the intercollicular nucleus. Projections from the striatum are directed to GP. From here, fibers run caudalward in the lateral forebrain bundle (lfb) where they distribute varicosities to Ent. Terminal fibers are also distributed to the region of the suprapeduncular nucleus. Further caudally SNr is densely innervated. This terminal field continues caudally and dorsomedially into the intercollicular nucleus. Only the most caudal part of the striatum projects to the laminar nucleus of the torus semicircularis and the adjacent periventricular stratum griseum, as well as to the caudal mesencephalic and the rhombencephalic tegmentum. With the exception of the globus pallidus, which has a dense enkephalin immunoreactive plexus, and the suprapeduncular nucleus, all the presently identified pathways and terminal fields coincide with areas of dense substance P immunoreactivity. This makes this peptide, or a related tachykinin, a likely transmitter candidate for these projections. Thus, the organization of the outputs of Acc and Str in the gekko shows major similarities with that in mammals. Further investigations are necessary to determine whether homologies exist between the suprapeduncular nucleus and the intercollicular nucleus of the gekko and the mammalian subthalamic and pedunculopontine nuclei, respectively.

417.5 MORPHOLOGY OF EFFERENTS FROM DORSAL VENTRICULAR RIDGE TO STRIATUM IN TURTLES. J. Nautiyal* and P. S. Ulinski. Dept. Anatomy, Univ. Chicago, Chicago, IL 60637

The anterior dorsal ventricular ridge (ADVR) is a subcortical telencephalic structure that receives sensory projections from the thalamus in reptiles and birds. In turtles, ADVR contains a visual region called dorsal area that receives visual information via the optic tectum and nucleus rotundus and has a subsequent projection to the striatum. Since the striatum can influence the tectum through its projections to the substantia nigra and prectectum (Reiner et al., 1981), understanding the organization of the projection from dorsal area to the striatum is an important step in determining the sequence of events by which visual information to the telencephalon modulates tectal activity during visual orienting behaviors.

We studied the organization of the projection from the dorsal area of ADVR to the striatum by placing small, iontophoretic injections of horseradish peroxidase in the dorsal area of red-eared turtles (Pseudemys scripta elegans). These injections produced an orthograde, Golgi-like filling of axons running from dorsal area to the striatum. Labeled axons issue from the injection site in concentric arcs, running ventrolaterally in approximately the transverse plane. They course through the dorsal, small-celled region of the striatum, continue ventromedially into the large-celled region of the striatum and ultimately reach the junction of the striatum with the diencephalon. Individual fibers are thick, about 2 μm in diameter, and smooth. They occasionally bear branches that leave the parent axon at oblique angles. These branches are thin, less than 1 μm in diameter, and bear fusiform varicosities (about μm 1 x 1.5 μm) at intervals of about 1-2 μm.

The projection from ADVR to the striatum is topographic in the sense that individual, restricted domains in dorsal area project to curvilinear trajectories within the striatum in an orderly manner. However, there is no indication that the thick parent axons are presynaptic to striatal neurons, so that information from a particular region of dorsal area is carried in a divergent manner to a relatively large region of the striatum by the thin, varicose collaterals. A given striatal neurons is, therefore, likely to receive convergent projections from several neurons located in different regions of the dorsal area of ADVR. (Supported by funds from the Brain Research Foundation of Chicago)

417.6 TWO-NEURON, TRANS-PALLIDAL CONDUCTION LINE FROM THE SUPERFICIAL LAYERS OF THE OLFACTORY TUBERCLE TO THE MEDIODORSAL NUCLEUS OF THE RAT THALAMUS. D.S. Zahm and L. Heimer. Dept. of Anatomy, St. Louis Univ. Sch. of Med., St. Louis, MO 63109 and Depts. of Otolaryngology - Head and Neck Surgery and Neurosurgery, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

On the basis of cytologic criteria and the immunocytochemical character of its synaptic input, a group of medium-sized cells in the polymorph layer of the olfactory tubercle (Tu) of the rat has been considered as a potential ventralmost counterpart of the thalamopetal projections of the globus pallidus and entopeduncular nucleus. In our previous studies these ventral neurons were examined at EM resolutions following retrograde transport of HRP from the mediodorsal nucleus of the thalamus (MD) and incubation in glutamate decarboxylase (GAD) antiserum with and without colchicine pretreatment. The results confirmed the thalamopetal nature of the "pallidal-like" cells in the Tu and demonstrated that some thalamopetal cells exhibited perikaryal GAD immunoreactivity.

Although it has been shown that pallidal-like neurons in the Tu receive a major input from the superficial layers of the Tu, it has not been rigorously confirmed that thalamopetal cells receive this projection. This important question has now been addressed by demonstrating degenerating boutons contacting HRP containing neurons following laminar heat lesions of the superficial layers of the olfactory tubercle and injections of HRP in the MD. Retrogradely labeled neurons were medium to large and, very frequently, almost entirely covered with boutons which made primarily symmetrical synapses. Large, straight dendrites which extended from the labeled neurons likewise were nearly covered by bouton "sleeves". Twenty-four hours after a superficial Tu lesion, the number of electron opaque, degenerating boutons was greatly increased in the ipsilateral polymorph layer of the Tu compared to the unlesioned contralateral side. The degenerating boutons almost exclusively contacted dendrites or perikarya of cells with "pallidal" character and input. Many of the neurons contacted by degenerating boutons were retrogradely labeled after injection of HRP into the MD. The results of these studies have confirmed that a two-neuron, trans-pallidal conduction line extends from the superficial laminae of the Tu to the MD. This finding supplements the growing body of evidence that the Tu is an integral component of the basal ganglia.

417.7 COLLATERALIZATION OF PALLIDAL EFFERENTS AS REVEALED BY FLUORESCENT TRACERS L.C. Schmued*, J.F. Fallon (SPON: S. Loughlin). Dept. of Anatomy, University of California at Irvine, Irvine, CA, 92717

The purpose of this study was to determine collateralization patterns of pallidal efferents in the albino rat. The pallidal regions examined included globus pallidus, ventral pallidum and entopeduncular nucleus. The first portion of this study involved determining the efferent termination sites of these pallidal regions. This was accomplished by injecting either PHA-L or WGA-HRP into a pallidal region, and subsequently processing the tissue for either immunofluorescence or HRP histochemistry. The efferent targets of the pallidum were then mapped. These efferent termination sites were found to include prefrontal cortex, caudate-putamen, ventrolateral thalamus, habenula, subthalamic nuclei, substantia nigra, and pedunculo pontine region.

The next phase of the study involved injecting the aforementioned pallidal efferent sites with a combination of different fluorescent retrogradely transported tracers. The most frequently employed combination of tracers was Fluoro-Gold, Propidium Iodide, and either Bisbenzamide or Nuclear Yellow. Thus, the presence of more than one of these tracers within the cell body of pallidal neurons indicates that these neurons send collaterals to the regions receiving the injections of the tracers.

Using this paradigm, we saw a number of examples of collateralized pallidal efferents. Collateralization of pallidal neurons to the ventrolateral thalamus and either the substantia nigra or the pedunculopontine nucleus was observed. In addition, collateral projections to both the substantia nigra, and the pedunculopontine nucleus was also present. Further studies employing three tracers indicated that a number of the neurons in each pallidal region project to the substantia nigra and pedunculopontine region. Some ventral pallidal and globus pallidus neurons also send a third collateral to the thalamus. With regards to ascending projections, a few neurons demonstrated branched projections to the caudate-putamen and pre-frontal cortex. Additionally, some large cells of the medial globus pallidus and ventral pallidum showed evidence of collateral projections to the prefrontal cortex and to the substantia nigra/VTA region.

These results support the findings of previous studies indicating that basal ganglia outputs from pallidal neurons reach many cortical, striatal, diencephalic and mesencephalic sites. Furthermore, individual neurons of the dorsal, ventral and internal pallidum may simultaneously affect extrapyradmidal motor strutures at multiple forebrain, midbrain, and pontine levels. Supported by NIH grant NS 15321 and NIMH predoctoral grant MH 09121-01.

417.8 CORTICONIGRAL PROJECTIONS FROM AREA 6 IN THE RACCOON. S. T. Sakai and D. Tanaka, Jr., Dept. of Anatomy, Michigan State Univ., East Lansing, MI 48824.

Although it is known that cortical area 6 projects strongly to a number of basal ganglia-related nuclei, the presence of a projection from area 6 to the substantia nigra (SN) is not well documented. Autoradiographic studies have shown that area 6 projects to SN in the monkey. Corticonigral projections have also been reported in the rat, but it is not clear if these projections emanate from a comparable cortical area. Findings regarding a corticonigral projection in the cat are equivocal and there are no studies that have demonstrated this system in any other species of carnivore. As part of our series of studies on area 6 projections in carnivores, we chose to investigate the extent and distribution of corticonigral projections arising from area 6 in the raccoon.

In the raccoon, the SN is composed of at least 3 cytoarchitectonic subdivisions: pars reticulata (SNr), pars compacta (SNc), and pars lateralis (SNl). The SNc is a well defined cell-rich area composed of densely packed cells containing darkly stained Nissl substance. It is located within the caudal 3/4 of the nucleus. SNr is a cell-poor zone bounded ventrally by the cerebral peduncle and extending throughout the length of SN. SNl is the smallest division of SN and forms a small wing-like extension lateral to SNr. It contains cells with a low to moderate packing density.

Following injections of tritiated leucine and proline into area 6, uniformly distributed silver grains of moderate density were observed overlying most of the mediolateral extent of the ipsilateral SNc. This label extended throughout the rostrocaudal extent of the subnucleus. Silver grains of low density were also observed overlying SNl while in SNr, silver grains indicative of labeled fibers were detected overlying the medial 1/3 of the subnucleus. In addition, the retrorubral area and the ventral tegmental area of Tsai contained silver grains. No label was noted overlying the contralateral SN.

The present findings confirm the existence of direct corticonigral projections from area 6. These data are interesting since area 6 has been implicated in the initiation of movement and as such may exert an unique influence on the SNc, a region intrinsically tied to other basal ganglia structures. (Supported by NIH NS 18551, NS 16991, and BRSG funds awarded to the College of Osteopathic Medicine).

417.9 PATTERNS OF SYNAPTIC CONTACTS UPON INTRACELLULARLY LABELED NIGROSTRIATAL NEURONS IN THE RAT. I. Grofova, H. Kita and S.T. Kitai. Depts. of Anatomy, Michigan State Univ., East Lansing, MI 48824 and Univ. of Tennessee, Memphis, TN 38163

Previous ultrastructural studies have noticed remarkable differences in the density of nerve terminals in the pars compacta (SNC) and pars reticulata (SNR) of the substantia nigra. While the robust dendrites in the SNR are richly invested by terminal boutons, synaptic contacts on dendrites in the SNC are infrequent. It is therefore assumed that the majority of synaptic afferents to the dopaminergic nigrostriatal SNC cells impinge on the ventral dendrites of these cells which penetrate deep into the SNR. Although the nigrostriatal cells are concentrated in the SNC, they can also be found within the SNR. We have previously reported that the dendrites of nigrostriatal SNR neurons establish a much more extensive dendritic field in the SNR than the nigrostriatal SNC neurons and may thus receive richer and possibly different synaptic input. To test this hypothesis we have performed quantitative morphometric analyses of the distribution of synaptic boutons on two SNR cells and two SNC cells which were physiologically and/or morphologically identified as nigrostriatal presumably dopaminergic neurons and intracellularly labeled with HRP.

Samples were selected from 50 um thick plastic-embedded sections which were further cut in 2-3 um thick serial sections and inspected in the light microscope. Each labeled profile was identified and indicated on drawings of reconstructed cells. Suitable semithin sections containing the soma, proximal dendrites (dendrites up to 100 um distant from the soma), distal dendrites (terminal dendrites in the SNR and SNC) and intermediate portions of an over 1000 um long sagittally oriented dendrite of one of the nigrostriatal SNR cells were remounted on a block face and thin-sectioned. The length of the soma-dendritic membrane apposed by synaptic terminals, dendrites and other non-synaptic profiles was measured from electron micrographs using the Bioquant Image Analysis System. Synaptic profiles related to the labeled cells were classified into 3 distinct types using previously established criteria. In both SNC and SNR nigrostriatal cells, the highest synaptic density was encountered on proximal dendrites and distal portions of ventral dendrites and the next highest on the somata. Although the synaptic density was somewhat higher on the somata and proximal dendrites of SNR cells than on the SNC cells, it sharply declined on long sagittal dendrites contained in the SNR and even more so on distal portions of dorsal dendrites contained in the SNC. Both SNC and SNR cells showed similar distribution patterns of the three bouton types.

The observations suggest that the extensive dendrites of SNR nigrostriatal cells are of minor importance for the reception of synaptic afferents and may be involved in other functions.

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417.10 INTRACELLULAR RECORDING AND HRP STAINING OF RAT NIGRAL NEURONS. J.M. Tepper, S.F. Sawyer*, S.J. Young*, and P.M. Groves. Dept. Psychiatry M-003, Univ. Calif., San Diego, La Jolla CA 92093.

Despite the abundance of data concerning the neuropharmacology and neurophysiology of substantia nigra dopaminergic (DA) neurons, relatively little is known about the role of afferent input in modulating the activity of these cells.

Intracellular recordings and HRP injections were obtained in urethane-anesthetized rats from nigral neurons, identified as DA or non-DA on the basis of established electrophysiological criteria. Most DA neurons responded to stimulation of the ipsilateral dorsolateral neostriatum with antidromic spikes, consisting usually of the initial segment (IS) spike only (63%), or occasionally of a full IS-SD spike (mean latency = 15.8 ms). A prominent, long-lasting hyperpolarization followed spontaneous or antidromic full spikes, which was not apparent following antidromically evoked IS spikes. Although non-DA nigral neurons in pars reticulata responded to striatal stimulation with pronounced IPSPs (mean onset latency = 8.5 ms), IPSPs were not apparent in DA neurons. Electrical stimulation of the ipsilateral globus pallidus also gave rise to antidromic responses in DA neurons (mean latency = 6.5 ms). These antidromic responses almost always consisted of the IS spike only (92%), which was observed riding on a short latency (mean onset = 3.7 ms) IPSP which could be reversed with injections of hyperpolarizing current. Non-DA reticulata neurons also responded to pallidal stimulation with IPSPs (mean onset latency = 3.5 ms). While many pars compacta neurons exhibited spontaneous activity typical of DA neurons in extracellular recordings, a significant proportion of neurons, later identified as DA on the basis of their intracellularly recorded action potential waveform and duration and antidromic responses, were silent prior to impalement.

At the light microscopic level, HRP-labelled DA compacta neurons were of medium size (15-25 μm) and emitted 3 or 4 primary dendrites, which branched very sparsely. Although most of the dendritic arborization appeared to follow the borders of the pars compacta in coronal section, one dendrite, usually the largest, descended ventrally into pars reticulata. DA dendrites were largely smooth and non-varicose, with occasional spine-like appendages, mostly on the distal regions of the dendrites. The axons of compacta neurons emerged from the cell body or primary dendrites and, within substantia nigra, were unmyelinated, of extremely fine caliber, and displayed irregularly spaced varicosities. The axon of one particularly well-stained non-DA pars reticulata neuron collateralized near the cell body and appeared to form autaptic connections on the proximal regions of two large, ventrally directed dendrites.

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417.11 DOUBLE-LABELING STUDY OF EFFERENT CONNECTIONS OF SUBTHALAMIC NUCLEUS IN THE SQUIRREL MONKEY. A. Parent and Y. Smith. Lab. de Neurobiologie, Fac. Méd., Université Laval, Québec, Canada.

The cellular origin and degree of collateralization of the subthalamostriatal, subthalamonigral and subthalamopallidal projections in the squirrel monkey (Saimiri sciureus) were studied using lectin-conjugated horseradish peroxidase (WGA-HRP), nuclear yellow (NY) and fast blue (FB), as retrograde tracers. In a first experimental group comprising 4 animals, WGA-HRP was injected in left putamen (PUT) and right caudate nucleus (CD). Fourty-eight hours after injections, numerous retrogradely labeled cells were found in subthalamic nucleus (STH), bilaterally. On PUT-injected side labeled neurons occurred in the dorsolateral two-thirds of STH, whereas on CD-injected side they were confined to the ventromedial third of STH. In a second experimental group, FB was injected in PUT and NY in substantia nigra (SN) in one monkey, whereas FB was delivered in CD and NY in SN in two other animals. FB and NY were injected on the same side, 48 and 18 hours before sacrifice, respectively. Again, neurons containing the tracer injected in PUT occupied the entire dorsolateral two-thirds of STH whereas those projecting to CD were confined to the ventromedial third of STH. Neurons labeled from SN injection also occurred in the ventromedial third of STH where they were closely intermingled with those projecting to CD. However, only a few scattered double-labeled cells were found after CD/SN injection, revealing that very few STH neurons send axon collaterals to CD and SN. In a third experimental group, FB was injected in SN and NY in globus pallidus (GP) on the same side in two monkeys. In these animals neurons containing the tracer injected in GP were significantly more numerous than those disclosed after PUT injection (second group), and they strikingly outnumbered neurons projecting toward SN that remained confined to the ventromedial third of STH. Neurons projecting toward GP occurred in virtually all regions of STH but they clearly prevailed in the rostral two-thirds of this nucleus. In contrast, neurons projecting toward SN were more abundant in the caudal third of STH and decreased progressively in number in the rostral two-thirds of the nucleus. It was estimated that 80% of all retrogradely-labeled neurons in STH contained the tracer injected in GP versus 20% of neurons labeled with the tracer delivered in SN. Approximately 10-20% of all positive STH neurons were found to be double-labeled after GP/SN injections and most of them were lying at the interface between the two populations of single-labeled neurons. These findings reveal that STH in primates projects much more profusely to GP and PUT than SN and CD, with only a minority of neurons sending axon collaterals toward striopallidal structures and SN. (Supported by MRC and FRSQ).

417.PO AN ULTRASTRUCTURAL STUDY OF THE ENTOPEDUNCULAR - LATERAL HABENULAR PROJECTION IN THE RAT: AN EM STUDY WITH TMB REACTED WGA-HRP ANTEROGRADE TRACING TECHNIQUE. Si Yun Shu* and Xi Min Bao* (SPON: R.J. Nelson), Dept. Neurobiol., Fourth Military Medical School, Sian, Shannsi, China

A major afferent source to the lateral habenular nucleus (LHb) arises from the entopeduncular nucleus (EP), however, the ultrastructural morphology of axon terminals arising from EP within LHb has remained unclear. We report here our observations on the morphology of EP axon terminals which are anterogradely labeled with WGA-HRP.

A 2% WGA-HRP solution in 0.05 M Tris buffer (pH 8.6) was injected iontophoretically into EP of the rat with glass micropipette. The WAG-HRP was delivered by passing a positive current of 3 uA for 6-10 min. The rat was perfused after 2 days with a fixative solution consisting of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was sectioned on a Vibratome at 100 um thickness. The sections were reacted with the TMB technique and examined with a light microscope. Subsequently, LHb was dissected out and postfixed with 1% osmic acid at 45°C for 1 to 2 hours and stained en bloc with saturated ethanolic uranyl acetate. The tissue was then dehydrated, embedded in Epon, cut with a LKB-1 ultramicrotome and examined with a DBX-12 electron microscope.

At the light microscopic level, dark blue, densely labeled terminals were found throughout LHb. At the electron microscopic level, the labeled terminals were identified by their content of one or more crystal-like HRP-TMB reaction products. Most of the labeled terminals formed axo-dendritic synapses with either symmetrical or asymmetrical synaptic contacts. Occasionally, labeled terminals forming asymmetrical axo-spinous, or symmetrical axo-somatic synapses were also observed.

The results of this study suggest that at least two types of synapses are formed by EP efferent axon terminals on LHb neurons, and that they may have different synaptic actions.

417.13 INTERRELATIONS OF THE SPINOTHALAMIC, CEREBELLOTHALAMIC AND THALAMO-STRIATAL PROJECTIONS IN THE INTRALAMINAR NUCLEI: AN ANTEROGRADE-RETROGRADE TRACING STUDY IN THE CAT. M. Bentivoglio, D. Minciacchi* M. Molinari, A. Sbriccoli* and G. Macchi*. Institute of Neurology, Catholic University, 00168 Rome, Italy.

Spinothalamic and cerebellothalamic fibers represent the main subcortical afferents to the anterior intralaminar nuclei. The intralaminar efferents are heavily distributed upon the striatum and are widely distributed upon the cerebral cortex. The present study was aimed at investigating in the cat the topographical interrelations between the intralaminar cells projecting to the head of the caudate and the afferents from spinal cord or deep cerebellar nuclei. A simultaneous anterograde-retrograde fluorescent tracing strategy was here adopted. The tracer Fast Blue (FB) was used to label anterogradely the afferents to the intralaminar nuclei. Evans Blue (EB) was used to label retrogradely the intralaminar cells projecting to the caudate. In one group of cats FB was injected in the grey and white matter of the cervical cord and EB was injected ipsilaterally in the head of the caudate. In another group FB was injected either in the superior cerebellar peduncle or in the deep cerebellar nuclei and EB was injected in the contralateral caudate. All of the injections were made under deep Nembutal anesthesia. The distribution of spinal or cerebellar terminals and thalamocaudate cells was carefully studied in the central lateral (CL), paracentral (Pc) and central medial (CeM) nuclei. In the first group of animals overlap between the anterograde labeling from the spinal cord was observed in the posterior part of the anterior intralaminar nuclei, especially in CL. In the second group of animals the overlap between the anterograde labeling from cerebellum and the intralaminar-caudate cells was heavier than in the preceding cases and was concentrated in the dorsal part of CL, throughout most of its anteroposterior extent.

In some of the previous cases the retrograde tracer Diamidino Yellow (DY) was also injected in the primary motor cortex. These cases allowed the study of the two populations of cells labeled from caudate and motor cortex and the afferents either from spinal cord or cerebellum. The anterograde labeling from spinal cord or cerebellum overlapped extensively the intralaminar cells labeled from the motor cortex. Further, it was observed in these cases that spinal or cerebellar afferents overlapped in CL the areas containing the EB-DY double labeled cells, i.e. the neurons branching to both motor cortex and caudate.

The present results indicate that the anterior intralaminar nuclei convey to basal ganglia as well as motor cortex the input from spinal cord and cerebellum. The intralaminar nuclei may thus provide the structural correlate for a powerful subcortical and subcorticocortical sensorimotor integration.

417.14 FLUORO-GOLD LABELS PATCHES OF STRIATAL NEURONS AFTER STRIATAL INJECTION INTO TWO-DAY OLD RATS. A.M. Snyder-Keller, M.J. Zigmond, and R.D. Lund. Dept. of Neurobiology, Anatomy and Cell Science and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

The use of the new tracer Fluoro-Gold (Fluorochrome, Inc.) to label neurons projecting to the region injected has been described by Schmued and Fallon (Soc. Neurosci. Abs. 11: 199, 1985). We have used this tracer to label neurons retrogradely in the raphe nucleus and substantia nigra after injections into rat striatum at two days of age. Four days after single injections of 20-30 nl Fluoro-Gold were made into the rostral striatum, numerous labelled cells were found in both regions. Nigral cells were densely labelled with considerable filling of dendrites, while raphe neurons were clearly labelled but less dense. Comparable numbers of labelled cells were still obtained after survival times of one month or greater.

An examination of the injected striatum either four days or four weeks after injection at two days of age revealed patches of cells that had concentrated the Fluoro-Gold. These patches were present in both dorsal and ventral striatum, and often at some distance from the pipette placement. When these sections were processed for immunocytochemistry using an antibody to tyrosine hydroxylase, dopamine "islands" were revealed which overlapped precisely the patches of Fluoro-Gold-labelled neurons. On other sections, AChE histochemistry revealed a partial contiguity of AChE-rich zones and Fluoro-Gold patches, which was most complete in the lateral and caudal portions of the striatum. In animals given intraventricular injections of 6-hydroxydopamine to destroy dopamine terminals either before or after the Fluoro-Gold injection, patches of labelled neurons were still seen. The patches of labelled neurons were not seen in animals given Fluoro-Gold injections after the first ten days of age.

Since patches of Fluoro-Gold-labelled neurons correlated directly with the distribution of dopamine terminals at the time of injection, early injections of this tracer can be used to mark the dopamine islands which otherwise are not easily revealed in older animals. Furthermore, since destruction of dopamine terminals did not affect the appearance of Fluoro-Gold-labelled patches, the ability of those neurons to concentrate Fluoro-Gold must represent an intrinsic property of those neurons to which dopamine terminals initially project.

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417.15 THREE-DIMENSIONAL RECONSTRUCTIONS OF A STRIOSOMAL NETWORK IN THE CAUDATE NUCLEUS OF ADULT CATS. M.Martone*, P.M.Groves, S.J.Young* and D.M.Armstrong (SPON: R.Katzman).Dept's of Neurosciences and Psychiatry, UCSD School of Med. San Diego, CA 92093.

The mammalian neostriatum has been compartmentalized based on patterns of histochemical staining, receptor localization and afferent and efferent connections. One series of compartments consists of zones approximately 300μ in cross section, termed striosomes, which can be identified in the light microscope by their high levels of enkephalin-like immunoreactivity (ELI) and low levels of AChE reactivity compared to the surrounding matrix (Graybiel et al, Neurosci., 6:377, 1981). Individual striosomes can be followed through several sections suggesting that they are parts of 3-dimensional structures within the neostriatum.

In the present study, the 3-dimensional organization of these compartments in the caudate nucleus of adult cats was investigated using computer-assisted 3-D stereo reconstructions. The striosomes were labelled using antibodies to leu-enkephalin. Serial 50μ coronal (N=2) or horizontal (N=1) sections were cut through the head and body of the caudate nucleus. Every other or every third section was incubated with antibodies to leu-enkephalin (Immunonuclear) at a dilution of 1:2000 and processed using peroxidase-anti-peroxidase immunocytochemistry with nickel chloride ($NiCl_2$) intensification. On individual sections, the striosomes appeared as discrete patches of intense ELI against a lighter staining background. Camera lucida drawings were made of each section showing the outline of these patches and the caudate nucleus. Each drawing was then traced into the computer using a high resolution digitizing tablet. Sections were aligned using the outline of the caudate and major blood vessels for registration.

Preliminary analyses of reconstructions of 3 brains, both right and left sides, indicate that the patches seen in individual sections overlap across sections to form a highly interconnected 3-dimensional structure within the head and body of the caudate. In some cases, individual patches could be followed for over 50 sections (5mm). A consistently observed pattern was the formation of branches radiating diagonally from the ventricular edge across the width of the caudate. Smaller branches were often seen extending from these branches to form ring-like structures of variable diameter. Comparisons between animals suggest that certain features of the overall pattern are similar from animal to animal. Additional reconstructions in both the cat and rat as well as more quantitative analyses are in progress.

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417.16 A STRIATAL MOSAIC REVEALED BY LECTIN BINDING AND TRITIATED FUCOSE INCORPORATION IN THE EARLY POSTNATAL MOUSE. T.F.O'Brien*, D.A. Steindler, and N.G.F. Cooper (SPON: R.Caldwell).Dept. of Anatomy and Neurobioloby, Univ. of Tenn./Memphis,Memphis,TN 38163

Numerous studies have examined the compartmental organization of the mammalian neostriatum, noting the presence of distinct mosaic patterns seen in neurochemical or axonal tracing preparations (Olson et al., Brain Res.,44:283,'72; Butcher and Hodge, Brain Res.,106:223,'76; Goldman and Nauta, J.comp.Neurol.,171: 369,'77; Herkenham and Pert, J.Neurosci.,2:1129,'82; Graybiel, Prog.Brain Res.,58:247,'83; Gerfen, J.comp.Neurol.,236:454,'85). 'Striosomes'(Graybiel and Ragsdale, PNAS,75:5723,'78) have now been demonstrated using histochemical detections of transmitters, enzymes such as acetlycholinesterase, or in autoradiographs following labeling of opiate receptors. In the present study, we have examined substructure within the neostriatum of early postnatal mice using lectin binding and incorporation of the glycan 3H fucose. Postnatal day 2 ICR mice were anesthetized and received intraperitoneal injections of 3H fucose (NEN), and survived 1hr-19days at which time they were cardiac perfused with mixed aldehydes. Parasagittal and flattened tangential vibratome sections were incubated in peroxidase labeled peanut agglutinin (PNA) or biotinylated PNA, and reacted for peroxidase ctyochemistry. Some sections were further processed for autoradiography. Histochemical labeling, indicative of PNA binding, was extremely pronounced in the striatum on postnatal day 6, and took the form of rings and oblong structures which contributed to a variegated mosaic. These structures are often doughnut-shaped, consisting of intense outer rims of labeling with lighter centers. Staining is generally more intense in the dorsal half of the striatum, with both the lectin-positive islands and surrounding areas becoming lighter approaching the ventral striatum. Autoradigraphic labeling of 3H fucose incorporation in the striatum is very similar, and the mosaic patterns are more subtle and labeling of fascicles more conspicuous in these preparations. We believe that the unique striatal labeling patterns described here relate to boundaries of striosomes, in keeping with similar observations made on hidden boundaries of developing 'barrels' in the somatosensory cortex of mice also using lectin recognition of particular glycosylated molecules (Cooper and Steindler, J.comp. Neurol.,249,'86). We also believe that both lectin binding and 3H fucose incorporation are predominantly associated with glial elements and related pattern formation molecules during this period (Steindler and Cooper, J.comp.Neurol.249,'86; Cooper and Steindler, Brain Res.,'86), and thus these patterns observed in the striatum might reflect an early postnatal stabilization of the neostriatal mosaic. Supported by NIH/NINCDS grant NS-20856, NEI grant EY-02708, and the UT Memphis Center for Neuroscience.

417.17 BRAIN ACETAMINOPHEN MEASUREMENT BY IN VIVO-DIALYSIS, IN VIVO ELECTROCHEMISTRY AND TISSUE ASSAY: A COMPARISON OF NEUROCHEMICAL TECHNIQUES IN THE RAT. K.E. Sabol* and C.R. Freed, (SPON: T. Audesirk). Depts. of Med. and Pharm., Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

The in vivo dialysis technique for monitoring neurochemical activity in brain has provided an improvement on push-pull methods by reducing tissue damage by the cannula. Wages and Justice (Analy. Chem., in press, 1986) have developed a concentric cannula and incorporated it into a system of online analysis of the dialysate using HPLC with electrochemical (EC) detection. With instrumentation similar to theirs, we have compared in vivo dialysis with in vivo electrochemistry and direct tissue assay using the EC detectable drug acetaminophen (APAP). Urethane anesthetized male Sprague-Dawley rats 300-450 g were implanted with dialysis cannulae (850 u diameter) in the left striatum. Ringer's solution with 10 uM para-aminophenol as an internal standard and 1.0 mM glutathione as an anti-oxidant was perfused through the cannula at a rate of 1.58 ul/min. The cannula outflow tube was connected to a computer controlled pneumatically activated Rheodyne 7013 HPLC injection valve with a 5 ul sample loop using low volume tubing (2.43 ul/ft). The column was 1 x 100 mm packed with 3 u Adsorbosphere C18 and the EC detector was an LC4B (Bioanalytical Systems). Samples were automatically injected every 10 min. After baseline recordings were made, 75 mg/kg APAP was injected i.p. and striatal levels were monitored by dialysis. In other experiments, rats were implanted with EC electrodes and injected with APAP. The EC peak for APAP was followed and compared to the dialysis peak. In a third group of experiments, anesthetized animals were injected with APAP and decapitated at intervals after the injection. Striatal tissue was dissected and assayed for APAP. In a fourth experiment, in vitro estimates of dialysis efficiency were made by placing dialysis cannulae in a beaker containing 100 uM APAP. The in vivo dialysis experiments showed that APAP reached a peak concentration of 17.1 $\pm$ 1.4 uM (n=6) 80 min after the injection of APAP. The EC peak for acetaminophen was maximal 70 min after drug injection. The shapes of the APAP curves measured by EC and dialysis were the same. Direct tissue assay of APAP showed a peak tissue concentration of 232 $\pm$ 16 uM (n=6) 50 min after drug injection. Thus, the dialysis concentration was only 7% of the APAP in the tissue and the peak tissue content occurred 20-30 min before the peak EC and dialysis measurements. In vitro recovery efficiency of APAP by dialysis was 28%. We conclude that dialysis and in vivo EC offer equivalent measurements of extracellular fluid APAP. Dialysis efficiency appears to be less in vivo than in vitro possibly because of slow tissue diffusion of APAP or concentration of APAP intracellularly.

417.18 DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF STRIATONIGRAL TERMINALS. Gord Fishell* and Derek van der Kooy (Spon. P. Stewart) Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8.

The striatum can be divided into two compartments, the patch and matrix, separated on the basis of afferent and efferent connections, neurochemicals and receptor types. Recently we have shown that the cell bodies of the earliest projections of the striatonigral pathway (E18-E20) provide an embryonic marker of what will become the adult patch compartment of the striatum. Matrix neurons (in a second wave of projections) send axons to the substantia nigra (SN) during the first postnatal week. The disparity in the developmental timecourses of striatonigral neurons originating from the patch and matrix compartments allows us to ask if the patch and matrix neurons terminate in different regions of the SN and whether these terminal patterns change during development. The SN distribution of striatonigral terminals was compared to that of tyrosine hydroxylase (a marker for dopamine perikarya). The anterograde axonal tracer Rhodamine isothiocyanate (RITC) was injected into embryonic (E) or postnatal (PND) striatum. After variable survival periods (in order to assess developmental changes) embryos or pups had their brains removed and SN sections were processed for immunofluorescent visualization of tyrosine hydroxylase. All cases involving both RITC injections and sacrifice during the first postnatal week indicate that the striatal projection is almost solely to the pars reticulata portion of the SN (as it is in the adult). Thus, postnatal striatonigral terminals are complementary to tyrosine hydroxylase which is located primarily in the pars compacta portion of this nucleus. However, in cases with embryonic RITC injections and sacrifice (E19-E20) a large overlap between striatonigral terminals and dopamine neurons was observed in the SN. E19-E20 represents the earliest time at which the patch compartment is discernible in the striatum. Remarkably, rats injected with the anterograde tracer RITC at embryonic times and sacrificed at PND2 and 10 revealed that the early striatonigral terminals had sorted themselves into a complementary relationship to dopamine perikarya identical to that observed in postnatally injected animals. The transient overlap of striatonigral terminals with dopamine neurons in the SN may play a role in the development and differentiation of both patch neurons in the striatum and dopamine neurons in the SN. In addition, the early dopamine nigrostriatal and early striatonigral projections may provide pioneer substrate pathways for each other. Although we observed no differences in the postnatal distribution of patch and matrix striatonigral terminals, an EM study may reveal quantitative or synaptic differences between the terminations of these two distinct striatal populations.

417.19 LONG-TERM CHANGES IN SYNAPTIC ORGANIZATION OF THE MOTOR THALAMUS OF THE CAT AFTER ITS DEAFFERENTATION FROM SUBSTANITA NIGRA. K. Kultas-Ilinsky, C. Cuthbert* and I. Ilinsky. Dept. of Anatomy, University of Iowa College of Medicine, Iowa City, IA 52242.

Substantia nigra pars reticularis (SNr) gives rise to a GABA-ergic inhibitory pathway that in the cat terminates in the ventral anterior (VA) and ventral medial (VM) thalamic nuclei. The ultrastructural features and synaptic sites of this pathway on thalamocortical projection neurons (TCPN) were described in detail earlier. In the present series of experiments long-term events caused by degeneration of nigrothalamic fibers were studied. Stereotactic injections of ibotenic or kainic acid were made in the SNr. Animals were sacrificed at 1 week, 2 weeks, 1.5, 3, 6 and 12 months postlesion. Samples from the VA and VM were processed for electron microscopy, and subjected to further qualitative and quantitative analysis. The initial measurements were done from the micrographs using the Bioquant image analysis system. The results were then processed by Wilbur IBM system using a program specially designed for the purpose. A large number of parameters were thus analyzed, including the types, sizes and number of boutons contacting different order dendrites of thalamocortical projection neurons, % of dendritic membrane length apposed by glia and the dendrites of local circuit neurons (LCN), density of different type boutons and synaptic contacts on the TCPN dendrites, etc. The results of the analysis indicate that: 1. Although the initial degeneration process and removal of debris is rather fast and complete by second week, single degenerating terminals can be observed as late as 3 months postlesion. 2. The sites originally occupied by nigral boutons become occupied by glia whose appositional length on the dendrites is still increased at 6 months postlesion. 3. The density of synaptic boutons with symmetrical contacts (characteristic of nigral boutons) keeps decreasing up to 1 year postlesion. 4. The signs of synaptic remodeling in individual subdivisions of the motor nuclei differ depending on the original composition of afferent and local inputs on TCPN dendrites i.e. zone of overlap of nigral and pallidal projections vs. pure nigral territory. Thus, in some regions the number of contacts made by LCN on TCPN dendrites appear to be increased whereas in others remaining intact terminals tend to expand. These data suggest that slow plasticity type changes occur in the adult motor thalamus after its deafferentation from the inhibitory nigral afferents. Supported by NINCDS grant RO119280.

417.20 CATACHOLAMINE NEURONS IN THE OPOSSUM MIDBRAIN. James C. Hazlett and George F. Martin, Dept. Anatomy and Cell Biology, Wayne State University, Detroit, Michigan and Dept. of Anatomy, The Ohio State University, Columbus Ohio.

As a prelude to ongoing studies of mesotelencephalic connectivity and development in the opossum, catacholamine neurons were identified in 4 adult opossums using tyrosine hydroxylase and processing by PAP immunocytochemistry. In the dorsal midbrain catacholamine cells were observed in medial portions of the dorsal raphe nucleus caudally and within the central gray, pars ventralis rostrally. More ventrally, at least six identifiable cell groups contained such neurons. Caudally a few of them were present in the deep tegmentum and in the adjacent pedunculopontine tegmental area (TgPP); whereas at slightly more rostral levels, they were found in the caudal end of the substantia nigra, pars compacta. From this point rostrally, the pars compacta expanded and was characterized by the presence of numerous catacholamine neurons. The pars reticulata and pars lateralis of the substantia nigra contained only a moderate number of such cells. Catacholamine neurons were also found throughout the entire longitudinal extents of the nucleus linearis and ventral tegmental area (VTA) as well as within the deep tegmentum ventral and lateral to the red nucleus.

Due to fortuitous staining of dendritic processes, many neurons in the above mentioned loci could be classified as isodendritic. Further confirmation of this, plus information pertinent to the connections of these cells, has been obtained from the results of HRP injections into various levels of the caudate nucleus and putamen. Although we presently lack the colocalization data for TH reactivity and HRP immunoreactive neurons, it is clear that neurons in all of the catacholamine cell groups listed above project to the neostriatum. Furthermore, it appears that the location of catacholamine neurons in the opossum's midbrain is comperable to that reported for the rat (Hokfelt et al.'84). While other aspects of the opossum mesotelencephalic system must be examined in order to establish further morphological and neurochemical similarities, we would predict that most of the substrates observed in other species will be recognized in the opossum.

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418.1 HETEROGENEOUS CORTICORETICULAR PROJECTIONS IN THE RAT. D.B. Newman. Dept. of Anatomy, Uniformed Services University, Bethesda, MD 20814.

Corticoreticular fiber systems were examined in adult albino or hooded rats using either anterograde transport of HRP-WGA (with TMB histochemistry) or an anterograde degeneration technique (the Wiitanen stain). HRP-WGA injections or lesions were made into the prefrontal cortex, the premotor (medial agranular) cortex, the cingulate cortex, various parts of the primary motor (MI) cortex, the face area of primary somatosensory cortex, primary auditory cortex, and primary and secondary visual cortices. The heaviest corticoreticular projections arise from the prefrontal and premotor cortices. The face and trunk areas of MI also give rise to relatively strong corticoreticular inputs. The arm area of MI gives rise to fewer corticoreticular fibers than the face or trunk areas, and the leg area fewer still. Corticoreticular fibers emanating from somatosensory, auditory, and visual cortices are rather sparse, and are chiefly directed to rostral brainstem levels. Within the upper brainstem the densest corticoreticular projections are to small-celled reticular nuclei (such as pedunculopontine and subcoeruleus) which in turn project extensively to the giant-celled rhombencephalic reticulospinal nuclei. Thus, corticoreticular projections to the upper brainstem target reticular "correlative" centers. Conversely, corticoreticular projections to the lower brainstem distribute not only to small-celled "staging" reticular nuclei, but also directly to giant-celled reticulospinal nuclei such as gigantocellularis and magnocellularis. Corticoreticular projections to the upper brainstem terminate predominately ipsilateral to the cortical injections site, whereas medullary corticoreticular projections distribute bilaterally. This agrees with previous findings that reticulospinal projections from the upper brainstem are ipsilateral, whereas reticulospinal projections from the lower brainstem are bilateral. Whereas corticoreticular fibers from premotor and trunk motor areas terminate heavily in somatomotor brainstem reticular nuclei, corticoreticular fibers from the prefrontal cortex send dense projections to autonomic brainstem reticular nuclei such as the raphe nuclei, parabrachial complex, and ventrolateral medulla. The face area of the motor cortex projects to medullary reticular nuclei closely associated with the trigeminal system, such as reticularis parvocellularis and dorsalis. Finally, corticoreticular fiber systems respect the reticular nuclear boundaries delineated previously on the basis of cytoarchitectonic or dendroarchitectonic criteria.

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418.2 THE ORGANIZATION OF THE MAGNOCELLULAR REGION OF THE RED NUCLEUS OF THE MACAQUE MONKEY: AN ELECTRON MICROSCOPIC STUDY. Diane D. Ralston, Antonia Milroy* and Henry J. Ralston, III. Dept. Anatomy, Univ. California, San Francisco, CA 94143

The fine structure of the magnocellular region of the macaque red nucleus (RNmc) has been examined in normal monkeys; in animals which have had cortical (Ctx) projections to RNmc labeled by degeneration following lesions of motor Ctx, or by injections of ^{3}H leucine into motor Ctx followed by autoradiography (ARG) of RNmc; and RNmc tissue stained for the immunolocalization of gamma-aminobutyric acid (GABA) identified by GABA antibody (ImmunoNuclear) coupled to peroxidase-antiperoxidase (PAP) using the method of Sternberger. All animals were anesthetized with intramuscular ketamine (10 mgs/kg) followed by intravenous pentobarbital (28 mgs/kg). Sterile surgical procedures were used in those animals having lesions or microinjections of ^{3}H leucine.

The fine structure of normal RNmc reveals numerous large neurons which frequently exhibit granules, probably lipofuscin. The neurons often are indented by capillaries, with only a thin (0.1um) glial process intervening between capillary basal lamina and neuronal cell body. The synaptic population is characterized by the heterogeneity of its constituent synaptic profiles, which are classified as follows: terminals of 2-4 um diameter with numerous round vesicles (R); profiles of similar size but with a pale axoplasmic matrix and numerous round vesicles (PR); large profiles (4-6 um) which contain round vesicles and which form the central element (C) in synaptic glomeruli; small profiles (1um) with rounded vesicles (SR); profiles of 2-4 um diameter with numerous granular vesicles (GV); profiles ranging from 2-5 um in diameter with numerous pleomorphic vesicles (PI); profiles which frequently can be seen to arise from dendrites, or to have ribosomes, and which contained scattered pleomorphic vesicles--these structures are identified as presynaptic dendrites (PSD).

The synaptic relationships of these various profiles are complex. R profiles are presynaptic to dendrites of all sizes, to neuronal somas and to PSDs. PR contact dendrites of all sizes and somas, but are not seen to contact PSDs. Cs are presynaptic to small dendrites, to spines and PSDs. SR synapses upon somas, all sizes of dendrites and upon PSDs. GV contact all sizes of dendrites. PI contacts somas, all sizes of dendrites, and PSDs. PSDs are synapsed upon by R, C, PI and SR, and contact somas and all sizes of dendrites. PSDs often participate in synaptic triads, being postsynaptic to R or SR, and presynaptic to dendrites or somas.

Following Ctx lesions, many Cs exhibit prominent neurofilamentous degeneration, indicating that this profile is of cortical origin. GABA antibody stains some PIs, PSDs and dendrites. The GABA positive PIs and PSDs synapse upon non-staining somas and large and medium dendrites. These findings indicate that Ctx projections terminate upon dendritic spines and PSDs, but that Ctx terminals are not postsynaptic to vesicle-containing profiles. GABA, which has been shown to be an inhibitory transmitter in RN, is contained in axons with pleomorphic vesicles, and in presynaptic dendrites. (Supported by NS-23347 from NIH).

418.3 **THE MORPHOLOGY OF THE RED NUCLEUS AND THE ORIGINS OF ITS SPINAL TRACT. A STUDY IN THE RAT.** **R.N. STROMINGER* AND N.L. STROMINGER. Department of Anatomy, Albany Medical College, Albany, NY 12208.**

Cytoarchitectural observations indicate that the red nucleus (RN) of the albino rat is comprised of three neuronal populations which can be classified according to characteristics of the Nissl substance. Neurons with coarse Nissl bodies occupy the caudal end of the RN and extend in diminishing number to the rostral tip. Neurons with finely granular Nissl material are the predominant type at the rostral end of the RN. They interdigitate with the coarse neurons except at the caudal end. Coarse neurons are multiangular in shape and tend to be larger than the fine ones, although there is broad overlap in areas. Fine cells tend to have smooth contours and often are surrounded by perineuronal glial satellite cells. The third neuronal population in the RN is referred to as achromatic because of scant cytoplasmic staining. These cells are almost uniformly smaller than the other types and are distributed throughout the RN. They are regarded as interneurons.

Injections of HRP at different levels of the spinal cord established that the coarse neurons on the contralateral side are the source of the rubrospinal tract. The RN is topographically organized with the dorso-medial part projecting to the cervical spinal cord, the ventral-ventromedial part to the lumbar cord and intermediate locations to thoracic levels. Further, coarse neurons from the entire rostro-caudal dimension of the RN contribute fibers to the rubrospinal tract.

Comparisons with data from studies in monkey, chimpanzee and human brainstems lead to the conclusion that in man the rubrospinal tract is, at most, small. In primates there is a progressive hypertrophy of the fine neurons coupled with a reduction in the number of coarse ones. There are fewer than 500 coarse neurons in the human RN and many of these may emit fibers that descend only to the level of the facial nucleus.

418.4 PONTOMEDULLARY RETICULAR FORMATION NEURONS RELATED TO HEAD MOVEMENT IN BEHAVING CATS: CONVERGING INPUTS FROM MOTOR CORTEX, DEEP SUPERIOR COLLICULUS, MIDBRAIN TEGMENTUM VESTIBULAR NUCLEI, AND SPINAL CORD. S. S. Suzuki and J. M. Siegel. V. A. Med. Ctr., Sepulveda, CA 91343 and Dept. of Psychiarty, UCLA Sch. of Med., Los Angeles, CA 90024.

Previous studies in this laboratory on the behavioral correlates of pontomedullary reticular formation (PMRF) neurons in unrestrained cats indicated that most of these neurons dischage maximally in relation to specific movements, particularly those of the head. Although a great deal is known about the efferent and afferent connections of PMRF cells based on a number of anatomical and acute electrophysiological studies, the relationship between the behavioral role of PMRF head movement cells and their connectivity is unknown. We have investigated this relationship by examining the orthodromic and antidromic responses of behaviorally identified head movement-related PMRF cells to electrical stimulation of several head movement-related structures in behaving cats.

Cats were implanted with PMRF microdrives containing moveable 32 um microwires as well as stimulating electrodes in the motor cortex (MC), deep layers of superior colliculus (SC), midbrain tegmentum (MT), vestibular nuclei (VN), and cervical/lumbar cord (C2/L1). EMG electrodes were implanted into three major neck muscles (splenius, biventer cervicis, complexus) bilaterally. The activity of each isolated PMRF cell was observed systematically during various movements and sensory stimulation to determine its sensorimotor correlates. Unit and EMG responses to single pulse stimulation of the above structures were processed by a computer.

The results may be summarized as follows. (1) Many PMRF head movement cells were antidromically activated by spinal cord stimulation at C2 and/or L1. Most had fast conduction velocities (about 100 m/s). (2) Head movement-related cells in the PMRF responded orthodromically with short latencies (including those within monosynaptic ranges) to stimulation of MC, SC, MT, VN, and C2/L1. Most of these cells were activated from more than one site. Bilateral activation was commonly observed from all these sites but contralateral dominance was evident with SC stimulation. (3) Short latency orthodromic unit responses usually occurred 1-4 ms earlier than neck EMG responses following MC, SC, and VN stimulation.

These observations support our hypothesis that head movement-related reticulospinal neurons in the PMRF mediate directionally specific head movements based on command inputs from the MC, SC, VN, and spinal cord. Our data on converging inputs also suggest that the same reticulospinal neurons are utilized for head movement generation in different behavioral contexts (e.g., voluntary head movements and head movements triggered by visual, auditory, somatosensory, and vestibular stimuli).

418.5 INTRACELLULAR HRP INJECTIONS REVEAL TWO CLASSES OF mPRF NEURONS WITH DIFFERENT CAUDAL PROJECTION PATTERNS. A. Mitani*, K. Ito*, Y. Mitani*, and R.W. McCarley. Lab. Neurosci., Dept. Psychiat., Harvard Med. Sch./Brockton VAMC, Brockton, MA 02401

The medial pontine reticular formation (mPRF) has been implicated in control of events related to the sleep-waking cycle and motor activity by a number of behavioral and physiological studies. However, any specialization of neuronal types/projection patterns that might mediate these effects has not been defined. In order to examine cellular morphology and projections we injected HRP intracellularly into electrophysiologically identified mPRF neurons in anaesthetized adult cats and visualized the HPR stained neurons in frontal, parasagittal, or horizontal sections.

The presence of two types of neurons was defined by caudal projection pattern. Type I neurons had axons that coursed medially and then turned caudally into the paramedian fasiculus that contains axons destined for the medial reticulospinal tract in cord; axons were traced for 5-7 mm (N=20 neurons studied). Mean axon diameter was 3.3 um. These neurons were large with multipolar, elipsoid somata with short and long axes averaging 44.9 and 69.7 um. Dendritic fields were large and nearly uniform in all three planes, with means: 1373 um, AP (N=11); 1745 um ML (N=16); and 1596 um dorsoventrally (N=13). Only 4 neurons had the preferential arborization perpendicular to the long axis of the brain stem ("poker-chip" arrangement) described in Golgi data from young animals. In contrast, Type II neurons had axons that coursed directly caudally to enter the bulbar reticular formation. Mean axon diameter was 2.5 um (N=4 neurons thus far analyzed; $p < .02$ vs. Type I axons, Mann-Whitney test). Type II neurons had medium-sized multipolar cell bodies with mean short and long axes of 36.9 and 46.9 um, significantly less than Type I neurons ($p < .02$ for short and $p < .001$ for long axis, Mann-Whitney tests). Type II neurons also had less extensive dendritic arborization: 1038 um, AP; 1688 um, ML; and 1131 um dorsoventrally. Both types had non-spiny or sparsely spiny dendrites that originated from 6-8 trunks and had extensive branching; in both types axons arose from cell bodies or proximal dendrites. These data suggest that mPRF has at least two caudally projecting neuronal groups: One group (Type I) is reticulo-spinal neurons characterized by large-sized somata, dendritic fields, and axons. The other (Type II) is reticulo-reticular neurons with medium-sized somata, dendritic fields, and axons. This typology should not be taken to be an exclusive catalog: in addition to these types, neurons with axonal projections rostrally and within mPRF were also observed.

418.6 COMBINED RETROGRADE-IMMUNOHISTOCHEMICAL EVIDENCE THAT THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS DOES NOT PROJECT TO EXTRAPYRAMIDAL MOTOR STRUCTURES. H. J. Lee*, D. B. Rye, B. H. Wainer. Dept. Pharm. and Phys. Sciences, Univ. of Chicago, Chicago, IL 60637.

Previous studies suggest that the pedunculopontine tegmental nucleus (PPTn) is reciprocally connected with the extrapyramidal motor system (EPMS) and may play a role in Parkinson's syndrome (Moon-Edley and Graybiel, '83). Others implicate the PPTn in rapid eye movement (REM) sleep (Sakai,'85) and diffuse cortical arousal (Shute and Lewis,'67). Many of these studies define the subprimate PPTn as an area of mesopontine tegmentum which is labeled from injections of anterograde tracers into the basal ganglia. Recently, we have defined the rat PPTn (Rye et. al., '85) as a large celled, cholinergic nucleus. Distinct from the PPTn is a medially adjacent group of smaller, noncholinergic cells which are also distinguished from the PPTn by their reciprocal connections with the substantia nigra. Given these contrasts, this area has been referred to as the midbrain extrapyramidal area (MEA) by Rye et. al..

We investigated additional efferent connections of the PPTn and MEA with the EPMS using combined choline acetyltransferase immunohistochemistry of the PPTn and WGA-HRP retrograde tracing from the EPMS. The MEA represents the major source of tegmental innervation to the motor cortex, neostriatum, both segments of the globus pallidus, and subthalamus. Conversely, the PPTn does not provide a major projection to these EPMS structures.

In a second set of experiments, the PPTn was found to represent a major source of tegmental innervation to the VA/VL thalamic portion of the EPMS. Moreover, PPTn projections were identified to other "specific" as well as "nonspecific" thalamic nuclei. This finding is in contradistinction to thalamic projections from the surrounding reticular formation which are identified only after WGA-HRP injections in "nonspecific" nuclei. This body of evidence suggests a possible role for PPTn pathways in global thalamic functions such as the "reticular activating system" and REM sleep, rather than a primary role in motor function. The adjacent MEA represents an additional component of the EPMS and may correspond to the "mesencephalic locomotor region".

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418.7 MORPHOLOGY OF VESTIBULOSPINAL AND RETICULOSPINAL AXONS TERMINATING IN THE UPPER CERVICAL SPINAL CORD OF THE CAT. A. Holmes, M. Neuber and P.K. Rose, Department of Physiology, Queen's University, Kingston, Ontario, K7L 3N6.

Most studies of the anatomy of descending spinal pathways have been concerned with their termination in the cervical or lumbar enlargements. In contrast, relatively little is known about the terminal distribution of descending systems at other levels of the spinal cord, particularly the upper cervical segments which innervate neck muscles. Electrophysiological studies have shown that neck motoneurons receive monosynaptic connections from at least seven brainstem descending pathways. The aim of the present experiments was to describe the structure of axons in the upper cervical spinal cord which originate from brainstem regions with known monosynaptic connections to neck muscle motoneurons.

PHA-L was iontophoretically injected into the vestibular complex or regions just ventral to the vestibular complex at the junction of the medulla and pons. Following a 14-19 day post-injection survival, the ABC method was used to visualize PHA-L labelled axons and axon collaterals. Small numbers of axons in the ipsilateral and contralateral lateral funiculus and ventral funiculus gave rise to collaterals which arborized and terminated in the grey matter. The quality of staining was equal to, if not better, than that seen in previous experiments using intra-axonal injections of HRP. Preliminary analysis of the termination zones has revealed two patterns. First, projections from the descending vestibular nucleus to the ventral horn were modest. The major termination zone was located in the intermediate zone and dorsal horn including lamina IV. These termination zones were seen ipsilateral and contralateral to the injection. Injections ventral to the medial vestibular nucleus in a zone previously reported to have few descending connections led to staining of axon collaterals throughout the ventral horn, ipsilateral to the injection. The greatest concentration of terminals were found in lamina IX and along the medial wall of the ventral horn. The contralateral projection was relatively sparse and was largely found in the central regions of lamine VII and VIII.

These results indicate that descending connections from the vestibular nuclei and immediately adjacent areas are more extensive than previously recognized. Some projections are confined to small regions of the ventral horn. Since neck muscle motoneurons have dendritic trees which project throughout much of the ventral horn, the connections of these descending systems may be restricted to a small region of the dendritic tree.

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418.8 SPINAL PROJECTING NUCLEI IN THE BRAINSTEM OF A GALEOMORPH SHARK, HETERODONTUS FRANCISCI. W.L.R. Cruce and R.G. Northcutt. Neurobiology Department, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Neurobiology Unit, Scripps Institute of Oceanography and Department of Neurosciences, School of Medicine, University of California San Diego, La Jolla, CA 92093.

Cruce and Newman ('84, Amer. Zool. 24:733-753) hypothesized that the reticulospinal nuclei of non-mammals exhibit a level of complexity comparable to that of mammals. The organization of reticular nuclei in the thornback guitarfish, Platyrhinoidis triseriata (Cruce and Northcutt,'85, Neurosci. Abs. 11:1313) supports this hypothesis, but a comparable study in a shark (Scyliorhinus, Smeets and Timerick,'81, JCN 202:473-491) only distinguished the three classical subdivisions (superior, middle, and inferior) of the reticular formation. Therefore we investigated these pathways in another galeomorph shark, Heterodontus, which exhibits a level of organization comparable to Scyliorhinus. We indicate suspected homologies by adopting, particularly for reticular nuclei, the mammalian names (using those of Newman,'85, J. Hirnforsch. 26:187-226, 385-418). These hypotheses of homologies must be tested by subsequent immunocytochemical and hodological studies.

Seven horned sharks (190-550 g., 30-43 cm.) received unilateral placements of horseradish peroxidase (combined with DMSO and/or lysolecithin and dried on a pin or into crystals) into the rostral spinal cord (segments 2-5). After survival periods of 4-12 days in sea water at 11-18 degrees C., the animals were perfused, the brains removed and sectioned, and sections were reacted with the tetramethyl benzidene procedure.

The following nuclei were identified and found to project to the spinal cord: reticularis dorsalis, reticularis ventralis pars alpha, reticularis ventralis pars beta, descending trigeminal, reticularis gigantocellularis, reticularis magnocellularis, raphe obscurus, raphe magnus, reticularis parvocellularis, reticularis paragigantocellularis lateralis, magnocellular octaval, descending octaval, reticularis pontis caudalis pars alpha, reticularis pontis caudalis pars beta, nucleus "B", locus coeruleus, reticularis subcoeruleus, reticularis pontis oralis pars lateralis, reticularis pontis oralis pars medialis, interstitial nucleus of Cajal, ruber, Edinger-Westphal, posterior tuberculum, ventromedial thalamic, central pretectal, and periventricular pretectal pars dorsalis.

These results are comparable to the mammalian level of organization including the presence of a rubrospinal pathway.

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418.9 CATECHOLAMINERGIC INNERVATION OF THE SPINAL CORD IN THE NORTH AMERICAN OPOSSUM, DIDELPHIS VIRGINIANA. R.R. Pindzola, G.F. Martin, and R.H. Ho. Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210

Catecholamine-containing axons and perikarya were visualized using antibodies to tyrosine hydroxylase in the indirect antibody peroxidase antiperoxidase technique (PAP) of Sternberger. Axons showing tyrosine hydroxylase-like immunoreactivity, hereafter referred to as catecholaminergic, were most abundant in the superficial layers of the dorsal horn (laminae I-IV), in the intermediolateral cell column, in the sacral parasympathetic nucleus and around the central canal (lamina X). The axons destined for the dorsal horn traverse the dorsolateral funiculi. Catecholaminergic axons were also found in the ventral horn (laminae VII-IX) where their densest distribution was at thoracic and lumbar spinal levels. The density of axons in the ventral horn, particularly in lamina IX, appeared to be less than in the rat (personal observations). The distribution of catecholaminergic neurons in the brain was similar to that in the rat (personal observations) except that few of them were present dorsal and dorsolateral to the superior olive (the A5 group of Dahlström and Fuxe, '64, Acta Physiol Scand. 62 (sup. 232): 3). Brainstem catecholaminergic neurons giving rise to spinal projections were identified using the PAP technique in combination with histochemical processing for cells retrogradely labelled by horseradish peroxidase injections of the cervical (C3) spinal cord (Bishop and Ho, '85, Brain Res. 331: 195). Double labelled neurons were located in the periventricular nucleus of the hypothalamus and related areas, the ventral part of the locus coeruleus, the locus coeruleus pars alpha, the lateral part of the pontine reticular nucleus, and several nuclei of the ventrolateral medulla. Our results suggest that the innervation of the spinal cord by catecholaminergic axons and the distribution in the brain of catecholaminergic neurons are similar in the opossum and rat, although differences in innervation density and the location of the A5 group may exist. These data provide the basis for interpreting the results of ongoing studies on the development of catecholaminergic projections to the spinal cord. (Supported by BNS-8309245 and NS-10165).

418.10 UNMYELINATED AXONS IN THE WHITE MATTER OF THE MAMMALIAN THORACIC SPINAL CORD: NORMAL MORPHOMETRY AND A QUESTION OF ORIGIN. A. R. Blight and M. Sobeih*. Depts. of Neurosurg. and Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

By comparison with large myelinated axons, unmyelinated fibers of the mammalian spinal cord are relatively little known and poorly understood. While studying the reaction of spinal axons to injury, it became necessary to examine some of the normal characteristics of unmyelinated fibers.

A line-sampling technique developed for light microscopy (*Neuroscience* 10, 521) was adapted for electron microscopic (e.m.) sampling. A normal adult female cat was fixed by perfusion with glutaraldehyde and a transverse section of the T9 cord was divided into sectors and embedded for conventional e.m. Contiguous sections from around the circumference of the white matter were sampled to a depth of 600 μm from the pial surface. In other experiments, horseradish peroxidase (HRP) transport was examined in dorsal and ventral tracts isolated from adult rat, guinea-pig and cat spinal cord, maintained *in vitro* for 12 to 24 hr. (*Neuroscience* 15, 1). One end of the isolated tract was exposed to 4% HRP solution while the rest was superfused with oxygenated Krebs solution.

In qualitative agreement with findings in the sacral spinal cord (Chung et al. 1985, *J.comp.Neurol*. 234, 117), large numbers of unmyelinated fibers were found in all regions of the cord cross-section, particularly concentrated in the dorsolateral funiculi. These fibers occurred mostly in small, often radially oriented bundles. The average caliber (defined here as the minor axis of an approximately elliptical cross section) was 0.42 ± 0.20 μm, yet, of the axons in the sample smaller than 0.6 μm dia., more than 35% were myelinated. Excluding the dorsolateral funiculi, the proportion of unmyelinated fibers in the total population was of the order of 20%, (though they occuppied less than 0.5% of the cross sectional area).

The HRP-stained tracts showed longitudinally running fibers that were stained continuously within a few millimeters of the end exposed to HRP solution, but that at greater distances were distinctly stained only at nodes of Ranvier. A significant proportion of such nodes showed a fine collateral branch of less than 1 μm dia., leaving the parent axon and coursing through the cord towards the gray matter. Such collaterals tended to associate in flattened, radially directed bundles, and were continuously stained with HRP, rather than showing nodal accumulations like their parent axons.

Comparing the HRP and e.m. data, it appears a large proportion of unmyelinated axons seen in transverse section are collaterals of myelinated fibers rather than true longitudinal elements. The size of this collateral contingent may be defined more clearly by further quantitative analysis. (Supported by NS-21122 from NIH, NINCDS).

418.11 AN AUTORADIOGRAPHIC STUDY OF ASCENDING PROJECTIONS FROM THE PONTINE AND MESENCEPHALIC RETICULAR FORMATION IN THE RAT. R.P. Vertes, R. Waltzer* and G.F. Martin. Mercer University, Sch. of Medicine, Macon, GA 31207 and Dept. of Anatomy, The Ohio State University, School of Medicine, Columbus, Ohio 43210.

Twenty-five rats received injections of tritiated leucine at various sites within the pontine and mesencephalic reticular formation (PRF and MRF). After a post-injection survival period of 12-14 days, the rats were killed, their brains removed and processed according to previously described autoradiographic techniques (Martin et al., JCN 187:373, 1979). The present analysis describes projections from the reticular nuclei, pontis caudalis (RPC), pontis oralis (RPO) and the mesencephalic RF.

The majority of labeled fibers from RPC ascended through the heart of the brainstem tegmentum within Forel's tractus fasciculorum (FTF) giving off profuse projections to the PRF and MRF in route. Terminal labeling within the pons was found in the lateral parabrachial nucleus, the pontine central gray (CG) and the pontine nucleus. In the midbrain labeled fibers coursed dorsally from FTF to innervate intermediate layers of the superior colliculus (SC) and the anterior and medial pretectal nuclei; and ventrally to innervate the retrorubral area (RR), VTA and the lateral mammillary nucleus. At the caudal diencephalon labeled RPC fibers divided into distinct dorsal and ventral bundles. The dorsal bundle distributed primarily to thalamic intralaminar nuclei and the ventral bundle to zona incerta (ZI), fields of Forel, the lateral hypothalamic-lateral preoptic areas (LH-LPO) and lightly to the lateral geniculate nucleus (LGN). All of these projections were bilateral. Labeled RPO fibers also ascended within Forel's bundle and distributed to many of the same sites which received axons from RPC. These included the MRF, the pontine and midbrain CG, RR, SC, the pretectal region, LGN, intralaminar thalamic nuclei, VTA, LH-LPO and zona incerta. In addition, labeled RPO fibers innervated substantia nigra pars compacta, the medial mammillary nucleus, the supramammillary region as well as the rhomboid (RH) and laterodorsal nuclei of the thalamus. Very heavily labeled areas included the anterior pretectal nucleus, SC, ZI, LGN and the central lateral intralaminar nucleus. RPO projections were strongest ipsilaterally. The bulk of labeled MRF fibers joined the FTF. The diencephalic distribution of MRF fibers was widespread. Labeled thalamic nuclei included the parafascicular, central lateral, central medial, paracentral, ventromedial, LGN, RH, reuniens as well as the thalamic reticular nucleus. The lateral, dorsal and anterior nuclei of the hypothalamus were moderately labeled and the SC was heavily labeled.

Major termination sites for PRF and MRF fibers included preoculomotor nuclei and diencephalic nuclei with widespread cortical and subcortical projections. Supported by NSF Grants BNS-8403544 to RPV and BNS-8309245 to GFM.

418.12 THE NEURONS OF THE INFERIOR OLIVE IN GUINEA PIG. B.E.Peterson* and R.E.Foster. (SPON: S. McMaster) U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010

The inferior olive (IO) of mammals has been divided into three primary subdivisions: The principal olive (PO), the medial accessory olive (MAO), and the dorsal accessory olive (DAO). The neurons in these three subdivisions are among the more unique cells in the brain in both their neurophysiological (Benardo and Foster, Neurosci. Absts. 11:1027) and morphological properties (Ramon y Cajal, 1909). We have studied the neurons of the inferior olive in adult guinea pigs using conventional Nissl-stain methods and a modified Golgi-Kopsch procedure. From the Nissl-stained preparations, the cytoarchitectonic characteristics of three common subdivisions were readily identified. We observed the PO to have darker staining cells than the MAO and DAO and to be the most densely populated with cells (28 - 55 x $10^3/mm^3$). In contrast, the packing density of cells in the MAO and DAO was in the range of 16 - 30 x $10^3/mm^3$. Further, it was observed that the somata of the neurons in all three subdivisions are oblique-spheroid in profile with the long cellular axis directed in the coronal plane (mean size: coronal= 18.3 µm, parasagittal= 15.8µm). Based on a survey of the Golgi material, it was apparent that the IO cells were of two unique radiate-cell types (I and II). Three dimensional computer-aided analyses of the dendrites of IO cells indicated that there was little difference among cell types when the parameters of dendritic length and surface area were calculated. However, the measures of the branching index and dendritic arbor volume (the smallest box that just circumscribes the dendritic arbor) were quite helpful in distinguishing cell types. A type I neuron had a relatively diffuse, unbranched dendritic arbor which occupied a volume of .13 mm^3. Type II cells, on the other hand, had dendrites which were highly branched and massed about the cell body, at times creating complex spirals. The spirals appeared to be of appropirate dimension to be encircling the soma of nearby neurons, perhaps making contact thru gap junctions. The compact dendritic fields occupied a volume of .007 mm^3. Within the type II category of IO neurons, there was a variation in dendritic arborization such that the cells were further divided into types IIa and IIb. Type IIb cells - transitional between type I and type IIa cells- had the high branching index of a type IIa cell, but dendritic volume measures similar to type I neurons. Finally, the cell types were differentially distributed across the subdivisions with type I neurons being encountered in the MAO and DAO, while type II cells were found in all three subdivisions. Thus, in comparison to previous descriptions of IO neurons in other mammals, these data suggested that both IO cell types and their distribution within the subdivisions are highly conserved across placental mammals.

418.13 THE VISCEROTOPIC ORGANIZATION OF THE NUCLEUS AMBIGUUS IN THE RAT
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The nucleus ambiguus (NA) has been reported to innervate both branchiomeric musculature and viscera including the pharynx, larynx, oesophagus, heart and stomach in the rat and other species. However, the literature is contradictory or confusing as to how different regions of the NA innervate specific structures or organs. Therefore, a systematic investigation of the viscerotopic organization of the NA was undertaken. In 70 rats 0.5-10.0 µl of HRP, WGA-HRP, cholera toxin-HRP or fluorescent tracers were injected into the IXth, Xth and XIth cranial nerves and their major branches as well as the organs supplied by these nerves. Serial sections in one of three planes were processed for HRP histochemistry (Mesulam, 1978).

The results demonstrate that the NA comprises a number of distinct subdivisions each of which innervates a specific organ or group of muscles. The compact formation of the NA or the so-called retrofacial nucleus, located dorsally and immediately caudal to the facial nucleus, contains closely packed fusiform cells with longitudinally oriented dendrites which remain largely within the boundary of this part of the nucleus. These cells innervate the oesophagus according to a crude rostrocaudal topography, such that neurons projecting to the proximal oesophagus via the superior laryngeal nerve form a cone around the rostral pole of the compact formation. Labeling previously reported in this subdivision after tracer injections into the stomach or heart can be attributed to spread to the oesophagus. Contiguous and caudoventral to the compact formation is a cylindrical column of neurons whose dendrites radiate ventrolaterally. This division constitutes the main source of pharyngeal motoneurons. The caudal aspect of the NA consists of 4-5 clusters of large motoneurons which innervate the voice box except for the cricothyroid muscle. The motoneurons supplying this muscle overlap with the rostral pharyngeal representation. Vagal preganglionic neurons projecting to the heart are located within an aggregate of loosely packed neurons occupying the ventral medullary tegmentum caudal to the facial nucleus and ventrolateral to the oesophageal, pharyngeal and laryngeal cell columns.

In conclusion, the results suggest that the NA innervates striated muscle groups prominently involved in swallowing and that its viscerotopic organization may hold clues to the functional organization of the deglutitive pattern generator.

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418.14 AFFERENT PROJECTIONS FROM THE UPPER ALIMENTARY TRACT IN THE RAT.
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It is well recognized that the nucleus of the solitary tract (NTS) is the principal recipient of topographically organized sensory inputs from thoracic and abdominal viscera. However, there is a dearth of anatomical information on the central termination of afferent fibers from the pharynx and oesophagus. In order to determine the distribution of such afferents, HRP and WGA-HRP were injected in 20 rats into the muscular tunics of the upper alimentary tract or cranial nerves supplying the tract. Following survival times of 1 to 2 days, brains were processed according to the method of Mesulam (1978).

Injections of tracer into the tunica propria of the cervical, thoracic or abdominal oesophagus resulted in dense anterograde labeling bilaterally of a cytoarchitectonically distinct subdivision of the NTS, irrespective of the oesophageal level. After pharyngeal injections anterograde label was observed in two distinct regions of the NTS and in the paratrigeminal islands (PTI). Terminal fields of pharyngo-oesophageal and gastric projections to the NTS displayed a lateromedial disposition with minimal overlap. Comparisons of afferent labeling after nerve versus muscle injections revealed that afferents from pharynx and oesophagus reach the NTS via the superior laryngeal, cervical vagus and glossopharyngeal nerves whereas pharyngeal afferents to the PTI were largely, if not exclusively, connected with the glossopharyngeal nerve. Transection of the latter nerve markedly reduced staining of the acid phosphatase-positive neuropil within the ipsilateral PTI. The pharyngeal branch of the vagus nerve was found to contribute a small projection to an area of the NTS receiving a heavier projection from the glossopharyngeal nerve.

We conclude: (i) there is a discernible viscerotopic segregation of pharyngeal, oesophageal and gastric afferents to the NTS; (ii) oesophageal afferents terminate within a subnucleus which in turn projects to oesophagomotor neurons of the compact formation of the nucleus ambiguus; (iii) fibres terminating in the PTI may represent nociceptive visceral afferents originating in the rostralmost alimentary tract.

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418.15 BARORECEPTORS AND SYMPATHETIC AFFERENTS ARE NOT REQUIRED FOR MEDULLARY INHIBITION OF MUSCLE TONE OR ITS REVERSAL BY BLOOD PRESSURE REDUCTION.
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Stimulation of the medial medullary reticular formation (MMRF) produces atonia of the antigravity muscles in decerebrate animals. Activation of this area is believed to be responsible for muscle atonia during REM sleep. We have found that MMRF induced muscle atonia can be inhibited or reversed by treatments which lower blood pressure (BP), including administration of hypotensive drugs, hemorrhage, and obstruction of the inferior vena cava. In the present experiment, we have investigated the role of baroreceptors, and sympathetic afferents originating below the cervical level, in MMRF induced inhibition.

The experiments were performed on precollicular decerebrate, unanesthetized cats. Bipolar electrodes were implanted into the splenius, occipitoscapularis, and biventer cervicis muscles bilaterally. A stainless steel monopolar microelectrode was placed in the medial medulla at P 8 to 14, L 1, and H -7 to -9. MMRF stimulation was delivered in 300 msec trains of 0.2 msec rectangular cathodal pulses at 100 Hz, with current intensity between 20 and 50 µA. Electromyograms were recorded and digitized for computer analysis.

Stimulation of the MMRF produced bilateral inhibition of muscle tone. During BP reduction, identical stimulation parameters produced no change or increased muscle tone, as we have previously reported. In three preparations both common carotid arteries, the internal and external carotid arteries the pharyngeal arteries and the Hering nerves were tied off to isolate the carotid sinus. MMRF induced inhibition and its reversal by BP reduction were unaffected. The addition of bilateral vagotomy did not interfere with either phenomenon. In two preparations, spinal transection at the cervico-thoracic junction combined with ligation of the common carotids and bilateral vagotomy was performed. This also did not alter MMRF induced inhibition or its reversal by blood pressure reduction.

These results lead us to hypothesize that the control of MMRF induced inhibition by BP does not require either peripheral baroreceptors or sympathetic afferents and may instead be mediated by direct effects of blood flow variations on the brainstem. Since we have found that large increases in heart rate precede cataplexy, the sudden loss of muscle tone characteristic of the narcolepsy syndrome, variations in brainstem blood flow may also be a factor in triggering and blocking these episodes (Sleep, 9:216-221, 1986).

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419.1 MEAL PATTERNS OF OBESE AND LEAN ZUCKER RATS TREATED WITH EOS, A GABA-T INHIBITOR. T. W. Castonguay*, D. V. Coscina, and J. S. Stern. Food Intake Laboratory and Nutrition Dept., U. C. Davis, Davis, CA 95616 and Section of Biopsychology, Clarke Institute of Psychiatry, Univ. of Toronto, Toronto, Ontario, Canada M5T 1R8.

Brain gamma-aminobutyric acid (GABA) plays an important, yet not thoroughly understood, role in the control of food intake and body weight. GABA appears to serve an important inhibitory function in ingestive behaviors. The GABA transaminase inhibitor ethanolamine-O-sulfate (EOS) reliably suppresses intake for several days in both normal and hyperphagic rats when injected intracisternally. Unlike rats given access to highly palatable foods, or hypothalamically lesioned rats, obese Zucker rats are refractory to EOS treatment. The present study set out to describe the feeding patterns of young, female genetically obese (and hyperphagic) Zucker rats treated with one of three doses (0, 50 µg, and 100 µg EOS). Feeding patterns of obese animals were compared to similarly treated lean female Zucker rat littermates.

After an 8 day baseline period, both obese and lean rats were lightly anesthetized with Metofane (methoxyflurane) and given an intracisternal injection of 100 µg EOS dissolved in 20 µl saline. Six days later, the same rats were injected with 50 µg EOS. Finally, 10 days later, each rat was injected with the 0 µg dose. Results from these trials indicated that EOS treatment was effective in suppressing the food intake of both lean and obese rats. The duration of the suppression was dose related, with the 100 µg dose suppressing food intake for 3 days, the 50 µg dose suppressing food intake for 1 day, and the 0 µg dose failing to suppress intake at all. Obese rats given the 100 µg dose decreased intake from an average of 24.4 g/day to 18.1 g/day. By contrast, lean rats given the 100 µg dose decreased intake from an average of 18.1 g/day to 10.5 g/day. Similarly, the 50 µg dose reduced the intake of the obese rats from 24.5 g/day to 20.5 g/day. Lean rats similarly treated reduced their intake from 16.3 g/day to 9.4 g/day. Quantification of the suppression in terms of meal frequency and size will be presented, as will neurochemical analyses confirming the efficacy of EOS treatment. Results will be discussed in terms of their potential use in the treatment of obesity.

419.2 THE PYRAZOLOQUINOLINE CGS 8216, A PUTATIVE BENZODIAZEPINE RECEPTOR INVERSE AGONIST: SELECTIVE EFFECTS ON INGESTION IN THE RAT. S.J. Cooper, L.B. Estall* and T.C. Kirkham*, Department of Psychology, University of Birmingham, Birmingham B15 2TT, U.K.

Since benzodiazepine receptor (BZR) agonists (e.g. diazepam, chlordiazepoxide) enhance the consumption of food in many mammalian species, we expected that BZR ligands which act as 'inverse agonists' (Polc, P. et al, N-S Arch. Pharmacol., 321: 260, 1982) should produce an opposite effect on food intake. The beta-carboline FG 7142 (N'-methyl-β-carboline-3-carboxamide) was shown to reduce feeding, but it also had an antidipsogenic effect (Cooper, S.J. and Estall, L.B., Neurosci. Biobehav. Rev., 9:5, 1985). We have now investigated the extent to which the pyrazoloquinoline CGS 8216 (2-phenylpyrazolo-(4,3-c)-quinoline-3(5H)-one), a weak BZR inverse agonist, has more selective effects on ingestional responses in the male rat.

In an extensive series of experiments, our results to date show that CGS 8216, administered intraperitoneally in doses up to 40 mg/kg, reduced the consumption of (i) palatable sweetened and oily mashes by nondeprived rats; (ii) milk and sucrose solutions by nondeprived rats; (iii) a 30% sucrose solution in sham-feeding rats with a gastric fistula; (iv) had only a modest effect on the consumption of standard lab. chow by food-deprived, water-deprived and nondeprived rats; (v) had little or no effect on the consumption of water or a 0.9% NaCl solution by water-deprived rats. The anorectic effect of CGS 8216 in nondeprived rats consuming a milk solution or in sham-feeding rats consuming a 30% sucrose solution was reversed by the BZR antagonist flumazepil (Ro15-1788). This indicates that the effect was most likely mediated at BZRs.

It has been shown that both FG 7142 and CGS 8216 increase plasma corticosterone levels in the rat. We found that adrenalectomy had no effect on the anorectic effect of either compound in nondeprived rats fed a palatable sweet mash. Furthermore, a detailed behavioural analysis using a time-sampling procedure showed that CGS 8216 (in doses up to 40 mg/kg) had no general behaviourally-disruptive effects. We conclude that the anorectic effect of CGS 8216 is not secondary to an increase in plasma corticosterone level or to behavioural impairment. The pyrazoloquinoline CGS 8216 appears therefore to have selective effects on ingestion, and may prove important as an anorectic agent which attenuates feeding responses to palatable foods.

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419.3 SEROTONIN TURNOVER IS ALTERED IN LIMBIC AND BRAIN STEM STRUCTURES AFTER INGESTION OF IMBALANCED AMINO ACID DIETS. D.W. Gietzen, M. Malek*, P.M.B. Leung* and Q.R. Rogers: Dept. Physiol. Sci. and Food Intake Lab. Univ. Calif. Davis, CA 95616.

Animals will reliably reduce their food intake if given a diet containing disproportionate amounts of amino acids. The brain has been shown to be involved in this response. Specifically, lesions of several limbic brain areas, prepyriform cortex (PPC), anterior cingulate cortex (AC), hippocampus (HIP), septum (SEP) and amygdala (AMYG) alter either the initial or adaptive phase of rat's responses to an imbalanced amino acid diet (IMB). Pharmacological manipulations have implicated serotonin (5HT) in the adaptive phase of these responses. In order to investigate further the role of 5HT in this phenomenon, we estimated 5HT turnover using the ratio of the 5HT metabolite, 5-hydroxyindole acetic acid (5HIAA), to 5HT after HPLC with electrochemical detection. Samples were taken as micropunches or dissected from 14 discrete brain areas of rats 3.5 hr after presentation of a mild imbalanced diet, limited in isoleucine (ILE-IMB), which was before the onset of the feeding depression, or 2.5 hr after threonine IMB (THR-IMB), a more severly imbalanced diet, at which time the food intake of the IMB group was significantly depressed. A parallel group fed ILE-IMB decreased their food intake by 6 hr. In the ILE-IMB group, killed before the onset of a significant decrease in food intake, 5HT turnover was increased to 155% of that in basal diet controls (BAS) in raphe, 153% in the nucleus of the solitary tract (NTS), 218% in LC, and 140% in HIP, and decreased to 71% in PPC. In the group fed THR-IMB, increased 5HT turnover was also seen: 133% of BAS in the PPC, 131 % in AC and 186% in the locus ceruleus (LC), with a decrease to 48% of BAS in SEP. Thus, in PPC, an area associated with the initial phase of the feeding response, 5HT turnover was reduced before the depression of feeding and increased afterwards. Lesions of medial and dorsal raphe nuclei have not affected the responses to IMB, but both SEP and HIP have been implicated in the adaptive phase. Parallel increases in 5HT turnover in the LC with both diets are interesting, since we have recently reported that concentrations of norepinephrine (but not 5HT) were decreased in PPC from rats fed both THR- and ILE-IMB. However, in NTS, LC and AC, values for the corrected diet control group were also increased relative to BAS. 5HT turnover was not altered in any of 7 other brain areas tested, including 3 hypothalamic areas and a section containing several amygdaloid nuclei. We conclude that alterations in the serotonergic activity of some limbic and brain stem structures may play a role in the rat's responses to IMB.

(Supported by USPHS-NIH Grants AM013252, AM07355 and AM07557)

419.4 INTAKE OF PROTEIN-FREE AND AMINO ACID DIETS BY RATS GIVEN INTRACEREBROVENTRICULAR INJECTIONS OF A THREONINE-DEVOID AMINO ACID MIXTURE. W.J. Hartman* and Q.R. Rogers. Food Intake Laboratory and Dept. Physiol. Sci., Univ. Calif., Davis, CA 95616.

Rats decrease intake of a diet devoid of threonine (thr) in 15 to 30 min or imbalanced with respect to thr by 2 h. The brain has been implicated as the critical site where the amino acid effect is sensed by the animal. This experiment was performed to examine the effect of a localized state of amino acid disproportion in the brain on food intake.

Eight male Sprague-Dawley rats were housed in meal pattern modules and trained to a 12:12 h light-dark cycle and 6 h daily feeding of thr basal (control) diet containing 12% amino acids. Chronic cannulas (22 ga) were implanted in the right lateral ventricle for daily injection (10 µl) 5 min before the feeding period. Each rat received injection of artificial cerebrospinal fluid (ACSF) on 6 non-consecutive days, a mixture of 12 amino acids (310 mM) (2 days) or the same mixture without thr (277 mM) (2 days). On test days protein-free (amino acid-free) diet was given for 1 h after icv injection and thr basal diet was fed for the remaining 5 h of the feeding period. Fifteen min cumulative food intakes were 3.7, 3.7, and 3.6 g ($p > 0.10$) following injections of ACSF, the complete and the thr-devoid amino acid mixtures, respectively. Intake of thr basal diet (after ACSF injection) was 4.0 g at 15 min. Injection of complete or thr-devoid amino acid mixture did not affect 30 or 60 min intake of protein-free diet ($p > 0.10$). Six hour food intakes after amino acid injection did not differ from those after ACSF injection, without injection or with control diet alone ($p > 0.10$). Within 5 days after the feeding experiment cisternal CSF was collected 7 min after injections of the amino acid mixtures. Thr concentrations in CSF were 103 to 1065 nmoles/ml following injection of the complete mixture and 21 to 69 nmoles/ml after injection of the thr-devoid mixture. CSF concentrations of the other amino acids present in the mixture were elevated 5 - 100 times normal by the injections. The elevated concentrations were similar after injection of the complete or the thr-devoid mixture ($p > 0.10$).

These results demonstrate that rats were able to tolerate an acute marked alteration from the normal proportions of CSF amino acid concentrations without decreasing food intake. Reduction of CSF and brain thr concentrations which have been associated with the depressed food intake response to thr deficient diets may be more important than marked disproportionalities among CSF or brain amino acid concentrations in affecting food intake. (Supported in part by NIH Grants AM13252 and AM07355.)

419.5 SWALLOWING DURING ONGOING INGESTIVE BEHAVIOR. J.M. Kaplan* and H.J. Grill (SPON. F.W. Flynn). Department of Psychology and Institute for Neurological Sciences, University of Pennsylvania.

Surprisingly little attention has been paid to the repetition of the discrete swallowing action during ongoing ingestive behavior in intact, unanesthetized rats. The swallowing pattern is a causal factor in the modulation of ingestion rate during mealtaking (ingestion rate = swallow frequency x average swallow volume). In addition, swallowing and other oromotor actions (licking, chewing, etc.) must be coordinated for efficient and uninterrupted ingestive behavior. We examined the oromotor and pharyngeal activity of non-deprived adult rats receiving controlled intraoral infusions of 0.1 M sucrose solutions delivered at 0.5, 1.0, or 1.5 ml/min. At least 1 week prior to testing, intraoral cannulae were inserted and two fine wire electrodes were implanted in the inferior pharyngeal constrictor muscle for chronic EMG recording. During test sessions, a video frame-splitter was used to simultaneously record the rat's oromotor behavior and an oscilloscope displaying the EMG acitivity associated with swallowing.

Intraoral infusions of the sucrose solution were actively ingested by all subjects. Mean swallowing frequency increased across infusion rates (21.48, 31.86 and 40.70 swallows per min, respectively, for the 0.5, 1.0 and 1.5 ml/min infusions). Average swallow volumes (= infusion rate/swallow frequency) also increased demonstrating that adjustment of both swallowing parameters combine to accomodate changes in infusion rate. The results of our intraoral infusion study agree closely with those obtained in a recent study of swallowing during voluntary drinking (Weijnen et al, 1984). In that study, thirsty rats were trained to lick at a spout which delivered water at different rates. In each study, swallow frequency and swallow volumes covaried across fluid delivery rates. Also, absolute values for these two swallowing parameters were in agreement across conditions of the two experiments.

Sucrose infusion also produces rhythmic movements of the jaw and tongue with an average interval between movements of approximately 160 msec. The inter-movement intervals associated with the onset of swallow-related EMG activity were lengthened by 20 msec or more. It is interesting that inter-lick intervals associated with swallow onset during voluntary drinking (Weijnen et al, 1984) were also prolonged by about 20 msec (both types of movements are emitted at about the same frequency).

We conclude that the intraoral infusion methodology provides a high degree of stimulus control while producing oropharyngeal behaviors typical of voluntary fluid ingestion.

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419.6 EFFECTS OF BRAIN NOREPINEPHRINE DEPLETION ON BURN-INDUCED CHANGES IN FEEDING AND METABOLISM. J.L. Nelson, W.T. Chance, T. Foley-Nelson, M.W. Kim* and J.E. Fischer*. Dept. of Surgery, Univ. of Cincinnati, Cincinnati, OH 45267.

Although hypermetabolism and weight loss has been associated with increased norepinephrine (NE) levels in major burn trauma, the effects of direct alteration of brain NE on these parameters have not been investigated. Therefore, brain NE was specifically depleted in 18 rats (297±2 g) by bilateral injections (30 ug/2 ul) of the neurotoxin, 6-hydroxydopamine (6-OHDA) into the locus coeruleus. Control (C) rats (n=18) were subjected to similar procedures, but no injections were administered. Two weeks later 12 of the 6-OHDA and 11 of the C rats were anesthesized (50 mg/kg, sodium pentobarbital) and subjected to a 30% body surface area full-thickness open flame burn (25 sec). This treatment produces a full-thickness burn, destroying the free nerve endings that mediate pain from cutaneous sources. Thus, the animals experienced minimal pain after the burn trauma. Only half of the 6-OHDA rats survived the burn, while only 1 of the nontreated rats died shortly after the burn trauma. Both groups of burned (B) rats exhibited a short period (3 days) of anorexia, that changed to hyperphagia by day 11 post burn. Thirty min assessment of resting energy expenditure on postburn days 10 and 17 revealed increased metabolic rates for both 6-OHDA-B and C-B groups, as compared to nonburned controls. No significant difference in metabolic rate was found between the 6-OHDA-B and C-B groups. However, the 6-OHDA-B rats gained body weight at a significantly ($p<0.01$) increased rate compared to C-B rats across the 28 days of postburn measurement, suggesting a decrease in hypermetabolism that was not observed in 30 min assessment of resting energy expenditure. As presented in the following table, assessment of amine neurotransmitter levels (ng/g) by HPLC on postburn day 28 revealed increased NE levels in the hypothalamus and nucleus accumbens of C-B rats, which were severely and selectively depleted by the 6-OHDA treatments.

	Hypothalamus			Nucleus Accumbens		
Group	NE	Dopamine	Serotonin	NE	Dopamine	Serotonin
C-Non B	1715±80	441±28	1220±121	516±46	5538±304	1302±79
6-OHDA-Non B	262±63*	479±30	1145±41	80±13*	4384±287	1208±161
C-B	2131±109*	460±22	1149±12	652±62	4627±223	1329±58
6-OHDA-B	332±28*	501±121	1222±121	54±8*	5072±536	1301±79

*$p<0.01$ vs C-Non B

Since half of the NE-depleted rats died shortly after the burn treatment, maintenance of normal NE levels is important for acute survival of burn trauma. However, reduction of elevated brain NE concentrations in B rats appears to reduce catabolism and may reduce hypermetabolism, allowing these rats to gain weight at faster rates.

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419.7 REGULATION OF BODY WEIGHT BY MONOAMINERGIC MECHANISMS IN THE RAT NTS. S.E. Spencer, P.J. Gordon,* A.F. Sved, W.T. Talman, Department of Neurology and Digestive Disease Center, University of Iowa, and V.A. Medical Center, Iowa City, IA 52242, and V.A. Medical Center, East Orange, NJ 07019.

Previous studies have suggested that the caudal medial nucleus tractus solitarius (cmNTS) is important in the regulation of body weight, but the chemical mechanisms of this regulation are not known. In this study, we sought to determine the effects on weight gain when noradrenergic and serotonergic mechanisms in the cmNTS were disrupted. Twenty-five adult male Sprague-Dawley rats were individually housed in an environmentally controlled room and weight gain (5-7 gm/d/rat) was monitored for 7-10 days. Five groups matched for weight were studied. Rats were anesthetized (halothane) and placed in a stereotaxic frame. Glass pipettes (O.D. 50 µm) were stereotaxically placed into the cmNTS through a partial occipital craniotomy and 0.5 µL of either 5,7 dihydroxytryptamine (5,7 DHT) (6 µg), 6 hydroxydopamine (6 OHDA) (2 µg) or vehicle (0.01% ascorbic acid) was instilled into each side of the cmNTS. In 5 rats, 5,7 DHT (6 µg/0.5 µl) was instilled into the lateral cuneate nuclei bilaterally. Each dose was given over 10 min and the pipette remained in place for 5 minutes after infusion. All rats receiving 5,7 DHT and their sham controls were pretreated with desmethylimipramine (DMI) (25 mg/kg I.P.) 30 minutes before central microinjections. The scalp was closed and animals returned to their home cages. At 7 and 14 days post-operatively, the mean gram weight change was -16.4 ± 5.0 (% ± SEM) and +19.2 ± 2.2 for rats receiving vehicle only, -18.8 ± 5.4 and +16.0 ± 5.1 for rats with vehicle and DMI pretreatment, -25.6 ± 5.4 and +5.4 ± 4.4 for 6 OHDA rats, -44.6 ± 6.3 and -3.8 ± 4.2 for rats with 5,7 DHT into the cmNTS, and -14.6 ± 5.6 and +20.8 ± 4.2 for rats with 5,7 DHT into the lateral cuneate nuclei. The 15-20 gm post-operative weight difference between 5,7 DHT and control groups persisted for 6 weeks of observation. Histologic evaluation of the brain stem of control and experimental animals showed no difference in the degree of tissue damage. Biochemical analysis of the cmNTS 42 days after injection of 5,7 DHT showed a 70% decrease in NTS serotonin content versus control (pg 5HT/µg protein). Previous studies indicate that 6 OHDA (4 µg total) in the NTS reduces noradrenaline by 60%. (Snyder, D.W., Circ Res 43:662, 1978). We conclude that bilateral injections of 5,7 DHT or 6 OHDA into cmNTS can elicit significant weight loss and that serotonin and noradrenaline may be involved in regulation of body weight by the NTS.

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419.8 Cholinergic and Alpha-Adrenergic Interaction With Glucagon in the Reduction of Food Ingestion. D.A. VanderWeele, D.R. Talbert* and B.L. Macrum* Dept. of Psychology, Occidental College, Los Angeles, CA 90041.

Pancreatic glucagon has been observed to reduce food intake in a number of species and feeding situations. Glucagon also possesses an ability to stimulate the release of pancreatic insulin and insulin has recently been shown to be capable of producing a suppression of food intake. The present experiments assessed the interaction of insulin secretion during exogenous glucagon administration in affecting food intake by either stimulating (alpha-adrenergic) or reducing insulin release (cholinergic).

Rats were adapted to 4-hr fasts beginning at light onset (0800). At 1150 separate groups of subjects (minimum of 6 per group) were given injections of saline, atropine methyl nitrate (125 ug/kg body weight), or phentolamine (300 or 500 ug/kg). Ten minutes later, separate groups received glucagon (400 ug/kg dissolved in DMSO) or DMSO alone such that the following groups of animals were tested: saline/glucagon, atropine/glucagon, phentolamine/glucagon, saline/DMSO, atropine/DMSO and phentolamine/DMSO. Food was immediately returned following the second injection and animals were allowed to feed for one hr, during which measurements of intake were recorded.

Glucagon by itself significantly reduced food intake in the hr following its injection by 32 to 42%. When glucagon was combined with a pretreatment of atropine, a nonsignificant reduction in food ingestion occurred (0 to 29%). When glucagon was combined with a pretreatment of phentolamine, significant reductions of 70 to 80% of ingestion were produced. When administered alone, atropine produced a reduction of 14 to 28% and phentolamine a reduction of 43 to 68%, neither result significant because of considerable within-group variability.

Taken together these results are consistent with the interpretation that raising the insulin levels contiguously with exogenous glucagon injection enhances the ability of glucagon to reduce eating (phentolamine) while reducing insulin release (atropine) renders glucagon less effective in reducing ingestion. It is certainly possible that other variables can contribute to the phentolamine effect (aversive conditions) and a taste aversion paradigm is currently under use to assess this possibility.

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419.9 DIURNAL VARIATIONS IN HYPOTHALAMIC MET-ENKEPHALIN CONCENTRATIONS DURING A MEAL. C.L. McLaughlin, C.A. Baile and M.A. Della-Fera . Washington Univ. Med. School, St. Louis, MO 63110.

Evidence suggests that hypothalamic met-enkephalin concentrations vary with the fed/fasted and obese/lean conditions of rats. The present experiment was designed to investigate the influence of the diurnal variation on met-enkephalin concentration changes with feeding. Groups of 9 Sprague-Dawley male rats initially weighing (255+3 g) were food-deprived during the 12-hr light or dark portion of the diurnal cycle, then sacrificed immediately or after a meal. Met-enkephalin concentrations were measured in extracts of the supraoptic nucleus (SON), paraventricular nucleus (PVN), and anterior (AH), ventromedial (VMH), dorsomedial (DMH) or lateral (LH) hypothalamus. Meal size in dark-deprived rats was 3 times that in light-deprived rats (15.9 vs 5.0 g, p<.01). Across fed/fasted conditions met-enkephalin concentrations were higher in light- than dark-deprived rats in the PVN (139 vs 110 pg/mg tissue, p<.05), AH (105 vs 89 pg/mg, p<.05) and VMH (185 vs 157 pg/mg, p<.01) and, across cycles, were higher in fasted than fed rats in the PVN (147 vs 103 pg/mg, p<.03). Analysis of a hunger x cycle interaction revealed that, after light deprivation, concentrations were higher in fasted than fed rats in the SON (100 vs 68 pg/mg, p<.01) and VMH (209 vs 161 pg/mg, p<.02) and concentrations were higher in fed than fasted rats in the SON (105 vs 59 pg/mg, p<.01). When meal size after dark deprivation was restricted to that after light deprivation, met-enkephalin concentrations were not different from those after a meal in light-deprived rats, except in the AH, where concentrations were more like those of fasted than fed rats (67 vs 68 pg/mg, NS). When the same estimated caloric load that the light-deprived rats consumed ad libitum was given by gavage in light-deprived rats, concentrations did not differ from those during an ad lib meal except in the VMH, where concentrations were more like those of fasted rats (216 vs 209 pg/mg tissue, NS). Thus, hypothalamic met-enkephalin concentrations change differentially with feeding within the hypothalamus and exhibit diurnal variation. Since met-enkephalin analogues stimulate feeding when admininstered in specific areas of the brain as well as the cerebrospinal fluid system, these findings provide further support for the role of opiate peptides in the control of food intake. Supported by grants from Monsanto and NIH 20000.

419.10 ETHANOL PREFERENCE IN ADULTHOOD IS AFFECTED BY BRIEF POSTNATAL EXPOSURE TO ETHANOL IN ARTIFICIALLY REARED RAT PUPS. B.S. Fisher* and W.C. Lynch. Department of Psychology, Montana State University, Bozeman, MT 59717.

Previous studies suggest that prenatal or early postnatal exposure to alcohol may predispose animals toward greater alcohol tolerance or preference later in life (e.g., Bond, N.W. and DiGuisto, E.L., Psychopharm 46:163, 1976; Reyes, E. et al., RSA Abst. 217:115, 1984). Due to numerous methodological problems, however, interpretation of these reports remains uncertain. In order to overcome problems of nutrition, cross-fostering stress, and uncertainty about alcohol dosage, we recently established the method of artificially rearing rat pups in isolation from postnatal maternal influences (Messer, M. et al. J. Nutrit 98:404, 1969 and Samson, H.H. and Diaz, J. In Fetal alcohol syndrome: Animal studies. CRC Press, 1982). Briefly, the method consists of implanting a chronic intragastric cannula in 4 day old rat pups that are then reared in individual incubator cups until weaning (postnatal day 18). During this period pups are fed by timed infusion of precisely controlled amounts of an artificial diet consisting of evaporated milk supplemented with vitamins and minerals.

A newly designed, inexpensive, syringe pump capable of feeding 10 animals simultaneously was developed in the course of these studies. Pumps include internal timing circuits for simple but accurate specification of the delivery period of each feed cycle. A Hurst LAS stepping motor provides precise control of formula volume pumped in each cycle. These pumps have proven highly reliable for the delivery of 0.2 to 10.0 ml of diet in each 23 hr period.

In preliminary studies, ten batches of Holtzman albino rat pups were studied. Each batch of 10 or 20 pups was treated with ethanol during all or part of the period from day 4-10 postnatally with doses in various batches ranging from 3 to 5% (v/v). During the preweaning period, growth rates were monitored and reflex tests were used to assess motor development and the acute effects of ethanol. At various times after weaning animals were tested using either single-bottle (intake) or two-bottle (preference) tests to determine the effects of early ethanol on later ethanol preference.

Growth rates were uneffected by ethanol. Reflex measures indicated wide variability in the acute response to ethanol with pups generally showing a depression of basic motor responses during the period of exposure and for 1 or 2 days longer at most. Subsequent alcohol intake and preference tests, nevertheless, suggested an enhanced ethanol tolerance/preference in previously exposed animals, lasting for a period of at least 3 months. Other findings included a highly significant effect of early ethanol on the development of cateracts and somewhat decreased brain/body weight ratios in pups 10 to 18 days of age.

419.11 ANOREXIA, WEIGHT LOSS, AND BRAIN SEROTONIN (5HT) IN OBESE RATS TREATED CHRONICALLY (OSMOTIC MINIPUMP) WITH LOW DOSES OF D-FENFLURAMINE. J.Carlton & N.E.Rowland Dept Psychol, Univ Florida, Gainesville FL 32611.

D-fenfluramine (FEN; Isomeride^{R}) is metabolized 6x more rapidly in rats than humans (approx. plasma half lives 3 vs 18 hr). Thus, single daily injections to rats may be a poor animal model of chronic FEN administration. In the present experiments, we examine the effects of chronic subcutaneous FEN administration by osmotic minipump (Alzet) to Sprague-Dawley (SD) rats.

Study A. 6mo old male rats (mean weight 600g) were infused with 0,3 or 6 mg FEN/kg/24hr for 6 days. Chow intake was suppressed by over 50% on the first day. This anorectic effect decreased across days, but was still significant after 6 days at the higher dose. The FEN-treated groups lost 5-10% body weight during the first 3 days, then maintained this weight loss.

Study B. 6mo old female rats (mean weight 400g) were infused with 0,3 or 6 mg FEN /kg/24hr for 6 days. Both doses of FEN produced a comparable suppression of chow intake throughout the 6 days, and marked weight loss during the first 3 days. (Half of the rats in this study were retired breeders, and the rest age-matched unbred controls: there was no difference between these groups.) Brain 5HT levels were unaltered by the 3 mg dose, but were 70% reduced by 6 mg/kg FEN.

Study C. 2mo old female rats were fed either chow or a "supermarket" diet consisting of sweetened condensed milk, cookies and chow for 11 wk. At the end of this time the chow groups weighed 396g and the supermarket groups 482g. Half of the rats in each group then were infused with FEN (3 mg/kg/24 hr for 4 wk), under the same dietary conditions. FEN treated rats initially lost weight, then tended to sustain this loss. The effect was greater in the supermarket group. The anorexia was modest, and was manifest against chow rather than sweetened milk. When the FEN was discontinued, body weights returned to normal within 1 week.

These studies show that 3 mg/kg/day FEN, a dose which in rats is comparable to the clinical dose of 0.5 mg/kg/day, can produce sustained weight loss. This seems to be more pronounced in older and/or obese females than in males or lean females. Further, this low dose is not associated with brain 5HT depletions.

Isomeride^{R} and financial support from Servier Labs

419.12 AUTORADIOGRAPHIC ANALYSIS OF THE NEURAL CORRELATES OF INGESTION AND BEHAVIORAL ACTIVATION IN 6-DAY-OLD RAT PUPS. L. M. Terry, C. B. Phifer and W. G. Hall. Department of Psychology, Duke University, Durham, NC 27706.

Infant rats deprived of food, water, maternal care, and the opportunity to suckle will ingest orally infused milk and become behaviorally active (locomote, probe, and roll) during ingestion. Ingestion depends on simple nutritional deprivation, while behavioral activation, which may be related to motivation and reward, requires both nutritional deprivation and maternal deprivation. We examined the effects of both deprivational states and ingestive stimulation on relative neural metabolic activity using [14-C] 2-deoxyglucose autoradiography.

At 5 days of age, pups were either: 1) deprived for 24 hr in a warm incubator (incubator-deprived, producing ingestion and activation), 2) placed with a non-lactating foster-mother (foster-deprived, producing ingestion alone), or 3) remained with their lactating mother (nondeprived, no ingestion or activation). At the end of the deprivation period, pups were placed in a warm test chamber and either fed milk through oral cannulas or not fed. 2-DG incorporation took place during the entire 1 hr ingestive test. At the conclusion of testing pups were sacrificed and brain sections were prepared by standard autoradiographic methods. Forty-eight frontal sections (olfactory bulbs through medulla) from each brain were matched to a set of reference sections. Using the Nissl-stained tissue as a guide, 140 neuroanatomical regions were identified (each on 3-10 sections) and their optical densities were measured. Measurements were transformed to Z-scores and averaged among brains within groups to provide an index of relative optical density for each region. These averages were used for between-group comparisons.

Nutritional deprivation alone or in combination with maternal deprivation caused changes in relative neural activity in hypothalamic and brainstem regions; the major differences in activity between the two deprivational states occurred in thalamic nuclei. Further, changes in neural activity associated with simple ingestion appeared in the basal ganglia and brainstem sensory and motor nuclei. For animals that were behaviorally activated in response to milk, neural activity increased in the nucleus of the horizontal limb of the diagonal band of Broca, and in certain thalamic, hypothalamic and brainstem nuclei. These results begin to reveal the complexity of the interactions between motivational states and appetitive behaviors in developing mammals.

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419.13 MODULATION OF GLUCAGON SATIETY BY PERIPRANDIAL EVENTS ASSOCIATED WITH PREVIOUS MEALS. S.C. Weatherford and S. Ritter. College of Veterinary Medicine, Washington State Univ., Pullman WA 99164.

The efficacy of glucagon (GL) as a satiety hormone appears to be subject to diurnal variation. Our results suggest that the diurnal distribution of spontaneous meals may contribute to this effect. We found that the satiety effect of GL is more consistent during the dark phase of the light cycle if animals are food deprived for 6-8 hr prior to the feeding test. However, during the light phase, GL suppresses feeding in nondeprived rats. These results, combined with the fact that rats normally eat frequent meals at night and fast spontaneously during the day, suggest that some consequence of recent food ingestion might modulate GL's effect on subsequent meals. We tested this hypothesis by allowing rats to eat two small meals of instant breakfast (5 ml) during a 7 hr fast, 4 and 6 hr prior to a feeding test conducted 1 hr after dark onset. We found that the satiety effect of GL was blocked by the ingestion of these two meals. We investigated possible periprandial events which might mediate this blockade. Insulin, GL, and CCK are gastrointestinal peptides released during a meal. Therefore, we gave insulin (100 mU/kg), GL (100 µg/kg), CCK (2 µg/kg) or saline in a counterbalanced design 6 hr prior to the GL test. Like the small meals, insulin and GL blocked GL's satiety effect, but CCK did not. Thus, food intake and its metabolic or endocrine consequences may modulate the efficacy of GL's satiety effect in tests conducted during the dark phase of the circadian light cycle.

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419.14 THE INTERACTION OF A FOOD PRELOAD AND HYPOTHALAMIC STIMULATION ON THE TIME COURSE OF CHOLECYSTOKININ-INDUCED SUPPRESSION OF FOOD INTAKE. *T. R. Maone and S. M. Feldman.* Department of Psychology, New York University, New York, NY 10003.

A discrete trials paradigm was used to investigate the time course of satiety caused by exogenously administered sulfated cholecystokinin octapeptide (CCK: 5-10 µg/kg *ip*) in rats. Daily feeding sessions consisted usually of 32 feeding bouts (*trials*), during which a dish of wet mash (3:2 w/w, water and rat chow) was available during delivery of 30-sec trains of electrical stimulation to the lateral hypothalamus (ESLH: 0.1 msec pulses @60 pps). The discrete trials paradigm revealed that the transition from pre-CCK ingestion rates to a state of virtually complete anorexia took place over at least one *transition* trial, which was *not* due to the pharmacokinetics of CCK (Maone *et al., Soc. Neurosci. Abstr.*, 10:301, 1984). The transition process was seen to occur during stimulation-induced feeding following changes in physiological state that had been caused by the introduction of exogenous CCK or brain stimulation (ESLH, *de novo* or via a contralateral feeding electrode). In addition, experience with ESLH-induced feeding within a session appeared to maximize the anorectic effect of subsequently administered CCK (Maone and Feldman, *Proc. Abstr. East. Psychol. Assn.*, 57:60, 1986).

We next investigated the separate influences of prior experience with brain stimulation and/or food, within a session, on the time course of CCK-induced anorexia, *i.e.*, on its onset (*transition*) and recovery. By varying the conditions of the initial 8 feeding trials, we established four different within-session "histories" of physiological states prior to injection of CCK or physiological saline: *food only* (no ESLH); *ESLH only* (no food); *food and ESLH*; *no food/no ESLH*. Results showed that a preload (*history*) of food prior to administration of CCK or saline had no effect on the initial rate of eating (*transition*) induced by ESLH. The effect of the preload was manifest only later in the session, retarding recovery from CCK, or accelerating the decrease in rate of intake following saline injection. By contrast, histories that included ESLH had a substantial attenuating effect on the initial rate of intake. When the preload was the result of ESLH-induced feeding, the effect of the load was evident throughout the feeding session, and the response to CCK was maximized.

We conclude that the initial introduction of ESLH within a session caused a motivational state that transiently overwhelmed satiety cues caused by a preload of food or exogenous CCK. It was only during the period of transition that the cues (presumably peripheral in origin) became centrally integrated to produce a change in behavior appropriate to the new physiological state. The combination of ESLH and food interacted with CCK to produce a maximum anorectic effect, probably due to the recruitment of central and peripheral satiety mechanisms.

419.15 EFFECTS OF GASTRIC LOADS ON SHAM FEEDING IN DOGS. T. J. Kalogeris*, R. D. Reidelberger*, T. E. Solomon*, and V. E. Mendel (SPON: P. H. S. Jen). Depts. of Medicine and Physiology, Univ. of Missouri Medical School and Truman V.A. Hosp., Columbia, MO 65201.

The stomach may play an important role in the short-term control of food intake. The importance of gastric factors -- distension, distension rate, and nutrient composition of food -- under different feeding conditions (e.g. length of prior food deprivation) in gastric-mediated satiety is unclear. To address these issues, we have developed an experimental model using dogs equipped with esophageal fistulas and gastroduodenal cannulas. The esophageal fistula can be closed or opened to allow normal or sham feeding. The gastroduodenal cannula permits either confinement of an ingested or infused load to the stomach or emptying of gastric contents into the duodenum. In preliminary studies, effects of gastric loads confined to the stomach on sham feeding of a liquified food (0.83 kcal/ml) were examined in dogs adapted to a single, daily 30-min feeding period. When ingested food drained from the esophageal fistula (sham feeding), food intake was 2 to 3 fold greater than that which occurred when ingested food entered the stomach. Gastric infusions of liquid food (250, 500, or 1000 ml) were administered during sham feeding at a rate similar to the dogs' feeding rate (200 ml/min). The 250 ml load had no effect on sham feeding; the 500 ml and 1000 ml loads decreased sham feeding by 37% and 78%, respectively. We then compared the effect of infusion of 750 ml of food versus 0.15 M NaCl on sham feeding. Both loads decreased meal size by 70%. These results suggest that gastric distension produces a dose-related satiety effect in dogs adapted to a single daily meal. (This work was supported by NIH training grant PHS AM07355 and the Medical Research Service of the Veterans Administration.)

420.1 CHOLINERGIC INPUT TO THE SUBFORNICAL ORGAN FROM THE LATERODORSAL TEGMENTAL NUCLEUS. R. Wallace Lind and Gwenda R. Gnadt*. Neural Systems Lab, Howard Hughes Medical Institute and Salk Institute, La Jolla, CA 92037.

For several years there has been interest in the source of an acetylcholine-containing input to the subfornical organ (SFO). The presence of acetylcholine was first indicated by the work of Lewis and Shute (1967) using cholinesterase histochemistry. The functional importance of this afferent connection was suggested by Simpson and Routtenberg's (1972) discovery that injection of a cholinergic agonist, carbachol, directly into the SFO elicits a short-latency drinking response. Mangiapane and Simpson (1979) demonstrated the independence of this effect from angiotensin-induced drinking, and extended the functions of acetylcholine in the SFO to include a pressor effect (1983).

The present work employed modern neuroanatomical techniques to determine the location of cholinergic neurons that send projections to the SFO. Injections of the retrogradely transported fluorescent dye, Fast Blue, into the SFO identified a number of putative neural inputs that have been described elsewhere. Of particular interest here was the identification of cells in the laterodorsal tegmental nucleus (LDT). Immunohistochemical staining with a monoclonal antibody to choline-acetyltransferase (kindly provided by Dr. Felix Eckenstein) indicated that many of the neurons in the LDT that send axons to the SFO contain the synthetic enzyme for acetylcholine. Injections of the anterogradely transported lectin PHA-L into the LDT reliably labeled fibers in peripheral parts of the SFO, where cholinesterase-positive fibers are concentrated. These techniques have not identified any other sources of cholinergic inputs to the SFO.

The LDT is thought to innervate parasympathetic pre-ganglionic neurons in the sacral cord, and recently it has been shown to give rise to broadly distributed forebrain projections. The present findings suggest a participation in central fluid balance regulation.

420.2 SYNAPTIC INPUT FROM ENKEPHALINERGIC AXONS TO GLUCORECEPTOR NEURONS IN THE HYPOTHALAMIC VENTROMEDIAL NUCLEUS(VMH). S. Inagaki, S. Kito, Y. Kubota*, M. Fukuda, T. Ono, M. Yamano* and M. Tohyama*, 3rd Dept. of Int. Med., Hiroshima Univ. Sch. of Med., Kasumi, Hiroshima, 734, Dept. of Physiol., Toyama Med. Parmaceu. Univ, Toyama, 930-01, Dept. of Neuroanatomy, Osaka Univ. Med. Sch., Kita-ku, Osaka, 530, Japan

A combination of intracellular injection of horseradish peroxidase (HRP) into the VMH glucoreceptor neurons and immunohistochemcial staining for enkephalin(ENK) allowed ultrastructural visualization of interrelations between such electrophysiologically identified neurons and ENK-immunoreactive(ENK-IR) terminals. Adult male rats were decapitated and about 500 - 700 μm thick coronal tissue slices were cut. To identify the glucoreceptor neurons, glucose was experimentally varied from 5.5 to 20 mM. After identification of glucoreceptor neurons by extracellular recording, HRP was injected intracellularly and processed for immunohistochemical procedures for ENK.

The effect of glucose level was tested on 114 VMH neurons in 18 slices. The spontaneous firing rate was 2.5 ± 1.7 spikes/s (mean ± SD, n=114). When glucose increased to 20 mM, 27 neurons (23.7%) responded, and the remaining 87 (77.3%) were unchanged. Of the 27 responding neurons, 16 (59.2%) increased their discharge rates upon glucose application and 7 (25.9%) showed an increase followed by a decrease. The discharge rates of four neurons (14.8%) decreased.

According to the description by Ono et al. glucoreceptor neurons were 10-20 um in diameter and multipolar with more than 3 primary dendrites ranging widely in the VMH and some projecting out of the VMH. With electron microscope glucoreceptor neurons had moderately developed cytoplasmic organs and an indented nucleus. They always received synaptic inputs from non-labeled terminals on their soma, and dendritic shafts and spines. A glucoreceptor neuron has a dendrite extending toward the third ventricle. This dendrite was observed to be closely apposed by 10 ENK-IR axonal boutons under light microscope. It was then determined by examining serial ultrathin sections that 7 of these sites received synaptic inputs directly from ENK-IR axonal boutons. This ultrastructural evidence well agrees with the direct excitatory effect of ENK or morphin on VMH glucoreceptor neurons.

420.3 ALTERATIONS IN REGULATORY BEHAVIORS INDUCED BY MEDIAL SEPTAL LESIONS AND SUPERIOR CERVICAL GANGLIONECTOMY. L.E.Harrell, D.S. Parsons*, A.Peagler* & T.S.Barlow*. Dept. Neurology, VAMC and Univ. Alabama Med. Ctr., Birmingham, Ala. 35294.

Studies from our lab suggest that peripheral sympathetic ingrowth following medial septal lesions (MS) is detrimental to recovery of performance in an appetitive spatial memory task but may allow subjects to regulate their body weights in a more normal manner. Body weight, food & water consumption were, therefore, studied under ad libitum and pharmacological stress conditions.

Thirty-four male rats were assigned to one of four surgical groups: control (CON), MS, superior cervical ganglionectomy (GX) or lesion + ganglionectomy (MSGX). Subjects were followed 8 days prior to and 38 days following surgery. Following this, pharmacological stressors (isoproterenol 25ug/kg, hypertonic NaCl (1M) 10ml/kg, 2-deoxy-D-glucose 500mg/kg, or epinephrine 120ug/kg; IP) were administered. Core temperatures were then measured.

Twenty-two animals completed the study: Con (n=7), MS (n=5), GX (n=6), MSGX (n=4). No differences were observed pre-operatively. Post-operatively, body weight fell following surgery, over time all groups gained weight. However, animals with MSGX were lighter than MS or GX animals (which were equivalent), who in turn were lighter than controls ($p<.0001$). Hypophagia was observed in the GX and MSGX animals when compared to the MS and CON groups ($p<.05$), while hyperdipsia was seen in the MS and GX groups ($p<.001$). Administration of both 1M NaCl and isoproterenol increased drinking in all animals ($p<.001$), with the MSGX group consuming significantly less than all others ($p<.025$). Food intake increased following 2-DG ($p<.0001$), while epinephrine treatment produced anorexia only in the MS group ($p<.05$). Hyperthermia was found in the GX and MSGX groups.

The results of this study suggest that both the MS region and SCG contribute to the maintenance of normal regulatory behaviors, with combined loss of these neural systems resulting in severe disturbances, both qualitatively and quantitatively different from either MS or GX. Although the MS group clearly regulated better than the MSGX group, it is unclear whether this is due to ingrowth or just the presence of the SCG.

420.4 EXTERNAL ACTIVATION OF INGESTIVE MOTIVATIONAL BEHAVIOR IN RATS WITH LATERAL HYPOTHALAMIC LESIONS. B. Roland, C.V. Grijalva, and N. Dess. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Lateral hypothalamic (LH) damage produces aphagia, anorexia, gastric pathology and sensorimotor dysfunctions (Grijalva & Lindholm, J. comp. physiol. Psychol. 94:164, 1980). Yet, behavioral deficits are generally not taken into account as contributing factors to the LH syndrome. Weingarten (Appetite 5:147, 1984) demonstrated the importance of behavioral cues in feeding regulation, in nonlesioned rats. He showed that the initiation of feeding is determined by both internal energy depletion cues and external food-related cues. Food intake was regulated within a 24 hour period, and conditioned cues (light and buzzer) had an influence on the initiation and distribution of meals throughout the day.

The present study was conducted to determine the effects of a preoperative food-related conditioned cue (buzzer) either alone or in combination with preoperative dieting on recovery of feeding in LH lesioned male rats. Prior to surgery one group was maintained at normal body weight by allowing them to eat two daily meals (10:30 a.m and 7:30 p.m.) preceded by a 4 min buzzer (Group NW, n = 6). A second group was dieted to 85% body weight over a 10 day period by giving rats access to one restricted meal, also preceded by the buzzer (Group D, n = 8). The third group was fed ad-lib and exposed to a random presentation of the buzzer (Group A, n = 7), and served as an unconditioned control group. Conditioning occurred over a 10 day period, after which all groups received bilateral anodal electrolytic LH lesions (1.2 ma/ 10 sec) under sodium pentobarbital anesthesia (50mg/kg, i.p.).

The results indicated that although the buzzer did not facilitate recovery of feeding, it did partially override the motivation/arousal deficit exhibited by LH damaged rats (Campbell & Baez, J. comp. physiol. Psychol. 87:142, 1974; Shoham & Teitelbaum, Physiol. Behav. 28: 323, 1982). Feeding was initiated in the presence of the buzzer by 83% of the NW rats, in contrast to 0% of the A rats. Rats in the dieted group either ate prior to presentation of the buzzer or showed little response to the buzzer. In addition, it was necessary to coax 50% of the NW rats to initiate feeding, while only 23% of the A rats needed the extra coaxing. This suggests that the preoperatively conditioned NW group may be relying on external food-related cues to initiate eating to a greater extent than the nonconditioned A group. The present results suggest that the rate of functional recovery of feeding behaviors in LH lesioned rats may be dependent on the extent to which motivational and behavioral processes are externally activated by food-related cues, and by the degree to which animals are affected by concomitant physiological disorders. (Supported by UCLA university research grant 3820).

420.5 EFFECTS OF β-CHLORNALTREXAMINE ON FOOD INTAKE. B.A. Gosnell and A.S. Levine. Dept. of Physiol., U. Texas Hlth. Sci. Ctr., Dallas, TX 75235 and V.A. Medical Ctr., Minneapolis, MN 55417.

β-chlornaltrexamine (β-CNA) is an ultralong-lasting opioid receptor antagonist which decreases the analgesic potency of morphine and inhibits the development of tolerance to morphine (Portoghese et al., J. Med. Chem. 22:168, 1979). Since opioid peptides and receptors are thought to contribute to the regulation of feeding, we have examined the effects of β-CNA on food intake, body weight and the responses to two drugs which stimulate intake.

All tests were performed on male Sprague-Dawley rats with cannulas implanted into the right lateral cerebral ventricle. In the first experiment, food intake was measured after rats were given intracerebroventricular (icv) injections of dynorphin A (DYN, 5 µg), neuropeptide Y (NPY, 5 µg) or saline. As expected, both DYN and NPY increased food intake. On the following day, a similar experiment was performed except that all rats were pre-treated with an icv injection of β-CNA (5 µg) 2 hrs before the injections of DYN, NPY or saline. In this trial, neither drug significantly increased food intake. When tested again in an identical manner on the next day (without further β-CNA treatment) DYN and NPY again stimulated intake. In another group of similarly treated rats, it was noted that 24-hr food intake after β-CNA injections was only 21-51% of the intake in the 24 hr period immediately prior to injections. During the second day after injections, intake was still 38-58% of pre-injection values. By the third day, intake had almost returned to normal. A corresponding drop in body weight was also observed.

Another experiment measured the effect of β-CNA on intake after 24 hr food deprivation. One group of rats received icv β-CNA injections (10 µg) 2 hrs before the deprivation period began; another group received β-CNA 22 hrs after deprivation. A control group received saline injections only. Two hrs after the final injections, food was returned to the cages and intake was measured. β-CNA reduced 4 and 24 hr food intake equally when given either before or after the deprivation period.

These results are consistent with the hypothesis that opioids are involved in the control of food intake and suggest that β-CNA may be a useful drug for further research in this area. Additional work is needed, however, to rule out the possibility that these effects are simply due to malaise or some other non-specific effect of the drug. (Supported by NIH Grant NS 23565 and the Veterans Administration).

420.6 EXAMINATION OF PYLORIC CHOLECYSTOKININ (CCK) RECEPTORS. T.H. Moran and P.R. McHugh. Department of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Previously, we have proposed that some aspects of CCK's satiety action derive from its gastric inhibitory actions. Specific receptors for CCK have been demonstrated in the circular muscle of the gastroduodenal juntion of the rat. Receptors are most dense in the distal pyloric sphincter. In order to furthur characterize these receptors, we examined the relationship of these receptors to vagal CCK receptors, whether a similar CCK receptor distribution could be demonstrated in monkey and man, and what role these receptors may play in the control of gastric emptying and feeding. Total subdiaphramatic vagotomies were performed in Sprague-Dawley rats and animals were sacrificed 1, 2, 4, and 6 weeks later. The pylorus and sorrounding duodenal and gastric segments extending 0.5 cm from the pyloric sphincter were rapidly removed and frozen. Slide mounted 25 um tissue sections taken from the tissue samples were incubated in the presence of 400pM I(125)CCK-33 as previously described. Alternate section were also incubated in the presence of 1 um unlabelled CCK-8 to determine the extent of non specific binding. Vagotomy did not reduce the overall number of CCK receptors at any postoperative time point. There was an apparent decrease in relative density but this coincided with a wider receptor ditribution which resulted from the loss in tissue tone following vagotomy. These results indicate that vagal and pyloric CCK receptors represent different receptor populations.

High densities of CCK receptors were present in the circular muscle layers of the pylorus sphincter in both monkey and man. In the monkey specific submucosal binding was also evident in the gastric antrum. Thus, as the inhibition in feeding is similar across species, so to is the distribution of gastric CCK receptors. In order to determine the role of these receptors in gastric emptying, the effect of IV CCK on gastric emptying was examined fluroscopically in rhesus monkeys. CCK clearly contracted the pyloric sphincter and distal gastric antrum preventing emptying from the stomach. Alterations in duodenal motility were noted as well. This pattern of CCK receptor distribution across species and physiological response to CCK support a mechanism for CCK induced satiety dependent in part on the inhibition of gastic emptying. (Supported by AM-19302)

420.7 EFFECTS OF CHOLECYSTOKININ, BOMBESIN AND TOXINS ON OXYTOCIN SECRETION AND GASTRIC EMPTYING: IMPLICATIONS FOR MECHANISMS OF INHIBITION OF FEEDING. Monica J. McCann*, Joseph G. Verbalis, and Edward M. Stricker. Departments of Psychology and Medicine, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Cholecystokinin (CCK) and bombesin (BBS) are known to reduce food intake in rats, and both decrease gastric emptying. These effects are comparable to those seen after delivery of nutrients to the intestines and therefore may reflect activation of pathways involved in mediating satiety. However, it has been suggested that CCK and BBS instead inhibit food intake by causing malaise. To resolve this controversy, it would be desirable to have procedures that could differentiate satiety from malaise.

To evaluate the specificity of the observed decreases in gastric emptying seen with peptides, we examined the effects of toxins that are known to produce learned taste aversions on gastric emptying in rats. Emptying of 10% glucose solution was virtually halted after ip administration of LiCl (3 mEq/kg) or $CuSO_4$ (5 mg/kg) for more than 1 hour, a duration which parallels the suppression of food intake. Thus, decreases in emptying are not unique to conditions associated with satiety and therefore gastric emptying does not distinguish satiety from malaise.

We have previously reported that $CuSO_4$ and LiCl stimulated dose-dependent secretion of oxytocin (OT) but not arginine vasopressin in male rats. Because these results suggested that OT levels might represent a quantifiable marker of visceral illness in rats when other stimuli to OT secretion are absent, we studied OT secretion in rats after administration of CCK and BBS. CCK (0.1-100 ug/kg, ip) caused dose-dependent increases in plasma OT measured 5 and 15 min after its injection, to levels comparable to those observed after injection of LiCl or $CuSO_4$. In contrast, BBS (1-100 ug/kg, ip) caused no significant elevation of plasma OT. Both peptides significantly reduced food intake at the higher doses. Thus, CCK but not BBS produced an elevation in plasma OT to levels similar to those observed after injection of nausea-producing agents.

Collectively, these results are consistent with CCK activation of central nausea pathways to account for at least part of its ability to reduce food consumption, but indicate that BBS likely acts via different mechanisms. However, the inhibitory effects of both peptides, as well as toxic agents, on gastric emptying implies that this may represent a common effect of all treatments that reduce food intake. (Supported by MH25140 and AM16166).

420.8 FEEDING INDUCED BY CCK ANTAGONIST, BENZOTRIPT: LOCALIZATION IN THE PVN. D. Schwartz, B. Dorfman* & and B. G. Hoebel. Dept. Psychology, Princeton Univ., Princeton, NJ 08544.

If CNS cholecystokinin (CCK) plays a role in satiety, then CCK antagonists should elicit feeding when injected in appropriate places in the brain, and the effect should be localized to specific regions.

In the first experiment, eight male rats with cannulas in the paraventricular nucleus (PVN) of the hypothalamus, were given 0.3 ul injections of benzotript unilaterally in the daytime. Benzotript at doses of 0.003, 0.015, 0.03, 0.18, 0.75, and 1.5 ug induced Purina pellet intake of 2.29, 2.95*, 2.77*, 2.66*, 3.31*, and 3.01* gm, respectively, during the three hours post injection (*p<.05). When CCK-8 was added to the 0.3 ul injection, benzotript-induced feeding was not statistically different from saline. Baseline food intake following 0.3 ul of saline was stable at 1.4 gm before and after the above tests. As a localization control, 0.75 ug benzotript injected 1.5 mm deeper in the brain of the same rats induced only 1.9 gm food intake.

In order to further test the anatomical specificity of the phenomenon, 1.5 ug of benzotript and saline were injected at 23 sites ventral, dorsal and lateral to the PVN and 6 sites in the PVN. At all sites in the PVN, benzotript-induced feeding exceeded control intake by at least 1 gm. Clusters of negative sites were observed in the lateral hypothalamus and the anterior hypothalamus below the PVN. These data lend support to the hypothesis that endogenous CCK normally suppresses feeding by an action on receptors in at least one discrete brain region, the PVN.

420.9 CAPSAICIN ATTENUATES THE SATIETOGENIC EFFECTS OF SOME INTESTINAL NUTRIENTS BUT NOT OTHERS. D. Yox and R.C. Ritter. Dept of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843.

Capsaicin is a neurotoxin that damages small unmyelinated sensory neurons. We have previously demonstrated that capsaicin attenuates suppression of sham ingested sucrose solution (15%) in response to systemic injections of cholecystokinin octapeptide or intestinal infusions of L-phenylalanine (Yox and Ritter, 1985). We have extended these studies to investigate further the extent to which capsaicin might interfere with suppression of food intake by specific nutrients infused separately into the small intestine. Isocaloric (1.3 kcal/10 ml) solutions (pH 7.2-7.4) of carbohydrate, fatty acid or protein were made isotonic (300 mOsm/kg) by adding appropriate quantities of NaCl. These solutions were infused into the upper small intestine of sham feeding rats treated with capsaicin or the vehicle. Sham ingestion of 15% sucrose solution was measured and the percent suppression of intake was calculated relative to a baseline established during intestinal infusion of isotonic saline on a previous day. The intake data are shown in the following table:

	% Suppression (Mean±S.E.)	
Infusate	Capsaicin	Vehicle
Oleic acid (C18)	12.6±6.8	55.3± 9.5 (p=0.001)
Octanoic acid (C8)	7.8±4.4	4.7± 7.5 (p=0.68)
Maltose	8.8±9.5	49.0±13.6 (p=0.02)
Casein hydrolysate	15.3±6.1	24.5± 6.1 (p=0.32).

These data show that intraintestinal infusion of oleic acid, maltose or hydrolyzed casein suppressed sucrose intake in vehicle treated rats, while octanoic acid did not. Capsaicin treatment attenuated the suppression of sucrose intake produced by infusions of oleate and maltose but not casein. Preliminary results indicate that inhibition of gastric emptying by intraintestinal nutrients is not impaired by capsaicin treatment. We therefore conclude that capsaicin sensitive neurons mediate some but not all intestinal satiety signals and that capsaicin's effect on ingestion does not depend on gastric distention or altered gastric emptying.

This work was supported by NIH grant RO1 NS20561.

420.10 DELAY OF GASTRIC EMPTYING BY SEROTONERGIC COMPOUNDS: DISSOCIATION WITH EFFECTS ON FOOD INTAKE. J.M. Miller*, R.S. Feldman*, and J.F. McElroy*. (SPON: J. MEYER). Division of Neuroscience and Behavior, Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Fenfluramine (FEN) is widely believed to exert its appetite suppressant effect through the release and/or depletion of brain serotonin (5-HT). Alternatively, it has been proposed that FEN produces its anorectic effect by slowing gastric emptying. However, it is unclear whether the pharmacologically active agent after systemic administration is the parent compound itself or the dealkylated metabolite norfenfluramine (NORFEN), since both compounds induce anorexia and lower brain 5-HT concentrations. The present study examined the effect of NORFEN and of other anorectic and hyperphagic compounds on gastric emptying.

Eighteen h food deprived rats were fed 5.0 g of wet mash (1:1, powdered chow:water). Drugs were injected I.P. 15 min after feeding, and 4 h later the rats were killed, stomachs removed, and the food remaining determined.

FEN and NORFEN delayed gastric emptying equipotently in a dose response fashion, suggesting that NORFEN may be the pharmacologically active compound. Alternatively the delay produced by NORFEN may be due to a pharmacological action similar to that of FEN. Maximal delay of gastric emptying was produced by non-anorectic doses of FEN (1-2.5 mg/kg), demonstrating that the delay of gastric emptying is in itself insufficient to produce anorexia.

Gastric emptying was similarly delayed by other appetite suppressants such as fluoxetine, 5-HT itself, and the 5-HT precursor 5-HTP. The delay in gastric emptying produced by 5-HTP was prevented by prior treatment with the peripheral decarboxylase inhibitor RO4-4062, indicating that gastric emptying can be delayed through increased peripheral 5-HT activity.

Surprisingly, gastric emptying was similarly delayed by the hyperphagic compounds (and 5-HT receptor antagonists) methysergide, cinnanserin, and cyproheptadine. Taken together with the FEN data, these results demonstrate that the effect of serotonergic compounds on gastric emptying can be dissociated from their effects on eating behavior.

420.11 AREA-POSTREMA-LESIONED RATS RESPOND TO INSULIN INJECTIONS BUT MAY SHOW DEFICITS IN INSULIN RESPONSE TO INFUSED GLUCOSE. Jon N. Kott, Nancy J. Kenney, Stephen C. Woods, & Daniel Porte, Jr., University of Washington, Seattle, WA 98195

Rats with lesions of the area postrema and subjacent caudal medial aspect of the nucleus of the solitary tract (AP/cmNTS) exhibit reduced food intake and body weight. As both peripheral and central insulin levels have been closely tied to body weight regulation and since considerable insulin binding occurs in the AP/cmNTs region, it seems plausible that ablation of this region may impair either the release of or the response to insulin.

To examine the effect of AP/cmNTS lesions on the response of rats to exogenous insulin, 7 lesioned and 7 sham-lesioned rats were given daily subcutaneous injections at gradually increasing doses (5-10 days at each dose level) of protamine zinc insulin (PZI). An additional 7 lesioned and 6 sham-lesioned rats received equal volume injections of isotonic saline. Injections were begun 45 days after lesioning. At doses of .5, 1, 2 and 4 U/kg, PZI had no effect on food intake of either lesioned or sham-lesioned rats. At 8U/kg, average daily intake of PZI-treated lesioned rats increased to 25.6 $\pm$ 0.9 g compared with an average of 20.9 $\pm$ 2.1 g for saline-treated lesioned rats ($t(12)=2.02$, $p<.05$). Sham-lesioned rats injected with this dose of PZI consumed an average of 28.6 $\pm$ 0.7 g per day while their saline-injected controls took 25.3 $\pm$ 1.2 g per day ($t(11)=2.25$, $p<.025$).

Plasma insulin levels of lesioned, sham-lesioned and weight-reduced neurologically intact rats during intravenous infusion of 50% glucose (.02 ml/min) are being measured. The infusion continues for 4 hr and blood samples are taken from the contralateral jugular vein at 30-min intervals beginning 45 min prior to the start of the glucose infusion and continuing for 1.75 hr after the cessation of the infusion. Plasma is assayed for glucose by the glucose oxidase method and for insulin by radioimmunoassay. Preliminary results indicate that, for rats tested within the first 2 wks after AP/cmNTS ablation, plasma insulin levels of normal-weight and weight-reduced neurologically intact rats increase rapidly and plateau within the first 15 min of the glucose infusion. Plasma insulin levels of the lesioned rats, on the other hand, may rise gradually and not peak for up to 2 hr after the onset of the infusion. Thus, while AP/cmNTS-lesioned rats increase food intake in response to administration of exogenous insulin, their insulin production and/or release in response to elevated plasma glucose may be impaired.

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420.12 EFFECTS OF AREA POSTREMA LESIONS WITH VARIED AMOUNTS OF SOLITARY NUCLEUS DAMAGE ON WATER INTAKE AND WATER, SODIUM AND POTASSIUM EXCRETION. Anita J. Bhatia, Douglas A. Fitts, Jon N. Kott and Nancy J. Kenney, University of Washington, Seattle, WA 98195

A role for the area postrema (AP) and subjacent caudal-medial aspect of the nucleus of the solitary tract (cmNTS) in water intake and water, Na+ and K+ excretion has been suggested by several studies. It has further been suggested that the critical brain structures for these disruptions is the cmNTS rather than the AP itself. To test this hypothesis, urine volume, and Na+ and K+ excretion of 5 sham-lesioned (control), 5 AP/cmNTS-lesioned (APX) and 6 AP/cmNTS-lesioned rats with more extensive cmNTS damage (APX+) were measured during the first 24 hr after the ablation. All rats were deprived of both food and water during this test period. In addition, water intake was monitored for all rats from day 2 to day 21 after the ablation.

There were no differences in urine volume among the 3 groups during the first 24 hr after the ablation. In addition, Na+ excretion (umol/100g/24 hr) and Na+ concentration (mmol/l) were similar for control, APX and APX+ rats during this time period. There was a tendency toward decreased urinary K+ concentration by the APX rats when compared to controls. The APX and APX+ rats did not differ in K+ concentration. All groups were similar in K+ excretion, urine osmolality and osmolar excretion.

On the first day of food and water access, APX+ rats drank less water (ml/100g) than did the controls. Rats in the APX group did not differ from either the APX+ rats or the controls in water intake on this day. Following this day, water intakes of the APX+ rats increased and no differences in daily water consumption were observed through day 21 after the ablation.

These data do not support earlier reports of diuresis immediately following AP/cmNTS ablation but do suggest that changes of water ingestion may result with increased damage to the solitary nucleus. Long term effects of small and large AP/cmNTS lesions on these parameters are currently being evaluated.

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420.13 EFFECT OF AREA POSTREMA LESIONS ON DIETARY CHOICE OF RATS FED DISPROPORTIONATE AMOUNTS OF DIETARY AMINO ACIDS. P.M.B. Leung*, D.W. Gietzen and Q.R. Rogers (SPON: R.L. Kitchell). Dept. of Physiological Sci., Sch. of Vet. Med., Univ. of California, Davis, CA 95616.

Electrolytic lesions were placed in Area Postrema (AP) of adult Sprague Dawley rats. Animals with AP lesions (APL) became chronically hypophagic and maintained body weight at a lower level. Earlier we reported that APL showed similar food intake depression as did intact controls (INT) when fed a threonine (thr)-imbalanced amino acid diet (IMB) with thr as the most limiting amino acid for growth, but exhibited facilitated adaptation when fed a high-protein (75% casein) diet (HP) (Leung, P.M.B. et al. Fed. Proc. 45:1091, 1986). In the present dietary choice studies, APL and INT were offered the choice between 2 identical low-protein (6% casein) diets (LP) for 3 weeks before they were allowed to choose, in sequence, between a protein-free diet (PF) and either IMB or a balanced thr basal amino acid diet (BAS). Following re-equilibration with LP presented in both food containers for 14 days, the animals were subjected to the choice between LP and HP for 2 weeks, then fed HP for 4 days and the choice repeated. The 2 identical food containers in choice regimens were rotated daily at random from side to side and among all animals tested. Foods and water were offered ad libitum. APL and INT ate randomly from 2 food containers containing LP showing no side preference. Both APL and INT preferred PF after the first day. PF comprised 80.6±4.2% (mean ± S.E.) and 75.5±10.0% of combined total food intake in choice regimen for APL and INT, respectively, during the entire choice period. When BAS replaced IMB after the PF vs IMB choice, BAS comprised 52.9±4.8% and 72.2±7.3% of combined total food intake in choice regimen for APL and INT, respectively, during the 14-day choice period. All animals again ate randomly during LP re-equilibration prior to the choice between LP and HP. APL consumed more HP in the choice regimen during the 4-week choice period. The protein-energy selected, expressed as % of total caloric intake, during each 2-week period before and after the exclusive HP feeding were 31.2±10.6% and 34.7±11.8%, respectively, for APL and 10.7±2.1% and 8.9±1.3%, respectively, for INT. Terminal hemi-forebrain concentrations of norepinephrine (NE), vanilylmandelic acid, dopamine (DA), homovanillic acid, 5-hydroxytryptamine (5-HT) (serotonin), and 5-hydroxyindoleacetic acid were assayed by HPLC in animals from the lesioned and the intact groups. No differences in turnover index were observed for NE, DA, and 5-HT between APL and INT. The observed feeding and dietary choice behavior differences between APL and INT when fed IMB and HP is consistent with our suggestion that different mechanisms control food intake of amino acid-imbalanced and high-protein diets. (Supported by USPHS NIH grant #AM13252 and AM07557.)

420.14 FAST AND SLOW DUODENAL GLUCOSE INFUSIONS INCREASE FOOD INTAKE IN MEAL-FED RABBITS. L. O'Farrell, A. Acevedo-Cruz*, and P.J. Geiselman. Neuroscience Program and Department of Psychology, UCLA, Los Angeles, CA 90024.

Slow intraduodenal glucose infusion (1 ml/min) has previously been shown to decrease subsequent chow intake, while fast infusion (3 ml/min) increases it. This feeding-enhancement effect may be due to increased intestinal absorption, increased insulin release and resultant hypoglycemia, and increased lipogenesis.

Animals given their food for restricted periods (meal-fed) absorb glucose more rapidly and gain more weight as fat than do free-feeding animals. Meal feeders also learn to increase their insulin secretion with the expectation of food. These data led to the hypothesis that different patterns of meal-taking may contribute to the satiating and feeding-enhancement effects of glucose.

Female New Zealand rabbits were chronically implanted with duodenal cannulae and were adapted to a schedule in which food was available for only 4 hours daily. Rabbits were infused intraduodenally (10 ml/3 kg) with 0.3 M glucose or 0.15 M saline at the rate of 3 ml/min in one group and 1 ml/min in the other. A mock condition was also tested.

Following rapid glucose infusion, meal-fed rabbits, like free-feeding rabbits, increased their food intake during the first 30 min postinfusion. However, unlike free-feeding rabbits, the meal-feeding rabbits that received slow intraduodenal glucose infusions also showed an increase in food intake during the first 30 min.

The present results suggest that meal-taking behavior determines whether glucose is satiating or hunger stimulating.

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420.15 BLOOD GLUCOSE LEVELS FOLLOWING INTRADUODENAL INFUSION OF GLUCOSE IN THE RABBIT. P.J. Geiselman, L. O'Farrell, and A. Acevedo-Cruz*. Department of Psychology and Neuroscience Program, UCLA, Los Angeles, CA 90024.

We previously found that, when infused into the duodenum at a slow rate (1 ml/min), glucose suppressed subsequent chow intake in the rabbit. However, when glucose was infused into the duodenum at a rapid rate (3 ml/min), the rabbits nearly doubled their food intake during the first half hour postinfusion. Elevated insulin levels may be involved in the mediation of this glucose-induced feeding-enhancement effect. The mechanism by which insulin produces the feeding-enhancement effect could be the resultant decrease in blood glucose. We are currently investigating this hypothesis.

Female New Zealand rabbits were chronically implanted with duodenal and jugular cannulae. Following recovery, rabbits were infused intraduodenally (10 ml/3 kg BW) with 0.3M glucose or 0.15M saline delivered at the rate of 3 ml/min or 1 ml/min. Rabbits were also subjected to a mock condition. Serial samples of blood were collected from the jugular cannulae beginning at 30 min prior to infusion or mock condition (baseline). Following infusion or mock condition, chow intake was monitored for four hours.

Within 30 minutes following fast infusion of glucose into the duodenum, blood glucose levels fell to their lowest point, which was approximately 72% of baseline. Both the hypoglycemic and the feeding-enhancement effects of rapid intraduodenal infusion of glucose were observed during the first half hour postinfusion. Following fast infusion of saline or the mock procedure, however, blood glucose values remained above baseline throughout the four-hour measurement period. Data following slow infusion of glucose will also be presented.

The above results are consistent with the hypothesis that the feeding-enhancement effect following rapid intraduodenal infusion of glucose is mediated by hypoglycemia or a metabolic correlate.

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420.16 FEEDING INDUCED BY LOW DOSES OF DYNORPHIN$^{1-17}$ INJECTED IN THE VTA. B. G. Hoebel and S. Scharfman. Dept. Psychology, Princeton Univ., Princeton, NJ 08544

The question was whether opiates would induce feeding at the same mesolimbic dopamine cell body site where they have been shown to induce locomotor and behavior reinforcement effects (P. W. Glimcher, A. A. Giovino, D.H. Margolin & B. G. Hoebel, Behav. Neurosci. 98:262-268, 1984).

Eleven female rats were prepared with 26 ga guide cannulas and 33 ga injectors in the VTA (A 2.2, L 0.6, V 6.5 perpendicular below the cortical surface). Bilateral injections of 0.3 ul were given in daytime at 1430 hrs, and Purina pellet intake was measured every 45 min for 4 hrs. Morphine sulfate (saline, 0.3 ug, 0.6 ug and 1.2 ug/side) was given every third day. Rats became hyperactive, then began eating about 90 min postinjection and consumed 4 gm ($p<0.05$) of food, compared to 1.9 gm for saline controls, by the end of 4 hrs.

Nine of the same rats received saline, 2 ug and 4 ug of d-ala^2-met^5-enkephalinamide (DALA). Food intake was similar to that with morphine but started sooner (45 min) and hyperactivity was less evident.

Eight of the rats then received dynorphin$^{1-17}$ (saline, 7 ug, 0.7 ug and 0.07 ug and saline again in that order). The highest dose (7 ug = 3 nanomols) caused catalepsy for about 1 hr followed by a mean food intake of 4.9 gm. The lowest dose (0.07 ug = 30 picomols) caused about 5 min of immobility followed by 4 gm of food intake (range 0 to 11 gm).

Eight rats given 10 ug of naloxone bilaterally ate the same amount as when given saline; naloxone did not suppress daytime eating.

These results show that opiates injected in the VTA induce feeding with a delayed time course similar to that seen after injections in the paraventricular hypothalamus (S. McLean & B. G. Hoebel, Peptides, 4:287-292, 1983) and lateral hypothalamus (P. Scott, K. Jawaharlal, & B. G. Hoebel, East. Psychol. Assoc. Abstr., 55:106, 1985). The large feeding effect of low doses of dynorphin in some rats suggests that kappa receptors are involved in this hyperphagia phenomenon.

420.17 D-1 AND D-2 RECEPTOR ANTAGONISTS REDUCE INITIAL RATE OF INTAKE IN SHAM FEEDING RATS. J.D. Davis, L.H. Schneider, J. Gibbs and G.P. Smith. Dept. of Psychology, Univ. of Ill., Chicago, IL, 60680 and Dept. of Psychiatry, Eating Disorders Inst. & Bourne Lab, NY Hospital-Cornell Med. Ctr., White Plains, NY 10605.

Dopamine (DA) receptor blockade has been shown previously to reduce the intake of pellets and sweet solutions and to reduce the sham intake of sucrose solutions by food deprived rats. This reduced intake caused by DA receptor blockade could be due to a change in palatability of the test diets or to an impairment of motor function. In order to distinguish between these two alternatives, the Davis and Levine model (Psych. Rev. 1977, 84, 379-412) was used to analyze the sham intake functions generated by rats ingesting a 10% sucrose solution following pretreatment with various D-1 and D-2 receptor antagonists. This model provides a measure of palatability by estimating through curve fitting the initial rate of ingestion and tests the motor function by estimating the rate of decay of ingestion.

Methods. Male Sprague-Dawley rats were chronically implanted with a stainless steel gastric cannula (n=22; 315-395g) and adapted to 30-min access to a 10% (0.3M) sucrose solution after 4h 45 min pellet deprivation and vehicle injection (i.p.). The D-2 receptor antagonists raclopride (0.1, 0.2, 0.4 & 0.8 $mg\text{-}kg^{-1}$), haloperidol (0.025, 0.05, 0.1, 0.2 & 0.4 $mg\text{-}kg^{-1}$), pimozide (0.25 $mg\text{-}kg^{-1}$), sultopride (10, 20 & 40 $mg\text{-}kg^{-1}$), and (-)-sulpiride (100 $mg\text{-}kg^{-1}$) as well as the D-1 receptor antagonist SCH 23390 (0.0125, 0.025, 0.05, & 0.1 $mg\text{-}kg^{-1}$) were injected (i.p.) prior to tests. Intake was measured at 3-min intervals and these data were fitted by the least squares method to exponential decay functions resulting in an estimate of the initial rate of drinking and rate of decay of ingestion.

Results. For at least one dose that significantly inhibited 30-min intake, all of the DA receptor antagonists reduced the initial rate of intake without affecting the half life of the rate of decay.

Conclusions. The reduction in the initial rate of intake without a change in the rate of decay after DA receptor antagonists is consistent with a change in the palatability of the sucrose solution, but not with an impairment of motor function. Since palatability of sucrose is positively related to the sensory and/or hedonic intensity of the sucrose solution, the results are further evidence that antagonism of dopaminergic mechanisms reduces the intake of sucrose by decreasing its sensory and/or hedonic effects rather than by interfering with ingestive movements. [Supported by MH09400 (LHS); MH15455, MH40010 & RSA MH00149 (GPS); RSDA MH70874 (JG)].

420.18 DOPAMINE D1 and D2 RECEPTORS IN RELATION TO PALATABLE FOOD CONSUMPTION IN THE RAT. I. Rusk* and S.J. Cooper. (SPON: L.J. Bindman) Dept. Psychology, University of Birmingham, Birmingham B15 2TT, U.K.

A distinction has been drawn between dopamine D1 and D2 receptors (Kebabian, J.W. and Calne, D.B., Nature, 277: 93, 1979). A number of drugs have been synthesised recently which are selective agonists and antagonists, respectively, for the two types of dopamine receptor. Our aim is to assess the relative contributions of the dopamine receptor subtypes to dopaminergic involvement in the control of feeding responses. Individually-housed male black-hooded rats (General strain, 250-350g) were familiarized with experimental procedures, and were adapted to 30-min daily presentation of a highly palatable sweet mash during the light period and in the homecage. The animals were neither food nor water-deprived.

The selective D2 receptor agonist RU24213 (injected i.p. 30 min. before the feeding test) produced a significant reduction in the amount of food consumed at 10 mg/kg but not at 0.1 or 1.0 mg/kg. Intake was reduced from 22.7g to 9.3g after treatment with the highest dose. Likewise, the potent selective D2 receptor agonist N-0437 (2-(N-N-propyl-N-2-thienylethylamino)-5-hydroxytetralin hydrochloride), when injected i.p. 15-20 min. before the feeding test, produced a marked reduction in palatable food consumption. At 1.0 mg/kg N-0437 reduced food intake by 60.4% and by 70.2% at 3.0 mg/kg. These data indicate that dopaminergic action at the D2 receptor can produce a pronounced anorectic effect in the rat. This conclusion was supported by the finding that the effect of N-0437 at 1.0 mg/kg was reversed by the selective dopamine D2 receptor antagonist YM 09151-2 (0.01 mg/kg). In larger doses, YM 09151-2 itself (0.1 and 1.0 mg/kg) produced dose-related reductions in food consumption indicating that blockade of dopamine's effect at D2 receptors can bring about change in feeding activity. D-amphetamine (0.3-3 mg/kg) had a dose-related effect on feeding, producing a virtually complete suppression of food intake at 3 mg/kg.

The selective D1 receptor agonist SK & F 38393 had only a relatively modest effect on palatable food consumption. At 30 mg/kg (i.p.) food intake was reduced by 29.5%. There were no reductions in food intake at 3 or 10 mg/kg. At present these data suggest that the dopamine D2 receptor may be more importantly involved in dopaminergically-mediated anorexia in the rat. Further studies are underway to assess whether there are interactions between drug effects mediated at dopamine D1 and D2 receptors.

N-0437 was kindly provided by Dr. A.S. Horn; YM 09151-2 and RU 24213 were donated by Yamanouchi Pharmaceutical Co. and Roussel UCLAF respectively.

420.19 NEUROANATOMICAL REGIONS OF THE CHICK BRAIN INVOLVED IN MONITORING AMINO ACID DEFICIENT DIETS. J. D. Firman* and W. J. Kuenzel (SPON: M.M.Beck). Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742.

Significant decreases in food intake are noted when chicks are fed a diet deficient or imbalanced with respect to amino acids (AA). Injections of the growth limiting AA into the carotid artery will restore food intake to normal levels while injections into the jugular vein do not restore the decreased intake. Several regions of the rat brain (amygdala and prepyriform cortex) have been implicated in this process. It has been proposed that receptors in these regions may monitor AA balance and thus regulate food intake. The objective of this study was to determine if homologous regions of the chick brain are involved in the ability of chicks to discriminate between a complete diet and one deficient in tryptophan.

Four regions of the chick brain were studied in a series of experiments. These regions included the n. taeniae (Tn), frontal archistriatum (FA), ventral archistriatum (AV), and the pyriform cortex (CPi). Procedures outlined below were similar for each experiment. Forty-eight chicks were placed on a commercial diet for 1 week. They were then caged in groups of two and adjusted to a complete semi-purified diet fed in two identical feeders. After this adjustment period chicks were placed on a diet 90% deficient in tryptophan (trp). After 3 days on the deficient diet chicks were given a choice of the two diets. Chicks generally selected 60-80% of total intake from the complete diet. After determining that birds could select the control diet over the deficient, surgery was performed on the 48 chicks (24 operated; 24 shams). The same sequence of diets was given post-operatively to determine if any differences in selection occurred. After completion of the experiment chicks were sacrificed and brains examined histologically to determine proper lesion placement. Data were analyzed using repeated measures ANOVA.

Comparisons of percent selection of diets by shams to operated birds indicated no significant differences in FA, AV, and Tn lesioned chicks. Results of an additional study comprised of knife cuts isolating the CPi and a respective control group showed that the latter selected significantly more (80.04% +/-6.92; $\bar{x}$ + S.E.M.) of the complete diet than the CPi knife-cut chicks (60.85% +/-6.92) at $P<.01$. These results suggest the pyriform cortex may be involved in monitoring AA balance.

421.1 VULNERABILITY OF THE DEVELOPING BRAIN IN EXPERIMENTAL GROWTH RETARDATION. S. Rees,* A. Bocking,* R. Harding,* (SPON: F. GÜLDNER) Department of Physiology, Monash University, Clayton, Victoria 3168 Australia.

It is now accepted that of all infants weighing less than 2.5 kg at birth, approximately one third are growth retarded or small-for-gestational age and not simply premature (Gruenwald, Biol. Neonate, 5:215, 1963). These infants have evidence of chronic intrauterine hypoxia and malnutrition at birth (Robinson Br.Med.Bull. 35:137, 1979). Most catch up physically within the first years of life, however a significant proportion suffer from persistent physical and neurological handicaps. At present it is not clear whether specific strucutural abnormalities underlie the intellectual and motor deficits observed in these children. In an attempt to answer this question we have been studying the fetal brain in an animal model of chronic placental insufficiency, the growth retarded fetus produced by the carunclectomised ewe.

In this procedure approximately 80% of the endometrial caruncles (potential attachment sites) in the ewe were removed prior to conception, thereby reducing the size of the placenta in a subsequent pregnancy and producing a growth retarded fetus. In these fetuses (n=8) at 139±1d(term = 147d) the weight of the brain & cerebellum was significantly reduced by 21% when compared with age matched controls ($p < 0.002$). Our initial histological studies have been on the cerebellum, motor and visual cortices and the hippocampus and we have shown that these structures are all markedly affected in growth retardation. Quantitative studies on parasagittal sections of the cerebellar vermis have shown a significant reduction in the areas of each of the layers in the cerebellum ($p < 0.05$), a decrease in the width of the molecular layer, ($p < 0.05$) a tendency for the linear density of Purkinje cells to be increased and for the width external granular layer to be decreased in growth retarded brains compared with controls. Neuronal densities in the visual and motor cortices and in the pyramidal layer of the hippocampus of the growth retarded brain were all significantly greater than controls ($p < 0.05$).

These results suggest that chronic placental insufficiency affects brain development by retarding the development of neuronal processes in these areas and in the case of the cerebellum, by reducing the proliferation of granule cells. Such structural abnormalities could affect the pattern of neuronal connectivity and could be associated with functional and behavioural deficits. Supported by the National Health & Medical Research Council of Australia.

421.2 CORPUS CALLOSUM DEFECTS AND MATERNAL ENVIRONMENT IN THE BALB/c MOUSE: EFFECTS OF OVARIAN GRAFTING. B. Bulman-Fleming and D. Wahlsten. Dept. of Psychology University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Mice of the inbred strain BALB/c have an hereditary defect of the corpus callosum (CC) in which about 20% of the animals show a severe reduction in size or a total lack of the structure at midline. Previous work has demonstrated that CC deficiency is completely recessive but is not due to a single gene (Wahlsten, J. Hered. (1982, 73: 281-285) and is sensitive to environmental influences (Wahlsten, Develop. Brain Res., 1982, 5: 354-357; Wahlsten, Brain Res. 1982, 239: 329-347). The present work used ovarian grafting to investigate the effects of an F1 hybrid maternal environment on the incidence of CC defects in BALB/c mice.

Reciprocal crosses between C57BL/6J mice and pigmented BALB mice produced the F1 dams used in the study. At between 6 and 10 weeks of age, 27 F1 and 27 BALB dams were ovariectomized and grafted with BALB/c ovaries from donors of like age using the method of Jones and Krohn (J. Endocrin., 1960, 20: 135-146) with minor changes. All grafted mice were paired with BALB/c sires 2 weeks after surgery, and the resulting litters were weaned at 30 days and sacrificed at 100 days of age. Litters from ungrafted BALBs were also included in the study. Dependent measures of interest included litter size, birth and weaning weights, and 100 day measures of body weight, brain weight, and areas of CC, anterior (AC) and hippocampal (HC) commissures at midline as determined from tracings made from frozen sections and quantified using a digitizing morphometry program.

The principal findings of the study were:

1) BALB/c offspring of F1 hybrid dams have heavier bodies at birth, weaning and at 100 days of age and heavier brains at 100 days of age than do BALB/c pups of grafted or ungrafted BALB dams.

2) The larger AC and HC areas of BALBs from F1 dams were attributable to their larger brains.

3) No significant differences were detected among BALB offspring from ungrafted BALB dams or grafted BALB or F1 dams in number of animals with defective CC (area less than 0.8mm^2).

4) Among animals having CC areas in the normal range, those from F1 dams had larger CC areas than those from BALB dams; however, this was probably a consequence of a very small but positive correlation between CC area and brain weight in BALB/c mice.

(This work was supported in part by Grant A4878 to D.W. from the Natural Sciences and Engineering Research Council of Canada and by an Ontario Graduate Scholarship to B.B-F. The technical assistance of Kathryn Blom, Glenna Smith and Walter Zagaja is gratefully acknowledged.)

421.3 PRENATAL PHENOBARBITAL EXPOSURE AND THE DEVELOPMENT OF POSTNATAL PTE-INDUCED SEIZURES. S. K. Sobrian, C.L. Brown*, D.L. Tuck* and H. James*. Dept. of Pharmacol., Howard Univ. Med. School, Washington, D.C. 20059.

We have recently shown (Sobrian & Nandedkar, Pharmacol. Biochem. & Behav., May, 1986) that in rat, prenatal exposure to phenobarbital (PB) protects male and female offspring from pentylenetetrazole (PTZ)-induced minimal and maximal seizures. To determine the age at which this protection develops and if sensitization to PTZ seizures occurrs in the pups the following study was conducted.

On gestation days 15-21, Sprague Dawley rats were exposed to 30 mg/kg/day of PB delivered by s.c. implanted osmotic pumps; controls received pumps containing 0.9% saline. Females delivered naturally; litters were culled to 10-12 at birth. On postnatal days (PND) 5,10,15,20 and 25 male and female offspring from both groups were injected with 20,35 or 50 mg/kg of PTZ and seven indices of convulsive behavior were scored. The 30 mg/kg dose of PB produced plasma levels of 26.28 ± 0.67 ug/ml in the mothers; at birth, plasma levels in female and male offspring were 23.28 ± 1.88 ug/ml and 18.39 ± 2.43 ug/ml, respectively. Prenatal exposure to PB did not alter the length of gestation, litter size, the male/female pup ratio or several indices of maternal behavior. Differences in pup body weights present at birth (PB offspring were smaller than controls) did not persist during the preweaning period but reappeared at PND 25. In control pups only 50 mg/kg of PTZ induced seizure-like behavior and only at PND 5 and 10. Prenatal exposure to PB eliminated PTZ-induced seizure-like behavior at PND 5. However, at PND 10, both 35 and 50 mg/kg of PTZ induced seizures in PB offspring. Moreover, the convulsive effect of PTZ was still seen at PND 15 and 20 with the higher dose of PTZ. Several other indices of neonatal convulsive behavior were also altered by prenatal PB exposure. Head/limb movements were increased by 50 mg/kg PTZ in both prenatal groups. However, only PB offspring showed an increase in this behavior following 35 mg/kg of PTZ at PND 20 and 25. With respect to Body Rotation, again only 50 mg/kg of PTZ enhanced this behavior in prenatal controls. In prenatal PB offspring, all 3 doses of PTZ increased Body Rotation but the effect was age related, with lower doses effective at PND 25. Similar changes were observed for Straub Tail.

These results indicate that in contral pups, PTZ acts as a convulsant during the first 10 postnatal days at a dose which induces maximal seizures in adults (50 mg/kg). Prenatal exposure to PB in doses that produce human therapeutic plasma levels appears to sensitize offspring to PTZ. Convulsive behavior is seen in PB offspring until PND 20 and PTZ can enhance other convulsive behaviors at doses ineffective in controls.

421.4 PRENATAL EXPOSURE TO PHENCYCLIDINE: EFFECTS ON BIRTH, DEVELOPMENT AND BEHAVIOR. T.A. Fico and C. VanderWende. Rutgers University, Department of Pharmacology, P.O. Box 789, Piscataway, NJ 08854.

Phencyclidine (PCP) abuse by pregnant women has been reported raising questions of safety for the fetus. The following studies were conducted to determine whether different periods of prenatal exposure to PCP modified (1) birth and litter development, (2) male postnatal aggressive behavior and (3) male postnatal sensitivity to PCP-induced locomotor activity and ataxia.

Pregnant, CF-1 dams were injected daily with either PCP (5, 10, 20, 40 mg/kg, sc) or saline during mid (E6-15) or late (E12-18) gestation. The number of live births, birth weight and anogenital distance were recorded. The litters were culled to six males and cross fostered to untreated dams. On day 35, the mice were tested for PCP-induced locomotor activity and ataxia or were individually housed to test for aggression. Beginning on the second day of isolation and every other day thereafter, a bulbectomized, conspecific was added to the home cage of the test animal for a 15 min aggression test. This continued until either the test mouse initiated a fight or 15 test days elapsed. Latency to first fight, number of fights and total fighting time were recorded. For the postnatal sensitivity experiment, each animal was injected with either PCP (2.5, 5.0, 7.5 mg/kg, sc) or saline and immediately placed in an activity chamber; locomotor activity and ataxia were recorded for one hour. The ataxia was scored for 20 sec periods at 5 min intervals using the rating scale of Castellani and Adams (Eur. J. Pharmacol. 73, 143, 1981).

There was a significant effect of treatment on body weight at P21 and P35 but not at birth. Male offspring exposed to 40 mg/kg during mid gestation had a significantly shorter anogenital distance than all other treatment groups. The number of pups/litter decreased with increasing doses of PCP. The mean latency in days to fight was decreased in all treatment groups. In pups prenatally exposed to 20 mg/kg during E12-18, 5 mg/kg PCP caused increased locomotor activity while 7.5 mg/kg caused a decreased response. This may result from an enhanced ataxia at the higher dose.

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421.5 ALTERATION OF BRAIN SEROTONIN METABOLISM IN RAT PUPS EXPOSED PRENATALLY TO ETHANOL. M.C. Yu and J.R. McGee, Dept. of Anatomy, New Jersey Medical School, Newark, N.J. 07103.

Considerable evidence has accumulated indicating that exposure to alcohol in utero results in certain cognitive and behavioral deficits. In this study, we report on alteration in the level of CNS neurotransmitter serotonin and its metabolite 5-hydrozyindole acetic acid (5-HiAA) in 1-day old rats exposed to ethanol during gestation.

Female rats were fed 10% ethanol for 2 weeks prior to conception in order to adapt them to alcohol consumption. These dams, rendered pregnant on the first day (positive vaginal smear), were then fed a graded regimen of ethanol of 10, 30 and 50%, successively, during the 3 weeks of pregnancy. Rat chow was given ad libitum. Another group of pregnant dams, the control, was given water. Alcohol consumption was monitored on alternate days. At birth, the body weights of neonates were recorded prior to sacrifice. Tissues from the hippocampus and the cerebellum were dissected out rapidly frozen at -80°C until use. The tissues were homogenized in 10 volumes of 0.1N perchloric acid containing 1ng/ul dihydroxybenzylamine as internal standard, centrifuged and filtered and the supernatant was used for quantitative assay by high performance liquid chromatography with electrochemical detection.

The body weight of the ethanol exposed pups was less than the control group, being 4.6g versus the 6.1g of the control. The amount of serotonin in the hippocampus of the pups exposed to alcohol was significantly greater than the control, being 71.2 ng/g as compared to 55.0ng/g of control. The level of 5-HiAA was also increased in the alcoholic pups, being 251ng/g versus 198ng/g of control. In the cerebellum, the values of serotonin and 5-HiAA were greater in the ethanol-exposed pups than the control, but the difference was not statistically significant.

The results of the present study indicate that chronic exposure to alcohol during gestation caused alteration in serotonin and its metabolite in the hippocampus, but had only a small effect on the cerebellum in 1-day old pups. Studies are in progress to correlate the neurochemical alteration with morphologic manifestation.

421.6 THE TIME OF ORIGIN OF NEURONS IN RAT MOTOR CORTEX IN EXPERIMENTAL FETAL ALCOHOL SYNDROME. M.W. Miller. Dept. of Anatomy, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine and R.W. Johnson Medical School, Piscataway, NJ 08854.

Prenatal exposure to ethanol has profound effects upon the developing central nervous system. Examination of human and rodent offspring of alcohol consuming mothers shows that they have behavioral deficits including motor dysfunction, a lack of fine motor coordination, mental retardation, and learning and memory deficits and structural alterations including microcephaly, abnormal cortical architecture, and dysmorphic pyramidal neurons. These clinical and experimental data indicate that ethanol affects the development of motor areas of cerebral cortex. The present study describes the effects of prenatal exposure to ethanol on the time of origin of cortical neurons.

Pregnant Long-Evans rats were fed a liquid diet containing 6.7% ethanol (v/v) or pair-fed an isocaloric liquid control diet. They were weaned onto a liquid diet beginning on gestational day (GD) 6 and maintained on it for the remainder of the pregnancy. Each female was injected with tritiated thymidine once during the period from GD 12-23. At 2-3 months of age, pups were sacrificed. The brains were processed by standard autoradiographic techniques so that neurons generated on the day of injection could be identified by the presence of silver grains over their nuclei.

In pups of control-fed animals, the generation of cortical neurons followed an inside-to-outside pattern, i.e. deep neurons were born prior to neurons located in superficial layers. Three changes were observed in ethanol-fed rats. (1) The period during which cortical neurons were generated was delayed by one day and lengthened by two days. In control rats the generation period was from GD 13-21 and in ethanol-exposed animals it was from GD 14-23. (2) The number of neurons born on a specific day was significantly less (5-50%) than in the control animals. (3) Early-generated neurons, i.e., those born on GD 13-19, followed the normal inside-to-outside sequence, however, late-generated neurons, i.e., those born on GD 20-23, did not. About 90% of the neurons born on GD 20 were not in layer II (as they were in control rats), rather these late-generated neurons were distributed in layers V and VI. Moreover, neurons born on GD 21-23 were located in layer VI and in the white matter. These ectopic neurons were not readily visible in Nissl-stained sections for despite these changes, cortical laminae remain distinct.

These data show that ethanol alters the generation and the migration of cortical neurons. Such developmental abnormalities may underlie the behavioral and structural changes characteristic of children with fetal alcohol effects.

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421.7 EFFECTS OF IN UTERO EXPOSURE TO ETHANOL ON SYNAPTOGENESIS IN LAYER I OF MOTOR CORTEX. M.J. Druse-Manteuffel, W.E.Rathbun*, J.A.McNulty* and C. Nyquist-Battie#. Departments of Biochemistry & Biophysics and Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153 and #The School of Basic Life Sciences, University of Missouri, Kansas City, MO 64110.

Previous work from this and other laboratories have shown that in utero exposure to ethanol alters the development of the cerebral cortex in terms of the concentration of neurotransmitters and neurotransmitter receptors (Kelly, G.M. et al. Exp. Neurol., 91: 219, 1986; Rathbun, W. and M.J. Druse, J. Neurochem., 44: 57, 1985) and the arborization of pyramidal dendrites (Hammer, R.P. and A.B. Scheibel, Exp. Neurol., 74: 587, 1981). However, at the time the present study was initiated, limited information was available about the influence of in utero exposure to ethanol on synaptogenesis in the cortex. Thus, the present study examined the influence of chronic maternal ethanol consumption, prior to parturition, on synaptogenesis in layer I of the motor cortex of 19-day-old rat offspring. The molecular layer (layer I) was examined because of the early timing of its development. The motor cortex was chosen because of evidence of motor problems in children afflicted with the fetal alcohol syndrome (FAS). The results of these studies demonstrated that the 19-day-old offspring of rats that were pair-fed, using control or 6.6% (v/v) ethanol-containing liquid diets on a chronic basis prior to parturition, had a comparable thickness of cortical layer I as well as a comparable density of synapses and distribution of paramembranous density lengths in layer I. Thus, evidence by this and other laboratories of cortical neurotransmitter and dendritic abnormalities are not reflected in synaptic changes in layer I of the motor cortex at 19 days. It is possible that synaptogenesis is affected in other areas of the cerebral cortex or at another stage of development.

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421.8 ADULT MALE AND FEMALE RATS EXHIBIT MICROENCEPHALY AND INCREASED LOCOMOTION WHEN EXPOSED TO HIGH BUT NOT LOW BLOOD ALCOHOL CONCENTRATIONS AS NEONATES. S.J. Kelly, D.R. Pierce and J.R. West. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

We reported previously that postnatal alcohol administered in a condensed regimen designed to produce high blood alcohol concentrations (BACs) can cause microencephaly that is still detectable in 90 day old female rats. We now report that this also holds true for male rats and furthermore, condensed alcohol exposure increases locomotion in both neonates and adults.

Alcohol was administered to rats via an artificial rearing technique (Samson and Diaz, in Fetal Alcohol Syndrome, Vol. III, E.L. Abel (ed.) 1982, pp. 131-150; West et al., Alcohol 1:213-222, 1984). Alcohol-exposed rats were given 6.6 g/kg of ethanol condensed into 8 hours (feedings every 2 hours) or spread uniformly over 24 hours every day from postnatal day 4 to 10. The condensed alcohol exposure resulted in mean peak BACs of 411 mg/dl and mean low BACs of 45 mg/dl. Uniform alcohol exposure resulted in stable mean BACs of 85 mg/dl. A gastrostomy control group was artificially reared on milk formula alone. A suckle control group was reared normally with dams. Some pups were sacrificed after the last feeding on postnatal day 10. Other animals were tested for activity on days 20, 30 and 90 and then sacrificed.

Severe reductions in brain weight were detected in the cerebrum, cerebellum and brainstem of both male and female rats given condensed alcohol exposure at 10 days after birth. These deficits were still present at 90 days after birth in both sexes. Mild microencephaly exhibited at day 10 by male and female rats exposed to uniform alcohol administration was even less robust at day 90. Locomotion was increased at day 20 in only those male and female rats given condensed alcohol exposure. This difference was no longer significant at 30 days after birth possibly because of an increase in variance. At day 90, male and female rats subjected to condensed alcohol exposure as neonates once again exhibited increased locomotion when compared to rats given uniform alcohol exposure, artificial rearing alone, or normal rearing.

Thus, a pattern of alcohol administration that induces high BACs in neonatal rats can produce permanent microencephaly as well as long-term alterations in motoric behavior in rats of both sexes whereas alcohol administration that results in low BACs does not. (Supported by NIAAA grant AA05523 to J.R.W.).

421.9 DIETARY EXPOSURE TO A SOY LECITHIN PREPARATION: ALTERATIONS OF SYNAPTOGENESIS IN DEVELOPING RAT BRAIN. J.M. Bell, W.L. Whitmore*, G. Barnes*, F.J. Seidler*, and T.A. Slotkin Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710

Commercially available soy lecithin preparations (SLP) have become a panacea for a wide variety of symptoms in the lay population. However, dietary ingestion of large amounts of SLP may be potentially harmful to developing organisms, either through high levels of lipid or choline components: either constituent can alter neuronal maturation or function. Recent animal studies have shown that neurological impairments occur following perinatal ingestion of SLP, effects which may arise from underlying abnormalities in the polyamine pathway which regulates cellular replication, as well as later changes in synaptic activity. If such biochemical and functional changes are related, then selective changes in synaptic dynamics should be occuring. Accordingly, the current study examined the effects of SLP exposure on the development of cellular replication as well as on pre- and postsynaptic measures of synaptogenesis.

Reduced cerebral cortex levels of DNA in SLP-exposed animals indicated a permanent reduction in number of cortical cells. To assess if this deficit was associated with a primary cholinergic effect, choline acetyltransferase activity, a marker for presynaptic nerve terminal activity was measured, and found to be significantly elevated in SLP-exposed animals during preweaning growth. If this index is indicative of hyperinnervation or increased activity of cholinergic neurons, then postsynaptic down-regulation of responsiveness should occur. Such was the case: carbachol's stimulatory effects on ^{33}Pi incorporation into phospholipids (an effect medjated by muscarinic receptors) was reduced in SLP-treated offspring. This effect was probably not due to a generalized interference with phospholipid metabolism, as basal (unstimulated) incorporation was significantly elevated and stimulation with another neurotransmitter (dopamine) was not reduced. Finally, since cholinergic tone is a modulator of catecholaminegic neurotransmission, norepinephrine and dopamine turnover were measured: again, SLP-exposed animals showed postweaning changes consistent with alterations in impulse activity. In conclusion, it appears that perinatal exposure to an SLP-enriched diet results in perturbations in the developmental profile of central synapses. It is likely that the net effect of the SLP dietary influence on development consists of both cholinergic-related effects as well as more generalized perturbation of CNS maturation.
(Supported by USPHS HD-09713 and MH-40939).

421.10 CONTROL OF BRAIN GROWTH DURING NEONATAL MALNUTRITION OR OVERNUTRITION: ORNITHINE DECARBOXYLASE (ODC) AND MACROMOLECULES. T.A. Slotkin, K. Queen*, A.P. Slotkin*, J.M. Bell and F.J. Seidler* Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Nutritional status of neonatal rats was manipulated by altering litter size to 5-6 pups (small litter) or 16-17 pups (large litter), compared to a standard litter size of 10-11 pups. In order to evaluate biochemical mechanisms which operate to buffer the brain from growth retardation, comparisons were made between spared brain regions (cerebellum, cerebral cortex, midbrain + brainstem) and a tissue which is not spared (heart): evaluations included assessment of ODC (an enzyme whose activity controls macromolecule synthesis during development) as well as DNA, RNA and protein. The redistribution of pups did not adversely affect maternal caretaking: there was no evidence for maternal deprivation, which is characterized by an acute (hours) decrease in ODC. Nevertheless, the long-term (days) ontogenetic pattern of ODC was affected specifically in the heart, where there was a sustained increase in the small litter and a reduction in the large litter, relative to the normal group. These two patterns are predictive of an acceleration and delay of macromolecular development, respectively. In the heart, growth enhancement and retardation were readily achieved by manipulation of litter size, with alterations of as much as 40% apparent by weaning; these effects were comparable to those on body weight. Acquisition of cells by cardiac tissue (DNA content) was similarly affected; specificity toward replicating cardiac cells was demonstrated by the predominance of effects on DNA compared to other macromolecules (RNA, protein). In fact, RNA per cell (RNA/DNA) was substantially increased in the growth-retarded group and suppressed in the growth-enhanced animals. In contrast, ODC in developing brain regions was generally uninfluenced by litter size and weights were within 10% of normal values. Cell acquisition was protected (small effects on DNA) and RNA was maintained at the same level regardless of nutritional status. Although all 3 regions were buffered from nutritional effects relative to the heart, the cerebellum, which undergoes major phases of replication and differentiation later than the other two regions (and coincidentally with similar events in the heart) was the least protected. These data support the view that intracellular control mechanisms involving ODC and its enzymatic products, the polyamines, participate in the adjustment of macromolecule synthesis and acquisition in developing brain tissue to nutritional deprivation or enhancement. Control mechanisms which protect the brain appear to be dependent upon the degree of differentiation of each brain region.
(Supported by USPHS HD-09713)

421.11 EFFECTS OF TAURINE DEFICIENCY ON THE RAT RETINA DURING DEVELOPMENT. Norma Lake, Naseema Malik*, and Luisa De Marte*, Depts. of Ophthalmology and Physiology, McGill University, Montreal, Canada H3G 1Y6.

The role of taurine in the growth and development of mammals is unclear, although there is much evidence that levels in fetal and neonatal CNS during development are higher than adult. High levels of taurine are present in milk and are transferred to neonatal organs before weaning. Taurine in milk is thought to be important for the nutrition of the neonate at a time when it may have a particularly high need for taurine due to rapid growth, and when its capacity for biosynthesis is immature. We have developed a method for depletion of taurine in the milk of lactating rats by treatment with the taurine transport antagonist, guanidinoethyl sulfonate (GES). Pups suckled by such mothers have retinal taurine levels 45% of control throughout nursing, indicating their dependence on dietary taurine during this time (Lake, Neurochem. Res. 1983, 8:881), and the inability of their biosynthetic capacity to compensate for dietary lack of taurine -- a mechanism which protects adult rats. In the present studies we have examined in albino and pigmented strains, rhodopsin and DNA content, and retinal morphology, measuring the width of retinal layers at 300 μ intervals across the retina along the vertical meridian passing through the optic nerve head, and observing fine structure in the electron microscope. The pups were suckled by mothers given taurine-containing (control) or taurine-free diets, and regular drinking water, or water containing 1% GES. We examined pups at 20 postnatal days (just prior to weaning), 29 days, and 41 days. Pups examined after weaning were maintained on the same diet as their mother and drank either regular water or water containing 1% GES.

Whereas in adult rats taurine-free diets are without effect, and GES-induced taurine deficiency primarily affects the photoreceptor cell layer, pups suckled by mothers on taurine-free diets and regular water had marked morphological abnormalities, not limited to photoreceptor cells. These were enhanced by addition of GES to the drinking water of the mother and/or the pup after weaning. Particularly striking were the reductions in the inner plexiform and ganglion cell layer. In many cases the superior retina was more affected than the inferior retina, consistent with our hypothesis that the damage observed in retinal taurine deficiency is mediated in part through increased vulnerability to light damage (Invest. Ophthal. Vis. Sci.1986, 27:suppl,57). It appears that the developing retina is more and differently sensitive to taurine deficiency than the adult, and that it is a prudent measure to add taurine to the low taurine formulations often substituted for breast milk in human neonates. Supported by the Medical Research Council and the National Retinitis Pigmentosa Foundation of Canada.

421.12 REHABILITATION IN RATS MALNOURISHED DURING LACTATION: DENDRITIC ARBORIZATION AND SYNAPTIC TERMINALS OF THE CEREBRAL CORTEX. A. G. Angulo-Colmenares and J. L. Colmenares. Centro de Investigaciones Biomédicas, Universidad Francisco de Miranda, Coro, Venezuela.

The effects of protein malnutrition and the possibility of rehabilitation have been studied in the somatosensory cortex of the rat. Pregnant Sprague-Dawley animals were given either a 24% (control, C) or 8% (malnourished, M) protein diet beginning at day 10 of gestation and continuing until 40 days. In addition, at day 20 of gestation, a group of malnourished animals were subjected to a rehabilitating treatment (rehabilitated, R) consisting of: a) feeding the animals a 24% casein diet, b) leaving the pups with their mother until they were 40 days old and c) reducing the litter size from 8 to 4 pups. Observations were made on tissue from animals 20 days (C and M groups) and 40 days (C, M and R groups) fixed by vascular perfusion of aldehydes. The somatosensory cortex from one hemisphere was embedded in Araldite, and that from the contralateral size was processed for Golgi staining.

Electron micrographs of layer I were taken in area 3 of the somatosensory cortex. Synaptic terminal density was measured in the micrographs and was found significantly reduced in the malnourished animals ($p < 0.005$) at 20 and 40 days. Following the rehabilitation treatment the synaptic density of the previously malnourished animals was not different from that of the control group at 40 days.

In Golgi preparations, Sholl analysis of the basal dendritic arborization of layer II/III pyramidal neurons showed significant differences from controls in the malnourished animals. In the rehabilitated group the dendritic arborization had partially recovered by 40 days. These observations support our theory that a normal structure of the cerebral cortex may be achieved following early malnutrition if adequate rehabilitation, including a delayed weaning, is provided following the stage of deprivation. (This research was supported in part by CONICIT grant CICS-4 and Fundacite Centro Occidente grant F1-24-01-83).

421.13 EFFECT OF A VITAMIN B-6 DEVOID DIET AND AGING ON BRAIN AND PLASMA PYRIDOXAL-5-PHOSPHATE AND ERYTHROCYTE ASPARTATE AMINOTRANSFERASE ACTIVITY IN RATS. E.F. Cochary*, S.N. Gershoff*, J.A. Sadowski*, (Spons: J.J. Tecce) The School of Nutrition and USDA Human Nutrition Research Center at Tufts University, Boston, MA 02111.

Pyridoxal-5-phosphate (PLP), the active form of vitamin B6, is required for over fifty biochemical reactions in the mammalian system. PLP-dependent reactions which occur in the CNS include neurotransmitter synthesis, glycogen degradation and protein metabolism. Nutritional surveys have revealed that the elderly may be at risk of developing a vitamin B6 deficiency. The biochemical consequences of such a deficiency have not been well studied, but will be addressed in this abstract.

Pyridoxine (PN) status was studied in male, F344 rats aged 3 weeks, 3 months and 12 months fed a semi-purified diet for 12 weeks. The dietary groups were 1) B6-Sufficient (7mg PN:HCl/kg diet) 2) B6-Devoid (0mg PN:HCl/kg diet) and 3) Pair-fed. Brain PLP was measured at 12 weeks and plasma PLP and erythrocyte Aspartate Aminotransferase Activity Coefficient (EGOT A.C.) were measured at weeks 0,1,2,4,6,9 and 11. There was an age-related difference in the brain PLP concentration of the B6-Sufficient rat as well as a significant decline in brain PLP when rats were fed a vitamin B6-Devoid diet. (The brain PLP values shown below are expressed as ng PLP/mg brain dry weight).

Age	B6-Sufficient ($\bar{X}\pm$SEM)	B6-Devoid ($\bar{X}\pm$SEM)	% Decrease
3 weeks	8.89 ± 0.12	4.29 ± 0.11	51.7%
3 months	9.22 ± 0.19	5.09 ± 0.22	44.8%
12 months	9.80 ± 0.42	5.57 ± 0.23	43.2%

Plasma PLP concentration dropped more rapidly and to a lower level in the 3 month B6-Devoid rat than in the 12 month B6-Devoid rat. The plasma PLP of the 3 week B6-Devoid rat dropped most rapidly. The change in EGOT A.C. was also the most severe in the young rat.

Thus, brain PLP, plasma PLP and EGOT A.C. demonstrated age-related differences with the most severe changes observed in the youngest B6-Devoid rats. The consequences of these differences may be important for the normal functioning of the CNS during aging and merit further investigation.

421.14 PLASMA ZINC TRANSPORT IN NORMAL AND KINDLED ADULT CATS. M.B. Sterman, M.N. Shouse* and M.D. Fairchild*. V.A. Medical Center, Sepulveda, CA and UCLA Sch. of Med., Los Angeles, CA 91343.

The importance of zinc uptake and transport regulation in relation to seizure mechanisms has been a topic of re-evaluation due to recent findings suggesting a protective rather than a pathalogical role for this cation (Porsche, E. IRCS Med. Sci., 11:599, 1983; Sterman, M.B. In: Kindling 3, J. Wada, ed., Raven, in Press, 1986; Howell, G.A. et al, Soc. Neurosci. Abs., 12: in Press, 1986). Since little is known about zinc regulation in the adult cat, the animal used in our studies, we sought to 1) determine this animal's plasma response to various levels of dietary loading, and 2) compare this in normal and previously kindled animals.

Twenty-four adult cats were assigned to three feeding groups of eight each. Each group consisted of four naive, control animals and four animals who had completed a standardized kindling process at least 30 days prior to entry into the study. The three groups were provided with normal laboratory food for four weeks, and then shifted to food and water containing 400, 800 and 1200 ppm zinc, respectively, for an additional four weeks. All were housed under special circumstances which assured their isolation from environmental zinc. Venous blood samples were drawn at weekly intervals across this eight week paridyme, and plasma derivitives subjected to atomic absorption spectrometry for determination of zinc and copper concentrations.

The results of this study can be summarized as follows: (1) Plasma Cu levels were not influenced by any of the Zn loading diets used here; (2) 400 ppm Zn in diet produced no significant changes in plasma Zn levels among both normal and kindled animals; (3) 800 ppm zinc in diet produced a significant elevation in plasma concentration lasting for one week in kindled animals only; (4) 1200 ppm Zn in diet produced a significant elevation of plasma Zn concentration in both normal and kindled groups and this effect was sustained. Kindled animals showed a trend towards higher levels.

These findings confirm our previous observations with 700 ppm loading, and indicate that intermediate levels of Zn supplementation are indeed accompanied by a selective increase in plasma concentration in animals made susceptible to seizures through the kindling process. We have interpreted this finding as suggesting an increased limbic tissue utilization of zinc under conditions of abnormal stimulation. (Supported by the Veterans Administration).

EFFECTS OF CHRONIC DRUG ADMINISTRATION AND NEUROTOXICOLOGY III

422.1 GLUTATHIONE DEPLETION MARKEDLY ENHANCES DRUG-INDUCED HEARING LOSS-POSSIBLE MECHANISM OF OTOTOXICITY. D.W.Hoffman, C.A.Whitworth*, N. Joyner-Triplett* and L.P.Rybak. Depts of Psychiatry, Pharmacology, and Surgery, SIU Sch. of Med., Springfield, IL 62708.

One of the most interesting and as yet unexplained phenomena in drug toxicity is the profound enhancement of the toxicity of loop diuretics such as ethacrynic acid (ETA) by the concurrent administration of an aminoglycoside antibiotic. This combination can result in irreversible deafness and kidney damage after a single combined administration of quite modest doses of these drugs. No mechanism has been proposed which can account for this potent synergy with important clinical implications.

Glutathione (GSH) is a tripeptide which plays a major role in the detoxification of electrophilic drugs and metabolites and active oxygen formed during drug metabolism. This reaction is catalysed by GSH S-transferases. ETA and related diuretics are known to be potent inhibitors of GSH S-transferases, and have been reported to bind irreversibly to these enzymes. Inhibition or inactivation of this important detoxification mechanism may be the central factor in the potentiation of the toxicities of the aminoglycosides and loop diuretics, and may also be involved in the toxicities of radiation therapy, cisplatin and other drugs.

Buthionine sulfoximine (BSO) is a compound designed to specifically lower GSH levels in tissue by the inhibition of GSH synthetase (Meister, A., Sci., 220:472,1983), without activity on other drug metabolizing pathways (Drew, R. and Miners, J.O., Bioc. Pharmacol., 33:2989,1984). When chinchillas are pretreated with (400-800 mg/kg, IP) to reduce intracellular GSH levels, the toxicity of a combination of 10 mg/kg ETA and 100 mg/kg KA, which causes no change in the auditory brainstem response (ABR) of the chinchilla, results in complete deafness and distinct morphological damage when a single dose of these drugs is given after a single dose of BSO. The kidney, which has a rapid GSH turnover and is therefore especially susceptible to GSH depletion by BSO, also demonstrates severe damage after this treatment. A similar rapid turnover of GSH and resulting limited capacity to detoxify reactive intermediates and free radicals may determine auditory vulnerability to this toxicity.

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422.2 EVIDENCE OF THERAPEUTIC, AS WELL AS BEHAVIORALLY TOXIC ACTIONS OF GANGLIOSIDES IN RATS. S.B. Sparber, R.B. Messing, B. Berra*#, G. Seran* and M.S. Huang*. Dept. of Pharmacology, Univ. of Minnesota Med. Schl., Minneapolis, MN 55455 and #Ist. Fisiol. Gen. e Chim. Biol., Milan, Italy.

Trimethyltin (TMT; 6 mg/kg, p.o.) produces cytotoxicity in hippocampus and olfactory cortex (Chang et al, Neurobeh Tox Ter 5:337,1983) and impairs learning of delayed reinforcement autoshaped behavior (Cohen et al, Neurosci Abs 10:1205,1984). Gangliosides (GS) have been reported to exert therapeutic effects after brain lesions (e.g. Toffano et al, Acta Physiol Scand 122: 313,1984), and the present experiment was therefore undertaken to assess the effects of GS in normal and TMT-treated rats.

Male Long Evans rats (N=40; mean b.w. 487±5 gm) were implanted i.p. with Alza osmotic minipumps (model #2002) and infused continuously with 1.2 mg/day of purified mixed GS extracted from calf brain, or with H_2O vehicle. Two days after implantation, half of the rats in each group (n=10) were treated with TMT and the other half with H_2O; 19 days later the pumps were removed. Food deprivation over the next week was followed by 12 trials/day learning sessions, for a total of 120 trials. Control rats (H_2O + H_2O) learned the task at the expected rate, while TMT + H_2O rats failed to do so. GS + H_2O rats learned significantly more slowly than controls, but GS treatment counteracted the learning deficit induced by TMT; both groups given GS performed similarly. These data indicate that the deficit induced by the neurotoxin TMT may be prevented or attenuated by one or more GS, but that GS may also produce a learning impairment. To further test the effects of these substances on behavior, the rats were shaped to lever press for food and tested (beginning 53 days after TMT treatment) in an operant progressive fixed ratio task (Lichtblau & Sparber, Neurobeh Tox Ter 4:557,1982) in which the number of lever presses required to obtain a food pellet was doubled daily until an FR128 schedule of reinforcement was attained. All groups responded similarly on continuous reinforcement, but animals treated with TMT did not increase responding as much as controls as the requirement was augmented, responding at lower levels for all schedules ≥ FR8. The two groups treated with gangliosides also responded less than controls for ratios ≥ FR16. These results thus paralelled those seen in the autoshaping test.

GM_1 ganglioside has recently been reported to produce opposite effects on blood flow, deoxyglucose uptake and phosphorylation of proteins on the lesioned side of rats' brains compared with the contralateral non-lesioned side (Agnati et al, Acta Physiol Scand 125:43,1985). It remains to be determined if such neurochemical changes are a reflection of GS potential for nervous system toxicity.

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422.3 LYSERGIC ACID DIETHYLAMIDE (LSD) ADMINISTRATION FOR 5 DAYS SELECTIVELY DECREASES SEROTONIN$_2$ RECEPTOR BINDING IN RAT BRAIN. N. S. Buckholtz, D. X. Freedman and W. Z. Potter.* National Institute of Mental Health, Laboratory of Clinical Science, Bethesda, MD 20892.

We previously reported that daily administration of d-lysergic acid diethylamide (LSD; 260 µg/kg) for 10 days decreased binding to serotonin$_2$ (5-hydroxytryptamine$_2$, 5-HT$_2$) but not 5-HT$_1$ receptors in brain membranes from rats killed 24 hr after the last injection, as measured by ^{3}H-LSD binding in the presence of cinanserin and 5-HT respectively (Buckholtz, N.S. et al., Eur. J. Pharmacol. 109:421, 1985). In the present series of studies, the selective 5-HT$_2$ ligand ^{3}H-ketanserin was used. We first found that the same injection schedule and dose as above produced a decrease in the number of receptors in cortex (Bmax: LSD = 188 fmol/mg protein, saline = 292 fmol/mg protein) without a change in affinity (K_D: LSD = .33 nM, saline = .34 nM). Since we were interested in the possible relationship of this change to behavioral tolerance to LSD, we switched to a dose (130 µg/kg) and injection schedule (5 days) previously shown to produce tolerance. We again found a decrease in Bmax in cortex (LSD = 279, saline = 358 fmol/mg prot) without a change in K_D (LSD = .34 nM, saline = .34 nM). A decrease was also present 24 hr after 3 daily injections but not 24 hr after a single injection. At 48 hr after the last of 5 injections of LSD, there was less of a decrease in 5-HT$_2$ binding, perhaps indicating recovery toward baseline. To evaluate the regional distribution and specificity of the change after 5 days of LSD, in addition to ^{3}H-ketanserin binding to 5-HT$_2$ receptors, we also measured binding to the following receptors: 5-HT$_{1A}$ [^{3}H-8-hydroxy-2-(di-n-propylamino) tetralin]; 5-HT$_{1B}$ (^{125}I-cyanopindolol in the presence of 30 µM isoproterenol); beta-adrenergic (^{3}H-dihydroalprenolol); α_1-adrenergic (^{3}H-prazosin); α2-adrenergic (^{3}H-rauwolscine, ^{3}H-para-aminoclonidine); D_2-dopaminergic (^{3}H-spiperone); and the 5-HT uptake site (^{3}H-imipramine). The only consistent receptor change was the decrease in ^{3}H-ketanserin binding in all brain areas evaluated (cortex, hippocampus, midbrain, hypothalamus, striatum, pons/medulla). These data are suggestive evidence for a selective involvement of 5-HT$_2$ receptors in the development of tolerance to LSD's behavioral effects. (Supported in part by the Howard Pack Foundation).

422.4 ACUTE IMIPRAMINE TREATMENT ENHANCES A CONDITIONED PLACE PREFERENCE FOR COCAINE IN A NOVEL TEST. Z. Pap*, V. Hinton*, R. Benarroch*, J. Rosecan* and N. Geary. Psychology Department, Columbia Univ., New York, NY 10027.

A novel apparatus was used to investigate cocaine's potency to support a conditioned place preference (CPP). On drug days, rats were injected i.p. with 5 or 10 mg/kg cocaine HCl 10 min before being placed in a white 45x45x30 lwh cm open-topped box for 20 min. On alternating control days they were injected with saline and placed in a black box. Control rats received saline each day. CPPs were tested in a 91x91x30 cm box. Three quadrants of the floor and adjacent exterior walls were black; one quadrant was white. An X-shaped partition with wall colors matching the facing quadrant was placed so its lower edge was 15 cm above the cage floor. Rats were put in the middle of the box and time spent in each quadrant was recorded for 10 min. After 4 conditioning cycles, the 5 mg/kg dose elicited a significant CPP, but the 10 mg/kg dose did not (see Table). Four additional conditioning cycles did not further increase the CPP for 5 mg/kg cocaine. Thus, under these conditions, an appropriate cocaine dose elicits a stable, robust CPP.

Tricyclic antidepressants have been reported by human cocaine abusers to attenuate cocaine's effects. Therefore, we injected 15 mg/kg imipramine 2 h before a CPP test. This acute imipramine treatment enhanced, rather than blocked, the expression of the cocaine CPP. Further experiments will examine the effects of chronic imipramine treatment on the formation and expression of cocaine CPPs.

Percent of CPP Test Spent in White Quadrant

White/Black Paired Drug	After 4 Cycles	After 8 Cycles	After Imipramine
sal/sal	12.9 ± 3.0	15.0 ± 3.6	14.5 ± 4.0
5 mg coc./sal	39.9 ± 4.0[a]	41.2 ± 3.1[a]	63.3 ± 9.8[ab]
10 mg coc./sal	21.6 ± 4.9	---	---

[a]Significantly larger than sal/sal
[b]Significantly larger than last test before imipramine

422.5 STRIATAL CHOLINERGIC DISTURBANCE INDUCED BY HALOPERIDOL: CAT & ACHE. S.P. Mahadik, H. Laev*, F. Vilim, A. Korenovsky*, S.E. Karpiak. Div. Neuroscience, NYS Psychiatric Inst., Depts. of Biochem. & Molecular Biophys, and Psychiatry, Coll. of Phys. & Surg., Columbia U., NY, NY 10032.

Administration of haloperidol to rats causes a striatal cholinergic disturbance by removing tonic inhibition of intrinsic cholinergic neurons as shown by increased acetylcholine (ACh) turnover. We earlier reported that the increase in ACh turnover is associated with an increase in the 10s ["functional"] form of acetylcholinesterase (AChE) after 7 and 21 days of haloperidol treatment. To study the mechanisms underlying this cholinergic disturbance following long-term (chronic) haloperidol treatment, we have measured choline acetyltransferase (CAT) & AChE activities, and examined the immunohistochemical localization of CAT. Male Sprague-Dawley rats (250gr) were injected with haloperidol (2mg/kg/day i.m.) for 7,14,20 & 40 days. Controls received saline. Two of the 40 day rats were used for CAT histochemistry. For measurement of CAT and AChE, striata were dissected and enzymes were extracted with buffer containing Triton X-100. CAT was determined by radiometric assay using ^{3}H-choline and AChE by Ellman's spectrophotometric assay in the presence of 0.1mM ISO-OMPA. Striatal CAT activity increased by 24% and 11% respectively after 7 and 14 days of treatment, and decreased by 26% after 40 days of treatment. No change in CAT was seen after 20 days. Light microscopic immunohistochemical analysis of CAT was done with both avidin-biotin and peroxidase-antiperoxidase using rat monoclonal antibody (Boehringer-Mannheim). Striatal CAT reactivity in 40 day haloperidol treated rats was markedly decreased in both neuronal perikarya and neuropil compared with saline controls. The decrease appeared to be larger than that detected by chemical analysis suggesting a pronounced ultrastructural change in striatal cholinergic neurons. AChE showed 10% to 35% increases after 7 to 20 days of treatment, returning to control levels or below (-12%) after 40 days of drug treatment. The initial increases in CAT & AChE after short-term treatment (7-14 days) reflect adaptive changes in response to increased cholinergic activity. The decreases observed at 40 days indicate a hypocholinergic state has been established in the striatum. Such a hypocholinergic state may be responsible for initiation and persistence of extrapyramidal syndromes that are associated with long-term neuroleptic treatment of schizophrenic patients.

422.6 EFFECT OF CHRONIC ANTIDEPRESSANT DRUG TREATMENT ON TRH RESPONSIVENESS AND RECEPTORS IN THE RAT CNS. M.A. Sills and D.M. Jacobowitz. Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

Thyrotropin-releasing hormone (TRH) has been suggested to act as a neuromodulator in the CNS, based in part on its widespread distribution in the CNS, and its ability to exert numerous extrahypothalamic effects. For example, TRH has been shown to affect dopaminergic, cholinergic and serotoninergic systems. During the past several years, there has been an increased interest in the possible involvement of TRH in antidepressant drug action. This was based in part on the results from several groups that demonstrated that TRH can potentiate the effects of imipramine. To investigate further whether TRH may be involved in the mechanism of action of antidepressant compounds, the present study examined the effect of repeated treatment with the tricyclic antidepressant desipramine (DMI), or the monoamine oxidase inhibitor, nialamide, on the ability of the TRH analog MK-771 (L-pyro-2-aminoadipyl-histidyl-thiazolidine-4-carboxamide) to elicit wet-dog shakes, and on TRH receptors in the rat CNS. Animals were treated repeatedly either with desipramine (10 mg/kg b.i.d. for 14 days), nialamide (40 mg/kg b.i.d. for 7 days) or saline (b.i.d. for 7 or 14 days). Twenty four hours after the last treatment dose, animals were either challenged with a test dose of MK-771 for behavioral experiments or were sacrificed for autoradiographic experiments. All injections were administered i.p.

In the behavior experiments, the number of head shakes that occurred during the 30 min time period after injection of MK-771 was determined. Repeated administration of desipramine or nialamide reduced the ability of MK-771 to elicit wet-dog shakes at doses of 0.7, 1.0, 3.0 and 10 mg/kg. The maximum response to MK-771 in the control group was approximately 62 wet-dog shakes whereas nialamide and DMI reduced the maximal response to MK-771 by 70 and 50 percent, respectively.

In the autoradiographic experiments, rat brain sections were incubated with 10 nM ^{3}H-(3-methyl-histidine^2)-TRH (^{3}H-MeTRH). After incubation for 2 hrs at 0°, the dried slides were exposed to LKB Ultrofilm for 2 months. The results from these experiments indicate that chronic treatment with both antidepressant compounds produced significant alterations in the binding of ^{3}H-MeTRH in rat brain. In particular, specific binding was reduced by 20% in the nucleus of the diagonal band (n. basalis) and by 15-25% in the posterior cortical nucleus of the amygdala. Thus, the present studies indicate that TRH responsiveness, as well as the binding of ^{3}H-MeTRH in certain brain areas, are reduced subsequent to chronic antidepressant drug treatment.

422.7 EFFECT OF CLONIDINE WITHDRAWAL ON CARDIOPULMONARY CONTROL DURING SLEEP. S.DeMesquita, S.A.Skolik,* and M.M.Griffith*. Department of Physiology, Marshall Univ. Sch. of Med., Huntington, WV 25704.

Abrupt cessation of chronic infusion of clonidine (CLON) in normotensive rats has been associated with the appearance of periods of labile hypertension (Thoolen, M.J.M.C., et al., J. Pharm. Pharmacol. 33:232, 1981). In order to determine the cause of these hypertensive periods, this study investigated the effect of various doses of CLON on sleep pattern and cardiopulmonary regulation during CLON withdrawal.

Four adult (6.7±0.8 months of age) (mean±S.E.) male Sprague-Dawley rats (540.7±16.1 g) were each implanted with a chronic indwelling arterial cannula, EEG and EMG electrodes and monitored for respiratory rate (chest pneumograph), blood pressure and sleep/wake state before, during and after s.c. infusion of 500 µg CLON/kg/day for seven days by an Alzet mini-osmotic pump. An additional 12 adult (4.4±0.2 months of age) male Sprague-Dawley rats (507.3±9.1 g) were implanted with electrodes only and monitored for sleep/wake state during and after s.c. infusion of saline, 30 or 300 µg CLON/kg/day for seven days by mini-osmotic pump. All rats were monitored for 3.5h (0900 to 1230). All records were scored into Wake, Rapid Eye Movement sleep (REM), or Non-REM sleep (NREM) states and tabulated. Cardiopulmonary data were analyzed from a total of 16 REM periods (32 min), 80 min of NREM sleep prior to the REM periods and 20 min of quiet Wake.

REM sleep was abolished at the 300 and 500 µg/kg/day CLON dose rates and showed a significant ($P<0.05$) rebound at 17h (33.4±6.3%; 30.6±7.3% resp.) and 41h (23.1±2.2%; 21.0±5.6% resp.) after CLON pump removal as compared to the saline control (13.3±3.1%) and the pre-CLON control (10.8±3.8%) respectively.

While CLON significantly ($P<0.05$) lowered systolic, diastolic, and mean blood pressures as well as heart and respiratory rates, there was no significant difference in cardiopulmonary data between the pre- and post-CLON conditions during either the Wake or the NREM states. Only the REM state at 17h post-CLON termination showed significant cardiopulmonary increases:

	REM SLEEP*		
Condition	Systolic Blood Pressure (mmHg)	Heart Rate (beats/min)	Respiratory Rate (breaths/min)
pre-CLON	136 ± 5	339 ± 15	96 ± 5
17h post-CLON	160 ± 5**	389 ± 10**	111 ± 7**

*N=4; **$P<0.05$ from pre-CLON, paired Student's t-test

The results of this study indicate that the periods of high blood pressure noted during clonidine withdrawal are associated with REM sleep periods and are exacerbated by the REM sleep rebound caused by abrupt clonidine withdrawal.

422.8 INFLUENCE OF PERINATAL EXPOSURE ON THE EFFECTS INDUCED BY DIPHENYLHYDANTOIN IN ADULT B6C3F1 MICE: BEHAVIORAL AND MORPHOLOGICAL CONSIDERATIONS. G.C. Haggerty, P.J. Kurtz*, B.D. Carlton*, A.C. Peters, and R.S. Chhabra*. Battelle Laboratories, Columbus, OH 43201 and National Toxicology Program, NIEHS, Research Triangle Park, NC 27709

Diphenylhydantoin (DPH) is a widely used antiepileptic agent known to cross the human placenta and induce a pattern of growth and developmental abnormalities which are known collectively as the Fetal Hydantoin Syndrome. Additionally, it has been reported that epileptic patients chronically treated with DPH exhibit effects such as ataxia and generalized movement disorders (Kooiker and Sumi, Neurology, 68-71, 1974). The objective of the present study was to determine whether DPH exposure during early development modifies the behavioral effects (including spontaneous motor activity, grip strength, thermal sensitivity, and acoustic startle reflex) produced by subsequent chronic DPH dosing in adult mice. In addition, since chronic DPH treatment is known to induce cerebellar degeneration in man and animals, cerebellar tissue from selected male and female mice was examined for ultrastructural changes.

Female mice were exposed to dosed or control feed for 60 days prior to breeding, and throughout breeding, gestation, and lactation. At two months of age, the young were assigned to chronic dose groups. Behavioral testing took place at 2, 11, and 26 months. The results of the chronic study (after 24 months of adult dosing) are presented here.

Female mice received higher concentrations of dietary DPH than males (600 versus 300 ppm high dose) and showed more obvious behavioral changes after 24 months of exposure. Qualitative observations of ataxic gait and tremors were augmented by quantitative measurements of forelimb and hindlimb weakness and decreased thermal sensitivity in the high dose females. These effects were attributed to adult dosing and were not exacerbated by perinatal DPH exposure. Despite these functional changes, ultrastructural abnormalities were not observed in the cerebellum of either the males or females exposed to DPH. In conclusion, it appears that the spectrum of behavioral changes observed in female mice, similar to that reported in humans exposed to long-term DPH, may occur without obvious changes in cerebellar morphology. Furthermore, susceptibility to the effects of DPH on behavioral function was not increased by perinatal exposure.

422.9 REGIONAL DIFFERENCES IN THE BINDING OF (^{3}H)PRAZOSIN IN RAT BRAIN FOLLOWING IMINODIPROPIONITRILE ADMINISTRATION. Tammy Braun*, Allan E. Johnson, Bruce S. McEwen and Jean Cadet (SPON: Christine T. Fischette). Neuropsychiatry Branch, NIMH, St. Elizabeth's Hospital, Washington D.C., 20032, and Rockefeller University, New York, N.Y. 10021

Chronic administration of Iminodipropionitrile (IDPN) has been shown to induce a complex behavioral syndrome which is similar to symptoms characteristic of neurodegenerative disorders. This syndrome consists of hyperactivity, spasmodic neck dyskinesias, circling behavior and increased startle responses. The behavioral effects of IDPN can be reversed by specific dopaminergic, adrenergic and opiate receptor blockers. In vitro binding assays have shown that the concentration of α_1-adrenergic receptors are increased in caudate following IDPN administration. In this study, we used tritium-sensitive film autoradiography to survey the effects of IDPN on α_1-receptors in several areas of rat brain with (^{3}H)Prazosin (PRAZ).

Adult male rats were treated with IDPN (100mg/kg, ip) or an equal volume of saline for 7 days and killed by decapitation 21 days after the termination of drug treatment. In vitro (^{3}H)PRAZ binding was assessed in 8µm coronal sections by previously published methods (Johnson et al., Neurosci. abstr., 1985). Briefly slices were thawed and preincubated for 30 min. in Tris buffer (50mM, pH7.7, room temperature) followed by incubation in buffer containing 1.0nM (^{3}H)PRAZ ± 10µM Phentolamine. Slices were then rinsed twice for 5 min. each in ice cold buffer, dipped in cold bidistilled water and rapidly air dried. Labeled slices were exposed to LKB Ultrofilm along with plastic tritium standards for 6 weeks. Binding was measured using a computer assisted spot densitometer that converted optical density to fmoles/mg protein using a standard curve derived from tritium standards (Berck and Rainbow, J. Neurosci. Meth., 13: 231, 1985).

In this study, IDPN treatment enhanced PRAZ binding in specific layers of cortex and in specific regions of the thalamus (lateral dorsal geniculate, lateral posterior nu.) and in the paraventricular hypothalamic nucleus.

This study further implicates the adrenergic system in the involvement of the behavioral effects of IDPN and identifies additional structures which may play a role in this model of neurodegenerative diseases. The effects of IDPN on other adrenergic receptors (α_2 and ß) have yet to be determined. Supported by NS07080 and NS07911.

422.10 ACUTE HEXAMETHONIUM: EFFECTS ON BEHAVIOR IN RATS GIVEN CHRONIC DOSES OF NICOTINE. Edward Levin, Gaylord Ellison, Ciema Salem*, Murray Jarvik and Ellen Gritz* Dept of Psychology, Dept. of Psychiatry and Cancer Control Center, UCLA, Los Angeles, CA 90024.

Rats chronically given nicotine and their controls were given acute doses of 0, 10, or 20 mg/kg of hexamethonium (HEX), a peripheral nicotine receptor blocker, on the 4th and 8th days of nicotine administration and on the day after withdrawal. Food and water consumption and spontaneous behavior was observed for two minutes every fifteen minutes for the 2 hour period after HEX injection. Nicotine significantly decreased the amount of food consumed and decreased observed feeding behavior. It also decreased exploratory sniffing and increased time spent resting. No significant effects of hexamethonium on the nicotine intoxication were noted of the 4th day, at the height of the nicotine effects. On the 8th day, the rats given 10 mg/kg of HEX showed significantly increased exploratory sniffing ($p<.001$) and significantly increased observed feeding behavior ($p<.05$). However, despite the significant increase in observed feeding behavior, there was no change in the amount of food actually eaten. Rats given the higher dose of 20 mg/kg did not show these effects. After withdrawal from nicotine, both observed eating behavior and the amount of food consumed returned to control levels. These data show that blockade of peripheral nicotinic receptors can alleviate some of the behavioral effects of chronic nicotine intoxication. The effect of HEX in increasing observed feeding behavior without affecting the amount of food consumed, shows that these behaviors can be pharmacologically differentiated and that possibly some aspects of their neural control may be different. The peripherial mechanisms involved could include sympathetic and vagal innervation of the liver. (The research was supported by a grant from the MacArthur Foundation. Ed Levin was supported by NIMH training grant 15795.)

422.11 CONTINGENT TOLERANCE TO DIAZEPAM'S ANTICONVULSANT EFFECT ON AMYGDALOID KINDLED SEIZURES IN THE RAT. M.J. MANA, J.P.J. PINEL and C.K. KIM. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada. V6R 1W5.

Contingent tolerance is tolerance that develops preferentially to the effects of a drug on responses that have occurred during periods of drug exposure. Most studies of contingent tolerance use the before-and-after design; on each trial, the subjects in one group (Before Group) are injected with the drug before the test response, whereas the subjects in the other group (After Group) are not injected until after the response. On the test trial, all subjects receive the drug before the test response so that the level of tolerance to the effects of the drug on the response can be assessed. Subjects in the Before Group frequently demonstrate significantly more tolerance than do the subjects in the After Group. Such tolerance is termed "contingent tolerance" because its development is contingent upon the occurrence of the test response during the periods of drug exposure.

In the present study, we demonstrated contingent tolerance to diazepam's anticonvulsant effect. During the tolerance development phase, each of the 23 kindled rats serving as subjects received 10 bidaily diazepam injections (2 mg/kg, I.P.), and 10 bidaily bipolar amygdaloid stimulations (400 A, 60 Hz, 1 s). The rats in the Before Group (n=11) received diazepam 1 hr before each stimulation, whereas those in the After group (n=12) received the drug 1 hr after each stimulation. On the test trial, the rats in both groups received diazepam 1 hr before stimulation, so that the effect of the drug on the duration of the forelimb clonus elicited by the test stimulation could be assessed.

There was a striking difference in the tolerance displayed by the groups on the test trial ($F(1,42)=39.9$, $p<.0001$). The rats in the After Group displayed no tolerance at all to diazepam's anticonvulsant effect; the test dose completely blocked forelimb clonus in every rat. In contrast, the rats in the Before Group were almost totally tolerant; the mean duration of clonus elicited by the test stimulation (M=35.3 s) was almost as long as that elicited in the absence of drugs.

This demonstration is important for two reasons. First, it provides support for the idea that tolerance develops to a drug's effects rather than to its presence; tolerance does not develop to drug effects that are not allowed to manifest themselves. Second, it identifies a factor potentially important in the development of tolerance to antiepileptic drugs in therapeutic situations.

422.12 A CHRONIC METHOD FOR DRUG ADMINISTRATION INTO LOWER BRAINSTEM IN AWAKE RATS: BEHAVIORAL EFFECTS OF SURGERY. Gery Schulteis* and Joe L. Martinez Jr. (SPON: H. Rigter). Dept. of Psych., Univ. Calif., Berkeley, 94720.

Using a new technique for injecting drugs into dorsal hindbrain regions of unanesthetized rats, we assessed the effects of the surgical procedure on two behaviors. Our technique involves implantation of a 25mm stainless steel guide cannula (GC), 23 gauge, according to the following stereotaxic coordinates: AP -6.0mm from Bregma, carrier angled 46-47.5° from the vertical, 11.2mm below skull surface. At the time of injection, an inner cannula (IC) of variable length is inserted. Precise ML placement, carrier angle, and IC length are determined by the specific structure to be cannulated. Accessible structures include the area postrema, nucleus tractus solitarius, XII nucleus, dorsal motor X, medullary reticular formation, nucleus gracilis, and nucleus cuneatus.

Behavioral studies were conducted on 7 operated (OP) subjects (GC coordinates: carrier angle 47°, ML 0.8mm bilaterally), 6 sham operated (SHAM) subjects, and 9 unoperated controls (UNOP). The first study assessed the effects of surgery on open field locomotor activity, beginning on the first day after surgery and continuing every 3 days for 5 weeks. Both SHAM and UNOP groups showed significant decreases in activity over days ($F[11,143]=4.99$, $p<.001$), but the SHAM group crossed significantly more lines than the UNOP group throughout testing ($F[1,13]=7.52$, $p<.03$). Only OP subjects exhibited ataxia on Day 1, and this rapidly disappeared. OP and SHAM groups showed no differences in overall activity ($F=.681$), although there was a significant interaction between groups and days ($F[11,121]=2.53$, $p<.012$). The second study measured active avoidance performance over an 8 trial training session. There were no significant differences among any of the groups on either average latency to reach the safe compartment or total number of avoidances. Thus, surgery did not affect shock-motivated learning, despite elevating open field activity over UNOP baselines.

Our new technique has several advantages. It allows administration of drugs to unanesthetized animals, in acute or chronic studies, and enables injections into multiple regions of the same nucleus using the same GC placement. The angle of entry allows for cannulation of structures caudal to obex. Finally, the indwelling cannula results in no lasting ataxia. (Supported by ONR N00014-83-K-0408)

422.13 RELEASE OF ENDOGENOUS AMINO ACIDS FROM SLICES OF MOUSE BRAIN FOLLOWING METHYLMERCURY POISONING. J.N. Reynolds* and W.J. Racz* (SPON: C. Romero-Sierra). Dept. of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Methylmercury (MM) poisoning results in a characteristic pattern of neurological symptoms which may reflect loss of specific cell types and/or alterations in specific neurotransmitter systems within the central nervous system. The purpose of these experiments was to examine the release of endogenous amino acid neurotransmitter substances regionally in the mouse brain following a neurotoxic dose of MM.

Male, Swiss white mice (25 g) received oral doses of methylmercury chloride at a dose of 8 mg Hg/kg/day for 7 days. Animals were monitored for symptoms of MM intoxication. At 1, 5 and 10 days after the last dose of MM, animals were sacrificed and slices (300 μm) of cerebellum and cerebral cortex prepared for superfusion experiments. The spontaneous and 35 mM K^+-stimulated release of aspartate, glutamate, glutamine, glycine, taurine and γ-aminobutyric acid (GABA) were determined by high performance liquid chromatography coupled with fluorescence detection.

Animals which received MM developed symptoms of intoxication (decreased locomotor activity, decreased body weight, ataxia, weakness or paralysis of hindlimbs) which ranged from negligible to severe. Twenty-four hours after the last dose of MM, the 35 mM K^+-stimulated release of glutamate and GABA were decreased to 75% of control in cerebellar slices obtained from treated animals. However, 5 and 10 days after the last dose of MM, the K^+-stimulated release of glutamate from cerebellar slices increased to 150% of control, whereas GABA release was unchanged. Also 5 days after the last dose of MM, the spontaneous release of taurine was significantly elevated (127% of control) in slices of cerebral cortex obtained from MM-treated animals. Thus, MM can produce alterations in specific amino acid neurotransmitter systems at different times during intoxication. Persistent changes in the release of glutamate in the cerebellum is consistent with the proposal that the cerebellar granule cell, which utilizes glutamate as its transmitter substance, is particularly sensitive to the neurotoxic effects of MM. Alterations in cerebellar function are proposed to underlie some of the neurological symptoms (e.g. ataxia) which characterize MM poisoning.

422.14 INTRACEREBROVENTRICULAR CORTICOTROPIN-RELEASING FACTOR (CRF): EFFECTS ON EXPLORATORY BEHAVIOR AND SOCIAL INTERACTION. A.J. Dunn, C.W. Berridge* and S.E. File. Dept of Neuroscience, University of Florida, Gainesville, FL 32610, and School of Pharmacy, University of London, London WC1N 1AX, England.

CRF exists in a number of extrahypothalamic brain sites, and CRF-binding sites are distributed throughout the brain. ICV injection of CRF causes a general behavioral activation in rodents and potentiates the behavioral effects of exposure to a novel environment. Results obtained in the open field-conflict test were opposite to those observed after administration of benzodiazepines, suggesting that CRF augments the anxiogenic or stressful aspects of a novel environment. These and other data have inspired the hypothesis that CRF acts as a general activating factor during stress, altering the activity of the pituitary-adrenal axis, the sympathetic nervous system, and the cerebral catecholaminergic systems.

In experiments with mice, we used a novel multicompartment chamber (MCC) similar to that used previously for rats (Arnsten et al., Pharm. Biochem. Behav. 22:803, 1985). Morphine (1.75 mg/kg) reduced the mean time per contact with environmental stimuli, and tended to make the animals hyperactive. Naloxone (0.75 mg/kg) prevented the effect of morphine, with no observable behavioral effect by itself. A higher dose (1.25 mg/kg) of naloxone significantly increased the mean stimulus-contact time. Likewise, a 30 min period of restraint decreased the mean stimulus-contact time. This effect of stress was prevented by a dose of naloxone that had no effect in the absence of stress (0.75 mg/kg). These results replicate previous studies in rats (Arnsten et al., 1985).

ICV CRF (75 ng) reduced the mean stimulus-contact time, without significantly altering locomotor or grooming behaviors. This reduction in mean stimulus-contact time was prevented by naloxone.

Thus, opiates, stress, and CRF all decreased the mean stimulus-contact time in the MCC. Furthermore, the effects of all three treatments were completely antagonized by naloxone.

In rats, ICV CRF (100 and 300 ng) decreased social interaction and increased grooming in a familiar environment, without affecting locomotion or rearing. Decreased social interaction has been interpreted to indicate anxiogenic behavior, and has been observed previously with ACTH (File & Clarke, Pharm. Biochem. Behav. 12:711-715, 1980) and caffeine. This effect of CRF was counteracted by chlordiazepoxide (CDP 5 mg/kg IP), but the effect of CDP on locomotor activity and rearing was not altered by CRF.

These results in both rats and mice support further the idea that cerebral CRF is anxiogenic and that it might mediate certain behavioral responses in stress. The pharmacological data implicate both opioid and benzodiazepine receptors in the responses to CRF.

This research was supported by NIMH (MH 25486).

423.1 **SUICIDE TRANSPORT ACTIVITY OF ABRIN (A), MODECCIN (M) AND VOLKENSIN (V) IN RAT PERIPHERAL NERVOUS SYSTEM.** T.N. Oeltmann, F. Stirpe, and R.G. Wiley (SPON: G. Patterson) Laboratory of Experimental Neurology, VAMC, Nashville, TN 37203.

The suicide transport technique, anatomically selective destruction of neurons using axonally transported cytotoxin, has proven useful in anatomical, neurochemical and neurophysiological studies. The toxic lectin, ricin, is an efficient, unselective suicide transport agent in peripheral nerves. The present study sought to determine if the toxic lectins, **A**, **M** and **V** are effective and/or selective suicide transport agents in the vagus, hypoglossal and sciatic nerves. **A**, **M** and **V** (0.2 - 2 ug) were dissolved in phosphate buffered saline containing 0.1% Fast Green dye and pressure microinjected subepineurally into nerves of anesthetized adult, male Sprague-Dawley rats. One nerve was injected with one toxin in each of 26 rats. After 33 hrs - 5 d, animals were reanesthetized and perfused transcardially with aldehyde fixative followed by 30% sucrose. Brainstems, spinal cords and ganglia were sectioned in a cryostat and sections stained with cresyl violet and Luxol Fast Blue/cresyl violet. At the doses studied, all three toxins were effective and unselective in destroying sensory and motor neurons projecting axons through the injected nerves. The order of potency was **V>M>A**. Concurrent administration of lactose (1 g/kg, i.p.) was useful in protecting animals from the systemic toxicity of **A** and **V** but not **M**. The time course and sequence of histopathological changes appeared identical to the effects of ricin except that **A**, and to lesser degree **M** produced greater leukocyte infiltrates around perikarya of poisoned neurons than either ricin or **V**. We conclude that **A**, **M** and **V** are effective, unselective suicide transport agents in the rat peripheral nervous system. They do **not** appear to offer any clear advantage over ricin in this setting. (This work supported by the Veterans Administration Research Service, the Consiglio Nazionale delle Ricerche and the Ministero della Pubblica Istruzione, Rome, Italy.)

423.2 **MODECCIN AND VOLKENSIN ARE EFFECTIVE SUICIDE TRANSPORT AGENTS IN RAT CNS.** R.G.Wiley, F.Stirpe and T.N.Oeltmann. Laboratory of Experimental Neurology, VAMC, Nashville, TN 37203.

Ricin is an effective suicide transport agent in the peripheral nervous system but not within the CNS. It has been hypothesized that ricin does not work in the CNS because it is not bound to nerve terminal surface membranes of CNS neurons. In the present study, we sought to determine if the toxic lectins abrin (**A**), modeccin (**M**) and volkensin (**V**) which each have different oligosaccharide binding specificities are effective suicide transport agents within the CNS. The toxins dissolved in phosphate buffered saline were pressure microinjected under stereotactic guidance into the caudate nucleus of 23 anesthetized adult male Sprague-Dawley rats. One caudate nucleus was injected with one toxin in each rat. All injections were 0.1 ul and contained 0.017-0.2 ug of **A**, 0.003-0.4 ug of **M** or 0.002-0.05 ug of **V**. Lactose (1 g/kg, i.p.) was given to all animals at the time of the toxin injection. After 2-13 d, animals were reanesthetized and transcardially perfused with aldehyde fixative. For routine histology, brains were sectioned in a cryostat and stained with cresyl violet. For catecholamine histofluorescence, fixed brains were sectioned in a Vibratome, dried onto slides and mounted in mineral oil. **M** and **V** at all doses produced cell loss in the ipsilateral substantia nigra, ventral tegmental area and intralaminar thalamus within 48 hrs. At all but the lowest dose, the above lesions were complete and accompanied by a severe neurological syndrome developing between 36-48 hrs postinjection and characterized by forced contraversive circling. All three toxins produced necrosis at the injection site but **A** did not produce distant lesions at any dose. We conclude that **M** and **V** are efficient suicide transport agents within the rat CNS. These agents should prove extremely useful in making a variety of unique lesions for anatomical, neurochemical and neurophysiological experiments. (This work was supported by the Veterans Administration Research Service, the Consiglio Nazionale della Ricerche and the Ministero della Pubblica Istruzione, Rome, Italy.)

423.3 **COLLOIDAL GOLD FLUORESCENT MICROSPHERES: A NEW RETROGRADE FLUORESCENT/EM PROBE.** J. Quattrochi, R. Madison, I. Kljavin* and R. L. Sidman (SPON: B. Seckel). Dept. of Neurology and Neuropathology, Harvard Medical School, and Dept. of Neuroscience, Children's Hospital, Boston, MA 02115.

Rhodamine latex microspheres recently have been introduced as a retrograde tracer for superior neuronal labeling with epifluorescent microscopy. However, microspheres do not allow ultrastructural analysis of fluorescent labeled cells. We have developed a new colloidal gold fluorescent microsphere which now permits accurate ultrastructural identification of fluorescent retrograde labeled neurons.

A reaction suspension of activated microspheres (25 mg/ml, 0.02 - 0.2 um) was incubated with various dilutions of protein A-colloidal gold (15 nm, Janssen Pharmaceutica) in 0.01 M phosphate buffer, pH 8.0, at 25 degrees C for 6 hrs, then at 4 degrees C for 24 hrs. The suspension was layered onto a gradient of 20% (wt/wt) sucrose overlayered with 5% sucrose buffered at pH 8.0 with 0.01 M glycine and centrifuged at 200,000 g for 10 min. Microspheres were recovered, resuspended in buffer, monodispersed on a mesh copper grid, and conjugation with colloidal gold confirmed with the electron microscope at 60 kV.

Aliquots of 0.5 to 1.5 ul were assayed for retrograde transport by extracellular micropipette pressure injection into the left motor cortex of mice at 60 postnatal days of age. At four days postinjection, animals were anesthetized and perfused with 4% buffered paraformaldehyde, 0.5% glutaraldehyde. The brain was removed and cut into a coronal series of 200 um sections placed in cold 20% buffered sucrose. Fluorescent neuronal profiles were identified with a compound epifluorescent computer microscope in the contralateral motor cortex following retrograde transport of microspheres by commissural axons. After sections were processed with a routine EM protocol, labeled neurons demonstrated intracellular colloidal gold allowing ultrastructural in vivo confirmation of retrograde labeling by colloidal gold microspheres. We have begun to apply this new tracer technique to the bulbospinal projection network of the respiratory brainstem using microspheres individually conjugated with different sizes of colloidal gold. Our purpose is to examine ultrastructural connectivity between separate respiratory assemblies each retrogradely labeled with a different size gold marker and previously inaccessible with existent neuroanatomical methods.

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423.4 MODIFICATION OF TRANSPORT SPECIFICITY OF HORSERADISH PEROXIDASE: ANTEROGRADE AND TRANSNEURONAL PROPERTIES. T.L. Neal and R.G. Carey. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Horseradish peroxidase (HRP), an enzyme commonly used in neuroanatomical tracing, exhibits predominantly retrograde as opposed to anterograde transport properties following cortical or subcortical electrophoretic injections. Intraocular injections of a 30% solution of buffered HRP result in visualization of projections to the known retinal recipient areas (e.g., superior colliculus, pretectum and lateral geniculate nucleus), but with no evidence of transneuronal activity. On the other hand, HRP that has been conjugated to the lectin wheat germ agglutinin (WGA) using a modification of the protocol of Nakane and Kawaoi (1974) is known to produce anterograde and retrograde transneuronal transport from intraocular injections (Carey and Neal, 1985) and to exhibit dense anterograde as well as retrograde transport from electrophoretic brain injections. We have found that the unreacted HRP isolated from the conjugation mix demonstrates similar transneuronal, anterograde, and retrograde transport properties.

Conjugation of HRP to the lectin WGA is achieved using a modification of the Nakane and Kawaoi (1974) protocol. HRP is treated with dinitrofluorobenzene and sodium periodate followed by reduction with sodium borohydride in the presence of WGA. Separation of the ensuing conjugate from unreacted HRP and WGA is accomplished by Sephadex G-150 column chromatography. The conjugate obtained from this procedure exhibits strong anterograde, retrograde, and transneuronal transport properties. Intraocular injections of a 10% solution of buffered WGA/HRP conjugate produce intense labeling of cells and/or terminals in such areas as the superior colliculus and parabigeminal, lateral posterior, pretectal, and lateral geniculate nuclei. Terminal labeling also is observed in striate cortex. HRP isolated from the reaction mix and injected intraocularly as a 10-30% buffered solution demonstrates anterograde and retrograde transport properties identical to the WGA/HRP conjugate. Cortical injections of the modified HRP result in dense terminal labeling in addition to the cellular labeling routinely elicited by native HRP. The mechanisms by which the Nakane and Kawaoi procedure modifies the transport characteristics of the unconjugated HRP are currently under investigation. Hypotheses based on alterations in carbohydrate configuration or modifications in charge are being critically evaluated.

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423.5 IMPROVED HRP DETECTION IN THE LIGHT AND ELECTRON MICROSCOPES. A. Foerster, Department of Neurosciences, McMâster University, Hamilton, Ontario, Canada L8N 3Z5.

A method that uses the reaction of glucose and glucose oxidase (GOD) to generate hydrogen peroxide in a TMB-HRP reaction mixture has been found to provide a greater yield of crystalline TMB-HRP product and less background labelling than the addition of hydrogen peroxide to the standard Mesulam reaction mixture. To measure this improvement the two reactions were compared on adjacent sections of the superior colliculus labelled by anterograde transport of HRP from an intraocular injection in the anesthetized rat, fixed with 1% paraformaldehyde, 1.25% glutaraldehyde, 2% DMSO in 0.1 M phosphate buffer (pH 7.4), and immersed in the same fixative for 2h at 4°C. Vibratome sections, 80 µm, were reacted in 0.05 M acetate buffer (pH 3.3) for light microscopy and phosphate buffer (pH 6.0) for EM. For TMB-GOD, preincubation for 20 min in 0.08% ammonium bromide, 0.1% sodium nitroferricyanide, 2% glucose, 0.48% TMB, was followed by incubation with the addition of 100 units/100 ml of GOD (Sigma #G-6500). The Mesulam mixture contained 0.03% H_2O_2. 20 min incubations were generally optimal. For light microscopy, sections were briefly washed in the buffer and mounted. For EM, sections were osmicated for one h at room temperature in 2% OsO_4 in 0.1 M phosphate buffer (pH 6.0), dehydrated in 80%, 90% and 3 x 100% acetone for 7 min each, and embedded in Spurr's resin. Ultrathin coronal sections of the upper 300 µm of the SC were cut and grid stained with lead citrate. In the light microscope the GOD reaction produced much less artefact and a deeper staining of HRP than seen in the adjacent Mesulam material, especially noticeable in sections that had been stored for days. In the EM the number of membrane-bounded profiles that contained TMB-HRP crystals was counted in the superficial region of the stratum griseum superficiale (SGS), i.e. major zone of optic terminations. In the TMB-GOD-reacted material there was approximately twice the number of such profiles as in the Mesulam-reacted section. Even more striking, crystals in myelinated axon profiles were seen only rarely in the Mesulam material, but were relatively common in the TMB-GOD.

In the sections there were on average about 20 labelled profiles (almost all terminals) per 900 μm^2 of the upper 60 µm of SGS, which receives a dense optic projection. If the "diameter" of these terminals is taken to be 2 µm or less, then one can estimate a lower limit for their total number in this zone as about 3×10^6. If all of the approximately 120,000 axons in the optic nerve had contributed to this population, then on average each axon would provide at least 25 boutons. Since many unlabeled presumptive optic terminals were seen, this is a gross underestimate.

(Supported by MRC Canada)

423.6 TRACT-TRACING WITH HRP AND CORALT IN POST-MORTEM HUMAN BRAINSTEM. T.G. Beach* and E.G. McGeer (SPON: P.C.K. Leung). Kinsmen Laboratory of Neurological Research, Dept of Psychiatry, Univ. of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

The introduction of anterograde and retrograde tracers has revolutionized experimental neuroanatomy. Unfortunately, these techniques usually involve the injection of a compound into the living brain and so cannot be used for the elucidation of human neuroanatomy. Certain of these methods have been adapted to in vitro preparations, however, and we have found that modifications of these published methods (J.F. Iles, B. Mulloney, Brain Res. 30:397, 1971; P. Ekstrom, J. Neurosci. Meth. 15:21, 1985) are successful in demonstrating connections in the post-mortem human brainstem and adjacent cervical spinal cord. Five human autopsy cases were used; their ages ranged from 70 to 92. All had died without evidence of neurological disorder. Post-mortem delay ranged from three to five hours. Three of these were used for HRP studies (using the method of Ekstrom cited above), and two for cobalt studies (using a liberal modification of Iles and Mulloney, cited above). For the HRP method: HRP crystals were applied directly to the right dorsal columns at the C1 level, rinsed away with PBS after 5 minutes, and the entire brainstem incubated in Dulbecco's modified Eagle medium at 4°C for 24-48 hours. Two mm transverse slices were taken and immersed for one hour in 2.5% glutaraldehyde at room temperature, then left overnight in 15% sucrose/PBS at 4°C. 50-100µm sections were cut on a freezing microtome and were reacted with a diaminobenzidine/nickel ammonium sulfate solution for 20 minutes. The cobalt method involved backfilling the stumps of cranial nerves III and IV by trailing the nerves into Eppendorf tubes filled with 0.3M $CoCl_2$ and sealed with vaseline. The entire brainstem with attached Eppendorf was incubated in PBS at 4°C for 48 hours, sliced into 2mm transverse segments for 400 µm sections on the freezing microtome, reacted with 1% ammonium sulfide for 20 minutes at room temperature, fixed in 2.5% glutaraldehyde at room temperature, and either mounted directly onto slides (400µm sections) or embedded in paraffin and sectioned at 10µm (2mm slices). Upon examination, the HRP-treated sections showed heavy terminal-like labeling in the ipsilateral dorsal horn up to the spinal cord-medullary transition (about 3-4 mm). The substantia gelatinosa was especially heavily labeled. A few labeled neuronal perikarya were seen in the ipsilateral dorsal horn. Labeled axons were seen in the ipsilateral fasciculi gracilis and cuneatus up to 1 cm from the application site. Cobalt-treated sections displayed labeled perikarya and fibers within the central courses of the treated nerves. This represented a travelled distance of 1-2.5 cm. As these observations are entirely consistent with knowledge regarding these pathways, both methods appear to be suitable for tract-tracing in post-mortem human brainstem.

423.7 DENSE FILLING OF NEURONS AND GLIA CELLS AND THEIR PROCESSES WITH HRP APPLIED TO PRE-FIXED TISSUE. G.H. Kageyama and R.L. Meyer, Dev. Biol. Center, Univ. of Calif., Irvine, CA 92717.

Horseradish peroxidase (HRP) has proven to be a valuable tracer for neurobiological research in live tissue. The present study examines its usefulness for labeling cells in pre-fixed tissue. Of several methods of fixation and HRP application, the following worked well: HRP + 2% DMSO was applied to tissue that had been pre-fixed with 2.5% paraformaldehyde and 1.5% glutaraldehyde for a period of 1 hour to several months, either by immersion or best by transcardial perfusion. After thoroughly rinsing the tissue in 0.1 M PO4 buffer, HRP was either injected into fixed brain slices of mice, rats or cats with a Hamilton syringe, or was applied to the optic nerve of goldfish with a short piece of polyethylene tubing glued into place and capped with tacky wax. After 1-14 days at 4°C, vibratome or frozen sections were cut at 20-60 µm and reacted with either Adams' ('77) intensified DAB reaction, or Hanker-Yates reagent. Three conditions were particularly important for successful filling: good fixation to preserve membranes, long application period to allow for intracellular diffusion, and minimal extracellular diffusion to reduce background. When these conditions were met, we found solid filling of neurons, including dendrites, axons and terminal arbors for distances of several hundred µm's. In mammals, selective injection into the desired nucleus or fiber tract allowed visualization of single neurons, neuronal clusters, dendritic bundles, bundles of glial processes, and terminal arborizations of axons. In goldfish, optic nerve axons and terminal arborizations were densely filled to posterior tectum (a distance of about 5 mm's). Larger diameter axons and/or dendrites tended to be preferentially filled in short-term injections. Occasionally, transcellular labeling of neurons and glia was also observed, presumably by diffusion across breaks in the axon-neuron or axon-glia membranes. Electron microscopy revealed clearly localized electron dense reaction product and excellent ultrastructural detail of both labeled profiles and unlabeled surrounding neuropil.

This method has a number of potentially useful applications: Biopsied or immersion fixed tissue may be used. Several regions of one brain may be independently labeled. By dissection, injections may be made under direct visual control. Rapidly occurring events can be "frozen" in time by fixation. Efforts at injecting single cells in fixed tissue are now in progress with the hope of examining both the gross morphology and ultrastructure of single cells.

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423.8 NONSPECIFIC ASSOCIATION OF DIAMINOBENZIDINE-HORSERADISH PEROXIDASE CHROMAGEN WITH SUBCELLULAR ORGANELLES OF THE NERVOUS SYSTEM. Michael R. Wells and Urmi Vaidya. Neurochemistry Research Laboratory(151S), Veterans Administration and Department of Physiology, George Washington University, Washington, D.C. 20422.

The horseradish peroxidase (HRP)induced oxidation of 3', 3'-diaminobenzidine (DAB) is often used in attempts to localize molecules in the nervous system. As control procedures for immunocytochemistry usually focus on the nonspecific interactions of the primary and/or secondary antisera within tissues, the adsorption of the chromagen itself within the tissue to subcellualr organelles is not often considered. At the ultrastructural level, however, nonspecific adsorption and diffusion of chromagen even over short distances could obscure the prospective subcellular localization of a molecule (Novikoff, J. Histochem. Cytochem. 28: 1036, 1980). To examine the possible artifacts which could arise from nonspecific reaction of DAB chromagen with CNS tissues, HRP was infused into sections of cerebral cortex and immediately reacted. Adult rats (300 g. body wt.) were perfused through the heart with 4.5% paraformaldehyde, 0.5% glutaraldehyde in 0.1 M cacodylate, pH 7.4. Coronal sections of cerebral cortex were made at 30-40 um on a vibratome. Sections were stored for 12 hours at 4°C in phosphate buffered saline containing 2 mg/ml glycine to quench all free aldehyde groups. HRP (Sigma type VI) was introduced into sections by two methods: (1) sections were soaked in a 100ug/ml solution of HRP in phosphate buffered saline in 0.3% Triton X 100 overnight; (2) sections were sonicated 30 minutes in 100 ug/ml HRP in phosphate buffered saline. In both instances, sections were reacted immediately with DAB according to the method of Graham and Karnovsky (1966). Tissues were postfixed with 2% osmium tetroxide, flat embedded in Epon and sectioned on an ultramicrotome. Sections were examined unstained or stained with uranyl acetate. In both infusion procedures, HRP-DAB reaction product was formed in the tissue matrix and associated nonspecifically with subcellular organelles. The reaction was exaggerated in sonicated tissues. Chromagen could be seen associated in a nonrandom manner with cell membranes, ribosomes, mitochondria, microtublules, postsynaptic densities, presynaptic vesicles, cell nuclear chromatin and nucleoli. The binding in some instances mimicked "specific" patterns of binding in immunocytochemical preparations. This indicates that while the presence or absence of a molecule in a particular region of a cell may be detected with DAB chromagen, its precise ultrastructural localization may not be reliable. The data suggest that nonspecific adsorption of DAB chromagen may produce serious artifacts which must be considered in the interpretation of the subcellular localization of molecules at the ultrastructural level in nervous system.

Supported by the Veterans Administration.

423.9 COMPARATIVE STUDY OF QUINOLINE COMPOUNDS AS REAGENTS FOR THE HISTOFLUOROMETRIC DETECTION OF ZINC IN HIPPOCAMPUS AND OTHER BRAIN REGIONS. E. J. Kasarskis, D. Ringo*, and C. J. Frederickson*. Dept. Neurology, VA and Univ. Kentucky Med. Ctrs., Lexington KY 40536 and Univ. Texas at Dallas, Richardson TX 75080.

Studies from our laboratories have established 2-methyl-8-hydroxyquinoline (M-HQ) as a histoflurometric probe of the Zn pool localized to the hippocampal mossy fiber projection (Soc. Neurosci. Abstr. 11:438, 1985). The present investigation determined the specificity and intrinsic fluorescence of a series of quinoline chelates with cations of neurobiological relevance (Zn, Ca, Mg).

The following compounds were studied: 8-hydroxyquinoline (HQ); M-HQ; 8-hydroxyquinoline-5-sulfonic acid (SA-HQ); and N-(6-methoxy-8-quinolyl)-p-tolunesulfomanide (TS-HQ). Excitation/emission maxima were determined for the chelates formed by addition of each quinoline and equimolar amounts of either Zn, Ca, or Mg (1mM in EtOH). For histological studies, sections of frozen brain were cut at 40 um, thaw-mounted, stained with quinolines (0.01% w/v in acetone), and examined with a Leitz fluorescence microscope.

The quinoline chelates of Zn exhibited the greatest fluorescence compared to either Ca or Mg. On an equimolar basis, the intesity series for the Zn-quinolines was: TS-HQ > M-HQ > HQ > SA-HQ (100:6:3:1). Moreover, both TS-HQ and M-HQ showed a great selectivity for Zn with a fluorescence intensity ratio of approximately 10:1 comparing either Zn:Ca or Zn:Mg. Fluorescence varied linearly over a wide range of Zn concentrations in vitro.

As with M-HQ, the hippocampal mossy fiber projection was intensely fluorescent after staining with TS-HQ. However, the greater TS-HQ fluorescence identified other brain regions positive for Zn in the following rank order: hippocampus > amygdala > cerebral isocortex excluding layer 4 > bed nucleus of stria terminalis > lateral septum > striatum. Sensitivity to changing Zn concentrations was demonstrated by observing increasing fluorescence during postnatal development of the mossy fiber system. These results indicate the potential utility of TS-HQ in investigating the neuroanatomic distribution and function of Zn in the CNS. (Supported by the VA Research Service and Grants Nos. AA05931, NS00768).

423.10 MICROPHOTOMETRIC DETERMINATION OF SUCCINIC DEHYDROGENASE (SDH) ACTIVITY FROM TISSUE SECTIONS USING AN IMAGE PROCESSING SYSTEM. C.E. Blanco*, G.C. Sieck, R.D. Sacks*, and V.R. Edgerton. Dept. of Respiratory Diseases, City of Hope Medical Center, Duarte, CA 91010 and Depts. of Anatomy and Kinesiology, UCLA School of Medicine, Los Angeles, CA 90024.

A method for quantifying the SDH activity of muscle fibers in cross-section was developed, which uses: the reduction of nitroblue tetrazolium (NBT) to its diformazan as the reaction indicator; 1-methoxy-phenazine methosulfate (mPMS) as the exogenous electron carrier; and sodium azide to inhibit cytochrome oxidase. The reduction of one mole of NBT to its diformazan is chemically equivalent to the formation of one mole of fumarate. Densitometric measurements were made using a microscope interfaced to an image processing system (IPS). Measurement error attributable to the uneven reception of light by the scanner was reduced to 4% by arithmetically subtracting an "empty" image from the sample image. Since NBT diformazan has a maximum absorbance at a wavelength of 560 nm, an interference filter was used to limit the spectral range of the video scanner. The grey levels (0 to 255) of the IPS were converted to optical density (O.D.) units based on calibration with a photographic density stepwedge. A linear relationship was observed between the measured O.D. and the NBT diformazan concentration of a series of gels.

The incubation medium composition which optimized the enzymatic reaction included: 80 mM succinic acid, 1.5 mM NBT, 1 mM mPMS, 0.75 mM sodium azide, 5 mM EDTA in a 100 mM phosphate buffer (pH=7.60). The substitution of phenazine methosulfate and cyanide with mPMS and azide significantly reduced the amount of nonspecific staining. The enzymatic reaction (i.e. change in O.D.) was linear with respect to: 1) the time of incubation (for at least 10 min); and 2) section thickness (from 4 - 20 um). The addition of malonic acid to the incubation medium inhibited the reaction. These data suggest that the SDH activity of single muscle fibers can be measured in tissue sections.

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423.11 THE POTATO AS MODEL NEURON: or COUNTING SECTIONED CELLS. R.D. Rose & Daniel Rohrlich*. Dept. of Neurobiology and Behavior & Institute for Theoretical Physics, SUNY, Stony Brook NY 11794.

Typically cell count corrections (e.g. Abercrombie 1946 Anat. Rec.) depend on the ability to distinguish subcellular structures (e.g. nucleoli) and fragments of these structures produced during sectioning. While it is easy to distinguish intensely labeled somal profiles in sectioned material, it is often difficult to recognize subcellular structures and particularly some of their smaller fragments.

We use the histogram of profile sizes and section thickness to reconstruct the number of somata present. Profiles are recognizable somata or somal fragments. The largest profiles come from the largest cells. Large cells will typically produce smaller profiles as well. Assuming cells to be spherical, the relative frequency, i.e. kernel, of such smaller profiles can be calculated. From the number of profiles in the right-most histogram bin, which contains the largest profiles, the number of cells of that size is determined. The right-most bin of the profile histogram is thus translated into a number of cells of that size. The kernel then prescribes the number of profiles to be subtracted from all the other bins to correct for smaller profiles generated from the largest cells. This procedure is repeated for the new profile distribution, recursively. Thus the measured profile histogram is translated into a cell size histogram. Summing the number of cells in each bin gives an accurate reconstructed cell number (see e.g. Fig. 1 below). Note that a cell size histogram is a by-product of this method (Fig. 2).

Most somata, however, are not spherical. The ability of the technique to reconstruct, from slices, non-spherical cells was tested using Long Island potatoes (a variety chosen for its particularly gnarled morphology). 92 potatoes were sliced at 25mm. The slices were used for the reconstructions illustrated.

Reconstruction from (i) a sample of 30 neurons intracellularly labeled with Lucifer, and (ii) a computer simulation of those same cells, showed that missing small or unrecognizable profiles does not significantly affect results. Still other simulations indicate that this method is robust in that it is insensitive to section thickness, sample size and somal morphology. The method is especially useful for densely labeled cells or cases where the cell size distribution is desired. Supported by NIMH 08323 (RDR), NSF-PHY 81-09110A (DR) & NIH NS14889 & NS16996 to L. Mendell.

Figure 1: Effect of Bin Width Used for Analysis

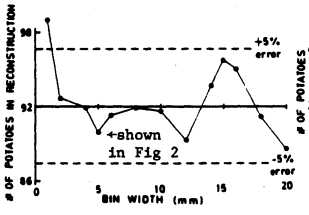
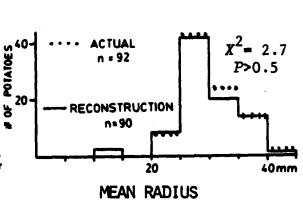


Figure 2: A Sample Reconstruction Bin Width = 5 mm



423.12 MORPHOLOGIC APPEARANCE AND PROJECTIONS OF NEURONS IN CAT LUMBAR PARAVERTEBRAL CHAIN GANGLIA. W.H. Percy,* J. Walsh* & J. Krier. Departments of Physiology and Biomechanics, Michigan State University, East Lansing, MI 48824-1101.

Experiments were performed to study in vitro the morphologic appearance of neurons in the cat lumbar paravertebral ganglia and to determine if this could be correlated with identifiable electrophysiological characteristics. Left or right L3, L4 or L5 ganglia were pinned out in vitro and cells impaled with 60-80M Ω resistance electrodes filled with a 5% horseradish peroxidase(HRP)/3M KCl solution. HRP was injected into cells by passage of depolarizing current pulses (1.5-2.5nA, 200msec, 0.5Hz, for 5 min) through the recording electrode. Cells were classified as "gradeable" or "non-gradeable" on the basis of the number of fast synaptic potentials evoked during electrical stimulation of preganglionic fibers. Cells filled by intracellular HRP injection were found to have two characteristic appearances. [50% : n=5] were spherical with a cell radius of 15-20μ and surrounded with a dense dendritic network consisting of upwards of 12 fibers. The remainder [50% : n=5] were fusiform being 10-30μ in the short axis and 40-80μ in the long axis, with a total of fewer than 10 long (up to 600μ) projections from the poles. Projections from the fusiform cells were always seen to run in a caudal to rostral orientation while projections from the spherical cells showed no obvious polarity. There was no apparent correlation between either type of cell and the number of fast synaptic potentials, action potentials, transmembrane potential or the action potential discharge of the cell in response to the passage of depolarizing current pulses. Secondly, to investigate in vivo visceral, ascending and descending projections of these neurons either lumbar colonic and hypogastric nerves were sectioned bilaterally or both paravertebral chains were sectioned between L3 and L4 or between L5 and L6. The appropriate cut ends were placed in HRP for 1.5-2 hours. At 72 hours both left and right chains were removed. Retrograde axonal transport experiments showed (a) that the proportions of cells projecting to the vicera were:

Left : L1(0),L2(14%),L3(75%),L4(10%),L5(1%): Total cells 2376
Right: L1(0),L2(26%),L3(65%),L4(8%), L5(0) : Total cells 1720

and (b) that projections of cells descend within the chain over four (T13-L3) and three (L2-L5) segments and ascend for at least two (L6-L4). (AM29920)

423.13 AN IMPROVED GOLGI STAIN FOR FORMALIN-FIXED ADULT HUMAN MATERIAL. E. Armstrong and B. Parker*, Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112-1393.

Dichromate Golgi techniques are widely used to visualize dendritic shapes. Optimal impregnation of neurons in tissue which has been fixed by immersion in 10% neutral buffered formalin is problematic, and this poses serious problems for visualizing neurons in human tissue. We have modified Braitenberg et al.'s technique (Stain Technol. 42, 1967) to increase the number of fully impregnated neurons and decrease the time required for processing adult human tissue.

Tissue blocks were taken from human and other primate brains fixed by immersion in 10% neutral buffered formalin. The blocks are loosely wrapped in cotton gauze and placed in a 3% potassium dichromate solution into which a second oxidizer, 3% H_2O_2, is added. A wooden stick holds the gauze down and centers the tissue in the solution. The solution is microwaved for 6-8 hours. The tissue is put into a fresh solution of equivalent concentrations overnight. The next morning tissue blocks are submerged in a 3% $K_2Cr_2O_7$ solution without any H_2O_2 and again microwaved for 6-8 hours. Following this the block is placed into a fresh 3% dichromate solution overnight. The next morning the tissue is rinsed and then placed in 100 ml 0.75% $AgNO_3$ for 2-4 days. We section without embedding the tissue in celloidin (we routinely cut at 100-150 um). The dehydration and clearing steps follow standard Golgi procedures.

Pyramidal cells, particularly those in the lower cortical layers, are the most frequently impregnated neurons. Spines are easily visualized on their dendrites. Except for the proximal ends, axons are not seen. Small stellate-shaped neurons are occasionally stained. Both glia and blood vessels are also impregnated, but generally not in sufficient profusion to obscure the neurons.

Supported by NSF Grant BNS-8204480.

423.14 IMMUNOCYTOCHEMICAL EVIDENCE FOR GM1 GANGLIOSIDE IN LARGE, CARBONIC ANHYDRASE CONTAINING DORSAL ROOT GANGLION CELLS OF THE RAT. B. Robertson* and G. Grant. Dept. of Anatomy, Karolinska Institutet, S-104 01 Stockholm, Sweden.

In a previous study in which either WGA-HRP or choleragenoid-HRP (B-HRP) was injected into the rat L5 dorsal root ganglion (DRG) markedly different labelling patterns were found in the spinal grey matter with the two tracers (Robertson, B. and Grant, G. Neurosci. 14:895-905, 1985). The marginal zone and the substantia gelatinosa were the most intensely labelled regions following WGA-HRP injections. These regions were almost devoid of labelling following B-HRP injections, after which the deep dorsal horn and the ventral horn showed dense labelling. A greater number of large than small cells appeared to be labelled in the injected ganglia. Since it is generally accepted that large-diameter DRG cells have myelinated processes which terminate in the deep dorsal horn and ventral horn, these results suggest that B-HRP is taken up and transported primarily by large-diameter DRG neurones.

In the present study the localization of the GM1 ganglioside, the receptor for choleratoxin and choleragenoid, the binding subunit of choleratoxin, was studied in the rat L5 DRG. Female Sprague Dawley rats were perfused with 4% paraformaldehyde and 14% of a saturated solution of picric acid in 0.1 M PBS. Five microns thick, adjacent cryostat sections were cut from the L5 DRG. Half of the sections were first incubated with choleragenoid and treated immunocytochemically. Choleragenoid immunoreactive cells were then examined for the presence of one of the following enzymes or neuropeptides: carbonic anhydrase, fluoride-resistant acid phosphatase (FRAP), substance P (SP) and somatostatin (SOM).

The results show that a subpopulation of the DRG neurones exhibit choleragenoid immunoreactivity. The majority of these were large-diameter neurones. The strongest immunoreactivity was found in association with the neuronal plasma membrane, but intense staining was also seen in the cytoplasm. A large part of the choleragenoid positive cells also showed carbonic anhydrase immunoreactivity. A few cells were immunoreactive to choleragenoid and positive to FRAP and a few were immunoreactive both to choleragenoid and SP. Immunoreactivity both to choleragenoid and to SOM occurred only in one cell.

This study provides evidence for the localization of the GM1 receptor predominantly in large-diameter DRG neurones and for the presence of carbonic anhydrase immunoreactivity in a large part of these neurones.

423.15 DIFFERENTIAL STAINING TECHNIQUES FOR SENSORY AND MOTOR NERVE AXONS AND TERMINALS. R. Butler and I.M. Payk#, Department of Anatomy, McMaster University, Hamilton, Ontario, L8N 3Z5.

We modified Ungewitter's (Stain Technol. 26, 73 (1951)) and Beermann and Cassens' (Stain Technol. 51, 173 (1976)) non-selective silver methods for peripheral nerve axons and their terminals by lengthening the time for the prestaining treatment of sections, adjusting the times of various stages of the staining procedures and selecting reagents with minimal chloride and sulphate impurities. These methods were applied to Bouin's fixed material embedded in wax with serial sections up to 100 µm thick, cut longitudinally. The Ungewitter modification stained sensory axons and their terminals very well but failed to give good impregnations of motor fibres. In addition, it marked intrafusal muscle fibres with a peppery silver deposit while sparing extrafusal fibres. The Beerman and Cassen method yielded excellent details of motor axons and their terminals but results were very poor on sensory fibres. Both sensory and motor methods worked regardless of whether the neural elements were mature, regenerating or neonatal, and in a wide variety of animals. Both methods consistently yielded pale backgrounds in these thick sections. For both methods, slides with wax sections should be left in dewaxing xylene overnight and moved to fresh xylene the following day. Each of the subsequent alcohols should be at least one hour in duration. We feel that the long dewaxing is important in achieving pale backgrounds when working with skeletal muscle. We also feel that xylene has an adverse effect on silver stains and as a result, extended times in alcohol is required to remove the dewaxing xylene. Final clearing before mounting should be kept as short as possible. For the Ungewitter method, the urea for the technique must have chloride levels of 2 ppm or less and sulphate levels of 3 ppm or less. The Ungewitter impregnation process (silver-urea followed by urea) has to be repeated 2 or 3 times before nerve fibres turn black. The backgrounds remain very pale when restaining times are kept to 15 min. and reducing times are kept to 3 min.

Supported by grants from the Ontario March of Dimes and the Muscular Dystrophy Association of Canada.

423.16 PEROXIDASE CYTOCHEMICAL APPROACHES REVEAL THE VASCULATURE OF THE CNS AND OF NEURAL AND NON-NEURAL GRAFTS. R. Broadwell, H. Charlton#, B. Balin#, and M. Salcman. Univ. Maryland Sch. Med., Balto., MD, 21201, and Univ. Oxford, Oxford, England.

The vascular network of the fetal/adult primate and subprimate CNS, including circumventricular organs (e.g. median eminence, pituitary gland, choroid plexus, etc.), and solid CNS neural and non-neural allografts placed into the brain parenchyma or cerebral ventricles of an adult host is visualized by applying peroxidase cytochemistry in three different ways. Native HRP (type VI) is injected into the jugular vein (1 mg/2 gm body wt) just **prior** to perfusion-fixation of the brain or is delivered into the aorta (0.5%) **subsequent** to perfusion-fixation. The third approach utilizes brains from animals not receiving exogenous HRP; these brains are fixed by immersion, and vibratome sections are incubated for endogenous peroxidase activity in red blood cells retained within the cerebral vascular tree. Cerebral vessels in animals receiving intravenous HRP are outlined strikingly with peroxidase reaction product as seen in bright and dark field light microscopy when TMB, as opposed to DAB, is used as the chromagen; vessels of the circumventricular organs are not discernable due to the dense concentration of reaction product, a consequence of blood-borne HRP that has exited fenestrated capillaries supplying these sites. Ultrastructurally, reaction product coats the luminal surface of the cerebral endothelia and/or is sequestered in endocytic organelles and dense bodies within the endothelial cells. HRP infused into the aorta of fixed animals likewise obscures the circumventricular organs but fills the lumen of non-fenestrated vessels throughout the brain when incubated in DAB or TMB solutions; the cytoplasm of all endothelia and that of cells and processes in circumventricular organs are inundated with reaction product which suggests that the normally impermeable plasmalemma becomes permeable to protein in the fixed brain. Endogenous HRP activity of red cells, revealed best with DAB, readily outlines the microvasculature throughout the cerebral gray and white matter and all circumventricular organs. The three approaches applied to transplanted tissues demonstrate that the vascularization of 16-19 day fetal/1 day neonatal neural grafts is not well-defined at light and EM levels prior to seven days following intracerebral placement; conversely, the vascularization of non-neural grafts (anterior pituitary gland) is prominent prior to three days. This difference may reflect the inherent low vascularity of brain versus that of pituitary tissue. Neural grafts secured from donor CNS sites expected to possess a blood brain barrier, regardless of where within the host CNS the allografts are placed, contain blood vessels that are not leaky to blood-borne HRP and, therefore, contribute to the formation of a blood-brain barrier. Pituitary grafts exhibit fenestrated vasculature that permits blood-borne HRP to enter the graft and surrounding host parenchyma. In this fashion, circumvention of the blood-brain barrier is possible. The results suggest that microvasculature indigenous to the allograft is retained and establishes continuity with cerebral microvasculature of the host. The peroxidase cytochemical approaches for visualization of CNS vasculature have additional potential application to studies of stroke, trauma, and neoplasia. Supported by NIH/NINCDS grant #18030.

423.17 HISTOCHEMICAL STAINING AND X-RAY MICROANALYTICAL IMAGING OF ALUMINUM IN AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSONISM-DEMENTIA OF GUAM. P. Piccardo*, R. Yanagihara*, R.M. Garruto*, C.J. Gibbs, Jr.* and D.C. Gajdusek. Laboratory of Central Nervous System Studies, National Institutes of Health, Bethesda, MD 20892.

We previously demonstrated the deposition of aluminum in the perikarya and dendritic processes of neurofibrillary tangle (NFT)-bearing hippocampal neurons in Guamanian patients with amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD) by using wavelength-dispersive spectrometry coupled to a computer-controlled electron beam x-ray microprobe. While this novel technique affords a high degree of sensitivity and specificity, the processing time required to generate a single computerized image of aluminum for an area measuring 1 mm^2 is 16 hours. We therefore investigated the suitability of histochemical staining for the localization of aluminum prior to x-ray microanalytical imaging of areas of specific interest within a given tissue section. Cryostat-cut, 10 µm thick serial sections of formalin-fixed brain and spinal cord tissues from 3 Guamanian patients with ALS and 6 with PD and from 6 neurologically and neuropathologically normal Guamanian and Caucasian controls were stained for aluminum, using solochrome azurine and morin. Adjacent 20 µm thick sections were mounted onto spectroscopically pure carbon discs for x-ray imaging to verify the sensitivity and specificity of the histochemical stains.

Histochemical staining for aluminum using solochrome azurine or morin provided a rapid, simple and reliable means of identifying areas and structures of interest for closer scrutiny by x-ray microanalysis. Neuronal perikarya, dendritic processes and the walls of some blood vessels were aluminum-positive. In some cases, the deposition of aluminum was rather diffuse, particularly in the white matter. Fluorescent staining for aluminum using morin proved to be equally sensitive and specific, but provided less morphological detail than solochrome azurine. Although the minimum detectable limits for aluminum with the staining procedures are not known, the lower detection limit of our x-ray microanalytical technique is 10-100 ppm dry weight. We believe solochrome and morin staining, as verified by x-ray microanalysis, provides a useful and reliable means of surveying multiple anatomical regions for aluminum deposition in naturally occurring and experimentally induced neurodegenerative disorders.

423.18 METHODS FOR EDITING AND EVALUATING ACCURACY IN A SEMI-AUTOMATIC CELL RECOGNITION SYSTEM FOR NEUROANATOMICAL STUDIES.
S.A. Hayden*, B.G. Border, W.K. Smith, S. Askari*, D.C. German, D.S. Schlusselberg and D.J. Woodward. (SPON: W.J. Gonyea). *Depts. of Cell Bio. and Anat., Psychiat., and Physiol., Univ. of Texas Hlth. Sci. Ctr. at Dallas, Texas 75235.*

A routine task in neuroanatomical research is the identification and graphical plotting of locations of neurons in sections of brain tissue examined in the light microscope. Significant progress has been made toward developing a visual recognition system suitable for recognizing clearly stained neurons observed in video images obtained from a light microscope. An important step in the evaluation of a practical system is the development of efficient means for manual editing of acquired information and for evaluating the sources of error.

Through the **CARP** system (Computer **A**ided **R**econstruction **P**ackage), the host computer controls stepper motors on a light microscope stage to scan a user-defined region of a given section. This region is divided into scanning rectangles which are sequentially digitized into a video frame buffer and analyzed by a high-speed microprogrammable graphics processor. The basic cell recognition program, which requires about one second per video field, scans the digitized image for shades between a pair of intensity thresholds and subsequently identifies the region bounded by pixels outside the threshold range. Regions are accepted as cells if the size and shape parameters are within user-determined values. Positions of cells and descriptors of size and shape are stored in a database for numerical analysis and two- or three-dimensional graphical imaging.

Editing options include first the ability of the user to decide whether each region is a cell or an artifact, both from the video image and from the view in the microscope. This allows a direct evaluation of error for repeated automatic scanning at different parameter settings until optimum accuracy in counting is attained. Video images of multiple cell regions which are connected or overlapping can be edited manually so that the blob recognition programs will resolve separate regions. The database can be scanned to select large, small or oddly shaped regions for sequential inspection by the user. More complex edge detection and region examination routines can be applied to selected regions.

Initial applications have involved counting of neurons in the primate *substantia nigra*, using either neuromelanin pigment or tyrosine hydroxylase staining. Preliminary trials have shown an overall factor of ten improvement in speed over manual counting with greater than ninety percent accuracy in comparison with a human observer. The method may be applicable to a wide range of immunochemically stained neuronal populations.

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CELL BIOLOGY: AXONAL TRANSPORT II

424.1 EFFECT OF PARATHYROID HORMONE ON INTRA-AXONAL ORGANELLE TRANSLOCATION. A.C. Breuer, M.B. Atkinson* and M.P. Lynn*. Departments of Brain and Vascular Research and Neurology, Cleveland Clinic Foundation, Cleveland, OH 44106.

Both primary and secondary hyperparathyroidism (HP) may result in a reversible neuromuscular syndrome characterized by muscle weakness, atrophy, fasciculations and neurogenic changes on muscle biopsy, all resembling Amyotrophic Lateral Sclerosis (ALS). In HP the syndrome is associated with high parathyroid hormone (PTH) levels, not with uniformly high serum calcium levels (Patten et al., Ann Int Med 80: 182-193, 1974) and its mechanism remains unknown. We report the effect of direct application of PTH on fast axonal transport in excised axons from rat sciatic nerves. Isolated fascicles were teased apart and bidirectional organelle translocation visualized in individual axons by differential interference contrast optics using analog and digital image enhancement techniques. Organelle translocations were measured by computer methods. Controls were observed in oxygenated buffer and test samples in identical buffer to which 0.05 µg/ml PTH was added. PTH increased mean anterograde organelle speed by 14.1% (PTH 1.63 ± 0.06; Control 1.40 ± 0.04 µm/sec, $p < 0.01$) and increased mean retrograde speed by 14.7% (PTH 2.11 ± 0.03; Control 1.80 ± 0.03 µm/sec, $p < 0.001$, Student's t-test). The PTH augmented mean anterograde speed is of interest as increased mean anterograde speed of organelles is also seen in motor nerve from ALS patients, a finding recently demonstrated in our laboratory. The PTH effect on retrograde organelle speeds is different from that seen in untreated ALS axons where mean retrograde speed is decreased. Our findings provide evidence on a cellular level of an effect of PTH on fast axonal transport.

424.2 DIRECT OBSERVATION OF AXON FUNCTION IN-VIVO. W. Levy, R. Rumpf, T. Spagnolia. University of Missouri Health Science Center, Columbia, Missouri 65212

We are using a system for the study of axon function and regeneration in-vivo, where the sciatic nerve of a rat is passed through a chamber over an AVEC/DIC type microscope. For axon transport studies in fifty rats, a surgical exposure of the sciatic nerve was carried out with removal of some of the adjacent muscles and preservation of blood supply to the nerve. Under an operating microscope, in Hank's solution with 4 millimolar glucose and carboxygen bubbling, the epinerium was dissected free of the nerve and the perinerium incised. The nerve was then passed through the chamber where enzymatic dissociation was carried out, with Worthington CLS collagenase solution and a multi-roller peristaltic pump to provide circulation of the enzyme. One percent thrombin was added to the chamber, and norepinephine to the nerve proximal and distal to the chamber to prevent bleeding during this time. At the end of the approximately 2 hour dissociation period a DeFonbrune micromanipulator was used to pare the nerve leaving only a single layer of axons without morphological damage evident. Microscopy was done using an AVEC/DIC system and a Hughes image processor. Axonal transport in this preparation is not significantly different from transport rates in teased preparations. A high percentage of axons, 80-90%, transport in good preparations. HRP applied distal to the chamber is present in axons proximal to the chamber several hours later. Morphological damage to axons is substantially less in this system than in teased preparations. Axon injury effects on transport are currently being assessed.

A separate version of the chamber chronically implanted in the rat sciatic nerve, shows in growth from a proximal stump at 0.8 mm/day with repeated observations. Axons can be identified in the chamber along with cells resembling tissue culture in appearance. This may be useful for investigating regeneration conditions in vivo, where individual axons can be observed and their conditions of growth manipulated.

424.3 STIMULATION OF DENDRITIC TRANSPORT BY ANTIMITOTICS
V.J. Roberts and C. Gorenstein. Department of Pharmacology, University of California, Irvine, CA 92717.

A single injection of colchicine into the lateral cerebral ventricle of the rat causes a rapid redistribution of lysosomes from their normal localization in neuronal cell bodies into dendrites. This redistribution can be seen within 2 hours and persists for at least 66 hours (Roberts et al., Soc. Neurosci. Abst. 11; 1305, 1985). Since colchicine binds to tubulin and inhibits microtubule polymerization, we speculate that the movement of lysosomes into dendrites, following colchicine treatment, is due to the disruption of microtubules.

In order to test this hypothesis further, the localization of lysosomes was examined following treatment with a variety of microtubule poisons. Although the Vinca alkaloids, vinblastine and vincristine, bind to a different tubulin site than colchicine, they cause a redistribution of lysosomes similar to that seen with colchicine, a tropolone derivative. Podophyllotoxin, a non-tropolone alkaloid, has been reported to cause a more rapid loss of microtubules and to be more potent in inhibiting mitosis and axonal transport than colchicine. We observe that podophyllotoxin is less potent than colchicine in causing a redistribution of neuronal lysosomes. Colcemid and colchicine have nearly identical structures, bind to the same tubulin site and are equivalent microtubule poisons. Nevertheless, treatment with colcemid results in a relatively weak and short lived redistribution of lysosomes into dendrites. Lumicolchicine, an inactive analog of colchicine, does not bind tubulin nor does it induce a redistribution of neuronal lysosomes.

We speculate that the mechanism of the selective redistribution of neuronal lysosomes from the cell body into dendrites is dependent upon drug binding to tubulin and the subsequent disruption of microtubules. However, the mechanism is different from that which inhibits mitosis and axonal transport and may involve a novel dendritic transport system.

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424.4 A ^{3}H NSP STUDY OF RETROGRADE AXONAL TRANSPORT FOLLOWING NERVE CRUSH INJURY.
D.J. Fink, D. Purkiss* and M. Mata. Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105.

In response to crush injury, neurons whose axons project from the CNS into the periphery undergo a sequence of morphologic and biochemical alterations culminating in nerve regeneration. It has long been assumed that the signal or signals triggering and to some extent controlling the reaction of the cell body to the distant injury must involve a biochemical signal which ascends from the axon to the cell body.

Using the ^{3}H N succinimidyl propionate (^{3}H NSP) method to study retrograde transport from the axon to the cell body, we have found that there is a specific rate of accumulation of labeled proteins in the cell bodies of motor and sensory neurons after radioactive in vivo covalent labeling of axonal proteins. In order to further delineate the role of these retrogradely transported proteins in the response to injury, we undertook the current study.

Male Sprague Dawley rats were anesthetized with chloral hydrate and the sciatic nerve crushed with a Jeweler's forceps. At 1, 3, 5, or 7 days after injury the animal was reanesthetized and the nerve injected with 1 microliter of saline containing 120 micro-Curies of ^{3}H NSP at point 1-2 cm proximal to the injury. One day or 7 days after ^{3}H NSP injection the animals were sacrificed and the amount of TCA precipitable radioactivity in the L4, L5 and L6 dorsal root ganglia and in the ventral horn of lumbar spinal cord determined. The amount of transport was defined as the amount of radioactivity ipsilateral to injection minus contralateral.

Transport to the cell bodies 1 day after ^{3}H NSP injection was not changed from control at all the time points studied, although there were trends which were not statistically significant. Accumulation at the cell bodies 7 days after ^{3}H NSP showed significant alterations. Transport to the sensory motor neuronal somata tended to increase shortly after crush before returning to normal levels, while transport to the motor neuronal perikarya was reduced early after crush.

We have previously found that the accumulation in the cell body at 7 days after ^{3}H NSP injection is reduced in intoxication with IDPN and also early in experimental diabetes. The differences between those changes and the changes seen after nerve crush suggest that the retrogradely transported labeled components may play a role in the regulation of the cellular response in these different conditions.

424.5 AN ANTISERUM AGAINST TUBULIN, WHICH INHIBITS AXONAL TRANSPORT, UNIQUELY DEFINES A SUBSET OF CLONES FROM A RAT BRAIN cDNA LIBRARY.
K.M. Johnston, D. van der Kooy, J.A. Connolly and R. Dunn*, Dept. of Anatomy, *Dept. of Medical Genetics, Univ. of Toronto, Toronto, CANADA, M5S 1A8

In two previous reports, we demonstrated inhibition of bidirectional axonal transport in the presence of NS-20 polyclonal antiserum against tubulin. This effect was described both in vivo, in rat brain, and in vitro, in extruded squid axoplasm. Other antisera against tubulin, for example PC-5, did not have this inhibitory effect. We suggested that NS-20 antibodies may be unique to a specific epitope on the tubulin molecule critical in the mechanism of axonal transport. Alternatively, it is possible that NS-20 antibodies to tubulin react with a unique form of tubulin that plays a major role in axonal transport.

In order to further investigate the unique effect of NS-20 antiserum, we have screened a rat brain cDNA library with both NS-20 and PC-5 antisera to determine if both antisera have the same binding activities with each tubulin clone. Specifically, a λgt11 expression library was used to infect E. coli Y1090 and recombinant plaques screened using the antibody probes. NS-20 positive clones were then identified by transfer to nitrocellulose filters, incubation with primary antiserum, followed by incubation with an alkaline-phosphatase conjugated secondary antibody. Duplicates of each filter were tested with PC-5 antiserum.

We found that, of the 24 NS-20 positive clones, none were cross-reactive with PC-5 antiserum, even though the PC-5 antiserum strongly reacted with a number of clones from the complete library. These preliminary results suggest that NS-20 binding to tubulin is distinctly different from that of PC-5. The question of whether this distinction is based on one unique tubulin epitope or a unique tubulin gene sequence is now being investigated. Further experiments will examine the relationship of NS-20 specificity with its inhibitory effect on axonal transport.

424.6 ISOLATION OF VESICLES FROM BOVINE BRAIN THAT CONTAIN RAPIDLY TRANSPORTED PROTEINS.
Benson, R.J.J., E. Hirsch* and R.E. Fine*, Depts. of Anatomy and Biochemistry, Boston Univ. Sch. of Med., Boston, MA 02118

Isolation of coated vesicles from bovine brain using sucrose density gradient centrifugation (Pearse, B.M.F. 1975. J. Mol. Biol. 97:93-98) results in a mixed population of vesicles, both coated and smooth. We have assayed these crude preps for the presence of acetylcholinesterase (AChE) and muscarinic acetylcholine receptors (AChR's) and found the vesicles contain both molecules. The predominant form of the AChE is the G4 form although the G1 form is also present. Both AChE and the muscarinic AChR undergo rapid axon-transport.

Using a density shift technique that allows us to separate AChE-containing vesicles (Helmy, S. et al.1986. Cell 44:497-506), we show that although we shift 80-90% of the AChE, we shift only 20-30% of the protein in the preps. Since coated vesicles comprise the majority of the protein in the prep, it appears that the AChE is contained in the relatively protein-poor smooth vesicles. If we compare preps made from axon-rich regions of the brain to ones from axon-poor regions, again most of the AChE shifts, but a consistently smaller percentage of the protein shifts in the axon-rich preps. Coated vesicles from these regions (largely from glial cells since axons do not contain many coated vesicles) probably do not contain AChE to any great degree.

In order to determine the source of the AChE and AChR's we find in our material, we employed agarose gel electrophoresis which has been used to purify coated vesicles to homogeneity (Rubenstein, J.L.R. et al.1981. J. Cell Biol. 89:357-361). Crude vesicles from brain, when electrophoresed on the gels yield two bands migrating toward the anode: a faster migrating band containing homogeneous coated vesicles, and a more slowly migrating band containing smooth vesicles ranging from 70-150 nm in diameter. Staining of the gels for AChE activity reveals that most of the AChE co-migrates with the smooth vesicles. Crude vesicles assayed for muscarinic AChR's show 90% specific ^{3}H-QNB binding. When these vesicles are subsequently run on agarose gels, the radioactivity co-migrates with the smooth vesicles.

In addition to their containing two rapidly transported proteins, these smooth vesicles are of interest because they are seen on agarose gels of coated vesicles prepared from brain only, and not from other tissues we have examined. These include liver, adipose tissue and skeletal muscle. We therefore propose that they are unique to nervous tissue, are involved in the processing of rapidly transported proteins, and may be intermediaries in rapid axonal transport.

This work was supported by NIA AG05894-13.

424.7 THE TRANSPORT RATE AND ORGANIZATION OF NEUROFILAMENTS DIFFER IN SOMATIC MOTOR AND PARASYMPATHETIC OCULOMOTOR AXONS
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Studies of the axonal transport of radiolabeled cytoskeletal proteins can provide information about the transport characteristics of the cytoskeletal polymers in axons (Lasek, R.J., J.Cell Biol. 99:212s, 1984). We compared the transport kinetics of the cytoskeletal polymers in the functionally distinct axon populations of the oculomotor system in chickens -- somatic-motor (M) axons and preganglionic parasympathetic (P) axons.

Three week old chickens received injections of ^{35}S-methionine in the cerebral aqueduct and were sacrificed from 1 to 93 days after injection. Labeled proteins were analyzed in segments of the oculomotor nerve, which contains the M and P fibres; in the ciliary ganglion, where the P terminals are located; and in the somatic-motor branches of the oculomotor nerve where M axons course. The radiolabeled proteins were subjected to one and two-dimensional SDS-PAGE, fluorography and were quantified by counting the excised bands.

The rate of neurofilament (NFP) transport was 0.26 mm/day in P axons and 0.73 mm/day in M axons. In M axons, most of the tubulin moved coordinately with the NFP and a small fraction moved with SCb. By contrast, in P axons most of the tubulin moved with SCb and a relatively small fraction moved with the NFP. These results suggest that the dynamic structural relationships that determine the rate of microtubule and neurofilament transport differ in the M and P axons.

Ultrastructural analyses of the M and P axons show that neurofilaments are more densely packed in the P axons than in the M axons. The average packing density of the neurofilaments was 496/um^2 in P axons and 347/um^2 in M axons. The tight packing of the neurofilaments in the P axons may restrict their mobility and contribute to the 3-fold slower rate of neurofilament transport in P axons than in M axons.

424.8 SOME STABLE AXONAL MICROTUBULES DO NOT UNDERGO SLOW AXONAL TRANSPORT.
D.F. Watson, J.W. Griffin, and P.N. Hoffman. Dept. of Neurology and Dept. of Ophthalmology, Johns Hopkins University, Baltimore, MD 21205.

Axonal tubulin is present as unassembled subunits and as assembled microtubules (MT), including MT that are unusually stable to cold and calcium exposure. In order to determine whether there might be multiple pools of intra-axonal MT, we measured the total unassembled tubulin, cold-labile MT and stable MT in nerve and compared them to the tubulin and MT undergoing slow axonal transport. Rat sciatic nerve segments, containing ^{35}S-labeled tubulin moving with either subcomponent a or b of slow axonal transport, were homogenized in 100 mM PIPES/50% glycerol/5% DMSO/1 mM EGTA/0.5 mM $MgCl_2$. A high-speed supernatant (425,000 g for 15 minutes), containing unassembled tubulin was prepared. The pellet was extracted 1 h at 0°C in buffer without glycerol/ DMSO, and again was centrifuged to produce a pellet containing stable MT. Total alpha or beta tubulin in aliquots of the unassembled, cold-labile, and stable MT fractions was measured by an enzyme-linked immunoassay system employing specific monoclonal antibodies. Transported tubulin was measured in aliquots of the same fractions by scintillation counting of the label in tubulin bands cut from gels. The fraction of tubulin present as stable MT was the same in subcomponents a and b of slow axonal transport (48.6±3.3 % stable) while a larger fraction of total tubulin by immunoassay was stable (56.4±2.6 %; n=16 nerves, $p<0.01$). The amount of alpha and beta tubulin was equal in all fractions. Tubulin and stable MT in non-axonal structures were measured by local protein labeling of intact nerve trunks, and by immunoassay on distal nerve stumps after Wallerian degeneration of axons. Non-axonal MT do not account for the difference in results between total and transported tubulin. The amount of non-axonal tubulin is small, and the fraction of stable MT in non-axonal structures is less than in total nerve. These data indicate that axons contain highly stabilized MT that do not move at normal rates of slow axonal transport. The results suggest that axons contain a pool of stationary MT which may slowly exchange with the MT undergoing axonal transport.

424.9 CHARACTERIZATION AND DISTRIBUTION OF PHOSPHORYLATED AND NONPHOSPHORYLATED SUBUNITS OF SQUID NEUROFILAMENTS.
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Monoclonal antibodies to squid neurofilament (aNFP) and intermediate filament (aIFA) proteins were used as probes for the biochemical and immunocytochemical analyses of neurofilament structure, distribution, and function in the squid giant axon (GA) and stellate ganglion (SG). On Western blots the aNFP antibody stained the 220 kDa and high molecular weight components of neurofilaments in the GA, while the aIFA antibody labeled the 60 kDa protein in the GA and the 60 kDa and 65 kDa proteins in the SG. Dephosphorylation of axoplasmic proteins by alkaline phosphatase resulted in a decrease in the molecular weight of both the 220 kDa and HMW components and a concomitant loss of reactivity with the aNFP antibody on Western blots, suggesting that this antibody is specific for the phosphorylated epitope in the neurofilament. Increased dephosphorylation of the 220 kDa protein led to an enhanced immunostaining of the resultant 190 kDa polypeptide by the aIFA antibody, indicating that the phosphorylated epitope may mask the conserved epitope recognized by aIFA. Light and electron microscopic immunocytochemistry, using colloidal gold as a probe, was performed on sections of LR White embedded GA and SG. Silver enhancement of the gold was performed to visualize it at the light microscope level. Intense labeling by the aNFP antibody was seen in the GA. The aIFA antibody labeled the glial cells around the GA intensely, while labeling of the GA, itself, was lower than that with the aNFP antibody. In the SG, the aIFA antibody predominantly labeled glial cells. Nuclear membranes of ganglion and glial cells, "islands" of filaments within ganglion cells, and the cytoplasm of ganglion cells were also labeled by the aIFA antibody. The aNFP antibody labeled only the small axons within the SG. The aNFP antibody is specifically associated with the "bead-like" portions and cross-bridges on the axonal neurofilaments. The aIFA antibody is associated with relatively smooth filaments in ganglion and glial cells. No reactivity for either antibody was seen in synapses. The 65 kDa protein may represent glial filaments and the 60 kDa protein may represent the low molecular weight subunit in the neurofilament, which may be formed and/or organized in "islands" of filaments within ganglion cells. These results indicate that the formation, phosphorylation, and degradation of neurofilaments are regionally segregated within the neuron. Supported by NIH Grant 15889.

424.10 AXONALLY TRANSPORTED PROTEOGLYCANS IN GOLDFISH OPTIC NERVE.
J. Elam and J. Ripellino, Dept. Bio. Sci., Florida State Univ. Tallahassee, FL 32306.

We report on further analysis of proteoglycans found to be axonally transported to the 100,000 x g fraction of goldfish optic tectum. (see Ripellino & Elam, Trans Am Soc Neurochem 13:224 (1982)). Goldfish were injected in the right eye with $^{35}SO_4$ and their left tecta removed 17h later. Tecta were homogenized in 1mM phosphate buffer containing 0.25M sucrose and a mixture of protease inhibitors. Homogenates were centrifuged at 100,000 x g for 1h, then the pellets resuspended in homogenization buffer and recentrifuged. Supernatants were combined and dialyzed to remove unincorporated $^{35}SO_4$.

The dialyzed supernatant, containing 21% of axonally transported $^{35}SO_4$, was subjected to ion exchange chromatography on DEAE cellulose utilizing a linear NaCl gradient. A sulfated glycoprotein fraction was eluted at 0.2M NaCl and a fraction that contained 5% of the supernatant protein and 53% of the supernatant radioactivity was isolated at 0.5M NaCl. The material in this latter fraction was dialyzed, digested with pronase and analyzed for the presence of glycosaminoglycans (GAGs) by fractionation with cetylpyridinium chloride. 50% of the radioactivity was recovered in a GAG fraction reprecipitated with ethanol-acetate. Cellulose acetate electrophoresis showed 69% of this radioactivity comigrated with standard heparan sulfate (HS) and disappeared following treatment with heparatinase. The remaining 31% of the radioactivity co-migrated with standard chondroitin sulfate (CS) A or C and disappeared following treatment with chondroitinase AC.

Gel exclusion chromatography of the intact proteoglycan fraction on Sepharose CL6B yielded a single radioactive peak. Addition of 4M guanidine HCl still yielded a single peak but a broader size distribution. No subfractions of either the DEAE or CL6B peaks were found to contain exclusively HS or CS, suggesting that the two GAGs may be attached to the same protein core.

An additional 8-10% of axonally transported $^{35}SO_4$ was released from the 100,000 x g pellet by washing with hypotonic buffer lacking sucrose. The fraction, putatively released from sequestered sites by lysis, was enriched in proteoglycan relative to sulfated glycoproteins and contained more HS relative to CS than the original supernatant. Of the 70% of $^{35}SO_4$ remaining in the 100,000 x g pellet, approximately 1/3 was recovered in HS & CS, in proportions similar to the supernatant.

424.11 POWERING OF AXONAL BULK TRANSPORT BY HYPERDENSE PARTICLES AND DIFFERENTIAL SENSITIVITIES OF ANTERO- AND RETROGRADE TRANSLOCATOR MECHANISMS. B. Edmonds* and E. Koenig. Dept. Physiology, Div. Neurobiology, SUNY at Buffalo, Buffalo NY 14214.

In a previous report (Koenig, et al., 1985 J. Neurosci. 5:715) a form of axonal bulk transport was described for goldfish retinal ganglion cell (RGC) axons regenerating in vitro, which was characterized by bidirectional saltatory behavior of large varicosities containing cytoskeletal and cytomembranous elements; however, movements of varicosities were generally infrequent. Transport of small particles, called intervening phase-dense inclusions (IPDIs), was also described which had a 10-fold greater velocity. Behavior of IPDIs included forming varicosities de novo, as well as IPDIs passing through, fusing with, or being released from preexisting varicosities. We now report a mechanism for the motility of varicosities that appears to be mediated by a dynamic interaction between hyperdense particles (HDPs), a subclass of IPDIs, and varicosities.

Alkalinization, using ammonium chloride, increases frequency of movement of varicosities and reduces phase density of varicosities so that internal inclusions become visible. Time-lapse, phase-contrast microscopy reveals that one or more HPDs are generally positioned in the leading pole of moving varicosities, where they may cause an excressence to form. The correlation of movement with requisite location of motile HDPs suggests that HDPs become "entrapped" in varicosities, and if local constraints on varicosities, which may involve transmembrane interactions with substratum, are overridden or removed, varicosities are mobilized. These results indicate that a simple kinesin-like translocator mechanism described for single organelles (Vale, et al., 1985 Cell 41:39) is sufficient to explain bulk transport as characterized by motile axonal varicosities.

Alkalinization also reduces mean velocity of IPDIs by almost 30-fold, and virtually halts anterogradely directed IPDIs. In mildly permeabilized preparations at neutral pH, orthovanadate (5 uM) selectively blocks retrograde transport of IPDIs, including HPDs, while 100 uM vanadate reversibly inhibits all bidirectional movements. These results suggest that two translocator mechanisms for directional movements may exist in RGC axons which possess different vanadate and pH sensitivities. These findings confirm and extend those reported for dispersed and reconstituted squid axoplasm (Vale, et al. 1985 Cell 43:623).

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424.12 ORGANELLES ORGANIZE MICROTUBULES INTO TRANSIENT BASKETS: A SERIAL EM 3D RECONSTRUCTION OF SYSTEMS OF AXONAL MICROTUBULES M.M. Greenberg and J.K. Stevens. Playfair Neuroscience Unit, Toronto Western Hospital; and Depts. of Anatomy and Surgery (MMG) and Dept. of Physiology (JKS), University of Toronto, Toronto, Canada.

Lasek (Brain Res 7:630; et al, JCB 99(1):212s), Allen et al (JCB 100:1763), Martz et al (Cell Mot 4(2):77, and Vale et al (Cell 40:449) have characterized the relationship of organelles in axonal transport to microtubules. At the EM level, we (and many others) have often observed organelles situated in a "basket" of microtubules. The question arises of whether microtubules intrinsically configure themselves into these baskets or whether organelles confer this organization upon local microtubules. To answer this question we serially reconsructed microtubules and organelles in unmyelinated murine sciatic axons. Using computerassisted techniques, we created a system of vectors which both specified the relative positions of microtubules in each basket and identified their sequential order. The individual microtubules in these baskets were then followed through serial sections, and vectors were drawn for all of them. By comparing the sequential vectors for each original basket, we could trace the fate of the microtubules. Results indicate that microtubules of a given basket disperse after the organelle in the basket has passed through. They often reform themselves into new smaller or larger baskets according to the size of an organelle that may appear, and otherwise weave a more random course through the axon. Baskets of microtubules could be seen in the sub-axolemmal spaces as well; the members of these also moved centripetally to join other baskets around organelles. Other than these subaxolemmal configurations, no baskets seen in the axon were unassociated with an organelle either in the same or in an immediately preceding section. We conclude that microtubules do not form baskets by themselves, but rather that it may be the organelles which confer these conformations upon local microtubules. Since microtubules are observed lining the internal rim of the axolemma, it is possible that a product in or on membranes is responsible.

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424.13 BIOCHEMICAL ALTERATIONS INDUCED BY 2,5-HEXANEDIONE: COMPARATIVE EFFECTS OF NEUROFILAMENT PROTEIN CROSSLINKING, PHOSPHORYLATION AND PROTEOLYSIS. D.M. Lapadula*, E. Suwita* and M.B. Abou-Donia (SPON: M.M. Abou-Donia), Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

Several biochemical mechanisms have been proposed to account for the accumulation of neurofilaments present in axons after administration of 2,5-hexanedione. These include protein-protein crosslinking, blockage of protein phosphorylation and inhibition of proteolytic breakdown of neurofilaments. In addition, 2,5-hexanedione has been demonstrated to affect axoplasmic transport. Animals were treated with 1% 2,5-hexanedione in their drinking water for 2 weeks. Spinal cord neurofilament proteins were isolated by the axonal floatation method and protein phosphorylation (15 µg protein) was assessed in a 50 mM Pipes buffer (pH 6.5) in the presence of Mg^{++} (10 mM), Ca^{++} (50 µM), [γ^{32}P]ATP (5 µM) and either calmodulin (1 µg) or trifluoperazine (50 µM). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to autoradiography. Microdensitometry was used to quantitate differences between control and treated animals. Protein phosphorylation of all three neurofilament triplet proteins was significantly decreased (68K, 50%; 160K, 36%; 210K, 33% of control) in treated animals. At the same time, neurofilament proteins from a separate set of control and treated animals were found to be crosslinked, as demonstrated by reactivity to anti-neurofilament antibodies of higher molecular weight proteins (greater than 250K) in SDS-polyacrylamide gels. In a separate experiment, rats were treated for 63 days with 0, 0.1, 0.25, 0.5, or 1% 2,5-hexanedione in their drinking water and spinal cord cytoskeletal proteins were isolated. High molecular weight crosslinks as well as a diminished amount of proteolytic degradation products were evident in a dose dependent manner in treated animals. These data suggest that a single mechanism may not account for the accumulation of neurofilaments in hexacarbon induced neuropathy but several mechanisms may combine to produce the accumulation of neurofilaments which are pathognomic of this neuropathy. Supported in part by NIOSH grant No. OHO 0823.

424.14 DENDRITIC TRANSPORT OF RNA IN HIPPOCAMPAL NEURONS IN CULTURE. L. Davis, G. Banker, and O. Steward. Depts. of Neuroscience and Neurosurgery, University of Virginia School of Medicine, Charlottesville, Virginia, 22908, and Dept. of Anatomy, Albany Medical College, Albany, New York 12208.

Although the properties of axonal transport have been well characterized (Grafstein and Forman, 1980, Physiol. Rev., 60:1167, 1980), dendritic transport is less well understood. Because dendrites contain protein synthetic machinery, the transport of labeled proteins into dendrites is obscured by local synthesis. However, RNA synthesis is restricted to the nucleus so that the transport of newly synthesized RNA can be readily followed. In this study we determine the rate of transport of 3H-RNA into dendrites following pulse-labeling with 3H-uridine.

Hippocampal cells were obtained from 18 day embryonic rats, plated onto polylysine-treated glass coverslips and maintained in serum free medium for 15-17 days. At this time, dendrites can be distinguished from axons on the basis of a number of criteria (Bartlett and Banker, J. Neurosci., 4:1944, 1984). Cells were incubated for one hour with 3H-uridine, then briefly rinsed in medium to remove the 3H-labelled precursor. Some cultures were fixed immediately; others were returned to a medium containing an excess of unlabeled uridine as a chase for 3, 6, 9, 12, 18, 24, 36, or 48 hours. Following the chase period, the cultures were fixed with paraformaldehyde and processed for autoradiography. Labeled neurons from each chase condition were selected randomly using predetermined microscope stage coordinates. The distance of migration within each dendritic process was determined, and the mean distance of migration was calculated at each time interval.

When cells were fixed at the end of the labeling period, label was found only over the nucleus. With increasing time, labeled RNA traveled progressively further into the dendrites, reaching the tips of the dendrites after 12-24 hrs. There was no detectable labeling of the axons at any time. Our results indicate a selective transport of RNA into dendrites at a rate comparable to that of slow axoplasmic transport. Supported by NS23094.

424.15 EFFECT OF INTRAOCULAR TETRODOTOXIN (TTX) ON AXONAL TRANSPORT OF Na,K-ATPASE IN RAT OPTIC NERVE. S.C. Specht, A.E. Colón-Mage*, E. del Valle* and S. Castro-Hernandez. Lab. of Neurobiology and Dept. of Pharmacology, Univ. Puerto Rico Sch.of Med., San Juan, P.R. 00936.

Axonal transport of Na,K-ATPase was evaluated in rats which had received intraocular injections of citrate-buffered TTX (15 µl of 1 mM), citrate alone or a puncture wound into the vitreal chamber. The retinae were labeled in vivo by intraocular injection of {^{35}S}methionine (250 uCi) at 6 h after treatment. Incorporation was stopped at 2 h with an intraocular injection (15 µl) of solution containing cycloheximide (20 mg/ml) and unlabeled methionine (0.8 mM). Axonal transport was inhibited at 6 h with intraocular colchicine (5 µl of 5 mg/ml). For axonal transport studies, animals were killed 6 or 24 h after {^{35}S} methionine. Labeling was evaluated by SDS-PAGE of enzyme isolated from the retina, optic nerve and tract, and lateral geniculate bodies and superior colliculi. Retinal synthesis of enzyme was assessed in animals killed 2 h after labeling. Enzymatic activity of the Na,K-ATPase and {^{3}H}ouabain binding were also evaluated in treated retinae.

After 6 hours of treatment, enzymatic activity and {^{3}H}ouabain binding were increased in the retina and axonally transported Na,K-ATPase recovered from optic nerve endings was enriched in the alpha(+) isozyme. {Supported by NIH grants NS 07464 and SO 6RR 08102.}

424.16 FAST AXONAL TRANSPORT IN CERVICAL AND LUMBAR ANTERIOR HORN CELLS IN WOBBLER MOUSE MOTOR NEURON DISEASE. K. Kurahashi*, H. Mitsumoto, G. Bunge* Neuromuscular Res Lab. Cleveland Clinic Foundation, Cleveland, Ohio 44106

Motor neuron disease in the wobbler mouse is clinically characterized by paralysis, atrophy, and contractures in forelimb muscles. However, hindlimb muscles are nearly spared. We have been investigating the mechanism of selective neuronal involvement in this model, since the reason(s) why certain motoneurons are preponderantly affected, while others are not, may explain a common pathogenetic process in motor neuron diseases. Regenerative capacity after nerve crush is markedly impaired in wobbler cervical motoneurons (Mitsumoto: Muscle Nerve, 1985), whereas that in lumbar motoneurons is found to be normal (Mitsumoto et al: Soc Neurosci Abstr, 1985). Since the axonal regeneration is sustained by axonal transport in addition to neuronal protein synthesis, we studied fast axonal transport to analyze differences between the two systems. Horseradish peroxidase (HRP) was injected in forelimb or hindlimb muscles in both groups of animals and HRP was reacted with tetramethyl benzidine in serial frozen sections of spinal cord. The earliest appearance of HRP in the anterior horn cells was examined at different times after injection under a bright/dark field microscope. In the forelimb system, HRP was found at 2 ½ hrs. in both controls and wobbler mice. Their estimated rates were 210-240 and 190-210 mm/day, respectively. In the hindlimb, the rate was identical in both groups, estimated as 220-240 mm/day. Thus, there seems to be no significant differences in retrograde transport between the two groups of animals in both systems. To study anterograde fast transport, a mixture of tritiated leucine and proline, adjusted to 160 uCi/ul, was stereotactically microinjected into the cervical ventral horn and the transported radioactivity was counted in 1 mm consecutive nerve segments, at 1 and 2 hrs. after injection in controls, and 2½ and 3½ hrs. in wobbler mice. The wobbler forelimb nerve had a delayed transport appearing into the nerve segments and the rate was estimated as 140-190 mm/day, whereas it was 340-380 mm/day in controls. An abnormal fast anterograde transport may play an important role in selective neuronal involvement of cervical motoneurons in this motor neuron disease. The study in the hindlimb is under progress.

424.17 **TRANSMITTER SPECIFIC RETROGRADE TRANSPORT IN APLYSIA.** L.M. Olson and J.W. Jacklet, Dept. of Biol., SUNY-Albany, NY 12222

Transmitter specific retrograde transport was tested in Aplysia using the paired, serotonergic metacerebral giant neurons (MGN's) of the cerebral ganglion. Each neuron has a single process that bifurcates within the cerebral ganglion. One axonal branch travels to the buccal ganglion through the ipsilateral cerebral-buccal (C-B) connective where it forms extensive synaptic contacts with buccal neurons. The other axonal branch travels in the ipsilateral lip nerve to innervate buccal musculature.

The buccal ganglion, containing MGN terminals, was isolated from the cerebral ganglion by a vasoline seal between a two-chambered incubation chamber and exposed to tritiated transmitters [serotonin (^{3}H 5-HT), ^{3}H DOPA, and dopamine (^{3}H DA)], for 18 hours at 15° C. The lip nerve, containing the other MGN axonal branch, was cut close to the cerebral ganglion. After washing, ganglia were processed for autoradiography and slides were exposed for one month.

After incubation of the buccal ganglia in ^{3}H 5-HT, label was prominent in the MGN soma and its axon in the C-B connective. The MGN's were the only cerebral ganglion neurons labeled, suggesting that uptake and/or transport of serotonin is limited to serotonergic neurons. The lip nerve axonal branch did not contain label. ^{3}H DOPA also was transported by the MGN, so these serotonergic terminals do not discriminate between DOPA and 5-HT. However, no other cerebral ganglion neurons were labeled, so DOPA is also excluded by non-aminergic neurons. Preliminary experiments indicate that DA is not taken up and/or transported by the MGN.

5-HT uptake may occur at serotonergic synaptic membranes, but not at somatal membranes. Exposure of the cerebral ganglion to ^{3}H 5-HT labels 30-40 cerebral ganglion neurons (similar to those described by serotonin immunofluorescence, Goldstein et al., 1985) but the MGN do not label. Labeled neurons appear to be serotonergic neurons that have terminals within the cerebral ganglion, and accumulate label by retrograde transport after synaptic uptake. The MGN have few or no terminals within the cerebral ganglion, and so could not accumulate label.

The anatomical separation of MGN terminals and soma in separate ganglia makes Aplysia an ideal system in which to examine the specificity and characteristics of transmitter specific retrograde transport. Our results show that retrograde transport of ^{3}H 5-HT may be limited to serotonergic neurons, but that its uptake/transport system does not exclude the related amine, DOPA. NSF BNS 8206245

424.18 NEUROFILAMENT PROTEINS IN THE AXOPLASM OF CRAYFISH. S. Halls[1]*, C.R. Wang[2]*, R.A. Sheller[1], W.J. Mandy[2]* and G.D. Bittner[1]. Departments of Zoology[1] and Microbiology[2], University of Texas at Austin, Austin, Texas 78703.

Axonal transport is thought to occur by mechanisms involving neurofilaments (NF) and microtubules (MT) (see Ochs, S. (1982) Axoplasmic transport & its relation to other nerve functions, J. Wiley & Son, NY). However, it has generally been stated that the axons of arthropods do not contain NF's, although such axons have large numbers of MT's and exhibit axonal transport (see Lane, N.J. (1985) Comprehensive Ins. Physiol., Biochem., & Pharmacol. 5, 9-21, G. Kerkut & L. Gilbert, Pergammon Press, NY). In the crayfish ventral nerve cord (VNC) there are axoplasmic matrix filaments (AMF) associated with the MT's. These AMF's are thought to be homologous with mammaliam microtubule associated proteins (MAP's) and may be involved in axonal transport.

We report that the axoplasm of crayfish VNC's not only contain MAP's and MT's, but also NF-like material that have epitopes that are recognized by monoclonal antibodies directed against the 200, 160 and 68 kd mammaliam NF's. VNC's were dissected from crayfish (Procambarus clarkii) in the presence of Ca^{++}-free van Haarevelds solution containing 5mM EGTA and 0.5 leupeptin (VH buffer). The axoplasm was extruded into a vessel containing 100 µl of VH buffer and homogenized. Aliquots of the homogenate were serially diluted and applied to a nitrocellulose membrane for dot-blot analysis. Non-specific binding sites were blocked by incubating the membrane overnight in 5% BSA-PBS buffer at 4°C. Primary antibodies were applied to the membrane at the optimum concentration and incubated for 2 hrs at 20°C, then washed (4X) with PBS-1% Tween 20 for 30 mins. The second antibody coupled to HRP was then applied to the membrane and for 2 hrs at 20°C and then washed as before. The HRP reaction product (HRP-RP) was developed using the DAB procedure. HRP-RP axoplasmic dot-blots revealed positive reactions to α and β tubulin, MAP's, NF (200kd), NF (160 kd) and NF (68 kd) proteins, suggesting the presence of MT and NF components in the axoplasm. HRP-RP dot-blots of the axoplasm gave negative results to glial filament acidic proteins (GFAP) antibodies, suggesting minimal glial contamination of the axoplasm during extraction. Electrophoretic and Western transfer analyses of axoplasm support the data from dot-blot analyses. We are currently pursuing the location and distribution of the NF-like material in the axons of the crayfish by immuno-electron microscopy.

Our results regarding the occurrence of NF-like material in the axons of an arthropod allows the generalization of the NF-MT hypothesis of axonal transport to be applied to this phylum.

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425.1 ANTENNAPEDIA, A HOMEOTIC REGENERATE IN THE WALKING STICK Carausius morsus: SENSORY PROJECTIONS. J.S. Edwards, G.R. Reddy,* and M.U. Rani,* Zoology Dept. Univ. of Wash. Seattle WA 98195.

It has been known for many years that the antenna of the walking stick insect Carausius morosus (Phasmida), when severed at the distal end of the second segment during an early instar, regenerates in the form of a leg (Schmidt-Jensen, 1913). In its most complete form the regenerate is composed of femur, tibia and a tarsus composed of 4 segments with a pair of claws flanking a terminal pad.

We have compared the central sensory projections of antennapedia regenerates in Carausius with those of the homeotically transformed antenna of the antennapedia mutant in Drosophila. In Carausis, as in Drosophila (Stocker et al, 1976, Stocker and Lawrence, 1981) the primary projection is predominantly to the olfactory lobe, where normal antennae afferents terminate. In Carausius some axons bypass the olfactory lobe and terminate beside the corpora pedunculata. Other axons wander extensively through both ipsi- and contralateral neuropile.

The projection of the normal tarsus to a thoracic ganglion includes a distinctive bilaterally symmetrical glomerulus that maps the pair of claws. No evidence of the characteristic claw projections was found in the brains of animals with homeotic antennae.

The morphogenetic rules for generating sensory projections of homeotic mutants in Drosophila appear to apply also to homeotic regenerates in Carausius.

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Stocker, R.F., Lawrence, P.A. 1981 Sensory projections from normal and homeotically transformed antennae in Drosophila. Devel. Biol. 82:224-237.

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425.2 LUCIFER YELLOW-CH DYE COUPLING IN NORMAL AND REGENERATED DORSAL GIANT FIBERS OF *Lumbricus terrestris*. A.W. Lyckman and G.D. Bittner, Department of Zoology, University of Texas, Austin, TX, 78712.

The dorsal giant fibers (DGF's) which mediate the escape response of the earthworm are segmental interneurons composed of electrically coupled giant axons. There are three DGF's, the medial giant fiber (MGF) and two lateral giant fibers (LGF's). The LGF's are electrically coupled and thus function physiologically as a single giant fiber. Using intracellular recording and dye injection, we are investigating the mechanisms and specificity of regeneration of transected DGF's.

Results from intracellular micro-iontophoresis and pressure microinjection of Lucifer yellow-CH (LYCH) into normal giant axons show: (1) LYCH rapidly fills the injected giant axon, its dendritic processes, neurites and cell body, completely highlighting the entire cell. (2) LYCH preferentially adheres to the axolemma, mitochondria, vesicles and cytoskeletal elements of the axon as visualized in plastic cross-sections of glutaraldehyde fixed tissue under epiflourescence. This indicates that LYCH binds to cytoplasmic elements and does not remain evenly distributed in the axoplasm. (3) LYCH dye coupling is far more rapid and complete between electrically coupled LGA's than between electrically coupled MGA's. This indicates that the gap junctions in both the septa of the LGF and in the area of contact between the neurites of contralateral LGF's are far more permeable to LYCH than are the gap junctions in the septa of the MGF. The marked difference between LGF's vs. MGF gap junction permeability to LYCH is due to differences in the intrinsic structures of the gap junctions of the LGF's vs. MGF and/or differences in the regulation of gap junction permeability in the LGF's vs. MGF.

Results from LYCH injection into regenerating transected DGF's show: (1) At early stages of regeneration (<1 wk post-transection) when regenerating DGF's have not restored electrical coupling across the transection site, there is also no LYCH dye coupling between DGF's across the transection site as seen in whole mount under epiflourescence. (2) At later stages of regeneration (>2 mos post-transection) when both the LGF's and MGF have restored normal electrical coupling across the transection site, there is strong LYCH dye coupling between MGF's across the lesion site and between LGF's across the lesion site as seen in whole mount under epiflourescence. LYCH dye coupling between the DGF's across the lesion is probably due to the formation of new gap junctions and/or membrane fusion between giant axons that become electrical coupled across the lesion site. (3) Plastic cross-sections of injected whole mounts show each giant axon crosses the transection site in bundles of up to four large diameter, LYCH-filled, regenerated axonal processes wrapped in a glial sheath. We have not detected LYCH-filled processes that project aberrantly in the nerve cord.

These results with LYCH injection confirm previous physiological results (Birse and Bittner, *J. Neurophysiol.* **45: 724, 1981**) that transected DGF's reconnect without misconnection. Our on-going experiments are investigating cellular mechanisms which contribute to cell-specific functional reconnection of transected DGF's, in particular the subcellular basis of electrical coupling between giant axons and the pathfinding ability of regenerating axonal processes. Our current results with intracellular injection of LYCH indicate that LYCH will continue to be a useful marker for identifying regenerating processes. Further, the differential permeability of gap junctions of LGF's vs. MGF's may indicate differences in the cellular and molecular properties of gap junctions of DGF's that are important to the specificity of electrical coupling seen in regenerating DGF's.

425.3 **Recovery of swimming following spinal transection is enhanced at lower temperatures in larval sea lamprey.** Joseph Ayers and Cricket Wilbur*. Marine Science Center, Northeastern University, East Point, Nahant, MA 01908

Larval sea lamprey, *Petromyzon marinus* can recover several behaviors following complete "cervical" spinal cord transection. We have utilized a quantitative behavioral assay *(Science 221: 1312-1314)* to determine the effects of reduced holding temperature on the dynamic organization of recovered swimming movements and the order of behavioral recovery. We found that *(1)*. The period and propagation time of swimming movements of ammocoetes which recover at lower temperatures (ca. 13°) closely approximates that of normal ammocoetes. In contrast, specimens which recover at room temperature (21°-23°), exhibit the shorter period and propagation time range characteristic of transformed adults. Intersegmental phase lag and body curvature were similar in all cases and the dynamic parameters of swimming are strongly related to specimen length. *(2)*. Lampreys take longer to recover when held at 13° than at 21°. The latency to achievement of the four stages of swimming, as well as crawling, burrowing and aversive withdrawal behaviors all are increased by 50 to 80%. *(3)*. Differences also occur in descending modulation of swimming. Greater than 90% of specimens held at 21°-23° are able to orient to gravity during swimming within 50-90 days following transection. The ability to orient to gravity recovers gradually in specimens held at 13° and by 120 days only ~70% of specimens orient 90% dorsal side up. We conclude that lampreys take longer to recover at lower temperatures, but recover more normal behavior.

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425.4 ELECTROPHYSIOLOGICAL PROPERTIES OF REGENERATED AXONS FOLLOWING LESIONS DURING EARLY AND LATE MATURATION. C.M. Bowe[1], C. Hildebrand*[2], J.D. Kocsis[1] and S.G. Waxman[1]. [1]Dept. of Neurology, Palo Alto VA Medical Center and Stanford University, Stanford, CA 94304 and [2]Dept. of Anatomy, Karolinska institutet, Stockholm, Sweden.

Following injury during adulthood, regenerated mammalian myelinated axons exhibit a prominent sensitivity to pharmacological blockade of potassium channels with 4-aminopyridine (4-AP) not observed in healthy, mature nerve fibers. This response to 4-AP, persisting beyond 2 years following injury, consists of a characteristic compound action potential late negativity with a distinctive "rippling" pattern and the presence of bursts of action potentials recorded from single fibers. It has been proposed that this response reflects a reorganization of ionic channels and the active participation of 4-AP sensitive potassium conductance in electrogenesis of the action potential in regenerated axons.

The present study examined the electrophysiological properties of regenerated rat sciatic nerves following lesioning during maturation. Sciatic nerve crushes were performed on 1 week to 4 month old Wistar rats. Following a recovery period of 6-21 months, animals were sacrificed, sciatic nerves excised and physiological properties of whole nerve responses examined in vitro during superfusion with Kreb's solution and Kreb's solution containing 1 mM 4-AP. While compound action potentials recorded from control nerves (N=24) showed only modest effects of exposure to 4-AP, those recorded from all regenerated nerves (N=45) developed a prominent delayed negativity following 4-AP. Nerves lesioned after 3 weeks of age demonstrated the characteristic "rippling" pattern previously described following adult lesions, while those lesioned at younger ages had a more continuous return to baseline. Examination of recovery cycle properties and ability to follow stimuli presented at fast frequencies indicated that function in the regenerated nerves from rats lesioned at the younger ages was less affected by 4-AP than that observed in the older lesioned groups. Morphological studies demonstrated a greater number of short internodal lengths in regenerated axons from the older lesion group compared to both controls and the younger lesion group.

These data suggest that 4-AP sensitivity may be related to abnormalities in the axo-glial organization of regenerated fibers and that the mechanisms underlying this organization are influenced by age at the time of lesioning.

This work was supported by grants from the VA and NIH.

425.5 DECLINE IN SYNAPTIC POTENTIALS OF IDENTIFIED AXONS DURING REGENERATION IN THE CRAYFISH. P.C. Prosser* and S.J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, NH 03755

The superficial flexor muscles of the crayfish Procambarus clarkii have been used to study regeneration of specific neuromuscular connections by identifiable neurons under a variety of experimental conditions (Ely & Velez, J. Neurophysiol. 47: 656-665; Hunt & Velez, J. Neurophysiol. 47: 666-676; Clement, Taylor & Velez, J. Neurophysiol. 49: 216-226). The motoneurons in this system innervate a field of around 40 muscle fibers from a medial-to-lateral direction forming a position-dependent connectivity map. We have reported previously the changes in connectivity patterns that are generated if regeneration proceeds when the target fibers are missing or when the nerve is transplanted to the middle of the muscle field. In these experiments the neurons were regenerating a complete set of new terminals after complete axotomy. We now report work in which neurons are left innervating part of the muscle field and some, due to a lack of target area, are forced to regenerate back into the same field. The superficial flexor nerve is cut at the center of the muscle field and all the lateral muscle fibers are removed. The medial field remains innervated by axons 1, 2 and 3 as regeneration begins. Crayfish were examined at regular intervals after the operation by monitoring the spontaneous activity of the nerve in isolated abdomens and matching individual spikes with intracellular recordings of muscle junction potentials. After a few days, the synaptic potentials recorded from the medial fibers for axons 1, 2 & 3 decline in amplitude. Cold treatment at this time reveals that synaptic potentials could still be recorded from individual fibers, an indication that connections that could not be detected under normal physiological conditions were still present. After six weeks, axon 4, which rarely innervates medial fibers, began to regenerate its contacts over the medial muscle population. At eight weeks, synaptic potentials recorded for axon 1 are still below normal values, those recorded for axons 2 & 3 approach control values and by now axon 4 is found innervating the entire muscle field. These results suggest that these neurons respond to partial axotomy by a decline in synaptic potentials and that axon 4 is able to expand into an innervated area in the absence of its normal target area.

425.6 **BEHAVIORAL RECOVERY IN SPINAL TRANSECTED LAMPREY DOES NOT REQUIRE SPECIFIC BEHAVIORAL FEEDBACK.** S. Park*. J.A. Snedeker* and M.E. Selzer. Dept. Neurology, Univ. of Pennsylvania, Philadelphia, PA 19104

Sea lamprey larvae recover several types of behavior following complete spinal transection (Rovainen, C.M., J. Comp. Neurol., 168:545, 1976; Selzer, M.E., J. Physiol., 277:395, 1978). This is accompanied by regeneration of functioning synaptic connections between neurons on opposite sides of the lesion (Mackler, S.A. and Selzer, M.E., Science, 229:774, 1985). The regeneration seems to be specific both in the direction of axon growth (Yin, H.S., Mackler, S.A. and Selzer, M.E., Science, 224:894, 1984) and in the selection of postsynaptic targets (Mackler, S.A. and Selzer, M.E., Soc. Neurosci. Abstracts, 1985). While this specificity might be mediated by chemoaffinity, it could also be "molded" or at least partially determined by behavioral feedback during recovery. In this case, assuming that the quality of the recovered behavior reflects the correctness of the synaptic reconnections, one might expect that the recovery itself would depend upon the ability of the animal to attempt the behaviors during convalescence. This was tested by comparing the rate of recovery of larval lampreys restrained in closed glass tubes with those allowed to move freely.

Larval sea lampreys 4-5 years old received a spinal transection at the level of the 7th gill. At weekly intervals the following were plotted: 1) staging of recovery of swimming on an ordinal scale (0-7); 2) swimming speed; 3) speed of crawling on dry surface; 4) ability to crawl forward out of a glass pipet; 5) ability to crawl backwards out of a pipet. There was no significant difference between the two groups in the rates of recovery of any of these parameters. Both groups achieved an asymptotic phase of recovery by 9 weeks. However, while control larvae took 0.3-0.6 sec to swim 20 cm, recovered animals took an average of 2-5 sec. Recovered behaviors were lost in all animals upon retransection.

We conclude that the observed behavioral recovery in spinal transected lamprey larvae is based on axonal regeneration and that whatever correctness of synaptic reconnection is required for this recovery is not dependent on behavioral feedback. (Supported by NIH Grants NS14837 and RR05415.)

425.7 **PROBABILITY OF REGENERATION AS A FUNCTION OF DISTANCE OF AXOTOMY FROM THE CELL BODY IN LAMPREY BRAIN NEURONS.** J.A. Snedeker* and M.E. Selzer (SPON. D.H. Silberberg). Dept. of Neurology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Previous data based on intracellular HRP injections suggested that giant reticulospinal axons of larval sea lampreys transected at the cloaca regenerated less well than those cut in the gill region (Yin, H.S. and Selzer, M.E., J. Neurosci., 3:1135, 1983). We wished to know if this was true of other cell types and whether it refected a continuous function of distance of axotomy from the cell body. In previously untransected larval sea lampreys 4-5 years old HRP-soaked Gelfoam plegettes were implanted at the site of a spinal cut. Wholemounts of the brain were prepared after 10-14 days and maps made of neurons with projections to each of four spinal levels. An average of 1037 neurons ± 51 SEM projected to the level of the 7th gill. Only 364 neurons ± 24 projected to the level of the cloaca. Intermediate numbers projected to levels at 25% and 50% of body length.

In the experimental group, animals were transected at the above levels and allowed to recover up to 210 days. HRP was then injected 5mm below the scar and wholemounts of brain examined for labeled neurons 12 days later. In animals injected 1 to 3 weeks post-transection (i.e. before regeneration occurs) virtually no brain cells were labeled, suggesting that HRP does not reach the brain cells by diffusion across or around the scar. Beyond 95 days after transection at the cloaca 27% of the projecting cells were labeled, while after transection at the 7th gill 41% were labeled. For the previously studied giant cells (Muller and Mauthner neurons) the numbers were 18% and 50%, confirming the previous study. However, at intermediate distances the proportion of labeled cells was not significantly lower than at the 7th gill.

We conclude that: 1) the regenerative capacities of the small spinal-projecting neurons of the lamprey brain are similar to that of the giant reticulospinal neurons; and 2) regenerative capacity is not a linear function of distance from the cell body. Instead the probability of regeneration drops off sharply at between 50% and 75% of body length. (Supported by NIH grants NS14837 and RR05415.)

425.8 ULTRASTRUCTURAL ANALYSIS OF AXONAL REGROWTH FOLLOWING LESIONS IN THE RAT VISUAL SYSTEM. S.E.Dyson*, A.R. Harvey* and S.K. Gan* (SPON:J.O. Willoughby). Dept. Anat.& Human Biol, Univ. of West. Australia., Nedlands,W.A. 6009.Australia.

It has generally been believed that axons of adult mammalian retinal ganglion cells do not regenerate after injury. Recently however,it has been shown that the conditions appropriate for optic fibre regrowth can be provided by peripheral nerve grafts (So K.F & Aguayo A.J. Brain Res. 328:349, 1985). It appears that the local non-neuronal environment of the grafts, in particular the presence of Schwann cells, is an important factor which facilitates regrowth. We have recently shown that retinal axons regrow through glial and connective tissue membranes which form over lesion sites in the rat visual system (Harvey A.R. et al Brain Res. 368:141, 1986). A detailed analysis of the cellular and non-cellular organization of the newly formed membranes may provide insight into the reasons why the membranes constitute a permissive microenvironment for the regrowth and maintenance of retinal axons. To this end we have studied the cellular composition of the membrane, its ultrastructural organization and the relationship between non-neuronal cells and regrowing axons.

Suction lesions were made in the left pretectum and rostral superior colliculus of anaesthetized, 10-18 day old hooded rats. Animals were sacrificed at postoperative days 15-256. The presence of optic axons in the membrane was established by the HRP-TMB technique in 40µm frozen sections. Adjacent frozen sections were osmicated, dehydrated and embedded for ultra-thin sectioning.

The membranes which formed over the lesion cavity had a set of consistent structural features. They possessed a limiting surface of fibroblasts and were composed of glial cells, macrophages, endothelial cells, pericytes and collagen. Dense interdigitating lamellae of glial cytoplasm were commonly found in close proximity to clusters of myelinated and unmyelinated axons. These glial cells contained numerous filaments and had a basal lamina on parts of their membrane surface, but this coating was not found on surfaces adjacent to the axons. The cells possessed the morphological features of reactive astrocytes (Munoz-Garcia D. & Ludwin S.K. J.Neuroimmunol. 8:237,1985).

The myelin sheaths surrounding larger axons were examined in order to determine their origin: (1) Ultrastructural features normally associated with peripheral myelin were absent, (2) The periodicity of myelin in the membranes (12.1 ± .12nm - mean ± S.E) was shown to be equivalent to that of other central structures (fornix 12.7 ± .16nm and superior colliculus 12.3 ± .11nm). In normal nervous tissue, the periodicity of peripherally derived myelin is 10-15% greater than that of central myelin. It appears therefore that in the membranes, the myelin surrounding the regrowing axons was centrally derived.

425.9 REGENERATION OF OPTIC AXONS IN THE MOUSE RETINA. M.B. Houston, C.F. Lagenaur, and R.D. Lund, Dept. of Neurobiology, Anatomy and Cell Science, and Center for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

The involvement of cell surface antigens in neurite outgrowth in vitro has been examined using a monoclonal antibody directed against the murine neuronal cell surface glycoprotein M6, (Lagenaur et al., Soc. Neurosci. Abstr., 10:759, 1984). The following experiment was undertaken to observe the effects of anti-M6 on neurite outgrowth in vivo.

Adult CD-1 albino mice received temporal retinal lesions while under tribromoethanol anesthesia. Some animals then received 0.5µl intraocular injections of either antibody (15µg) or saline immediately following the lesion, or 1 week post lesion (PL). Others received no further treatment following the lesion. Animals were perfused at various survival times (up to 37 days), and whole retinae were stained using a modification of Boycott's neurofibrillar stain for optic axons.

Peripheral to the lesion, in all cases, the population of cells in the ganglion cell layer became substantially reduced with time, but class I cells remained and became more intensely stained as compared with adjacent uninjured retina. Lesioned axons of the surviving cells in retinae untreated with antibody, exhibited profound regenerative capabilities, forming a dense polydirectional arborization proximal to the lesion site. Animals treated with antibody immediately following the lesion exhibited outgrowth, but at less than 10 days PL substantially less than that observed in untreated animals. Animals treated with antibody 1 week PL and allowed to survive for 1 to 2 weeks, also exhibited some outgrowth, but the extent of arborization was less than those untreated of the same survival time. Antibody did not affect ganglion cell density or fasciculi in unlesioned areas of surrounding retina.

Our results indicate that during the first five weeks following axotomy in the adult mouse retina, selective cell death occurs in the ganglion cell layer, with the class I cells remaining virtually unscathed. We propose that axonal regeneration in the murine retina is a selective phenomenon confined to the class I cells and that the cell surface glycoprotein M6 is important in this process.

Supported by NIH grant EY05308.

425.10 LONG-TERM CONSEQUENCES OF NEONATAL TRANSECTION OF THE INFRAORBITAL NERVE IN RAT ARE NOT INFLUENCED BY REESTABLISHMENT OF PERIPHERAL CONNECTIONS. N.L. Chiaia, P.R. Hess and R.W. Rhoades. Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine & Rutgers Medical School, Piscataway, NJ 08854.

Transection of the infraorbital (IO) nerve in newborn rats results in the death of numerous trigeminal (V) ganglion cells and a marked disruption of the central representations of the mystacial vibrissae. We sought to determine whether the long-term consequences of IO nerve transection reflect the connections that regenerate axons make with their peripheral targets. Two groups of animals were studied. In one group (N=6), the IO nerve was transected on the day of birth and the animals were allowed to grow to maturity without further manipulation. In a second group (N=13), the IO nerve was transected on the day of birth and again at weekly intervals. At 35-45 days of age, all rats had a mixture of HRP and WGA-HRP applied to the IO nerve proximal to the point of transection(s). Labelled cells in the V ganglion were counted and measured, and the transganglionic transport to the trigeminal brainstem nuclear complex (TBNC) was evaluated. The efficacy of repeated cutting of the IO nerve for the prevention of regeneration was evaluated by injecting diamidino yellow (DY) into the vibrissal pad 2 days prior to the HRP labelling experiment. The presence of DY-HRP double-labelled cells in the ganglion was taken as evidence that IO nerve fibers had reached a portion of the IO peripheral field. The results in the two groups were very similar. The average number of HRP-labelled IO ganglion cells in the rats lesioned only on the day of birth was 4,392 (sd.=1,316); that in the animals subjected to repeated cuts was 4,244 (sd.=864). The average cell diameter for 971 labelled neurons measured from the single transection group was 22.0 µm (sd.=8.2) and that for 935 cells measured in the animals subjected to multiple cuts was 21.5 µm (sd.=6.6). Numerous cells in the rats subjected to a single lesion of the IO nerve were labelled with both HRP and DY. No such neurons were observed in the animals subjected to multiple transections. The transganglionic labelling in the TBNC in the two groups was also virtually identical. Only very sparse label was observed in principalis, oralis, interpolaris and the magnocellular portion of caudalis. In contrast, very heavy labelling was visible in layers I and II of subnucleus caudalis. These results demonstrate that the long-term effects of neonatal IO nerve transection upon ganglion cell survival and innervation of the TBNC by this V branch are not influenced by regeneration of damaged axons.

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425.11 MORPHOLOGY OF AXOTOMISED MOTOR NEURONS WHICH REINNERVATE THEIR TARGET. E.H. Murphy(1), D.E. Klions*(1), R. DiRienzo*(1) and R. Baker(2). (1)Dept. of Anat., Med. Coll. PA, Phila., PA 19129; (2)Dept. of Physiol. & Biophysics, NYU Med. Ctr., New York, NY 10016.

Following axotomy, the soma of a neuron undergoes a retrograde reaction which may include breaking up of Nissl bodies and neuronal hypertrophy, along with changes in neuronal metabolism including an increase in RNA synthesis. When successful regeneration occurs, the metabolic changes reverse with time, but long-term changes in neuronal morphology have not been described.

We have sectioned the trochlear nerve unilaterally in adult cats. The section was made distal to the decussation at the site where the nerve courses between the cerebellum and inferior colliculus. Following a survival period of 2-3 months, HRP was injected into the superior oblique muscle, and the trochlear nucleus was examined for the presence of HRP filled cells indicating successful regeneration by these neurons to the original target.

In normal cats, the trochlear nucleus contains approximately 1200 neurons, most of which are filled with HRP following a superior oblique injection. In the trochlear nucleus of axotomised cats, the neuronal population was significantly reduced. Fewer than 200 neurons had successfully reinnervated the superior oblique muscle as indicated by HRP filling but a Nissl counterstain revealed other trochlear neurons which had not reinnervated the superior oblique muscle.

The soma size of those neurons which had successfully regenerated and reinnervated the superior oblique muscle was greatly increased. In the normal cat, the mean soma diameter of HRP filled cells was 26.4µm. In 2 axotomised cats the mean soma size of HRP filled cells was 53.2µm and 48.2µm. The results indicate that significant neuronal hypertrophy persists after successful regeneration has occurred. This suggests that the cell loss in the trochlear nucleus has reduced competition at the target resulting in hypertrophy of surviving cells.

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425.12 REGROWTH OF FACIAL AND HYPOGLOSSAL AXONS FOLLOWING CRUSH INJURY. S.K. Jacob, T.E. Durica and J.M. Kerns. Dept. of Anatomy, Rush Medical College, Chicago, IL 60612.

We have reported that facial and hypoglossal motoneurons survive crush injury as long as 60 days postoperative (dpo). Observations of behavior reveal a significant functional recovery in affected musculature by 21 dpo following a crush injury. This study utilized horseradish peroxidase (HRP) to retrogradely label these motoneurons after injury. This method will show what percentage of neurons have successfully regrown their axons beyond the crush injury site.

In four adult male hamsters the right facial and hypoglossal nerves were located, the perineurium was marked with a fine suture and crush was performed at the suture site with forceps for 60 sec. Animals were observed at 30 dpo for a return in facial and hypoglossal muscular function. These nerves were located, cut just distal to the suture point, and the proximal segment soaked in a 30% HRP solution within a capillary tube for 15 min. These animals were perfused 48 hours later and brainstems sectioned at 50µ on a Vibratome. Sections were prepared for HRP histochemistry, reacted with the TMB method, and counterstained with neutral red. All labelled profiles in every other section were counted throughout each nuclear group. No contralateral labelling was observed. Control data was obtained from a separate series of normal animals labelled with HRP in the same manner.

Preliminary results indicate a decreased number of labelled profiles in each nuclear group 30 days after nerve crush. Only 70% of the cells in the facial motor nucleus were labelled, while only 60% of the hypoglossal neurons were labelled. We stress that this is not due to neuronal cell loss, since previous work has shown no neuronal cell death at 30 dpo following crush injury in these nuclear groups. Routine histological examination of these neurons reveal that they have a relatively normal appearance and do not show any degenerative changes. There are some neurons that do not regrow their axons beyond the crush site and yet are capable of surviving without reestablishing their normal peripheral connections. It would seem that functional recovery of the affected musculature does not require reinnervation by all of the neurons of the nuclear group.

425.13 REGENERATION OF THE VIIIth CRANIAL NERVE IN THE BULLFROG. ANATOMIC AND PHYSIOLOGIC VERIFICATION. A. Newman,* A. Kuruvilla,* J. Eichel,* and V. Honrubia (SPON: L. Karaviti). Div. of Head and Neck Surgery, UCLA School of Med., Los Angeles, CA 90024.

A combined anatomical and physiological study was made of the capability of central regeneration of the vestibular nerve in the bullfrog Rana catesbeiana. Horseradish peroxidase labeling techniques were used to evaluate anatomical changes. Postural head deviation and the compensatory movement of the head in response to lateral tilts (roll) were measured to evaluate functional recovery after various surgical procedures in three groups of animals.

Group I animals, in which the left VIIIth nerve was surgically sectioned at the root, showed complete recovery of posture and response to tilt 75 days after surgery owing to CNS compensation or regeneration of neurons. Labeling of the VIIIth nerve peripheral to the site of surgery revealed that large numbers of regenerated primary afferents were present in the CNS and projected to the various vestibular nuclei in a pattern similar to that of normal animals (Kuruvilla et al., Laryngoscope 95: 692-707, 1985). Labeling of individual branches of the regenerated VIIIth nerve showed that regenerated fibers projected to the same specific areas in the various vestibular nuclei as in normal animals (Suarez et al., Laryngoscope 95: 1238-1250, 1985).

In Group II animals the left ganglion was surgically removed. Three months after surgery posture had recovered by about 75% and reflexes to tilt were almost normal owing to CNS compensation. After subsequent sectioning of the right VIIIth nerve the reflexes disappeared, then gradually returned to their preoperative levels. Either anatomical reinnervation, which was confirmed histologically, or CNS compensation could account for this recovery.

The left labyrinthine organ of Group III animals was surgically removed. The functional behavior of these animals was similar to that of Group II animals after the first operation. Removal of the left VIIIth nerve ganglion 150 days later did not produce significant changes in posture or reflexes to tilt. In a third operation, two months after the second one, the right VIIIth nerve and ganglion were removed. This led to loss of tonicity and absence of both postural head changes and reaction to tilt stimuli that persisted even after two additional months. In these animals with absent vestibular nerves, the recovery of function observed in Group II animals, whose right VIIIth nerve was allowed to regenerate, did not occur.

Thus, vestibular nerve regeneration in the adult bullfrog is accompanied by recovery of tonic and postural control.

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425.14 REGULATION OF CHLORIDE AND ACh CHANNELS IN MUSCLE MEMBRANE. R. David Heathcote and William J. Betz, Department of Physiology, U. of Colo. Sch. of Med., Denver, CO 80262.

Skeletal muscle membrane Cl channels and extrajunctional acetylcholine (ACh)-gated channels appear to be regulated in opposite directions. For example, in normal adult muscle chloride conductance (G_{Cl}) is relatively high, while extrajunctional ACh sensitivity is low. During development and after denervation, the reverse is true.

In order to investigate this anti-coordinate ion channel regulation we have developed an in vitro organ culture preparation of adult rat lumbrical muscle. Muscles were pinned through a stainless steel mesh to a sterile sylgard-lined dish, covered with a shallow layer of Trowell's T-8 medium, and incubated at 37° C on a rocker plate which alternately exposed the muscle to culture medium and to a 95% O_2/5% CO_2 atmosphere. Under these conditions the small lumbrical muscles remained healthy in vitro, responding with a vigorous twitch to electrical stimulation after 7-12 days in culture.

Following various periods of culture, G_{Cl} and the amount of extrajunctional ACh rceptors (AChR's) were measured. G_{Cl} was calculated as the difference between input conductance in normal Ringer and in Cl-free Ringer (Cl replaced by isethionate). AChR's were assessed by measuring the binding of ^{125}I-α-bungarotoxin, subtracting the signal due to endplate binding. Extrajunctional AChR's appeared and G_{Cl} fell with time in vitro in a fashion resembling that observed in vivo after denervation.

We have asked whether the inverse expression of the two channels is rigidly linked. Approximately 6 days after denervation in vivo, 48% of the fiber input conductance was due to chloride whereas in contralateral innervated muscles, G_{Cl} was 73% of the total conductance. Extrajunctional ACh binding in denervated muscle was 5 times greater than that seen at innervated endplates. Matched pairs of denervated and control muscles were placed in organ culture. Cycloheximide, a blocker of protein synthesis was added to the cultures and decreased extrajunctional AChR expression by approximately 50% each day. Yet the conductance to chloride remained low.

The fact that G_{Cl} did not rise after blocking AChR expression in denervated muscles shows that the usual anti-coordinate expression of Cl and ACh channels is disrupted under these conditions. The degree of independence of expression of Cl and ACh channels is not yet known.

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425.15 CONNECTIVE TISSUE CELLS THAT ACCUMULATE IN THE JUNCTIONAL REGION OF DENERVATED MUSCLE ARE ANTIGENICALLY RELATED TO FIBROBLASTS. Elizabeth A. Connor, Shlomo Rotshenker, and U.J. McMahan. Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305.

We previously demonstrated that denervation of both frog and rat skeletal muscles results in a selective accumulation of connective tissue cells in the junctional region of the muscles. Such cells may play a critical role in reestablishing neuromuscular function after nerve damage; connective tissue cells make and degrade extracellular matrix, and extracellular matrix of nerve and muscle is directly involved in regeneration of the neuromuscular junction. We hypothesized that these cells are derived from the fibroblasts that normally reside in the muscle and/or nerve. This hypothesis is based on two observations. First, the structure of the accumulated cells is strikingly similar to that of fibroblasts in normal muscle and nerve. Second, the increased cell number in the junctional region of denervated muscle resulted from cell division; the connective tissue cell nuclei incorporated tritiated thymidine. The aim of the experiment described here was to test whether the accumulated cells were in fact derived from fibroblasts.

We made a library of monoclonal antibodies (Mabs) against the junctional region of denervated frog muscle. Two of these Mabs stained connective tissue cells. In innervated muscles, the stained cells were few in number.. The stained cells had the same size, shape and distribution as cells previously identified as fibroblasts. In denervated muscles, the Mabs stained a high density of connective tissue cells in the junctional region of muscle fibers. The junctional staining pattern of the Mabs was detected by eleven days postaxotomy and persisted for up to ten weeks of chronic denervation. Mabs also stained a few cells in the severed nerve, which were probably fibroblasts. No other structures in nerve or muscle (including Schwann cells, perineurial cell or muscle satellite cells) were labelled. These observations are consistent with the hypothesis that the cell that accumulate in junctional regions of denervated muscle are fibroblasts.

425.16 ALTERATIONS IN THE MOLECULAR COMPOSITION OF MUSCLE FIBERS IN THE PERIJUNCTIONAL REGION OF DENERVATED FROG MUSCLES. S. Rotshenker and E. A. Connor. (SPON: R. Nitkin). Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

Denervation of a skeletal muscle results in several striking changes in the structure and function of muscle fibers. Some of these changes are confined to sites of the former neuromuscular junctions while others extend the full length of the muscle fibers. We demonstrate here a novel denervation-induced alteration which involves the region of muscle fibers that surrounds the former junctional sites, the perijunctional region.

We raised monoclonal antibodies against denervated frog muscles and used them to stain frozen cross sections of both normal and denervated muscles for fluorescence microscopy. Several of the monoclonal antibodies stained only neuromuscular junctions in normal muscles. In cross sections of the muscles, where junctional sites occupied approximately 10% of a muscle fiber perimeter, the stain lined that portion of the muscle fiber surface that was coextensive with the high concentration of acetylcholine receptors at the junction. On the other hand, in cross sections of denervated muscle the stain outlined the entire perimeter of some muscle fiber profiles. The staining was confined to those muscle fiber profiles sectioned through or near former synaptic sites indicating that while the staining on denervated muscle fibers covered a broader area than in normal muscles, it was centered on the junctional region and extended only several tens of microns beyond it. The altered staining pattern was first observed eleven days after denervation and persisted without change for ten weeks. No staining was observed in whole mounts of muscle indicating that the molecules recognized by the antigens are intracellular. This change in the molecular composition of the muscle fiber surface in its perijunctional region is coextensive with changes that occur in the connective tissue surrounding each muscle fiber (Connor et al., this volume). Experiments are currently underway to determine if these coextensive changes are causally related and what role, if any, they play in the regeneration of the neuromuscular junction.

425.17 **INHIBITION OF POLYAMINE SYNTHESIS IN REGENERATING NERVE.**

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In an earlier study (Fed Proc 44(5):1790,1985), we reported that following a crush injury to the rat sciatic nerve the concentration of polyamines (putrescine (PUT), spermidine (SPD) and spermine (SPM)) in nerve increased significantly. Daily i.p. injections of spermine 50 μmoles/kg/day were found to accelerate the initiation of functional recovery.

We have now determined the effects of inhibiting PUT synthesis using alpha-(difluoromethyl)ornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, on nerve polyamines and nerve regeneration.

Two days prior to nerve crush, male rats (200-225 g) were divided into two groups, those receiving drinking water containing 2% (w/v) DFMO and a control group. The sciatic nerve was crushed using hemostatic forceps. Functional recovery was determined by measuring the distance between foot digits when the spreading reflex was elicited; digit extension was recorded using an IBM photocopier. Polyamines and DFMO levels were determined using HPLC with a post-column o-phthaldehyde derivitization. DFMO decreased PUT in sciatic nerve of naive rats to less than one half of normal. In the nerve crushed group DFMO reduced PUT only proximal to the crush site; distal to the crush PUT was elevated. SPD was unaffected by DFMO in the naive group but elevated in the proximal segment of crushed nerve. DFMO treatment elevated SPM in naive and nerve crushed sciatic nerve. DFMO treatment depressed spinal cord levels of PUT to a greater extent than sciatic nerve PUT and also significantly reduced SPD but not SPM levels. DFMO had no significant effect on the rate of functional recovery from nerve crush relative to the water control group. These findings, in conjunction with our earlier studies, suggest that the concentration of SPM, or a SPM metabolite stimulates nerve regeneration. (Supported by Heineman Foundation. DFMO was a gift of Merrell Dow Research Institute.)

425.18 TIME COURSE OF CHANGES IN POSTTRANSLATIONAL PROTEIN MODIFICATION BY AMINO ACID ADDITION IN THE RAT SCIATIC AND OPTIC NERVES AFTER CRUSH INJURY

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Previous experiments have demonstrated that posttranslational protein modification by amino acid addition sharply increases in the sciatic nerve and decreases in the optic nerve of rats up to 2 hrs post-crush, when compared to normal intact controls. (Shyne-Athwal et al Science (1986) 231:603-605). The current study compared the posttranslational addition of ^{3}H-Arg, ^{3}H-Lys and ^{3}H-Leu to protein in these systems at time intervals up to 3 weeks post-crush. We have also examined histological changes in the nerve induced by the crush and partially characterized the proteins modified by ^{3}H-Lys using 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

The posttranslational incorporation of each amino acid tested increased significantly in the injured sciatic nerve at 6 and 14 days post-crush, while increasing only at 6 days post-crush in the optic nerve. Regional analysis of the regenerating sciatic nerve indicated that the increases in these reactions were associated with the portion of nerve containing the advancing axonal tips. Histological examination of the optic nerve showed that the increased modification activity coincided with the appearance of bulbous axonal enlargements. These structures were not present at earlier or later time points, times when modification reactions were also depressed. Autoradiograms from 2D-PAGE of sciatic nerve proteins modified by ^{3}H-Lys 6 days after crush injury showed labeling of basic proteins primarily in molecular weight ranges of 18 Mr, 30-40 Mr, 80-100 Mr). These results demonstrate that posttranslational modification of specific nerve proteins by amino acids occurs during regeneration of the sciatic nerve primarily in the region of the advancing tips of the growing axons, and in optic nerves, at a time coincident with the formation of bulbous axonal enlargements. (Supported by Grants NS07501 and NS19148 from NIH).

425.19 MOLECULAR CLONING OF cDNAs FOR INTERMEDIATE FILAMENT PROTEINS EXPRESSED IN THE GOLDFISH REGENERATING VISUAL PATHWAY.

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We are investigating the structure and expression of neuronal intermediate filament proteins synthesized by retinal ganglion cells in the goldfish visual system. We have shown that significant increases in the synthesis of neurofilament proteins ON_1 and ON_2 occur during optic nerve regeneration and such increases correlate with an elevation in the production of specific mRNAs for these proteins. A peak of ON_1/ON_2 mRNA synthesis occurs approximately 20 days after optic nerve crush.

We are isolating cDNA clones corresponding to the ON proteins to further characterize their involvement in the regeneration process. A cDNA library was prepared from RNA isolated from retinas during optic nerve regeneration. cDNA synthesis was performed by reverse transcribing poly $(A)^+$ RNA purified from 20 day post-crush retinas. After second strand synthesis utilizing RNase H and DNA polymerase, cDNAs were methylated, tailed with Eco RI linkers, and cloned in lambda gt 11. We have been able to generate an expression library of approximately 10^6 recombinant clones. This library is being screened with antisera specific for these neurofilament proteins. Positive clones are being confirmed by their ability to direct the in vitro production of transcripts encoding the ON proteins. The synthetic transcripts are translated in vitro into proteins and the products identified by immunoprecipitation and gel electrophoresis.

Certain intermediate filament proteins are associated with neurons undergoing development or regeneration. Included in this group is vimentin which is transiently expressed in neurons during embryogenesis and continuously expressed in the olfactory epithelium of adult mammals. The sequencing of ON cDNA clones will allow us to compare and contrast structural characteristics between the goldfish proteins and these other intermediate filament proteins. All intermediate filament proteins share common structural features located within a highly conserved 40 kdal core region. However, we expect structural features located within the variable amino- and carboxyl- terminal regions outside the core to be responsible for imparting certain intermediate filament proteins with functions related to nerve development and regeneration. Because of the unique characteristics of the goldfish visual system, i.e., its capacity for both plasticity and regeneration, we speculate that the variable regions of the goldfish intermediate filament proteins have evolved to meet the physiological demands of these processes. A molecular link is suggested between the regenerating goldfish visual pathway and neurogenesis in other systems in terms of intermediate filament expression.

This research is supported by a grant from the National Institute of Health (EY-05212) to N.S.

425.20 IMMUNOHISTOCHEMICAL LOCALIZATION OF INTERMEDIATE FILAMENT PROTEINS OF NEURONAL AND NON-NEURONAL ORIGIN IN THE REGENERATING GOLDFISH VISUAL PATHWAY.

P.S. Jones*, P. Tesser* and N. Schechter (SPON.: W. Quitschke). Depts. of Pharmacological Sciences, Biochemistry, and Psychiatry and Behavioral Science, SUNY at Stony Brook, NY 11794.

We prepared polyclonal antibodies to the 58Kd goldfish intermediate filament proteins (ON proteins) for histological studies to determine whether the ON proteins could be used as markers for different structures involved in nerve regeneration in the goldfish visual system. Previous biochemical studies showed that the ON proteins are the predominant intermediate filament proteins in the goldfish optic nerve (Quitschke, W. and Schechter, N., J. Neurochem. 42:569, 1984). These studies indicated that ON_1 and ON_2 are neuronal proteins while ON_3 and ON_4 are of non-neuronal origin. In addition, the levels of ON_1 and ON_2 are profoundly influenced by nerve degeneration and regeneration, whereas ON_3 and ON_4 are not influenced (Quitschke, W. and Schechter, N., J. Neurochem., 41:1137, 1983). Two sets of antibodies were used in immunohistochemical localization studies in goldfish optic nerve and retina under normal and regenerative conditions to confirm these biochemical observations and to demonstrate their specificities as histological markers with which to monitor regeneration.

Anti-ON_1/ON_2 reactivity on sections of normal optic nerve is consistent with intra-axonal staining. Staining with anti-ON_3/ON_4 results in a non-neuronal pattern similar to the astrocytic staining observed with anti-GFAP and mammalian optic nerve (Dahl, D. and Bignami, A., Brain Res., 61:279, 1973). During degeneration of the goldfish optic nerve the histological results parallel those of previous biochemical studies. At nine days after surgery, the levels of ON_1 and ON_2 decreased substantially in both enucleated and crushed tissue. A loss of axonal structure was also observed. Conversely, very little change in the anti-ON_3/ON_4 staining pattern is observed with respect to both morphology and staining intensity. This suggests that there is little or no gliosis in the goldfish optic nerve at this time point after nerve injury.

Very little anti-ON_1/ON_2 reactivity is observed in normal retina. However, at 20 days after optic nerve crush, when ON_1 and ON_2 synthesis is at its maximum, there is significant reactivity in the retinal ganglion cell layer, thus confirming the neuronal origin of these proteins. Incubations of both normal and regenerating retina with anti-ON_3/ON_4 results in the staining of cells in the inner nuclear layer. These cells appear to be the axoniess horizontal cells, a neuronal cell type which shares some properties of glial cells, and which has been shown to react with anti-vimentin antibodies in mammals (Drager, U., Nature, 303:169, 1983).

These results demonstrate the development of immunological probes with which to study novel intermediate filament proteins associated with nerve regeneration in the goldfish visual system.

This research is supported by a grant from the National Institutes of Health (EY-05212) to N.S.

426.1 THE POND SNAIL, AN ANIMAL MODEL FOR THE STUDY OF AGING PROCESSES AT THE NEURONAL LEVEL. C. Janse*, M. van der Roest*, J.J.M. Bedaux* and W. Slob*. (Spon: European Neuroscience Association). Department of Biology, Vrije Universiteit, 1007 MC Amsterdam, The Netherlands.

Survival characteristics and changes of cellular functions with age were studied in the pond snail *Lymnaea stagnalis*. Survival characteristics. Laboratory-bred *Lymnaea* has survival characteristics specific for aging animals. At an age of about 200 days death rate increases considerably indicating the start of senescence. The median age (50% survival) is reached at about 330 days, 10% survival is reached at about 460 days of age and maximum age is about 600 days. Egg laying ceased at an age of about 450 days. Cellular functions, recovery after damage. Recovery of the tentacle reflex after damage of sensory input was studied. With age the time needed for recovery of reflex function increases from about 20 weeks (at an age of about 80 days) to 40 weeks (at an age of about 300 days). Recovery of sensory connections was not impaired with age and the reflex could always be evoked normally via undamaged pathways. It is concluded that restoration of synaptic connections in *Lymnaea* is impaired with age. Neurophysiological studies indicate that similar processes occur at the cellular level. Cellular functions, biophysical properties. In two electrically coupled identified neurosecretory cells age-related changes were found in animals between 60-360 days of age. Coupling efficiency decreased from 60% in young animals to 30% in old animals. Indications were obtained that this decrease is due to an increase of the resistance of the electrical synapse between the cells. In young animals the cells fired nearly synchronously (delay about 5 msec). The delay increased to 30-40 msec in old animals. The firing frequency decreased with age. This decrease was correlated with changes in the repolarization phase of the action potentials.

From the above findings it is concluded that in *Lymnaea* neuronal functions change with age. The underlying mechanisms of these changes will be studied.

426.2 AGE-RELATED HEARING LOSS IN BDF_1 MICE AS EVIDENCED BY THE BRAINSTEM AUDITORY EVOKED POTENTIAL. D.W. Shucard, M.W. Church. Department of Neurology, State University of New York at Buffalo, and Research Institute on Alcoholism, Buffalo, NY 14222.

Age-related hearing loss was studied in unanesthetized female BDF_1 mice using brainstem auditory evoked potential (BAEP) latency-intensity (L-I) profiles and BAEP thresholds. Five different age groups were studied: 3-4 months, 5.5-7 months, 9-10 months, 12 months and 18 months.

While BAEP audiometry has been used previously to characterize age-related hearing loss in several mouse strains, the present study differs from these studies in several ways. First, previous mouse studies used anesthetized animals. However, recent data have indicated that anesthetics can influence the BAEP. In the present study, using a technique developed in our laboratory, we were able to record BAEPs from unanesthetized mice. Second, most studies have not evaluated animals across the natural adult life span. In this investigation animals as old as 18 months were examined. Finally, in the present study we used the techniques of BAEP L-I profiles and BAEP thresholds to examine age-related hearing changes. Most previous animal investigations of aging did not systematically examine these variables.

The findings indicated that relative to the youngest group, BAEP threshold data showed shifts in auditory sensitivity ranging from +6 dB for the 5.5-7-month-old group to +71 dB for the 18-month-old group. The BAEP L-I profiles of older mice were consistent with recruitment-type sensorineural (cochlear) hearing losses. No evidence of retrocochlear involvement was observed.

426.3 MORPHOLOGICAL EFFECTS OF HOUSING ENVIRONMENT AND VOLUNTARY EXERCISE ON CEREBRAL CORTEX AND CEREBELLUM OF OLD RATS J.E.Black, R.Parnisari,* E.Eichbaum,* & W.T.Greenough College of Medicine, Depts of Psychology & Anatomical Sciences, Neural & Behavioral Biology Program, University of Illinois, Urbana, IL 61801

Previous reports of experience-induced changes in dendritic branching of mature and senescent animals have neglected the potential contribution of physical exercise obtained by rats in a complex environment. This report describes different patterns of synaptic plasticity in rats housed in a complex environment vs. rats allowed voluntary exercise in an otherwise simple environment. Thirty-nine rats in the laboratory of G. Winocur were reared in groups of 4-6 until 22 months old and then placed individually for 3 months in either a mesh cage with toys, an empty cage in a relatively noisy room, or a quiet empty cage. They were then given 3 weeks of behavioral testing and sent to Illinois. After acclimation, these 26-month-old rats were either placed in a complex environment (EC) with other rats and changing sets of toys, or placed individually with a running wheel in an isolated-exercise (IX) cage for 80 days. Golgi-Cox stained layer IV stellate and layer III pyramidal cells of occipital cortex and cerebellar Purkinje cells were analyzed for numbers and lengths of dendritic branches and the amount and location of dendritic material with respect to the soma. The stellate cells were subcategorized by soma shape and branching pattern. The apical and basilar dendrites of the pyramidals and the main and spiny branches of Purkinje cells were analyzed separately.

EC neurons generally had more dendritic material than baseline animals in three of the four stellate cell types as well as the apical dendrites of the pyramidal cells. The basilar dendrites of the IX animals had significantly more dendritic material in the middle region than both the baseline and EC animals. The amount of dendritic material in this region was significantly correlated with the mean amount of wheel running per day ($r=.75$ Spearman rank, $p<.025$). Spiny and main branches of Purkinje cells generally showed a significant degenerative effect of aging for both EC and IX rats relative to the baseline rats. Spiny branches of EC rats had more material in their middle region, but less at their ends than those of IX rats. The main branches of EC rats had more material than those of IX rats in their outer regions. Overall, regions of some cell types were insensitive to both housing and exercise, while other regions were sensitive primarily to exercise or primarily to complex experience. These results, taken with previous work, suggest that the degenerative changes attributed to aging are not entirely inevitable, and may be influenced by extrinsic factors, including physical exercise and complex experience.

Supported by the Retirement Research Foundation.

426.4 CAPILLARY MORPHOLOGY IN VISUAL CORTEX OF MIDDLE-AGED, BUT NOT OLD, RATS IS ALTERED BY HOUSING ENVIRONMENT K.R.Isaacs, J.E.Black, M.Polinsky,* & W.T.Greenough College of Medicine, Depts of Psychology & Anatomical Sciences, Neural & Behavioral Biology Program, University of Illinois, Urbana, IL 61801

Post-weanling rats raised in complex environments (EC) with other rats and toys have significantly greater tissue volume and more synapses per neuron in visual cortex than rats paired in the social condition (SC), or reared in isolation (IC) (Turner & Greenough, Brain Res, 329, 1985). These same EC rats have larger fractions of tissue potentially filled with blood, closer packing of capillaries, and slightly larger mean vessel diameter in visual cortex than those of SC or IC rats (Black, Sirevaag, & Greenough, in preparation). The closer packing of capillaries, in the face of expanded volume of neuropil, suggests that new capillaries were formed under housing conditions that may have substantially increased local metabolic requirements. To determine whether similar effects occurred in older animals, one micron sections of expoxy-embedded visual cortex were obtained from 8 middle-aged (12 mos) and 9 senescent (22 mos) rats reared for 50 days in either a complex environment (EC) or a social condition (SC). Estimates of volume fraction, capillary density, and capillary diameter were obtained from drawings of layers I-IV at 1250X. Compared to the middle-aged SC rats, the middle-aged EC animals on average had significantly higher densities of small capillaries, lower densities of larger vessels, smaller blood volume fractions, and nearly equal distances from a random point in tissue to the nearest vessel. Preliminary estimates of neuronal density point towards greater cortical volume in the EC animals.

The higher density of small vessels of EC rats tended to maintain the distance between vessels in the face of expanding cortex volume, suggesting that mature rats can form new capillaries in visual cortex under conditions likely to increase local metabolic requirements. New capillaries may remain smaller because of an age-related impairment in vessel proliferation, which was reduced 35% in the skin of middle-aged rats (Yamaura & Matsuzawa, Exp Gerontol, 15, 1980). The expansion of neuropil, spreading out pre-existing blood vessels, may explain the lower density of larger vessels and the decreased blood volume fraction in the middle-aged EC rats. The senescent animals showed similar but non-significant trends in these measures. This is the first report of experience-induced morphological changes in brain microvasculature of mature animals.

Supported by the Retirement Research Foundation.

426.5 CHRONIC INTRACEREBRAL INFUSION OF NERVE GROWTH FACTOR (NGF) IMPROVES MEMORY PERFORMANCE IN COGNITIVELY IMPAIRED AGED RATS F.H. Gage[1], K. Wictorin[2], W. Fisher[2], L.R. Williams[1], S. Varon[3], and A. Bjorklund[2]. Dept. of Neurosciences[1], UCSD, La Jolla, CA, Dept. of Histology[2], Univ. of Lund, Lund, Sweden, Dept. of Biology[3], UCSD, La Jolla, CA.

We have previously demonstrated that a subpopulation of aged rats can be behaviorally characterized as being impaired in learning and memory performance. This impairment is correlated with a significant decrease in regional metabolic activity in the hippocampus and frontal cortex. Subsequently we have demonstrated that fetal grafts of basal forebrain rich in cholinergic neurons can improve the performance of these age-impaired rats, in an atropine-sensitive manner, suggesting the deficit and improvement are related to the cholinergic system. More recently we have demonstrated that approximately 70% of the cholinergic neurons in the septal diagonal band region die following fimbria-fornix lesions, and the cell death can be prevented by chronic infusion of NGF into the lateral ventricle adjacent to the septal region. With this background information we tested the hypothesis that the positive effects of chronically infused NGF on cholinergic neurons would ameliorate the apparent cholinergic related cognitive impairment in aged rats.

Out of 75 aged rats originally tested 25 were characterized as impaired in the Morris water-maze task. All 25 age-impaired rats had cannula implanted in their lateral ventricle. The cannulae were attached to Alzet osmotic mini-pumps, through which cytochrome C (N=10) or NGF (n=13) was infused for 4 weeks. Controls (n = 13) received vehicle or no treatment. NGF was infused at a rate of 0.5 ul/hr at a dose of 2 ug/wk. One week after surgery the rats were tested on the Morris water-maze for 5 consecutive days. The animals were then given a rest period of 11 days, and then were tested again for 4 days. At the end of the second test period the animals were processed for morphometric analysis using AChE and nissl staining. No significant difference between the two groups were obtained during the first test period, however, the rats which received NGF where significantly improved in the swim-maze performance during the second test. This was evidenced both in swim time and distance, thus it is unlikely the effect of NGF was on motor performance. Of particular note was the significant improvement that the NGF treated rats showed on the first trial of the second test indicating they remembered the task better than the nontreated animals. Histological analysis of the basal forebrain is now in progress.

These preliminary findings are suggestive of a role for NGF in the functional improvement of age-impaired animals.

426.6 A NEW STUDY OF CEREBROVASCULAR ACTIVATORS ON THE BLOOD FLOW AND SPINAL REFLEXES OF AGED ANIMALS. T.Shibuya[1], Y.Watanabe*[1], T.Matsuda*[1], H.Shimura*[1], N.Matsunaga*[1], N.Imanishi*[1] and T.Matsumiya*[2]. 1) Dept. of Pharmacology, Tokyo Medical College, Tokyo 160, Japan. 2) Dept. of Pharmacology, School of Medicine Tokai University, Isehara 259-11, Japan

In Japan, a large problem is that the usage of specific drugs (cerebrovascular activators) for aged persons has increased with the prolongation of the average span of life. Unfortunately the pharmacological properties of these drugs are not well known. Generally, large animals (primarily dogs) have been used for measuring the effects of these drugs on the blood flow of the carotid (CA), femoral (FA) and vertebral (VA) arteries. However, it is troublesome to use many large animals to investigate the pharmacological functions of drugs. We therefore developed new methods for simultaneously measuring the blood flow in VA, FA and CA, and further the neuronal activities of both lumbar roots of small animals (e.g. rats or guinea pigs). Using the transit-time ultrasonic volume flow meter, the blood flow of VA, FA and CA, in the rat were 1.45±0.44, 1.93±0.29 and 4.26±0.68 ml/min, respectively, and further, in a guinea pig, 2.46±0.34, 3.52±0.63 and 7.86±1.02, respectively. The blood flow of VA and FA in old rats (over 20 months old) was less than that in young rats (under 5 months old). The administration of papaverine increased the blood flow of CA, FA, and VA in rats and guinea pigs. These increases showed no differences between the several arteries tested. However, the cerebrovascular activators (e.g. ifenprodil and etc.) enhanced the blood flow of VA more than of FA in old rats and guinea pigs. The duration of this enhancement induced by the cerebrovascular activators was obviously longer (about 40 min) than that by papaverine. In the studies of spinal neuronal activities, the onset time of monsynaptic reflex (MSR) of old rats was obviously longer than that of young rats. For evaluating the pharmacological properties of cerebrovascular activators, anoxic model rats were prepared by the ligation of the left CA and the onset time and potencies of their MSR in the right and left lumbar roots were simultaneously measured using our modified method. These MSR were examined on the 1st, 4th, 8th and 15th days after the ligation. The onset time and potency of right lumbar MSR, which is contralateral to the ligation, were remarkably reduced unlike those of the left lumbar MSR. These reductions were reversed after the administration of cerebrovascular activators. These results on the studies of simultaneously measuring blood flow of VA and other arteries, and neuronal activities of both right and left lumbar roots in the small animals can be utilized as new screening tests for evaluating the new properties of cerebrovascular activators.

426.7 Differential Effects of Partial Denervation on Old vs. Young Soleus Muscle. J.M. Jacob* and N. Robbins (Spon: J.T. Mortimer). Dept. Develop. Genetics and Anat., Case Western Reserve Univ., Cleveland, OH 44106

Age changes reported at the neuromuscular junction (Cardasis 1983; Drahota and Gutmann, 1961) may reflect an inability of the motor neuron to maintain its field of innervation. After transection of the L5 spinal root, soleus motor neurons expand the nerve terminal field 5-fold to reinnervate the denervated fibers (Brown and Ironton, 1978; Brown, et.al., 1981). We used this paradigm to study the safety factor and strategy of reinnervation in the old (25-27m) vs. young (5-7m) male CBF-1 mouse soleus muscle at 30, 60 and 120 days after partial denervation.

The initial number of motor units, and the number remaining after L5 transection, were similar in young and old mice. Functional recovery (indirect isometric twitch tension) of the operated muscles was complete by 60d in young and old mice, with a similar number of muscle fibers per motor unit. However, in old (vs. young) mice certain physiological parameters did not recover. Latency (delay from stimulus to end plate potential (epp) onset) in young mice was less than 1300usec in 60% of the muscle fibers by 60d and 120d but in only 30% of fibers in old mice. Similarly, miniature end plate potential (mepp) frequency recovered by 60d in young, but not in old, operated muscle. In old, but not young mouse muscle, mepp amplitude dropped by 50% at 60 and 120 days. Further, in contrast to young operated muscles, in the old muscle there was a shift to low quantal contents in all fibers by 60d with a slight recovery by 120d. At that time, absolute quantal content was similar in young and old control. Morphological analyses of the nerve terminals are in progress.

In summary, after partial denervation, motor unit number, functional recovery and extent of reinnervation are similar in young and old mice. However, in old mice, transmitter release recovered more slowly and less completely, and latency and mepp amplitude did not recover at all. Thus, partial denervation reveals specific deficits in the functional reserve of aging motor neurons even though gross functional restoration is complete. Possibly, critical cellular components are re-allocated to maintain adequate transmitter output at the expense of other less critical synaptic functions (Supported by NIH grants AG00795 and T32-AG-00105.)

426.8 USE OF THE AGING MOUSE STERNOCLEIDOMASTOID MUSCLE IN THE SEARCH FOR GENERALITIES ABOUT NEUROMUSCULAR SYNAPTIC AGING. N. Anis* and Norman Robbins, Developmental Genetics and Anatomy, Case Western Reserve School of Medicine, Cleveland, Ohio 44106

Reported age changes at the neuromuscular junction vary from one muscle type to another, and no hypothesis has been offered to account for these differences. For instance, in the aged (27-30 mo) CBF-1 hybrid mouse, transmitter release is increased in soleus and ext. digitorum longus (EDL) but not in diaphragm (Banker et al., 1983). In old mice, nerve terminals in both soleus and EDL (Robbins & Fahim, 1985) but not in diaphragm (unpublished) are more complex and divided into small "regions". In order to test whether such regionalization was generally associated with increased transmitter release, morphologic and physiologic comparisons were made between young (5-8 mo) and old (24-27 mo) CBF-1 mouse sternocleidomastoid (SCM) muscle.

In zinc-iodide osmium-stained nerve terminals, neither nerve terminal area nor total length of terminals changed with age. However, the number of isolated boutons or branches ("regions") increased from 10 to 22 per junction and the longitudinal extent of terminals along the muscle fiber increased from 67 to 83 um (both age differences, n=20, p<0.01). Directly measured quantal content of transmitter release in low Ca/High Mg solution was 5.0 in young and 3.6 in old mice (n=40-42 in 5-6 mice, p<0.001). Thus, there was nearly a 30% age-related drop in transmitter release, in contrast to soleus in which release increased 2-3 fold (Kelly & Robbins, 1983). Therefore, regionalization of the terminal branches is not necessarily correlated with increased transmitter release. However, in soleus junctions, there were 6 regions per junction in young muscle and 9.4 in old, and the longitudinal extent increased only 3 um with age (Robbins & Fahim, 1985). Therefore, we suggest that in SCM, the already highly regionalized junction in young mice was more readily compromised (more passive decay of depolarization in the more complex terminal arborization).

Mepp amplitude did not change with age in SCM (0.44 mV young vs. 0.49 mV in old, 46-47 fibers in 5-6 mice), even though it increased in muscles of C57BL mice (Anis & Robbins, 1984). However, extracellular recording of miniature endplate currents in directly visualized SCM junctions revealed that the decay time-constant was significantly lengthened from 1.1 (young) to 1.25 msec (old) (p<0.001, 30-33 fibers in 4-6 mice at each age). This resulted from a shift in a unimodal population of data. Thus, changes in receptor channel properties may be present in many aging neuromuscular junctions (see also Smith & Chapman, 1984) but are not always large enough to affect mepp amplitude.

We suggest that age changes at the neuromuscular junction, once initiated, may be qualitatively similar in different muscles. Reported physiological differences may result from special features of the initial (young) condition, e.g. functional complexity, or from the sensitivity of detection, e.g. voltage vs. current recording.

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426.9 EFFECTS OF EXERCISE ON RAT SKELETAL MUSCLE ACETYLCHOLINESTERASE MOLECULAR FORMS J.A. Donoso[1,2] E.J. Gregory*[1] and H.L. Fernandez[1,3] Neuroscience Research Laboratory, Veterans Administration Medical Center[1], Kansas City, MO 64128, and Departments of Neurology[2] and Physiology[3], University of Kansas Medical Center, Kansas City, KS 66103 (U.S.A.)

We have previously shown that aging skeletal muscles undergo a gradual decrease in the activity of acetylcholinesterase (AChE), a suitable marker of nerve-target cell interactions. In Fisher 344 male rat gracilis muscles such decay starts at 6-9 months of age and continues throughout the animal's life. Since motor endplate AChE is partly maintained by nerve-evoked muscle activity, we studied the effects of enhanced motor activity on AChE molecular forms from various skeletal muscles of 12 months old rats. Motor activity was increased via treadmill training (8.5 mt/min; 15 mt/min sprints every 10 min for 1h; 5 days/week) during 4, 5, and 6 weeks. After 12-15h of the last training session gracilis (fast), tibialis (fast) and soleus (slow), muscles were processed for separation of AChE forms in sucrose gradients. Under these conditions all AChE forms remained relatively constant except for 10S AChE which significantly increased (100% over control) in fast, but not in slow, muscles. Additional tests showed that the 10S AChE increase was directly related to the number of training sessions performed (30%, 60%, and 100% over control after 1, 2 and 3 days of training, respectively). While the mechanism(s) underlying this dramatic change is currently under investigation, the present findings indicate that the 10S AChE increase is a specific response (in relation to other AChE forms) of fast muscles to enforced exercise. Additionally, our results implicate this particular form of AChE as the main enzyme responsible for terminating the action of the neurotransmitter acetylcholine at the neuromuscular junction. (Supported by the Medical Research Service of the Veterans Administration)

426.10 DIFFERENTIAL KINETICS OF ACETYLCHOLINE SYNTHESIS AND CHOLINE UPTAKE IN FUNCTIONALLY DIFFERENT MUSCLES OF MATURE ADULT AND AGED RATS. M.R. Chapman* and D.O. Smith (SPON: D. Gilboe). Department of Physiology, University of Wisconsin, Madison, WI 53706.

In diaphragm muscles, there is an age-related increase in acetylcholine (ACh) efflux. This is balanced by a correspondingly faster ACh synthesis rate and, therefore, a similarly faster choline (Ch) uptake rate. In physiologically different muscles, however, similar changes in ACh metabolism might not occur. Indeed, age-related changes in glucose uptake, calcium homeostasis, and end-plate architecture in diaphragm have been found to be quite different from those in extensor digitorum longus (EDL). Therefore, we have assayed choline uptake, ACh synthesis, and release in EDL of Fischer 344 rats aged 11 and 27 months ("mature adult" and "aged", repsectively).

EDL muscles were incubated 30 min in oxygenated Krebs Ringer bicarbonate buffer containing eserine (50uM) and were stimulated at 1 Hz for 10 min to attain steady-state stimulation conditions. ^{3}H-Ch tracer was subsequently added to the bathing medium, and stimulation was continued for various durations up to 20 minutes. The muscles and the media were then removed. Muscle ACh and Ch were extracted using formic acid/acetone; both muscle and media ACh and Ch were then further concentrated using dipicrylamine ion-pairing. Levels of endogenous and labeled ACh and Ch were determined using reverse phase HPLC coupled with electrochemical detection and scintillation counting.

At equilibrium, tissue ACh and Ch levels were lower but specific activities were higher in the aged animals. The time to half-maximal ^{3}H-ACh production was 2-fold longer in the 27-month rats, but there was no significant age-related difference in the rate of ^{3}H-Ch uptake. The rate of ACh released into the medium was less, but the specific activity was higher in the aged animals.

During aging in EDL, slower ACh efflux is apparently coupled to reduced ACh synthesis. However, this is not accompanied by a corresponding decrease in ^{3}H-Ch uptake. Since tissue Ch levels are lower, we expect that Ch efflux must be greater in the aged tissue. This age-related shift in the balance between ACh production and Ch availability in EDL is different from that in diaphragm.

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426.11 MUSCLE MEMBRANE PHOSPHOLIPID AND CHOLESTEROL COMPOSITION IN MATURE ADULT AND AGED RATS. K.D. Williams* and D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

In rat diaphragm muscles, there is an age-related increase in acetylcholine (ACh) efflux, resulting in lower steady-state ACh levels. Specifically, membrane permeability to acetylcholine (ACh) increases 2-fold between the ages of 10 and 28 months. To explore the possible causes for this increased leakage during aging, membrane composition was assayed in diaphragm muscle obtained from Fischer 344 rats aged 10 ("mature adult") and 28 ("aged") rats.

Immediately after dissection, muscles were weighed, freeze-clamped, and stored in liquid nitrogen for subsequent analysis. Upon thawing, they were homogenized in distilled water and lyophilized to constant weight. Lipids were then extracted with hexane:isoproponol (3:2 v/v). The washed extract was evaporated to dryness, and the lipids were resuspended in chloroform/methanol (2:1 v/v). Phospholipids were separated by thin-layer chromatography, with 92 to 95% recovery, and the amounts of the various separated components were determined by a colorimetric phosphorus assay. Total and free cholesterol were determined by an enzymatic, colorimetric assay.

There were no significant age-related changes in total lipid phosphorus content. Likewise, the relative proportions of sphingomyelin, phosphatidylcholine, phosphatidylserine/inositol, phosphatidylethanolamine, and cardiolipin were unaltered with age.

In contrast, average (±s.e.) total cholesterol levels increased from 7.8 (±0.1) to 8.9 (±0.2) µmol/g dry wt in the 10- and the 28-month animals, respectively; this increase is highly significant (0.01 level). Similarly significant increases in free cholesterol and cholesterol esters contributed to this age-related elevation. Changes in membrane cholesterol of this magnitude might be expected to decrease membrane fluidity, and this may be related to the observed changes in permeability.

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426.12 EFFECT OF AGING ON NORADRENERGIC INNERVATION IN RAT URINARY BLADDER. J.M. Johnson*, L.J. Wallace* and M.C. Gerald. Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210.

Urinary incontinence, a frequent clinical problem in the aged, might result in part from a decrease in bladder sympathetic tone. Accommodation of increasing fluid volume by the urinary bladder is dependent upon the sympathetic release of norepinephrine (NE) which relaxes the bladder BODY (β-receptor activation) and constricts the internal sphincter or BASE (α-receptor activation). Noradrenergic neurochemistry was studied in BODY and BASE in bladders from male Fischer 344 rats aged 6-7 (I), 15-17 (II), and 22-24 (III) months.

BODY size increased with age; wet weights (I) 62.8±3.2, (II) 78.6±3.2 and (III) 88.8±3.4 (mg±SEM). BASE size did not differ with age. Protein content was equivalent for all groups.

BODY NE ranged from 0.32-0.39 and BASE from 3.56-4.44 ng/mg tissue. NE concentration did not change significantly with age in either region. NE turnover rate was estimated by measuring tissue NE content 4 hrs after α-methyl-para-tyrosine (AMPT). AMPT decreased BODY NE by 17-30% indicating a turnover index of approximately 0.023 ng NE/mg tissue/hr with no difference due to age. NE in BASE was not altered by AMPT, suggesting a very low turnover rate in this region.

Tyrosine hydroxylase (TH) activity in BASE ranged from 202-243 pmol/mg prot/hr in all groups and did not differ with age. TH activity in BODY was below detectable limits. BODY MAO activities for Groups I, II and III were 139.6±8.9, 119.0±13.8, and 119.8±12.2 nmoles/mg prot/hr±SEM. BASE values were 89.3±5.9, 81.4±9.0 and 71.1±7.0. The data suggest a decrease in MAO activity in both regions with increasing age. As before, BODY increased in size with age.

The combined data suggest little if any alteration in noradrenergic innervation of the bladder with increasing age. This is of particular interest since the BODY in 22-24 month animals is 30-40% larger in size when compared to 6-7 month animals, and a comparable decrease in NE content and MAO activity based on mg tissue might be expected. It is apparent that, as the BODY increases in size with age, the new elements are innervated. It is clear, however, that both noradrenergic innervation and sympathetic tone are regionally specific. NE concentration in BASE was approximately ten fold greater than that in BODY, and MAO activity in BASE was lower than that in BODY. The finding of a greater NE turnover rate in BODY when compared to BASE suggests the importance of sympathetic tone in the former region. (NIA grant AG04622)

427.1 CHARACTERIZATION OF NEUROTRANSMITTER PRODUCTION DURING NEURONAL DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS. S. Sharma* and M.F.D. Notter (SPON: C. Phelps). Dept. of Neurobiol. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Mouse embryonal carcinoma cells can be induced to differentiate into neuron-like cells in vitro. We have studied neurotransmitter production in the neurons formed, by analysing catecholamine (CA) production during retinoic acid-induced (RA) differentiation of P19-O1A1 cells.

Cells were induced to differentiate by growing for 2 days in monolayer culture in αMEM, supplemented with fetal and normal calf serum, in the presence of $5X10^{-7}M$ RA. They were grown for 2 more days in the same medium but as aggregates, and on day 5 the aggregates were plated on coverslips coated with 0.1% gelatin. The cultures were 11-14 days old when tested. At this stage, many cells had emerged from the aggregates, and appeared as a layer of neuron-like cells growing on a background of glia and fibroblast-like cells. The cultures were tested in the following ways: By immunocytochemistry for neuron-specific enolase (NSE) and neurofillament protein (NF), both of which are specific markers of neuronal phenotype; by immunocytochemistry for the enzymes a) tyrosine hydroxylase (TH), one of the enzymes involved in dopamine production, b) dopamine B-hydroxylase (DBH), which converts dopamine to norepinephrine and c) phenylethanolamine-N-methyl transferase (PNMT), which converts norepinephrine to epinephrine; by histofluorescence, using the modified glyoxylic acid method, which shows the presence of the catecholamines dopamine, and norepinephrine.

The neuron-like cells stained positively for NSE and NF. These results support the neuronal phenotype of these cells. In both cases the non-neuronal cell types showed no staining. There was positive staining for TH, DBH and PNMT in some, but not all of the neuronal cells. Some neuronal cells showed positive histofluorescence. The non-neuronal cell types showed no TH, DBH or PNMT staining, and no histofluorescence.

The positive TH, DBH and PNMT results suggest that these neurons have the machinery for synthesizing dopamine, norepinephrine and epinephrine. The histofluorescence shows that they do synthesize and accumulate dopamine and/or norepinephrine, though it cannot be concluded whether either of these is released or whether they are precursors for epinephrine production. The presence of PNMT does suggest that epinephrine is expressed. Not all of the neuronal cells show positive histofluorescence, or positive TH, DBH or PNMT staining. Other researchers have reported evidence for acetylcholine production by neuronal cells differentiated from the P19 cell line, so it would be interesting to see if acetylcholine and catecholamines are produced in the same cells, or if acetylcholine production is restricted to the catecholamine-negative population of neurons. This system is a useful one for studying mechanisms which direct the development of different neuronal cell types.

427.2 APPEARANCE OF PNMT mRNA IN THE FETAL RAT ADRENAL GLAND. M. Ehrlich, M. Evinger, G. Teitelman and T.H. Joh. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

During development of the rat adrenal medulla, a discrepancy has been reported between the ontogeny and initial expression of tyrosine hydroxylase (TH) and phenylethanolamine N-methyl transferase (PNMT). Migrating cells from the neural crest invade the fetal adrenal anlage at embryonic day (E) 15-E16. Immunocytochemically, TH appears in the adrenal anlage on E16, and at E11-E12, in thoracic ganglia at the level of the primordial adrenal and in migrating cells. PNMT, however (Teitelman et al., PNAS 1979; Bohn et al., Dev. Biol., 1981), does not appear in the fetal adrenal until E17.5-E18. We sought to determine whether the interval between the appearance of the TH and PNMT proteins during development of the adrenal is due to regulation of PNMT at the transcriptional or translational level.

The full-length 1100 bp bovine PNMT-cDNA (Baetge et al., PNAS, in press), inserted in the "antisense" orientation in the pSP65 vector, served as a template for transcription of an RNA probe. Linearization of the plasmid with HindIII, followed by transcription with SP6 polymerase in the presence of ^{32}P-UTP, results in synthesis of a full-length, RNA probe of high specific activity for PNMT mRNA. Northern blot analysis of total cellular RNA prepared from adrenal glands of animals of E16, E17, E18, E19, E21, postnatal day 7, and adult stages produced strong hybridization signals to PNMT mRNA beginning at E17. A weak hybridization signal was present at E16. In addition, a strong hybridization signal was consistently seen at approximately 5500ntd, even when the cDNA nick-translated PNMT probe was used. This band was not observed when hybridization of the PNMT probes was performed with bovine ribosomal RNA.

The strength of the hybridization signal at E17 warranted re-investigation of the appearance of PNMT protein. With the preparation of a new, specific PNMT antisera, the sensitivity of our immunocytochemical techniques was significantly improved. We then repeated the immunocytochemical study of the developing rat adrenal at selected embryonic stages. In contrast to previous reports, a large number of cells in the adrenal medulla stained positively at E17, and a smaller, but significant, number were positive at E16. Immunocytochemistry of the adrenal and its precursors remained negative for PNMT on E14 and E15.

These results indicate that PNMT mRNA and protein are actually present in the fetal rat adrenal as early as E16, and therefore, that the appearance of PNMT at this stage is regulated at the transcriptional level. Since this precedes the normal rise in endogenous steroids at E17-E18 and the establishment of splanchnic innervation, these observations confirm that these stimuli are not required for initial PNMT expression. We are presently investigating 1) whether PNMT mRNA can be detected by in situ hybridization prior to E16, and 2) what the relationship is between the approximately 5500ntd band and the 1100ntd PNMT mRNA.
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427.3 NEONATAL BASAL FOREBRAIN LESIONS IN MICE INDUCE ABNORMAL CORTICAL MORPHOGENESIS. C.F. Hohmann, A.R. Brooks, M.L. Oster-Granite and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The maturation of murine neocortex begins several days before birth and continues into the second postnatal month. The most dramatic progress in differentiation and establishment of cortical connections occurs during the first week postnatal (PN). While cells of increasingly more superficial cortical layers undergo rapid differentiation, many different fiber systems ascend into cortex and begin to form connections. Among them are projections that are rather specific for certain neuronal populations such as thalamo-cortical and cortico-cortical afferents. It appears to be necessary for cortical cells to be properly differentiated in order to be contacted by these axons. Differentiation of mutually connected cortical input and output systems must also be properly timed. It has thus been suggested that cortical cell differentiation during the early PN period is induced and regulated by the ingrowth of one or more modulatory afferent system(s) which include the NE, 5HT and ACh projections to cortex. The effects of norepinephrine depletion on cortical morphogenesis has previously been investigated with mixed results. In the present study, we examined the impact of lesions of cortical afferents in the basal forebrain on maturation in neocortex.

Mice receiving unilateral electrolytic lesions to their ventromedial globus pallidus, the primary source of cortical cholinergic innervation, at birth exhibited dramatic alterations in the morphogenesis of their ipsilateral fronto-parietal cortex. By 3-4 days PN, a disorganization of cortical layer V was apparent in Nissl stained preparations. The large pyramidal shaped neurons characteristic for this layer were reduced in number, unevenly distributed and often lacked their appropriate radial orientation. Many small, darkly Nissl-stained cells, not normally found in the differentiated layer V, were interspersed among the large neurons. By PN day 7, the disturbance of the layer V cytoarchitecture had become less striking but disorganization of more superficial cortical layers became apparent. At PN day 14, all cortical layers were discernable but subtle disturbances in cell density and shape persisted in Nissl stained sections, particularly in layers IV and V. Analysis of Golgi impregnated sections from PN day 30 and adult mice suggest that cortical cytoarchitecture remains abnormal.

Experiments are currently under way to establish whether the observed changes in cortical morphogenesis are due to an error in migration, differentiation or cell survival. Furthermore, the specificity of the cortical dysgenetic effects to cortical cholinergic afferents from the ventral medial globus pallidus are discussed in an adjacent poster.

427.4 CORRELATION BETWEEN LESION INDUCED ABNORMAL CORTICAL DEVELOPMENT AND DEFICITS IN CORTICAL TRANSMITTER MARKERS. A.R. Brooks, C.F. Hohmann and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In the previous poster, we have shown that neonatal electolytic lesions of the ventromedial globus pallidus (VGP), the primary source of cortical afferents, lead to abnormal morphogenesis of the ipsilateral neocortex. However, electrolytic lesions of the VGP are not entirely specific for the cholinergic neurons and may damage noradrenergic (NE) and serotonergic (5-HT) fibers traversing the area. The aims of the present study were to define the neurotransmitter specificity of the lesion and to correlate reductions in cortical neurotransmitter markers to the observable morphologic alterations.

Acetylcholinesterase (AChE) histochemistry was employed to assess morphologically the extent of the cholinergic denervation of the fronto-parietal cortex between postnatal (PN) day 3 and adulthood. Initially (PN day 3-5), the AChE depletion in cortex ipsilateral to the VGP lesion was marked and overlapped with the areas of abnormal cortical development. With increasing age of the animal, the AChE depletion became more subtle and enzyme levels became comparable to the contralateral hemisphere by approximately 30 days PN. Lesions not resulting in an AChE depletion also did not induce abnormal morphogenesis in cortex. Choline-acetyltransferase (ChAT) was measured between PND 4 and 30 to verify the cholinergic depletion indicated by the AChE decreases. ChAT activity was reduced by at least 29% during the first postlesion week, reduced by 25% between days 7 and 10 and only decreased by 10% between days 12 and 15. Cholinergic fiber ingrowth into cortex thus appeared to be transiently impaired rather than eliminated. The time course of presynaptic cholinergic marker recovery corresponded with the attenuation of morphological disturbances in cortical organization in Nissl stains.

NE and 5-HT levels were measured by HPLC with electrochemical detection during the first postnatal week. NE decreases in cortex ipsilateral to the lesion were erratic and varied between 0 and 70% depletion. However, 5-HT levels, measured in the same animals, were reliably reduced by 50% ipsilateral to the lesion. In light of the evidence that 5-HT may regulate cell cycle in neurogenesis (Lauder, J.M., Psychoendocrinology, 1983), we are presently investigating whether 5,7-dihydroxytryptamine lesions are able to affect cortical morphogenesis in a way comparable to the basal forebrain electrolytic lesions. Thus, VGP lesions of the neonatal mouse disrupt cortical morphogenesis. Neurochemical studies suggest a role for cortical cholinergic and/or serotonergic projections in the cortical dysgenesis.

427.5 PRESENCE OF NEURONS IN THE VAGUS NERVE OF THE NEWBORN RATS AND THEIR RESPONSE TO THE CAPSAICIN INJECTION. Zarco de Coronado, I. Vargas, H.L.*, Contreras, B., M.A.* and Coronado, M.R.* Depto. de Fisiología, Facultad de Medicina, UNAM. Apdo. Postal 70250, México 04510, D.F.

The contact of the ingested food with the digestive mucosa from the mouth on, immediatly evoques the transmission of signals to the enteric and central nervous systems. These signals are followed by the reflex responses which form the basic substrate for the regulation of the digestive function including the ingestive behavior. Because the newborn rats exhibit complex and adequate ingestive behavior it is interesting to study the neural structure of the alimentary canal and some of their chemical properties.

Esophagi of just born rats were used to make rapid Golgi staining. The 50µ slices observed by light microscopy showed a submucous and mienteric plexuses and also a great number of Kultschitsky cells (Costero y Barroso Moguel) at all levels of the esophagus. The adjacent vagus nerve presented neuronal cells along its course till the proximity of the stomach. In order to find out whether some of this neurons are sensitive or not to capsaicin the acuose extract of Capsicum annum was applied around the cervical vagus looking for changes in the body weight, as a sign of disturbed afferent information to the central nervous system. In fact, a significant reduction of body weight as compared with control animals, during the postnatal development, was the result.

These data permit the verification of the already described motor and sensory plexus in the walls of the esophagus in the newborn rat. The presence of the neural elements in the vagus suggests that neurons, whose origin could be placed in the brainstem follow this way to reach other portions of the digestive system. It is surprising that in the rat the migration of neurons continue after the birth. In humans, chickens and mice it occours only during the intrauterine life. The response to capsaicin aplication indicate that important afferences, SP mediated, to integrate the ingestive and metabolic components, necessary to the normal somatic development, were altered or eliminated.

Costero, I., Barroso-Moguel, R. Hipertensión de origen neoplásico. I. Tumor de células biliares del ovario. Arch. Inst. Cardiol. Mex. 60 (1) 52-67, 1970

427.6 Quantitative analysis of Purkinje cell dendrites in normal and Lurcher chimeric mice. K.W.T. Caddy, G. Fieldman*, and K. Herrup. Dept. Physiol. Univ. Coll. London, and Dept. Human Genetics, New Haven.

Heterozygous Lurcher mice (+/Lc) suffer a cell-autonomous postnatal degeneration of 100% of their cerebellar Purkinje cells (Caddy & Biscoe, Phil. Trans. Roy. Soc. London, B 287:167). In Lurcher <-> wild-type chimeras, the number of these cells present is intermediate to the number found in +/Lc and +/+ animals and all surviving Purkinje cells in the chimeras are of +/+ genotype. Cell counts of such chimeric cerebella have demonstrated that the ratio of granule cells to Purkinje cells is increased (by as much as 4.5 fold) above the values seen in normal animals (Wetts & Herrup, Dev.Brain Res. 10, 41). The present study seeks to establish whether the morphology of the dendrites of the remaining Purkinje cells is altered by this novel environment.

The Golgi-Cox technique was used to study dendritic morphology using either cornally or sagittally sectioned material. Purkinje cell body diameter, width of the dendritic tree and number of branch points on the dendritic tree were all measured using a drawing attachment on a Reichert microscope (150X and 400X). Statistical analysis was performed using an unpaired two tailed Student's t-test (Tektronix Plot 50 Software). The results show no significant difference in the Purkinje cell body diameter between the Lurcher chimeras and normals ($P>0.2$). However, dendritic tree width measured in coronal sections, shows a significant increase in the chimeras over the normal values ($P<0.001$). This indicates that, in the experimental animals, the Purkinje cells have lost the planar orientation exhibited in the normal. The number of dendritic branch points counted in sagittal sections was significantly less in the chimera than in the normal ($P<0.001$)and the lengths of the spiny dendrites proved to be equal in both cases ($P>0.7$). This indicates both a reduced dendritic area and total dendritic length in the chimera Purkinje cells relative to the normal.

Clearly the altered environment of the Lurcher chimeric cerebellum can affect the morphology of the wild-type Purkinje cells. The observed differences may result from an unforseen chimeric artifact, although this seems improbable since other neurones in the CNS appear normal. Analysis of Purkinje cell dendrites in wild-type<->wild-type chimeras will resolve this question. Despite the increased granule: Purkinje cell ratio, the dendrites of the Purkinje cells in these chimeric brains show clear evidence of reduced size and branching complexity.

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427.7 INTER-SPECIFIC MOUSE CHIMERAS TO STUDY WEAVER MUTANT GENE ACTION AND CELL LINEAGE IN THE CNS. D. Goldowitz, Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107.

The use of chimeric or teraparental mice, made from aggregating 2 four cell embryos of different parental genotypes, is a powerful technique to examine cellular interactions and lineage. The availability of a perfect cell marker (i.e., cell autonomous and marking all cells at all developmental stages) has been a major limitation of this technique. The new tools of molecular biology present special solutions to this problem. In particular, satellite DNA specific to Mus musculus but not to Mus caroli has been cloned and used as a cell marker by the technique of in situ hybridization of the cDNA on musculus ⟷ caroli chimeric tissue (Siracusa et al., JEEM 73:163-178). The present work uses this marking system to study the site of weaver gene action in homozygous weaver (wv/wv) mutant ⟷ caroli chimeras, and to examine patterns of cell lineage in the CNS.

Chimeras were made in a standard fashion. Adult chimeras were perfused with saline followed by a 3:1 95% ETOH: glacial acetic acid solution. Brains were removed and processed for paraffin embedding and sectioning. Sections of chimeric brain were hybridized with a cDNA clone that was complementary to either M. caroli or M. musculus satellite DNA sequences.

The most obvious expression of the weaver mutant locus, the wv phenotype, is the diminutive cerebellum due in a large part to extensive granule cell death. A two-step analysis was performed on 3 wv/wv⟷caroli chimeras. First, cerebellar regions were scored as to a +/+, intermediate or wv/wv phenotype. Second, the genotypes of granule, Purkinje and Bergmann glia cells were examined in these different phenotypic areas. The only cell type that had a clear correlation with the wv phenotype was the granule cell. This strongly supports the notion that the granule cell is a primary site of wv gene action in heterozygous (Goldowitz and Mullen, J. Neurosci. 2:1474-1485) and, now, homozygous mutants. Examination of wv gene action in relation to Purkinje and Bergmann glia cell phenotypes requires a finer analysis that is presently underway.

The cerebellum of interspecific chimeras had like-genotype cells gathered in larger ensembles than the fine-grained mosaicism found in conspecific chimeras, making the correlation of genotype with phenotype an easier task to accomplish. In other areas of the CNS, both limited (within a distinct group of cells, e.g. hippocampal formation) and more global (e.g., diencephalon vs. telencephalon) ensembles of like-genotype cells are found. This finding suggests that interspecific chimeras present an experimental system wherein cell lineage and/or sorting events can be examined.

427.8 CLONES AND POLYCLONES IN RETINAS OF XENOPUS INTERSPECIFIC CELL-INJECTION CHIMERAE. S. O'Gorman* and R.K. Hunt* (SPON: G. Evans). The Salk Institute, La Jolla, CA 92037.

To prepare chimerae in which genetically marked CNS progenitors were introduced into the presumptive CNS at low densities, the blastocoel roof of a Xenopus borealis (Xb) donor was isolated and placed in Ca and Mg free saline and 10 to 50 dissociated donor cells were injected into blastocoels of albino Xenopus laevis hosts. Donors and hosts were stage 7-9 blastulae; all manipulations were completed before segregation of the primary embryonic layers. Chimerae were sacrificed at early larval or postmetamorphic stages and serial paraffin sections were stained with quinacrine and examined under epifluorescent illumination to identify derivatives of the injected Xb cells on the basis of nuclear staining. Coherent patches of Xb cells, representing clones or polyclones descended from injected cells, were distributed throughout the CNS. CNS patch number sometimes exceded the number of cells injected, implying that (1) following incorporation into the presumptive CNS Xb cells divided and mixed with host cells, (2) a period of coherent growth followed mixing, and (3) some patches were clones descended from progenitors isolated before the onset of coherent growth. Cell-injection chimerae therefore represent a valuable approach for defining terminal lineages in a vertebrate CNS.

Retinal cytology was normal in all chimerae. Most retinal Xb patches were small (5-100 cells), populated all three nuclear layers, and were located within the proximal retinal regions that undergo early mitotic withdrawal. A few patches consisted of many thousands of cells and extended into peripheral retina as "pie slice" domains. These represented progeny of Xb cells that became established in the peripheral growth zone that generates neurons throughout larval and juvenile life. Thus, descendants of injected Xb cells participate in normal retinal development and their tangential distributions conform to known patterns of mitotic activity during retinal histogenesis.

Five patches (in three retinas from two chimerae) were found in which Xb cells were restricted to the ganglion cell layer. All five were located in the dorsal quadrant of proximal retina and, in light of published autoradiographic data, may represent the first retinal cells to withdraw from the mitotic cycle. Barring interspecific selection (which is unlikely because most Xb patches comprised multiple phenotypes), the data establish that some retinal progenitors produce lineages whose fates are restricted to a single laminar phenotype. The restriction could be dictated by temporal constraints: the first progenitors to enter terminal series of mitoses may produce vangards of postmitotic neurons that respond to local cues and become ganglion cells, the earliest phenotype to appear in the retina. Alternatively, the lineages derived from a few progenitors may more directly specify the cellular phenotype of the earliest ganglion cells.

427.9 EARLY POSTNATAL DEVELOPMENT OF GLIAL CELLS IN THE CANINE CERVICAL SPINAL CORD. K.Lord* and I.D.Duncan.* (SPON: M. Behan). Dept. of Med. Sci., School of Vet. Med., Univ. of Wisconsin, Madison, WI 53706.

This study was undertaken to provide a detailed analysis of normal glial cell development in the dog, and in addition to provide comparative data for ongoing investigations of abnormalities underlying myelin and glial cell development in the canine hypomyelinating mutant, the "shaking pup". To study the qualitative and quantitative changes in the glial cell population of postnatal dogs, the cervical spinal cord of 20 beagle pups, ranging in age from 1-28 days, was prepared for light and electron microscopy. Glial cells in the lateral corticospinal tract were classified according to morphological criteria as glioblasts, oligodendrocytes, astrocytes and microglia, and quantified using a stereological method designed to correct for sampling bias.

Glioblasts predominate in the first few days after birth, comprising 45% of the glia, and then decline to 3% by 28 days. This group comprises a variety of cell types, including very immature, possibly multipotential cells, and others that are clearly astroglial or oligodendroglial precursors. Oligodendrocytes can be subdivided into light and dark types, which differ in size, electron density, and in the amount and organization of their organelles. Light oligodendrocytes increase slightly prior to myelination, peak at 8 days (39%), and decrease slowly up to 28 days. Dark oligodendrocytes continue to increase throughout the 4 week period, and are the predominant glial cell type at 28 days (66%). The astrocyte and microglial cell populations remain relatively stable throughout the 4 week period.

These findings show that very immature, indistinct glial cell precursors coexist postnatally with distinct cell types in the spinal cord of the dog. Glioblasts appear to be differentiating primarily into oligodendroglia, since there is relatively little change in the astrocyte population. The growth of the oligodendrocyte population correlates with myelination in the lateral corticospinal tract, and both light and dark oligodendrocytes appear to be actively involved in this process. In light of the present findings, detailed quantitative analysis of glial cell populations may demonstrate some fundamental differences in postnatal glial cell development in the "shaking pup".
(Supported by the Kroc Foundation and NMS grant 1791-A-1)

427.10 GLIAL CELL DIVISION IN THE OPTIC NERVE OF THE MYELIN DEFICIENT RAT. KF Jackson*, JP Hammang*, ID Duncan* (SPON: V. Miletic) School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706

The myelin deficient (md) rat is a mutant of the Wistar strain, characterized by failure of normal CNS myelination. Previous studies have reported an absence of myelin in the optic nerves and have suggested a slow decline in the oligodendroglial population, compared to controls (Dentinger et al, 1985. Brain Res. 344 255-266). This study was designed to investigate glial cell proliferation in the optic nerve of the md rat. 4 md rats and 4 normal male littermates were pulse--labelled with tritiated thymidine prior to perfusion with Karnovsky's aldehyde fixatives at 6, 8, 12, 18, and 25 days of age. The optic nerves were removed and the intracranial segments processed into epon blocks. 1μ sections were taken for EM, and adjacent thick sections were taken for LM autoradiography. Labelling indices (LIs) were determined by counting labelled and unlabelled cells in complete transverse sections of the optic nerve. There were no significant differences between total cell counts in the md and control rats at the ages studied. At 6, 8, and 12 days, LIs for md and controls were not significantly different. At 18 and 25 days, LIs were significantly higher ($p < 0.05$) in the md rats. Labelled cells were distributed throughout the parenchyma in all animals. Preliminary studies suggest that dividing cells are of both astrocytic and oligodendroglial origin, in md and control rats at 12 days of age. Full identification and differential counts of dividing cells at all ages are presently underway. Dentinger et al reported an absence of myelin in the md rat optic nerve. This study indicates that at 8, 12, 18, and 25 days, myelinated axons do occur, and in clusters similar to those reported in the jimpy mouse, although the myelin is poorly compacted and has few lamellae. The raised LIs in the older md rats suggest a trend of protracted proliferation of glioblasts in the CNS, analogous to the situation described in the jimpy mouse (Privat et al, 1982. Dev. Brain Res. 2 411-416. Skoff, 1982. Brain Res. 248 19-31). There is no evidence of an increase, or reduction, of the glial cell population in the optic nerve. One possible explanation is that glial cell death may parallel increased cell division, and preliminary observations of dying cells indicate that this may be the case in the md rat, as has been reported in jimpy (Knapp, Skoff and Redstone, 1986. In press, J. Neurosci).
(Supported by NMSS grant 1791-A-1 to IDD).

427.11 IMMUNOHISTOCHEMICAL LOCALIZATION OF α2_GLYCOPROTEIN (α2GP) IN CULTURED GLIAL CELLS OF RAT. K.Arimoto*°,N.R. Bhat°+,E.G.Brunngraber°and K.Warecka*++,Neurochemistry Res. Unit°,Missouri Inst. of Psychiatry,Dept. of Biochemistry,Univ. of Missouri-Columbia,Sch. of Med., St.Louis MO 63139,Dept. of Biochemistry+,Univ. of Kentucky Med. Ctr.,Lexington KY 40536_0084 and Klinik fur Neurologie++ Medizinische Hochschule Lubeck,Lubeck, West Germany.

The brain specific glial α2GP has been thought to be involved in the process of myelin formation.α2GP,glial fibrillary acidic protein(GFAP),galactocerebroside(GC) and myelin basic protein(MBP),all of which are glial markers were identified in cultures of new born rat brain cells using immunohistochemical technique.The percentage of the glial cells that expressed each of these markers was related to accompanying morphological characteristics.A population of glial precursor cells expressed α2GP at an early stage of development.This cell population consisted of small,phase dark cells that proliferated in culture and were overlaid on the surface of flat epithelial-like astrocytes.These astrocytes contained GFAP but did not react with antibodies to α2GP,GC and MBP.The percentage of α2GP_positive cells to the total number of overlying cells decreased from 88% at 6 days of culture to 44% at 23 days of culture.The heterogeneity of these overlying cells became especially marked after 10 days of culture.Although ambiguous cell forms were present,two predominant types of overlying cells were distinguished morphologically after fixation.The smaller cells contained a few short processes and a dark nucleus.Among this population the proportion of α2GP_positive cells decreased from 96.7% at 6 days to 31.7% at 23 days.The larger cells had larger and more abundant processes and an eccentric nucleus.These cells stained positively for GFAP and α2GP.Of these cells,61.4% and 85.4% stained positively for α2GP at 6 days and 17 days,respectively. We tested mechanically separated overlying cells from primary culture of new born rat brain and found that 42.5% of the cells were α2GP-positive and 23.9% GFAP_positive.We also examined a glial cell line(C6) which also expressed α2GP.

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427.12 A RAT ASTROCYTE-SPECIFIC CELL-SURFACE ANTIGEN IDENTIFIED WITH A MOUSE MONOCLONAL ANTIBODY. Jerome R. Wujek and Richard A. Akeson, Children's Hospital Research Foundation, Cincinnati, OH 45229.

Cell-cell interactions between neurons and glial cells have been shown to play an important role in the development of the nervous system. Some glial-neuronal interactions appear to be mediated by cell-surface constituents of astrocytes (Noble et al., J. Neurosci. 2: 1892, 1984). The strategy we have chosen for identifying the molecular basis for these and other interactions during development is to use monoclonal antibody (Mab) technology. We used the following protocol to generate Mab's that are specifically reactive with cell surface antigens of astrocytes: Cultures of glial cells were obtained by dissociation of the cerebral cortex of day 1 postnatal Sprague-Dawley rats according to a modification of the technique of Barkley et al. (J. Cell Physiol. 81: 271, 1973). The glial cultures were further enriched to 91% astrocytes (astrocytic enrichment was determined by immunofluorescence with antiserum to glial fibrillary acidic protein) by use of a modified immuno-adhesion protocol (Wysocki and Sato, P.N.A.S. 75: 2844, 1978). CB6F1/J mice were immunized with suspensions of live astrocytes that had been removed non-enzymatically from the culture dish. Later, dissociated spleen cells from these mice were fused with a non-secreting mouse myeloma line as previously described by Akeson and Warren (J. Neurosci. Res. 12: 41, 1984). Antibodies from the resulting hybridomas were screened by ELISA against cultures of live astrocytes and live skin fibroblasts. Three separate candidate hybridomas were obtained that registered positively against astrocytes and negatively against skin fibroblasts. These three hybridomas were dilution cloned and then grown in mass culture. Mab's from these hybridomas were further tested for astrocyte-specific immunoreactivity by indirect immunofluorescence on live cultures of CNS (brain and spinal cord) and PNS (dorsal root ganglion) neurons, non-astrocytic glial cells (oligodendrocytes and Schwann cells) and also non-neural cells (meningeal cells and skin fibroblasts). Two of the candidate Mab's cross-reacted with cultures of live meningeal cells and thus were eliminated from further consideration. However, one clonal hybridoma cell line (designated Mab 54-3C4) did react positively with astrocytes, but did not display cross-reactivity with any of the other cell types tested. Thus, by these criteria, Mab 54-3C4 appears to react with an astrocyte-specific cell-surface antigen. In a double-labelling immunofluorescence experiment with Mab 54-3C4 and anti-GFAP, it was found that only a subpopulation of cultured cerebral cortex astrocytes were immunoreactive with Mab 54-3C4. In addition, this Mab may recognize an antigen which is developmentally regulated. Glial cells that had been dissociated from day 1 postnatal rats and cultured for 3 days exhibited little or no reactivity by immunofluorescence. But glial cells cultured for 7 days or longer showed a marked increase in cell surface immunoreactivity with Mab 54-3C4. These results may reflect the acquisition and/or increased expression of a cell-surface antigen that is involved with differentiation or maturation of astrocytes. Further work is being done to characterize the molecular nature of this astrocyte-specific, cell-surface antigen and to determine its localization and developmental regulation within the mammalian CNS.

427.13 PLASTICITY AND COMMITMENT IN OLIGODENDROCYTE DEVELOPMENT
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The optic nerve (ON) of neonatal rats contains a bipotential glial progenitor cell with the ability to develop into either an oligodendrocyte or a specific astrocytic subpopulation, the Type 2 astrocyte (Raff, et al., Nature, 303:390, 1983). Oligodendrocyte-Type 2 astrocyte (O-2A) progenitor cells are induced to divide when cultured in medium conditioned by Type 1 astrocytes (Noble and Murray, EMBO J., 3:2243, 1984), which are the first differentiated glial cell-type to appear in the ON (and which represent a seperate glial lineage).

Earlier studies on oligodendrocyte development showed that cells recognized by the O4 antibody include both oligodendrocytes and their precursors (Sommer and Schachner, Dev. Biol. 83:311, 1981; Neurosc. Let., 29: 183, 1982). What is the relationship between (O-2A) progenitor cells and cells labeled by the O4 antibody? We have found in neonatal rat optic nerve (ON) that:

1) O4-positive cells appear in development after the first recognizable O-2A progenitors. However, in ON cell suspensions of 7 day old rats (the age at which the O-2A progenitor has been most intensively studied), most cells with the antigenic phenotype of O-2A progenitors are also O4 positive.
2) Depletion of ON cell suspensions of O4-positive cells reduces the number of oligodendrocytes and also reduces the number of type 2 astrocytes which appear in subsequent cultures.
3) Expression of the O4 antigen is a necessary step in the development of oligodendrocytes but not of type 2 astrocytes. However, many type 2 astrocytes are O4 positive in culture.
4) Many cells prelabelled with O4 antibody in suspension are able to develop into type 2 astrocytes when cultured for 2 days in appropriate conditions, whereas GalC-positive oligodendrocytes prelabelled in the same way only rarely do so.
5) However, O4-positive cells of the 7 day ON are not stimulated to divide by astrocyte conditioned medium (ACM). This is in striking contrast to the intensive proliferation of O-2A progenitors seen in ACM.

These results demonstrate the existence of an antigenically definable intermediate stage between the O-2A progenitor and the oligodendrocyte, with properties different from both progenitor and oligodendrocyte.

427.14 AN ABNORMALITY IN THE CELL CYCLE OF OLIGODENDROGLIA FROM JIMPY MICE.
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In the jimpy (jp) mouse, an X-linked recessive mutation, the CNS of affected males is severely hypomyelinated. Female carriers of the jp gene (mosaics) have myelin deficits at early ages but recover by 30D (Bartlett and Skoff, 1986, J. Neurosci. In Press). Autoradiographic studies show that oligodendrocytes from jp animals incorporate ^{3}H-thymidine into their nuclei in numbers up to 10 times that found in normal animals (Skoff, 1982, Brain Res., 248: 19). ^{3}H-thymidine uptake in the synthesis phase (S) of the cell cycle is considered to be an index of mitotic activity. Accordingly, the numbers of mitotic figures in jp CNS should increase in correlation with the increased ^{3}H-thymidine labeling.

We counted both ^{3}H-thymidine labeled cells and mitotic cells between late prophase and telophase in 1µ plastic sections of jp, mosaic and control spinal cords. In normal spinal cords, at all ages and at different labeling indices, the ratio of labeled:mitotic cells was always close to 3:1. This 3:1 ratio reflects the fact that the S phase of the normal glial cell cycle is much longer than the mitotic phase (M). In 4D jp animals the ratio of labeled:mitotic cells was close to normal, but by 14-15D it had increased to approximately 8:1. By 20D, jp spinal cords had 10 times more labeled cells than mitotic cells. At all ages examined, the labeled: mitotic cell ratio in spinal cords of female carriers was normal.

The increased ratio of labeled:mitotic cells indicates that as jp animals age, the normal relationship between DNA synthesis and mitosis is eliminated. A number of changes in the cell cycle could account for this phenomenon. We have recently shown that oligodendroglial death is increased in jp CNS (Knapp et al, 1986, J. Neurosci. In Press). We believe that the elevated labeled:mitotic cell ratio might be due to the death of jp cells between S and M. Whatever the mechanism behind the cell cycle abnormality and the premature oligodendroglial death, data from the female carriers show that they are most likely secondary effects of the jp mutation. As mosaics, the females contain roughly equal proportions of normal and jp cells. If a cell cycle abnormality were the primary defect in jp, mosaics should exhibit a labeled:mitotic cell ratio intermediate between that of normal and jp. However, mosaic animals maintain a normal temporal relationship between DNA synthesis and division. Furthermore, oligodendroglial death is not increased in mosaics. These observations support the idea that oligodendroglia may not be the primary target of the jp mutation, and that myelin compensation in the mosaic provides an environment more compatible with oligodendroglial survival. The timing of onset of these abnormalities indicates that the differentiation of cells in jp animals which are committed to the oligodendroglial cell line is compromised at an early stage. Supported by NS 15338 and NS 18883.

427.15 ORIGIN OF RADIAL GLIAL CELLS IN MOUSE CEREBRAL CORTEX: A STUDY USING COMBINED IMMUNOHISTOCHEMICAL AND [^{3}H]THYMIDINE AUTORADIOGRAPHICAL LABELLING.
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Radial glial cells (RGC) comprise a class of cells in the developing brain which have their somata in the subventricular or ventricular zones (VZ) and extend a radial process to the pia. They are believed to play a critical role in guiding the migration of postmitotic neurons and to be a progenitor of protoplasmic astrocytes. In the monkey, immunocytochemical staining with antibodies to the glial fibrillary acidic protein (GFAP) has revealed that RGC co-exist with neuronal precursors in the VZ from early stages of neurogenesis and that they express this marker protein at least as early as a terminal metaphase (Levitt, Cooper and Rakic, J. Neurosci. 1:27,'81).

The monoclonal antibody, 8C7, appears to recognize a carbohydrate epitope concentrated in RGC of the mouse brain from as early as embryonic day E10 (Edwards et al, Neurosci. Abst. 12,'86). The present study in the mouse neocortex uses this immunohistochemical marker in combination with short-term [3H]thymidine autoradiographic labelling to address additional questions about the proliferation of RGC: 1) do RGC themselves serve as progenitors for additional RGC? and, 2) if so, does the generative cycle involve the same sequence of somatic translocations within the VZ as shown by neuronal stem cells?

One or two hrs following maternal injections of [^{3}H]thymidine on E15, the embryos were fresh-frozen. Cryostat sections taken through neocortex were stained immunohistochemically with 8C7 (HRP-conjugated anti-IgM; DAB reaction) and processed for autoradiography. In cases with a survival time of 1 hr, autoradiographic silver grains are detected over nuclei in the outer but not the inner half of the VZ, whereas after 2 hr some labelled nuclei appear in the inner half of the VZ. Some of these cells showing DNA synthesis also exhibit 8C7-immunoreactivity. Thus, the antigens recognized by 8C7 are expressed by some cells as early as the S and early G2 phases of the cell cycle. For a few of double-labelled cells, it was possible to identify an apical radial process, a finding consistent with the hypothesis that RGC generate additional RGC. The appearance of double-labelled cells in the deep VZ after a survival of 2 hr suggests that mitotically cycling RGC show the same to-and-fro nuclear translocations as neuronal precursor cells.

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427.16 PROLIFERATION OF SCHWANN CELLS ASSOCIATED WITH UNMYELINATED FIBERS IN RESPONSE TO PARANODAL DEMYELINATION.
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Recent studies have shown that vigorous Schwann cell proliferation results from paranodal demyelination and giant axonal swelling produced by β,β'-iminodipropionitrile (IDPN). Using electron microscopic autoradiography, we have previously found that Schwann cells associated with unmyelinated fibers, Schwann cells associated with myelinated fibers, and, as they are formed, supernumerary Schwann cells (not apparently associated with any fiber) undergo division. The present study was designed to identify the predominant source giving rise to new Schwann cells at early stages of this lesion.

Using four different labeling protocols, we have systematically injected [^{3}H] thymidine during IDPN intoxication. Using light and electron microscopic autoradiography, we identified the type and frequency of proliferating cells in L4 and L5 dorsal roots six days after IDPN intoxication.

Schwann cells surrounding unmyelinated fibers were the predominant proliferating cell type in dorsal roots. During or shortly after mitosis, these Schwann cells often surround unmyelinated axons; however, these axons are no longer separated by intervening Schwann cell cytoplasmic processes. Some labeled Schwann cell perikarya formed long, thin, stalk-like projections separating the nucleus from the regions containing unmyelinated axons.

These results raise the question as to how paranodal demyelination gives rise to a signal stimulating proliferation of Schwann cells in nearby unmyelinated fibers. We postulate either a diffusible mitogenic factor originating from nerve fibers undergoing paranodal demyelination or axonal sprouting at the nodal regions of affected axons. In this case, contact between sprout axolemma and Schwann cells might provide the mitogenic signal. In either case, it is clear that paranodal demyelination, not associated with myelin or axonal degeneration, provides a strong signal for proliferation of Schwann cells associated with unmyelinated fibers.